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Guest Editorial

Anaerobic microbiology: Time to rejuvenate

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Anaerobes were first discovered by Louis Pasteur in 1862. They made their first appearance in the clinical microbiology laboratory in 1893 when the first clinical isolate, *Bacteroides fragilis*, was isolated.[1] Subsequently, over the next three decades, anaerobes were documented to be the major causative agents of puerperal sepsis, lung abscesses and intra-abdominal sepsis.[2] However, not much research was done on the non-sporing anaerobes in the next few decades and the scientific community concentrated on the spore-bearing organisms causing invariably fatal diseases like tetanus and gas gangrene. One of the reasons for this could have been the increasing number of these cases observed in soldiers during the world war. Difficulty in culturing the anaerobes and lack of standardisation in nomenclature prevented progress in the field of anaerobic microbiology.

The year 1965 marked the start of the Renaissance of anaerobic microbiology, largely spearheaded by Sidney Finegold, who is often referred to as the father of anaerobic microbiology. In India, interest in anaerobic microbiology started a little later but soon caught up, and by the 1980s, anaerobes had been cultured from all types of infections, starting with brain abscesses, otitis media, oro-dental infections, cutaneous abscesses, lung abscesses, intra-abdominal sepsis, pelvic infections, etc.[3-5] Anaerobic microbial bacteriology went from a period of intense neglect to a period of intense activity. Anaerobic infections were treated largely with clindamycin and metronidazole.[6] In India, the latter was the choice for treatment of anaerobes. As anaerobic infections were better diagnosed and treated, metronidazole was used for any infection where an anaerobe was detected. With the availability of intravenous metronidazole, it became a standard in the management of the critically ill patient also.

Once the aetiology and clinical manifestations of anaerobic infections were documented, anaerobic microbiology again took a backseat in most clinical microbiology laboratories. Several factors were responsible for this. Anaerobes required properly collected and transported samples before they could be isolated and often the persistence of the microbiologist was tested when growing and identifying anaerobic bacteria. Hospital administrators also felt that anaerobic microbiology was not cost effective. The cost of an anaerobic culture and sensitivity was five times that of an aerobic culture and sensitivity.[1] Empirical treatment for anaerobes became a routine practice.

Anaerobic bacteria, however, not to be outdone, made a comeback in healthcare settings. Firstly, in the form of hospital-acquired *Clostridium difficile* infections. It is the only endogenous anaerobe which is easily transmissible from patient to patient and has thus resulted in several outbreaks of *C. difficile* infections in Europe, USA and other developing countries over the last two decades.[7,8] The beginning of the current year witnessed a massive outbreak of *C. difficile* diarrhoea in 10 Canadian hospitals.[9]

The emergence of a new, highly toxic strain of *C. difficile* caused geographically dispersed outbreaks in North America and the United Kingdom.[10] It was felt that these outbreaks occurred due to increasing usage of fluoroquinolones in the hospital scenario. This epidemic strain has increased virulence, antibiotic resistance, or both. Reports of hospital-acquired *C. difficile* infection from India are few and infrequent. Does this reflect the fact that the organism is not a major hazard in Indian hospitals or is it due to clinical microbiology laboratories in the country relaxing their surveillance of anaerobic infections such as these?

In India, infrequent reports of *C. difficile* infections have been documented and the incidence of *C. difficile* diarrhoea has varied from 7.1 to 26.5%.[11,12] No outbreaks in Indian hospitals have been reported to date in spite of the fact that fluoroquinolones are extensively used in this country. However, we must keep in mind that very few centres are actually performing cultures for this organism and many of the present reports are based on toxin studies. Anaerobic

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stool culture is still the gold standard for diagnosis of *C. difficile* infection. It essentially provides a clinical isolate which can be used for typing to study the epidemiological pattern of the disease. It can also be used to monitor antimicrobial susceptibility patterns and develop vaccines.

Secondly, an upsurge of anaerobic infections has also resulted due to the development of resistance to the commonly used anti-anaerobic drugs. The drugs active against the majority of anaerobic bacteria are the nitroimidazoles, carbapenems, chloramphenicol, and the combination of β-lactam/β-lactamase inhibitors. Cefoxitin, clindamycin and broad-spectrum penicillins have also been reported to have activity against anaerobes, but are less efficacious.

Resistance among anaerobic pathogens was thought to be low. However, the susceptibility patterns of anaerobic bacteria are undergoing changes and decreased *in vitro* susceptibility to various antimicrobials has been reported in recent years.[13] The most frequently isolated antibiotic-resistant anaerobe is *B. fragilis*. However, resistance is also seen among anaerobes that were previously considered to be highly susceptible to antibiotics, raising concerns about appropriate empirical therapy.

Variability in resistance may be regional or temporal. β-lactamase production has been reported in many *B. fragilis* isolates. Lower susceptibility rates have been noted with penicillin G, clindamycin and cefoxitin. Up to 90% of isolates of the *B. fragilis* group have been shown to be β-lactamase producers.[14] These isolates may be reported as sensitive if β-lactamase production is not specifically looked for.

Resistance to metronidazole is also on the rise. At the turn of the century, only six metronidazole-resistant isolates were recorded.[1] The practice in many laboratories of identifying obligate anaerobes by susceptibility to metronidazole is a factor that contributes to probable underestimation of true resistance rates. A general decrease in susceptibility to metronidazole has been displayed among anaerobes. An increasing number of clinical failures with metronidazole treatment of *C. difficile* infection has been reported during the past few years. Resistant rates up to 63% have been reported with metronidazole in clinical anaerobic isolates.[15]

This poses a problem since metronidazole is a frequent choice for empirical anaerobic coverage over the other antibiotics. Low-level metronidazole-resistant strains may be overlooked because the breakpoint of 32 mg/L that was set by the Clinical and Laboratory Standards Institute (CLSI) is much higher than the 4 mg/L cutoff level for strains isolated in the community. In a short unpublished study from our institute, in which the resistance of metronidazole was evaluated using E-test, 40% of the anaerobes showed resistance to metronidazole, i.e. they showed minimum inhibitory concentration (MIC) of >32 μg/mL.

Specific resistance genes (*nim*) conferring resistance to nitroimidazoles have been isolated in different genera of gram-positive and gram-negative anaerobic bacteria, including *Bacteroides* species. The *nim* genes encode an alternative reductase that can convert nitroimidazole to a nontoxic derivative, thereby circumventing the toxic effect that causes breakage of the DNA.[16] So far, seven members of the genes – from *nim*A through *nim*G – have been detected. In the light of the emerging resistance in anaerobes, routine antibiotic susceptibility of clinical isolates is becoming important, especially in clinical settings where there is inadequate response to empirical therapy. Association between antibiotic-resistant *B. fragilis* and adverse outcomes has been documented.[17]

This should make us realise that it is high time susceptibility testing of anaerobes be undertaken by clinical microbiology laboratories. This has always been an arduous task. However, with a special set of guidelines for anaerobic sensitivity introduced by CLSI standardisation, it no longer remains an issue. Agar dilution methods are to be preferred to disc diffusion methods. This tends to be tedious in a clinical microbiology laboratory. The introduction of automated systems for testing anaerobic sensitivity would definitely expedite the results and benefit the patient. Easy availability of E-test strips could make the task of the clinical laboratory easier.

Interest in anaerobic microbiology has waxed and waned. However, in the light of emerging evidence, it is imperative that clinical microbiology laboratories remove the dust from their anaerobic jars and make a fresh attempt to isolate and identify anaerobes from clinical infections. Most clinical laboratories are not proficient in isolating and identifying anaerobes, and many do not even try or do only a minimal workup. Even fewer laboratories are doing antimicrobial susceptibility testing of anaerobes. We need to act soon to benefit from the advantage we still have over these organisms.

Routine sensitivity testing of clinical isolates of anaerobes seems to be the need of the hour. Unless judicious use of antimicrobials is planned for the anaerobic bacteria based on their sensitivity patterns, they will soon follow their counterparts, the aerobes, in developing into “super bugs” which do not respond to commonly used drugs. It is a critical time for clinical microbiologists. We must reinvigorate our interest in these pathogens to prevent future clinical disasters from resistant microorganisms.

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