

Non-Spore-Forming Anaerobic Bacteria causing Endodontic Infections: *Fusobacterium nucleatum*

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Abstract: Non-spore-forming anaerobic cocci and rods are a heterogeneous group of bacteria that form the predominant bacterial population on the skin and mucous membranes in humans. The most important anaerobic gram-negative non-spore-forming bacteria are in the genera *Bacteroides*, *Fusobacterium*, *Porphyromonas*, *Prevotella* and *Parabacteroides*. These microorganisms are primarily opportunistic pathogens, usually responsible for endogenous infections in humans, and are more often isolated in polymicrobial associations with other anaerobic and aerobic bacteria. Almost half of the chronic sinus and ear infections and almost all periodontal infections are polymicrobial associations of gram-negative anaerobes, the most frequently isolated being *Prevotella*, *Porphyromonas*, *Fusobacterium nucleatum* and *Bacteroides non-fragilis*. These microbes have stringent nutritional requirements and grow slowly in nutrient environments in laboratory conditions for 3 or more days. A major component of their cell wall is the lipopolysaccharides (LPS), which, in the genus *Bacteroides*, have little or no endotoxin activity, unlike the LPS molecules in aerobic gram-negative rods such as bacteria of the genus *Fusobacterium*. By applying a suitable therapeutic approach and treatment, life-threatening conditions are avoided.

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

1. Introduction

There are more than 50 types of gram-negative anaerobic bacteria living in the human body that can cause endogenous infections. Different rod-shaped and cocci anaerobic species are found in the mucous membranes of the mouth, the upper respiratory tract, the gastrointestinal tract and external genital organs – Table 1: (1, 2). Gram-negative bacteria appear to be the most common microorganisms in primary endodontic infections (32).

Table 1: Types of anaerobic gram-negative microorganisms

• Anaerobic gram-negative rod-shaped bacteria <i>Bacteroides</i>, <i>Prevotella</i>, <i>Porphyromonas</i>, <i>Fusobacterium</i>, <i>Bilophila</i>, <i>Desulfomonas</i>, <i>Mitsuokella</i>, <i>Tissierella</i>	• Anaerobic gram-negative cocci <i>Veillonella</i>, <i>Acidaminococcus</i>, <i>Megasphaera</i>
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Under certain conditions though as, for example, in the case of traumatic or surgical damage to the protective barriers of the macro-organism (mucous membranes, skin, enamel, cement); in avitaminosis; in the presence of other infections with bacterial and viral etiology, they find beneficial conditions for growth and reproduction both independently (osteomyelitis) and in polymicrobial associations with other microorganisms of the normal flora, causing purulent and gangrenous diseases (e.g., acute necrotizing ulcerative gingivitis). Bacteria in the root canal system may exist as planktonic (unattached) cells, suspended in the liquid phase of the main root canal, or more often organized into a biofilm that adheres to the walls of the root canals. The biofilm is the main form under which bacteria are found in nature. Biofilms are observed not only on the walls of the main root canals but also on the walls of the lateral canals, the isthmuses and the dentinal tubules (9, 10, 13, 16, 20, 21, 27, 32).

General characteristics

The genus *Fusobacterium sp.* (*F. nucleatum*, *F. necroforum*, *F. varium*, *F. gonidiaformans*) encompasses polymorphous rod-shaped or spindle-shaped (fusiform) bacteria that are broader at the center and have pointed ends. Fusobacteria are normal inhabitants of the organ cavities in humans and animals.

Routes of infection penetration

Under normal conditions, the dental pulp and the dentin of the teeth are sterile and isolated from the oral microorganisms by the hard dental tissues covering them – the enamel and the cement. Due to caries, cracks and fractures on the hard dental structures, various restorative manipulations, tartar removal, attrition dentis and/or abrasio dentis, these protective barriers of the teeth do not function well or are naturally absent in some congenital anomalies (dens invaginatus, dens evaginatus, defects of the palatal groove), leading to exposure of the dental pulp. As a result, the pulp-dentin complex is exposed to the impact of the oral environment and the risk of infections by oral microorganisms (25, 32). The permeability of the dentin near the pulp is increased due to the larger diameter and the greater canal density (17). The dentinal tubules pass through the entire dentin width and have the shape of inverted cones, whose smallest diameter is at the periphery near the enamel or cement (0.9 µm on average) (11). The smallest canal diameter is fully compatible to the cell diameter of most oral bacterial species, which typically ranges from 0.2 µm to 0.7 µm. Bacterial invasion of the dentinal tubules occurs more rapidly in non-vital teeth than in vital ones (15).

In vital teeth, the outward movement of the dentinal fluid and the canal content affect the permeability of the dentin and may eventually delay the intracanal invasion by bacteria. Other factors, such as the layer of reactive protective dentin formed under a chronic carious lesion, the smear layer, as well as the intracanal accumulation of host

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defense molecules, such as antibodies, play a role in limiting and even inhibiting the bacterial progression to the pulp through the dentinal tubules (12, 17). Microorganisms from subgingival biofilms associated with periodontal health could also reach the pulp through dentinal tubules or lateral and apical foramina, and possibly through a systemic route (12, 32).

Morphology of fusobacteria

Fusobacteria are gram-negative, motile or non-motile peritricha. They have both short and very long, thin filamentous forms. The anaerobic bacteria, including *Fusobacterium nucleatum*, grow in an oxygen-free atmosphere; they are fastidious and develop on special, enriched nutrient media for 2-3 or more days. Their optimal temperature for development is 37°C at pH = 7.0. They decompose carbohydrates to gas-free acids. They do not have enzyme systems involved in the metabolism of oxygen that are characteristic of aerobic and facultative-anaerobic bacteria, such as: **respiratory chains**, **superoxide dismutase** and **catalase**. Obligate anaerobes lack those enzymes, whereas facultative anaerobes may have small amounts of **superoxide dismutase** and **catalase** and are more common causative agents for infections in humans as compared to the obligate ones (1, 2, 4, 5).

Virulence factors

Virulence reflects the degree of pathogenicity of microorganisms. Virulence factors are products without microorganisms and include a number of their structural components such as, for example, peptidoglycans, outer membrane proteins, lipopolysaccharides, exopolysaccharides, fibers in the filamentous ones (1, 2, 4).

Fusobacterium nucleatum's virulence depends on the presence of pathogenicity factors facilitating the adhesion of microbes to tissues such as **lectins**; factors protecting bacteria from the macroorganism's protective mechanisms, such as **lipopolysaccharides** which have anticomplementary and antiphagocytic activity; **volatile fatty acids** with antiphagocytic activity and factors leading to tissue necrosis – various **enzymes**: *protease*, *collagenase*, *neuraminidase*, *phospholipase*, etc. Another virulence factor in fusobacteria is **spheroplast transformation**, in which bacteria are transformed into spheroplasts, release endotoxin and can cause endotoxic shock (1, 2, 3, 4)

Gram-negative bacteria produce **endotoxins**, which are, inherently, lipopolysaccharides. They are a structural component of the cellular bacterial wall and are released in the destruction of bacteria. **Lipopolysaccharides (LPS)** are an important virulence factor. They are thermally stable – not activated at 80°C to 100°C (some even at 121°C) for 1 hour. They are moderately toxic, not acting selectively but causing similar pathophysiological effects (1, 2). They are slightly antigenic and immunogenic. They can affect many biological functions when freed from microorganisms (e.g. they have a pyrogenic activity). A bacterial cell can contain approximately $3.5 \cdot 10^6$ LPS on an area of $4.9 \mu\text{m}^2$. In the dental structures, the separated endotoxins have the ability to diffuse into the dentin. Various studies have indicated the relationship between endotoxins and periapical inflammation (3, 26). Lipopolysaccharides may cause:

- Activation of macrophages/monocytes with a subsequent biosynthesis and release of cytokines, prostaglandins;
- These substances are chemical mediators of inflammation and most of them can stimulate bone resorption;
- They can increase vascular permeability;
- They can accelerate the production of bradykinin – an important chemical mediator of inflammation;

Bacterial components stimulate the cells of the body to start to release chemical mediators of inflammation and pain (3). The concentration of **LPS** in infected root canals, such as they are in acute apical periodontitis, increases proportionally to the increase in the number of gram-negative microorganisms (*Porphyromonas endodontalis*, *Fusobacterium nucleatum*). Studies done by Siqueira, J. F. (2001) indicate that the content of endotoxins or **LPS** in infected root canals is higher in teeth with symptomatic apical periodontitis, teeth with periradicular bone destruction or teeth with persistent exudation (26).

Facultative-anaerobic (rarely aerobic) bacteria, present in bacterial associations, exhaust oxygen at the site of inflammation and create conditions for the development of anaerobic causative agents (2).

The role of fusobacteria in endodontic infections

Endodontic infections (acute and chronic periodontitis) and respiratory tract infections (chronic sinusitis, middle ear infections) are caused by associations consisting of *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Bacteroides* (without *Bacteroides fragilis*), including peptostreptococci and aerobic cocci. These combinations increase pathogenicity of fusobacteria. Some studies of these associations report the presence of specific symptoms in the endodontic area such as pain, purulent exudate and swelling (12). Sinusitis and otitis can be complicated by multiple brain abscesses. About 5% of the sepsis cases these days are caused by anaerobes – *B. fragilis*, *Fusobacterium* (2, 3, 28, 29, 30, 31). Anaerobic infections are often accompanied by an unpleasant odor resulting from the production of short-chain volatile fatty acids and anaerobic metabolism, sometimes with tissue breakdown. Gas may often accumulate in inflamed tissues (2). Gram-negative bacteria, which are common in primary intraradicular infections, are usually eliminated by endodontic treatment, because they are very sensitive to strong oxidizing agents, such as sodium hypochlorite. Most studies on this subject have clearly shown higher occurrence of gram-positive bacteria (streptococci, lactobacilli, *Enterococcus faecalis*, *Olsenella uli*, *Propionibacterium propionicum*, *Parvimonas micra*, *Pseudoramibacter alactolyticus*) in both post-instrumentation and post-medication samples (4, 7, 8, 29, 32). This supports the thesis that gram-positive bacteria can be more resistant to antimicrobial treatment and have the ability to adapt to the harsh conditions of the surrounding environment in instrumented and medicated root canals (32). Evaluation studies with samples taken in re-treatment cases indicate that clearly well-treated canals may contain between 10 and 30 bacterial species, which is very similar to what has been found for the untreated canals (19, 23, 28, 32). The microbial counts in treated canals vary from 10^3 to 10^7 cell equivalents (6, 18, 22, 24). Studies have shown that

fusobacteria can also be isolated from the periapical region (31).

Microbiological examination

Microbiological samples are collected and transported to a laboratory in an oxygen-free transport environment. They must immediately be inoculated in an anaerobic nutrient medium, usually on selective agars enriched with bile. As most anaerobic infections are endogenous, it is important that the samples are not contaminated by the normal microbial flora present on adjacent mucosal surfaces. Samples should be stored in a damp environment as drying causes significant bacterial losses. In addition to traditional biochemical microbiological identification tests, molecular biology methods could also be used, such as sequence analysis of species-specific genes (e.g. 16S ribosomal RNA gene) or mass spectrometry for spectral analysis of species specific protein profiles (proteomic analysis). These methods are reliable but they are still quite expensive and time consuming (1, 2)

Therapeutic effect on anaerobic infections involving *Fusobacterium*

Therapeutic effect on anaerobic infections involving *Fusobacterium* in dental medicine is local but it can also be general – in the case of complications. Local treatment involves mechanical root canal treatment using oxidizing agents such as sodium hypochlorite. Antibiotic therapy, sometimes combined with surgical intervention, is an approach to the treatment of complicated anaerobic infections. Virtually all members of the genera included in the group of non-spore-forming, anaerobic gram-negative bacteria produce the enzyme β -лактамаза. This enzyme makes bacteria resistant to penicillin and many of the representatives of cephalosporins. Antibiotics with the best activity against gram-negative anaerobic rods are metronidazole, carbapenems (e.g. imipenem, meropenem) and β -lactamase inhibitors (e.g. piperacillin, tazobactam) (1, 2, 3, 4, 5, 14, 32).

2. Conclusion

Knowing endodontic bacterial pathogens in detail, 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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