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Microbiological Characteristics of *Bacteroidesspp.* in the Composition of Endodontic Infections

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Abstract: ***Anaerobic non-spore-forming gram-negative bacteria colonize the human body by a large number, their primary function being to stabilize the normal resident microbiota. They prevent colonization by pathogenic microorganisms from exogenous sources, participate in the digestion of food and stimulate host immunity. These microorganisms are primarily opportunistic pathogens involved in endogenous infections, usually in polymicrobial associations of aerobic and anaerobic bacteria. About 10 – 12 strains of anaerobic bacteria are most commonly isolated from infected root canals and associated with apical periodontitis (Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Veillonella). In this review are presentedthe microbiological characteristics of anaerobic gram-negative, non-spore forming bacteria of the genus Bacteroides that are commonly isolated in endodontic studies.***

Keywords: non-spore-forming bacteria, anaerobic infections, gram-negative bacteria, endodontic infections, periodontitis, Bacteroides

1. Introduction

At the root of the infected necrosis of the dental pulp is a mixed bacterial infection with up to 10^6 microorganisms, 90% of which are obligate anaerobes. These microorganisms penetrate into the dentinal tubules as deeply as 200 μm, and others, such as *Enterococcus faecalis*, even as deeply as 700 μm (19). The most important gram-negative rod-shaped anaerobes in infected root canals are of the genera *Bacteroides*, *Parabacteroides*, *Porphyromonas*, *Prevotella* and *Fusobacterium.* These obligate non-spore-forming rods are the predominant bacteria on most mucosal surfaces in humans, exceeding the microbial count of aerobic bacteria by 10 to 1000 times (20).

The genus *Bacteroides* consists of more than 90 species and subspecies, the most important representative being *Bacteroides fragilis.* It is one of the main microbes in the contents of the lower intestinal tract. It is also often found on the mucous membrane of the oral cavity. Other representatives of this genus are *B.serpens*, *B. niger*, *B. buccae*, *B. oris*, *B. oralis*, *B. gingivalis*, *B. denticola*, *B. pneumosintes*, *B.coagulans*, *B. asaccharoliticus*, *etc. Bacteroides oralis* is usually isolated from the gingivalsulcus, the respiratory tract and the urogenital system. It's role in human pathology has not been fully studied yet. *Bacteroides melaninogenicus* is normally found in the oral cavity, the faeces and the urine. Individually or in association with other types of microorganisms, it is found in oral, respiratory, intestinal and urogenital infections (2, 3).

B. buccae, *B. intermedius*, *B. denticola*, *B. oris*, *B. oralis* and *B. gingivalis* are some of the most commonly isolated microorganisms in endodontic infections compared to other representatives of the genus *Bacteroides.* Studies show that their presence is associated with teeth with acute symptoms. Acute symptoms can persist for up to a week after the treatment was started (16).

Representatives of *Bacteroides* are mainly isolated from teeth with a necrotic pulp, as well as in apical periodontites with abscesses (23). In such cases, the predominant isolates are *Fusobacterium nucleatum* (60%), *Porphyromonas endodontalis* (53%), *Parvimonas micra* (51%), and *Streptococcus species* (45%) (22).

The incidence of the black-pigmented gram-negative anaerobe isolates in endodontic infections varies between 25% and 50% (17).

Prevotella and *Porphyromonas* are black-pigmented gram-negative obligate anaerobes belonging to *Bacteroides* and are also isolated mainly from teeth with a necrotic pulp and a primary endodontic infection (21).Their identification is significantly higher through the use of PCR methods in comparison to methods using cultures (11, 15).

2. Physiology and Structure

Bacteroides species are pleomorphic in size (2 to 200 μm) and in shape, non-motile or motile peritricha and resemble a mixed population of microorganisms in a Gram stain. Most Gram-negative anaerobes respond poorly to Gram staining, so stained samples should be carefully examined. Often, they exhibit bipolar staining. They reproduce at an optimum temperature of 37°C, but can propagate within the range of 25° – 45°C. The pH of the medium optimal for their development is 7.0. A common characteristic of most species in the *Bacteroides* genus is that their growth is stimulated by bile. On solid nutrient media (blood agar), they create 1 to 3 mm big circular, transparent or translucent colonies. Although *Bacteroides* species grow rapidly in cultures, the other anaerobic gram-negative rods are demanding with regard to cultures and cultures may need to be incubated for 3 or more days before bacteria are detected (2, 3, 20). They decompose carbohydrates to a gas-free acid and they do not liquefy gelatin.

Bacteroides have a cell wall structure that is characteristic of gram-negative bacteria and is surrounded by a ***polysaccharide capsule.*** The capsule is a major virulence factor, it has antiphagocytic activity and is the cause of

abscess formation. A major component of the cell wall is surface lipopolysaccharides (LPS). Unlike the LPS molecules in aerobic gram-negative rods, the LPSs of *Bacteroides* have little or no endotoxin activity. This is because the lipid component A of LPSs does not contain phosphate groups on the glucosamine residues and the number of fatty acids associated with amino-sugars is reduced. Both factors correlate with the loss of pyrogenic activity (20). Some strains of *Bacteroides fragilis* produce *enterotoxin* (25). *Bacteroides spp.* release collagenase, neuraminidase, DNase and proteases. *Bacteroides fragilis* forms β-lactamases.

Pathogenesis and immunity

Bacteroides fragilis, as well as other *Bacteroides* species, can be attached to epithelial cells and extracellular molecules (e.g. fibrinogen, fibronectin, lactoferrin) via fimbriae. Fibrinolysin and collagenase are ***invasion factors*** in *Bacteroides spp.*

The capsule of the members of *Bacteroides* is also antiphagocytic, similarly to other antibacterial capsules, and is the major virulence factor of *Bacteroides fragilis.* Short-chain fatty acids (e.g. succinic acid), produced during anaerobic metabolism, inhibit phagocytosis and intracellular death (20).

Bacteroides fragilis is the most commonly isolated anaerobic microorganism in soft tissue infections. It is found individually or in microbial associations in surgical wounds, blood, heart valve infections, urogenital tract infections, rectal abscesses, etc. (2, 3). Injected subcutaneously into laboratory animals (rabbits, guinea pigs and mice), *Bacteroides fragilis* causes abscesses.
Endogenous infections, involving *Bacteroides*, are characterized by polymicrobial associations. Studies have found that microbial populations or the microbiomeof healthy mucosal surfaces differ in composition from those found in a medical condition. In a pathological condition, microbial associations undergo changes and are characterized by lower species diversity and predominance of microorganisms that are most relevant clinically. For example, *Bacteroides fragilis* is usually associated with pleuropulmonary, intra-abdominal (abscesses and peritonitis) and urogenital infections. *Bacteroides fragilis* comprises less than 1% of the normal intestinal microbiota. It is rarely isolated from the mucosa of the oropharynx or the genital tract in healthy individuals, and this is only when highly selective techniques are used (8, 20).

Microbiological test

The clinical study materials are secretions and punctates. Materials are transported in anaerobic media to exclude the possibility of experiencing the damaging effect of oxygen (Amies and Stuart transport media) or sterile syringes with aspirates are used for transportation and immediately sent to a microbiology laboratory. In some cases, the plating is done on site. Direct microscopy is done if the material is taken from a sterile area. Cultivation is carried out under laboratory, anaerobic conditions, on nutrient media (Zeissler and Schaedler, a fluid thioglycollate medium) at 37°C for 7 – 10 days. The identification is performed on the basis of the morphology of the microorganisms, their oxygen tolerance, biochemical activity, antibiotic resistance, etc. (2, 3, 8).

Management of endodontic infections

In endodontic treatment, the most important step is to remove the pain symptoms of the patient and reduce root canal and peri radicular tissue infection. To do this, the inflamed and infected root canal content is removed. If necessary, a surgical intervention with an incision and drainage is initiated. After the pain symptoms have subsided and the acute phase of inflammation has passed, the mechanical and chemical treatment of the dentin on the root walls is performed. There is enough evidence from clinical practice and the literature in favor of the timely and properly performed removal of the infected canal content alleviating the patient's condition. In order to achieve a greater reduction of the microbial count during the mechanical root canal treatment, the use of antiseptic solutions – hypochlorite, chlorhexidine, 3% hydrogen peroxide – is of particular importance (4, 5, 6, 8, 14). Calcium hydroxide is a medication recommended for the treatment of apical periodontitis. Its antimicrobial mechanism of action is influenced by the rate of the chemical dissociation into calcium and hydroxyl ions. As a result, a high pH of the medium is achieved, which in turn inhibits the enzyme activity, which is of particular importance for the metabolism and growth of the bacterial cell (10, 13, 18).

The literature data on the use of antibiotics in endodontics is contradictory. Prophylactic endogenous use of antibiotics in endodontics is recommended in patients at risk of infective endocarditis (9, 24). Systematic administration of antibiotics in endodontics is limited and indicated mainly in: (1) Patients who have symptoms of periapical infection (periodontitis), but in whom no drainage of the exudate through the root canal or through the soft tissues can be achieved, who suffer from physical malaise and elevated body temperature; (2) in all cases of patients with diffuse edema, no matter whether drainage has been achieved or not (1, 6, 24). Antibiotic administration should not replace incision like a manipulation pf draining the inflammatory exudate (1).

In one of their studies, Ehrmann, E.H. et al. (2003), report topical application of a ***Ledermix*** paste (containing tetracycline) in root canals in cases where other intracanal drugs have failed (12).

The ideal antimicrobial agent should only affect the microorganism and not the macroorganism. The following antibiotics are used for targeted general treatment of infections with proven *Bacteroides'* participation (2, 3, 8):

3. Antibiotics in endodontic infections

1) Beta-lactam antibiotics – bacterial cell wall synthesis inhibitors

The active principle of all β-lactams is based on their β-lactam ring that inactivates (inhibits) irreversibly important bacterial enzymes by acylating them. They have bactericidal activity. In order to exhibit their antibacterial activity, β-lactams must penetrate well through the bacterial outer wall,

have high affinity for *penicillin-binding proteins* of bacteria and have high stability with respect to inactivating enzymes, β-lactamases. β-lactams include: Penicillin, Cephalosporins, Carbapenems, Penems, Carbacephems, Monobactams, β-lactamase inhibitors. Beta-lactams are non-toxic as there are no targets in eucites for their action. Some of the patients are allergic to them. Nowadays, Penicillins make up 60% of all antimicrobial agents on the pharmaceutical market (2, 3, 8).

Penicillins with β-lactamase inhibitors applied in β-lactamase-forming anaerobic infects (e.g. Augmentin).

Cephalosporins – derivatives of 7-aminocephalosporanic acid. Only its semisynthetic derivatives, arranged in different generations, find clinical application.

Carbapenems (imipenem) have a very wide range of action. They are used to treat severe, life-threatening infections – sepses, endocarditides, infections caused by polyresistant bacteria.

Monobactams – active only against gram-negative bacteria.

2) Non-beta-lactam antibiotics – Protein synthesis inhibitors

Their mechanism of action is to inhibit or destroy by preventing the binding of transfer RNA to ribosomes. They are applied i.v. or i.m. because they are not absorbed by the intestinal tract. They do not penetrate well into the bones and do not cross the blood-brain barrier. They are excreted by the kidneys.

Chloramphenicol - It has inhibitory activity against human mitochondria, because they contain 50S ribosomal subunits, which explains the dose-dependent toxicity on the bone marrow. Today, the application of chloramphenicol is very limited due to its severe side effects – dose-dependent reversible bone marrow suppression, a rare but fatal aplastic anemia (1 in 30 000 cases), etc. Thiamphenicol has fewer side effects.

Macrolides – Erythromycin, Clarithromycin (Klacid™, Fromilid™), Azithromycin

Lincosamides – The most important preparations in the group are lincomycin and clindamycin. They inhibit protein synthesis by a mechanism similar to that of the macrolides by interacting with 50S ribosomal subunits. There are *Bacteroides* strains which are resistent to clindamycin.

3) Nucleic Acid Synthesis Inhibitors

Nitroimidazoles – These are chemotherapeutic agents with strong antiprotozoal activity (metronidazole and tinidazole). Their mechanism of action works through their damaging the structure and function of the bacterial DNA.

4. Conclusion

In the first place, endodontic treatment is related to solving the microbiological problem in endodontic infections, and then to solving anatomic-morphological and technical difficulties. Knowing endodontic bacterial pathogens in details, and the interactions between them also, is of particular importance for the choice and application of the most effective therapy possible.

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