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Letter to Editor

Microbiology Section

Isolation, Identification and Antimicrobial Susceptibility of Anaerobic Bacteria: A Study Re-emphasizing Its Role

RITU GARG[1], NEELAM KAISTHA[2], VARSHA GUPTA[3], JAGDISH CHANDER[4]

Dear Editor

Now-a-days, anaerobic infections are showing evidence of increasing virulence, rising incidence, unresponsiveness to metronidazole therapy and worse outcomes. Some of these infections are serious and have high mortality rate and can no longer be overlooked as in the past and need to be properly identified [1]. Anaerobic microbiology has fallen out of the spotlight of infectious disease, due to extraordinary efforts required to recognize these infections as also the availability of generally effective antimicrobials against these organisms [2]. Anaerobic culturing can be made cost efficient by strict adherences to several principles, including the selective culturing of only appropriate general specimens that are uncontaminated by normal flora; Rapid transport of specimens and use of appropriate transport system; use of a system of rejection when inappropriate and multiple samples have been received [3].

This was a prospective study conducted at a Department of Microbiology, Government Medical College and Hospital Chandigarh, India over a period of three years from June 2009 to June 2012. The study comprised analysis of 100 pus samples from patients suspected to have anaerobic infections from Diabetic foot ulcers, Head and neck, Breast, Brain, Psoas abscesses, Peritoneal aspirations, Vaginal swabs received from different Departments of hospital. These included 5 samples in 2009, 14 samples in 2010, 40 samples in 2011 and 36 samples up to June 2012.

The specimens were collected by aspiration in a sterile syringe or in the form of swab which were further transferred to transport media, Robertson cooked meat media (RCM). These samples were sent to the laboratory and processed immediately within 1-2 h of collection. Cultures were put up on brain heart infusion agar supplemented with haemin and vitamin K, L-cysteine, yeast extract with preliminary disks like metronidazole (5 µg), vancomycin (5 µg) and colistin (10 µg) sodiumpolyanethol sulphonate (SPS) discs for anaerobic incubation, Blood agar and MacConkey agar were put up for aerobic incubation. Incubation of brain heart infusion agar was done at 37°C for 48-72 h in anaerobic jar. Anaerobiasis was created by automated anaerobic system (Anoxomat). Aerobic plates were examined after 24 h. Anaerobic plates were examined after 48-72 h and observed for any growth. Colony characteristics were noted and staining was done and those organisms which were sensitive to metronidazole were considered as anaerobes. However some colonies also showed resistance to metronidazole. These individual colonies were inspected and aerotolerance test was done for each of them. Those organisms which failed to grow aerobically after 24 h on blood agar are considered as anaerobes. Pure culture isolates were identified by standard biochemical methods [3]. Antimicrobial susceptibility testing was done with various commonly used antimicrobial agents that are recommended by CLSI for anaerobes by the disc diffusion method [4]. Approval for study has been taken from ethical committee of the department.

Isolation rate of anaerobic bacteria was 19%. The predominant anaerobic bacteria were *Peptostreptococcus* species. (11%), followed by *Eubacterium* species (3%), *Clostridium* species (2%), *Veillonella* species (1%), *Bacteroides fragilis* (1%), *Prevotella* species (1%). Anaerobic isolates are shown in [Table/Fig-1].

The susceptibility of these bacteria was as follows: all the isolates were found to be sensitive to penicillin, clindamycin, imipenem, amoxycillin – sulbactam, piperacillin – tazobactum. Sensitivity to metronidazole was 84% (n-16/19). All the three isolates resistant to metronidazole were found to be *Eubacterium* species.

The importance of isolation and characterization of anaerobes has long been overlooked. Certain reasons being the difficulty in isolation, time required and low levels of drug resistance. In our own institution we initially found great reluctance in sending samples but over a span of 3 y the number of samples increased from a mere 5 to a maximum of 40 in 2011 and until June 2012 already 36 samples had been received. This has given a new impetus to our anaerobe section and has been indeed encouraging. Additionally, good isolation results have been achieved.

The prevalence of anaerobes in this study was 19%. The most commonly isolated anaerobe was Peptostreptococcus 11% followed by *Eubacterium* species 3%, *Clostridium* species 2% and *Veillonella* species, *Bacteroides* species, *Prevotella* species making up 1% each. The studies of Eslami et al., Colaycoet et al., reported *Peptostreptococcus* as the most frequently isolated anaerobe [5,6].

Most of the anaerobes were 100% sensitive to majority of the drugs; however, sensitivity to metronidazole was 84%. The resistance to metronidazole was shown by all the three *Eubacterium* species which are usually resistant to this drug despite the generally excellent activity of this drug [3]. The most frequently isolated antibiotic resistant anaerobe is *Bacteroides fragilis* due to beta-lactamase production [7]. The another study showed, metronidazole had the highest rate of resistance among anaerobes at a high 48% [8]. This poses a difficulty since metronidazole is a frequent choice for

Organism	Number	Total	Percentage (%)
Gram positive cocci			
Peptostreptococcus species. *Peptostreptococcus anaerobiasis*	9 2	11	11
Gram negative cocci			
Veillonella species.	1	1	1
Gram positive bacilli			
Clostridium species.	2	2	2
Eubacterium species.	3	3	3
Gram negative bacilli			
Bacteroides fragilis	1	1	1
*Prevotella*species.	1	1	1
Total	**19**	**19**	**19**

[Table/Fig-1]: Anaerobic isolates from samples

empirical anaerobic coverage over the other antibiotics. Another cause of concern is the high resistance rate to clindamycin. But in our present study, no resistance to clindamycin was noted by any of the isolates.

There is necessity for precautions to be taken for proper collection and transport of specimens where ever anaerobic bacteria are suspected to be the cause. For this trained paramedical staff is required. Therefore, the greatest challenge is to sensitize clinicians to send samples followed by the second hurdle of isolation, characterization and identification and routine sensitivity testing. Hence, clinicians and microbiologists working in tandem can go a long way in decreasing mortality due to anaerobic infections.

REFERENCES

[1] Finegold SM. Anaerobic infections in humans: an overview. *Anaerobe.* 1995;1:3-9.
[2] Bharadwaj R. Anaerobic microbiology: Time to rejuvenate. *Indian J Med Microbiol.* 2012;30:3-5.
[3] Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM. Wadsworth-KTL anaerobic bacteriology manual. 6th ed. Belmont, California: *Star Publishing Company,* 2003.
[4] CLSI. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria;Approved Standard - Eighth Edition, M11A8E standard 2012.
[5] Eslami G, Fallah F, Goudarzi H, Navidinia M. The prevalence of antibiotic resistance in anaerobic bacteria isolated from patients with skin infections. *Gene Ther Mol Biol.* 2005;9:263-68.
[6] Colayco CAS, Mendoza MT, Alejandria MM, Ang FC. Microbiologic and clinical profile of anaerobic diabetic foot infections. *Phil J Microbiol Infect Dis*. 2002;31: 151-60.
[7] Morya F, Lozniewskia A, Blandb S, Sedallianb A, Grollierc G, Girard-Pipaud F, et al. Survey of anaerobic susceptibility patterns: A French multicentre study. *Int J Antimicrob Agents.* 1988;10:229-36.
[8] Raymundo M, Mendoza MT. The microbiogic features and clinical outcome of diabetic foot infections among patients admitted at UP-PGH. *Phil J Microbiol Infect Dis.* 2002;31:54-63.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Government Medical College Hospital, Sector 32, Chandigarh, India.
2. Associate Professor, Department of Microbiology, Government Medical College Hospital, Sector 32, Chandigarh, India.
3. Professor, Department of Microbiology, Government Medical College Hospital, Sector 32, Chandigarh, India.
4. Professor and Head, Department of Microbiology, Government Medical College Hospital, Sector 32, Chandigarh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ritu Garg,
Assistant Professor, Department of Microbiology, Government Medical College Hospital,
Sector 32-B, Chandigarh-160030, India.
Phone : 09646121667, E-mail : dr_rittu07@yahoo.co.in

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