Review Article *ISSN: 2277-8713*
Biswajit Batabyal, IJPRBS, 2012; Volume 1(5):533-538 *IJPRBS*

ANAEROBIC DENTAL INFECTIONS AND ADVANCE LABORATORY DIAGNOSIS: A MINI REVIEW

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Accepted Date:
22/09/2012
Publish Date:
27/10/2012

Keywords
Anaerobes,
Dental infections,
Culture,
Molecular techniques

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Abstract

Anaerobes occur as major components of bacterial flora of the human skin and mucous membranes. They are responsible for a variety of serious and life-threatening infections. Anaerobes make up a significant part of the oral and dental indigenous and pathogenic flora. Their role in periodontal disease, root canal infections and infections of the hard and soft oral tissue, as well as their importance as foci for disseminated infectious disease is well established. This paper will highlight the molecular techniques used to identify anaerobic bacteria from anaerobic dental infections. Recovery from an anaerobic infection depends on adequate and rapid management. The main principles of managing anaerobic infections are neutralizing the toxins produced by anaerobic bacteria, preventing the local proliferation of these organisms by altering the environment and preventing their dissemination and spread to healthy tissue.

INTRODUCTION

Anaerobic infections are caused by anaerobic bacteria. Anaerobic bacteria do not grow on solid media in room air (10% carbon dioxide and 18% oxygen); facultative anaerobic bacteria can grow in the presence as well as in the absence of air. Microaerophilic bacteria do not grow at all aerobically or grow poorly, but grow better under 10% carbon dioxide or anaerobically. Anaerobic bacteria can be divided into strict anaerobes that cannot grow in the presence of more than 0.5% oxygen and moderate anaerobic bacteria that are able of growing between 2 to 8% oxygen.[1] Anaerobic bacteria usually do not possess catalase, but some can generate superoxide dismutase which protects them from oxygen.

The clinically important anaerobes in decreasing frequency are:[2] 1. Six genera of Gram-negative rods (*Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila* and *Sutterella* spp.); 2. Gram-positive cocci (primarily *Peptostreptococcus* spp.); 3. Gram-positive spore-forming (*Clostridium* spp.) and nonspore-forming bacilli (*Actinomyces, Propionibacterium, Eubacterium, Lactobacillus* and *Bifidobacterium* spp.); and 4. Gram-negative cocci (mainly *Veillonella* spp.).

The frequency of isolation of anaerobic bacterial strains varies in different infectious sites.[3] Mixed infections caused by numerous aerobic and anaerobic bacteria are often observed in clinical situations.

Anaerobic bacteria are a common cause of infections, some of which can be serious and life-threatening. Because anaerobes are the predominant components of the skin's and mucous membranes normal flora, they are common cause infections of endogenous origin.[4] Because of their fastidious nature, anaerobes are hard to isolate and are often not recovered from infected sites. The administration of delayed or inappropriate therapy against these organisms may lead to failures in eradication of these infections. The isolation of anaerobic bacteria requires adequate methods for collection, transportation and cultivation of clinical specimens.[2] The management of anaerobic infection is often difficult because of the

slow growth of anaerobic organisms, which can delay their identification by the frequent polymicrobial nature of these infections and by the increasing antimicrobial resistance of anaerobic bacteria to antimicrobials.[1]

Anaerobes make up a significant part of the oral and dental indigenous and pathogenic flora. Their role in periodontal disease, root canal infections, infections of the hard and soft oral tissue, as well as their importance as foci for disseminated infectious disease is well established. Despite the ubiquitous involvement of bacteria, significant progress in our understanding of specific microbial etiologies has occurred only in the past decade. Common anaerobic isolates include Fusobacterium, Bacteroides, Actinomyces, Peptococcus, Peptostreptococcus, Selenomonas, Eubacterium, Propionibacterium, and Treponema. Recently, several significant advances in our knowledge have set the stage for future research. First, circulating levels of hormones in pregnant women were shown to be stimulatory to Bacteroides species, which were associated with increased levels of gingival infection. Second, bacterial invasion of the soft and hard periodontal tissues has been documented in gingivitis, advanced periodontitis, and localized juvenile periodontitis. The frequency and identity of invading bacteria will determine the implications for diagnosis and treatment. Third, antibacterial "probes" aimed at anaerobic (and capnophilic) bacteria have had promising results in controlling and arresting oral, dental, and peridontal anaerobic infections.

Culturing anaerobes

Since normal microbial culturing occurs in atmospheric air, which is an aerobic environment, the culturing of anaerobes poses a problem. Therefore, a number of techniques are employed by microbiologists when culturing anaerobic organisms, for example, handling the bacteria in a glove box filled with nitrogen or the use of other specially sealed containers, or techniques such as injection of the bacteria into a dicot plant, which is an environment with limited oxygen. The Gas Pak System is an isolated container that achieves an anaerobic environment by the reaction of water with sodium borohydride and sodium bicarbonate tablets to produce hydrogen gas and carbon dioxide. Hydrogen then

reacts with oxygen gas on a palladium catalyst to produce more water, thereby removing oxygen gas. The issue with the Gaspak method is that an adverse reaction can take place where the bacteria may die, which is why a thioglycollate medium should be used. The Thioglycollate supplies a medium mimicking that of a Dicot, thus providing not only an anaerobic environment but all the nutrients needed for the bacteria to thrive.[5]

New diagnostic methods for anaerobic bacteria

The use of DNA probes and monoclonal antibodies as new diagnostic methods are described in this review. For each technique, the principles for preparation of reagents and the different immunological methods, in which monoclonal antibodies are used, are described. Specificities and sensitivities are discussed with special reference to anaerobic bacteria or their toxins. The two groups of methods are compared as rapid diagnostic methods for direct detection of anaerobes in patient samples. If we consider DNA probes, the radiolabelled are more sensitive and more specific, but results are obtained only after some days. Non-radioactive probes are less sensitive, but amplification procedures greatly enhance their sensitivity. Monoclonal antibodies give rapid results but their high specificity may be a disadvantage when the technique is used for rapid diagnostic purposes on patient samples. This problem may be reduced by the use of pools of several monoclonal antibodies[6].

In order to assess the rapid laboratory diagnosis of anaerobic pyogenic infection, that compared the results of Gram stains, ultra-violet fluorescence and gas chromatography, all performed directly on pus, with those of anaerobic culture. Fluorescence was most rapid but there were many negatives unless *Bacteroides melaninogenicus* was present. Gas chromatography was rapid and sensitive but there were some false negatives, often in pure *Bacteroides fragilis* infection, and a few false positives. Gram-staining was also rapid, but only helpful on its own when there were large numbers of organisms of mixed or characteristic morphology. The three methods together almost always provided a reliable and rapid presumptive diagnosis of anaerobic pyogenic infection[7].

CONCLUSION

There is a need to urgently address deficiencies in the diagnostic service for anaerobic dental infections. There have been many advances in methodology for anaerobic diagnosis and earlier diagnosis is of value clinically, and through the early institution of appropriate drug therapy is of public-health benefit.

While, at present many of these techniques are only economically viable in the developed nations, it is to be hoped that recent advances will lead to the development of novel diagnostic strategies applicable to use in developing nations, where the burden of anaerobic dental infections is greatest and effective intervention most urgently required. Recovery from an anaerobic infection depends on adequate and rapid management. The main principles of managing anaerobic infections are neutralizing the toxins produced by anaerobic bacteria, preventing the local proliferation of these organisms by altering the environment and preventing their dissemination and spread to healthy tissue.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance and guidance provided by Dr. ChandraNath Majumder and Prof. (Dr.) T.K. Saha, Director cum Principal of Gurunanak Institute of Dental Science and Research, Panihati, Kolkata-700114, West Bengal for permission to do the work in Gurunanak Institute of Dental Science and Research.

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