ORIGINAL ARTICLE

Identification and Antimicrobial Susceptibility Testing of Anaerobes Isolated from Clinical Specimens: A Study Re-emphasizing Role of the Neglected Bacteria Causing Significant Infections

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Abstract

Background: Anaerobic bacteria cause significant human infections but isolation, identification and antimicrobial susceptibility of these bacteria in routine microbiology is given least importance. Because of neglect towards this branch of microbiology, there is emergence of resistance to commonly used antibiotics. ***Purpose:*** To know the prevalence, isolation, identification and antimicrobial susceptibility testing of anaerobes from clinical samples in our institute. ***Material and Methods:*** A prospective study was done for identification and antimicrobial susceptibility testing of anaerobes by following standard methods. ***Results:*** Isolation rate of anaerobic bacteria found in our study was 28.96%. Predominant organism was *Peptostreptococcus species* (26.20%). Sensitivity to metronidazole in our study was 88.64%. *Bacteroides fragilis* showed resistance to penicillin in 22.22% isolates. There was no resistance observed to rest of the antimicrobials tested to any other organism isolated. ***Conclusion:*** This is the high time for microbiologists to show their interest in isolation with antimicrobial susceptibility testing with cooperation of clinicians to prevent anaerobic organisms to become "Super-bugs".

Key Words

Anaerobic pathogens, Emerging drug resistance, Anaerobic infections, Neglected bacteria

Introduction

Anaerobes and the infections caused by anaerobic bacteria were recognized since more than hundred years. But unfortunately, very less progress has been attained in isolation and identification with antimicrobial susceptibility of anaerobes from infections caused by these organisms that can be accredited to negligence and ignorance among the clinicians and clinical microbiologists towards anaerobes (1). Another reason which is contributing in neglect is long turnaround time of the isolation of the organism and treatment with broad spectrum antibiotics by the clinicians because resistance amongst pathogenic anaerobes is assumed to be low (2). But in today's scenario we cannot overlook anaerobic bacteriology as we are doing in the past because there is evidence for the increase in incidence as well as severity

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of infections, and emergence of drug resistance in the anaerobic bacteria which leads to therapeutic failure and poor outcome (3,4).

So, there is dire need to be properly identified the anaerobes with routine antimicrobial susceptibility testing of the anaerobes seems to be the need of the hour. This can be achieved by awakening interest and curiosity of microbiologist for anaerobic bacterial identification and their susceptibility testing. Clinical microbiologists should work together with clinicians to go a long way in reducing mortality and morbidity resulted due to anaerobic infections (5). By keeping in mind the above facts, we had planned a study with the aim to know the prevalence, isolation, identification with antimicrobial susceptibility testing of anaerobes from clinical samples in our institute.

Material and Methods

It was a prospective study conducted over a period of one year from November 2016 to October 2017. Ethical clearance was taken from Institutional Ethical Committee. The study was done on clinical samples collected from the patients suspected to have anaerobic infections. The samples were collected from different body sites affected like diabetic foot ulcers, head and neck infections, breast abscess, brain abscess, psoas abscesses, osteomyelitis, blood cultures, peritoneal aspirations. Samples received from different departments of hospital in the department of microbiology of tertiary care hospital of North India. Specimens including tissue, pus aspirate, body fluids, wound swabs. For diabetic foot ulcers the level of the lower extremity involved in infection were assessed based on Wagner's classification at the time of collection of samples (6). Specimens were transferred to laboratory in transport media i.e., Robertson cooked meat media (RCM) and processed as soon as possible as per standard protocol. Blood cultures for anaerobic bacteria were processed in automated blood culture system.

Gram staining of the smears was examined under the microscope and findings were recorded. Subculture was done from RCM after 24 hours of incubation. Anaerobic cultures were put up on Brain Heart Infusion agar which was supplemented with haemin and vitamin K, L-cysteine, yeast extract along with preliminary disks like metronidazole (5 ìg), vancomycin (5 ìg) and colistin (10 ìg) Sodium Polyanethol Sulphonate (SPS) discs. Incubation was done by using Gas Pack anaerobic culture method. Blood agar and MacConkey agar were put up for aerobic incubation as well. Aerobic plates were examined after 24 h while anaerobic plates were examined after 48-72 h and observed for any growth. RCM was kept reserved for backup cultures. Aero tolerance test was done from isolated colonies. Those organisms which failed to grow aerobically after 24 h on blood agar are considered as anaerobes. Further, processing was done and pure isolates were obtained as per standard procedure and these pure culture isolates were further identified by standard biochemical methods for anaerobic organisms (5,7). Antimicrobial susceptibility testing was done as recommended by Clinical Laboratory Standard Institute for anaerobes (8).

Results

The Study was conducted on 145 specimens with suspected anaerobic infections. Isolation rate of anaerobic bacteria found in our study was 28.96%. But predominant organism was *Peptostreptococcus species* (26.20%). All the anaerobic organisms which were isolated in the present study are shown in *Table-1*. Sites of isolation of anaerobic organisms are given in *Table-2*.

All the diabetic foot ulcers in our study fit in to Wagner's classification grade III, IV, V. But all the anaerobic isolates from diabetic foot were from Wagner's grade IV and V. Monomicrobial anaerobic growth was obtained from diabetic foot ulcers; burn wound infections, brain abscess, breast abscess, psoas abscess. Polymicrobials anaerobic growth and mixed aerobic and anaerobic growth was obtained from intraabdominal infections. (*Figure 1*)

Table-1: Anaerobic Organisms Isolated from Clinical Samples

Name of the Isolate	Number	Percentage
Gram positive cocci		
Peptostreptococcus species	38	26.20
Peptostreptococcus anaerobius	30	20.68
Peptococcus species	12	8.27
Gram negative cocci		
Veillonella species.	3	2.06
Gram negative bacilli		
Bacteroides fragilis	23	15.86
Prevotella species	12	8.27
Porphyromonas species	6	4.13
Gram positive bacilli		
Clostridium bifermentans	6	4.13
Clostridium septicum	1	0.68
Clostridium histolyticum	1	0.68
Bifidobacterium species	2	1.37
Propiniobacterium acne	2	1.37
Eubacterium species	9	6.20

Table-2: Various Sites of Isolation of Anaerobic Organisms

Sample site	Number of specimens	No. of samples with positive growth	Positivity Rate (%)
Diabetic foot ulcer	20	06	30
Burn wound	20	07	35
Brain abscess	3	01	33.33
Psoas abscess	5	02	40
Breast abscess	2	01	50
Appendicitis, pancreatitis, liver abscess	30	05	16.66
Peritoneal fluid	53	20	37.73
Bone tissues	3	0	0
Blood culture	9	0	0
Total	145	42	28.96

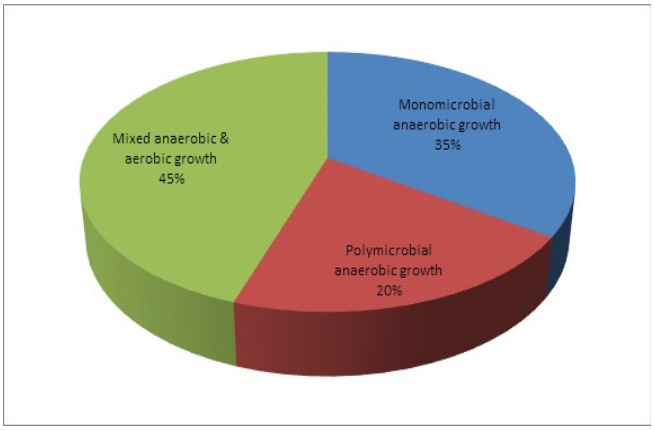

Figure 1: Showing Percentage of Mixed Anaerobic and Aerobic Growth from Clinical Samples

Antimicrobial susceptibility Sensitivity to metronidazole in our study was 88.64%. All the *Eubacterium species* and 19% of *Bacteroides fragilis* showed resistance to metronidazole. *Bacteroides fragilis* showed resistance to penicillin in 22.22% isolates. No resistance was observed by any other organisms to rest of the antimicrobials tested.

Discussion

The significance of identification and antimicrobial susceptibility of anaerobes has long been unheeded by the microbiologists as being the difficulty in culturing, long turnaround time and dearth of literature on drug resistance in anaerobes that prevent the progress of anaerobic bacteriology. Another reason was reluctance in sending samples by clinicians due to the routine use of antimicrobials with broad spectrum against anaerobes. Our study reemphasis the role of this neglected bacteria causing significant infections in humans and there is emerging antimicrobial resistance to anaerobic bacteria.

The prevalence of anaerobes in this study was 28.96%. Gram positive anaerobic bacteria predominant over gram negative anaerobic organisms. Same observations were reported by other studies also (9,10). But predominant organism was *Peptostreptococcus species.* The findings were in concordance with studies done by Garg *et al.* (11), Eslami *et al*. (12), and Murphy *et al.* (13). While there are studies which showed the *bacteroides fragilis* is the predominance anaerobic bacteria (14,15).

In our study, most of the anaerobes are isolated from intraabdominal infections, diabetic foot ulcers; burn wound infections, brain abscess. Other studies done from India showed varied isolation rate of anaerobic infections in their studies (16,17,18). All the anaerobes from diabetic foot ulcers were from Wagner's grade IV and V. It is observed that anaerobic pathogens are isolated from infections of the deeper tissue whereas aerobic pathogens are associated with superficial ulcers (11).

Monomicrobial anaerobic growth was obtained from diabetic foot ulcers; burn wound infections, brain abscess, breast abscess, psoas abscess. Most of the polymicrobial anaerobic growth and mixed aerobic and anaerobic growth was obtained from intraabdominal infections.

Most of the anaerobic isolates showed 100% sensitive to majority of the drugs in our study. Sensitivity to metronidazole in our study was 88.64%. All the *Eubacterium species* and 19% of *Bacteroides fragilis* showed resistance to metronidazole. In anaerobes, acquired resistance to metronidazole may be due to a combination of various and complex mechanisms. *Eubacterium species* is considered to be inherently resistant to metronidazole according to the literature search (7). In *Bacteroides fragilis* nim genes are now described with variable expression ranging from phenotypically silent to low-level or high-level resistance to metronidazole (19). *Bacteroides fragilis* also showed resistance to penicillin in 22.22% isolates.

Resistance to Metronidazole and penicillin showed less resistance in our study as compared to study done by Pednekar *et al.* (20), Reymundo *et al.* (21) and Cobo *et al.* (22). Isolation rate and antimicrobial susceptibility pattern can be varied in different geographical locale which depends upon numerous environmental factors.

In the present study, no resistance was observed by any other organism to rest of the antimicrobials tested (5).

So now a day, rise of anaerobic infections has resulted due to the development of resistance to the commonly used antibiotics against them. However, resistance is too developing amongst those anaerobic bacteria that were previously well thought-out to be highly susceptible to routinely used antibiotics which are further adding our trepidations about the emergence of multidrug resistance which we are facing for aerobic bacteria.

Conclusion

Routine culture and sensitivity of anaerobes isolated from clinical samples is the need of the hour. Resistance among anaerobic pathogens is emerging but it is still low. We need to act soon by wake up our interest for research in anaerobic bacteriology. Now is the critical time to prevent these organisms to become superbugs as their counterparts i.e., aerobic bacteria.

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Conflicts of Interest

There are no conflicts of interest.

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