

Important Microorganisms Responsible for Dental Infections - Actinomycetes (*Actinomyces spp.*)

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Abstract: *The unequivocal role of microorganisms in the etiopathogenesis of periodontitis was established about 40 years ago. Endodontic infections develop after pulp necrosis or in cases where the pulp has been removed for the purposes of endodontic treatment. After pulp necrosis, bacteria colonizing the root canal system come into contact with the periradicular tissues of the tooth through the apical and lateral foramina. They can develop and propagate uninhibited by the defense mechanisms of the macroorganism by forming polymicrobial biofilms in the endodontium of the teeth, which leads to inflammation of the periradicular tissues. Actinomycetes (Actinomyces spp.) in humans are part of the normal mucous microbiota of the oral cavity, the intestinal tract, and the urogenital tract in women. Actinomycetes have been isolated from tooth plaques, carious lesions (A. odontolyticus), tonsillar crypts, the pharynx. The infections caused by them are endogenous, with no evidence of person-to-person mode of transmission or origin from an exogenous source. Most actinomycotic infections are believed to develop in the area of the face and neck, in patients with poor oral hygiene or in such that have undergone invasive, surgical interventions (e.g. tooth extraction) or trauma. Actinomyces spp. have been isolated in 10% of the cases of infected root canals, and mostly in those of them that have undergone failed primary endodontic treatment.*

Keywords: Gram-positive bacteria, endodontic infections, periodontitis, actinomycetes, actinomycosis

1. Introduction

In 1877, Bollinger isolated actinomycetes from cattle for the first time. Harz gave them their name. In 1891, Wolff and Israel isolated an anaerobic species and named it *Actinomyces israeli* (5). Actinomycetes (genus *Actinomyces*), also called “ray fungus” (from Greek *actis*, ray, beam, and *myke*, fungus), are obligately anaerobic or facultatively anaerobic non-spore-forming gram-positive rods. They occupy an intermediate place between bacteria and fungi. They resemble bacteria in their prokaryotic composition, a bacterial type of cell wall with muramic acid and their susceptibility to antibacterial preparations, and the formation of mycelia likens them to mushrooms. They are related to bacteria of the genus *Corynebacterium* and *Mycobacterium*. *A. israeli*, *A. naeslundii*, *A. viscosus*, *A. odontolyticus*, *A. pyogenes* and *A. meyeri* can cause actinomycosis in humans, and *A. bovis* in bovine animals (1, 2, 5, 10, 11, 15, 17). Bacteria are slightly pathogenic.

Morphology and physiology

Actinomycetes have a prokaryotic composition, a gram-positive cell wall, pronounced polymorphism – from filamentous (branched, unicellular) to rod-shaped and cocci forms. They are nonmotile, non-spore-forming and are acid-unstable (5). They are fastidious about their food environments and their temperature optimum is 35°C ± 2°C. The conditions for *A. israeli* and *A. bovis* are anaerobic, whereas for the other species they are aerobic. They can grow as S or R colonies after being cultivated for 10-14-days. In thioglycollate media they form flocs (5). Their basic cellular unit is the **hypha**, having the form of long, branched rods or filaments and being 1µm wide. Upon cultivation in laboratory conditions, **mycelia** are observed – a network of hyphae that spread over the surface of the nutrient medium – ‘**airy mycelium**’, but it can also penetrate into the medium – ‘**substrate mycelium**’. Hyphae can easily break into short rod-like forms (1, 2).

Etiopathogenesis and clinical presentation

Although there is evidence that anaerobic gram-negative bacteria are the most common microorganisms in primary endodontic infections, gram-positive bacteria, some of which having such high prevalence rates as those of the most common gram-negative species, have also been found in the mixed endodontic bacterial consortium. Gram-positive bacteria, often isolated in primary infections, include: *Actinomyces* (e.g. *A. israeli*), *Filifactor* (*F. alocis*), *Streptococcus* (*S. anginosus*), *Peptostreptococcus* (*P. anaerobicus*, *P. stomatis*), *Propionibacterium* (*P. propionicum*, *P. acnes*), *Parvimonas* (*P. micra*), *Pseudoramibacter* (*P. alactolyticus*) и *Olsenella* (*O. uli*) (7, 8, 9, 16, 18, 19, 22, 23, 24, 26, 27). In 75% of the cases of periostitis, the cause of the inflammatory process is an anaerobic mixed infection (gram-positive and gram-negative cocci, actinomycetes, fusobacteria, etc.). In more than 70% of the cases, periodontitis with infectious etiology is usually developed as a complication of exacerbated chronic periodontitis and rarely as a complication of its acute forms (4).

Actinomyces spp. have been isolated in 10% of the cases of infected root canals, and mostly in those of them that have undergone failed primary treatment (3). The infection can also spread to the facial, faciocervical, thoracic areas (after aspiration), and rarely to the central nervous system. *Actinomyces israeli* is believed to be one of the types of microorganisms involved in the development of root caries (15). Actinomycosis, which is caused by *Actinomyces israeli*, is characterized by the development of chronic, granulomatous lesions that form abscesses in the affected tissues. In chronic development and non-treatment, they fistulize to the skin. The fistulas produce a bloody purulent secretion, where, as in the abscesses and the fistula canals (draining sinus tract) **actinomycotic druses** (also called **serum granules**), which are typical for the disease, are

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found. These are yellow or orange beans (actinomycete colonies) with the size of a millet seed (2, 6). They have a dense center containing a mycelial mass surrounded by filamentous organisms located radially and bound together by calcium phosphate. The inflamed area is surrounded by a fibrosing granulomatous tissue that gives a solid consistency to the surface covering the corresponding tissues (2, 17). The illness may occur as an acute painful pyogenic infection or as a slowly developing and relatively painless process. The establishment of a tissue swelling with fibrosis and scars on the skin of the face in the area of the lower jaw and neck angle, as well as the presence of a suppurating fistula intraorally or extracorally, points to the likelihood of actinomycosis.

Microbiological examination

For decades, the identification of endodontic microorganisms has been performed using culture techniques. It appears, however, that only about 10% of the human microbiome can be cultivated (20). To detect actinomycetes, purulent secretion from the fistulas, purulent root canal exudate and biopsy material should be examined. The following must be performed:

- Direct microscopy – the typical actinomycotic druses are identified in the isolated samples and are treated with 10% sodium hydroxide (NaOH) or potassium hydroxide (KOH) solution, followed by Gram and Ziehl-Neelsen staining. Druses can be pressed between two slides and examined microscopically. The presence in the preparations of a thick center of mycelial mass, surrounded by hyphae radially located and thickened like bats at their ends, confirms the diagnosis.
- Culture test – plating of liquid (thioglycollate broth) and solid (blood agar) nutrient media is done. Plating is cultivated aerobically and anaerobically. Growth can take 2 weeks. Isolated pure cultures are identified by their morphological, cultural and biochemical properties.
- Biochemical tests – Unlike *A. bovis*, *A. israeli* hydrolyzes starch. *A. israeli* are anaerobes with an optimum temperature for development of 37°C. They are fastidious about their nutrient media, requiring blood, serum, glucose. On solid media, they form smooth or granular white colonies with a flat or uneven periphery within 7-14 days. In thioglycollate broth, *A. israeli* grow in the form of cotton-like flocs. They decompose different carbohydrates to a gas-free acid. *A. israeli*, as well as other actinomycetes of the normal microbiota, is sensitive to environmental factors and disinfectants (1, 2, 5).

Treatment

Good hygiene of the oral cavity and teeth is important. Treatment is topical and general. Topical treatment includes surgical incision and evacuation of the purulent-bloody abscess collection (6).

The microorganisms that have entered the root canals should be minimized. Endodontic treatment consists of extensive irrigation of the root canals with strong oxidants such as sodium hypochlorite at various concentrations (0.5% – 5.25%) and subsequent instrumentation. Antiseptics such as chlorhexidine gluconate, which has a wide range of action, can also be used (3). Concomitant antibiotic treatment is

required, particularly with medically compromised patients who are more susceptible to complications emerging from odontogenic infections and in whose treatment antimicrobial agents play a more specific role (21). Actinomycetes are sensitive to antibiotics from the groups of: penicillins, macrolides, sulphonamides, etc., which have to be administered according to a schedule, for months (5). Penicillin at a dose of 10 to 20 million units / 24 hours may be administered for 2 – 4 weeks, followed by 5 – 10 million units / 24 hours for 3 – 4 months (6). Although penicillin is usually the first antibiotic chosen to treat infections of endodontic origin, there is one disadvantage associated with its use. Approximately 8% of the population has a history of penicillin allergy (13, 14). The unchecked penicillin allergy is increasingly recognized as a major public health problem. It must be taken into consideration that some patients may report some symptoms of intolerance, that is, diarrhea or upset stomach, as allergy (21). For patients with a confirmed history of penicillin allergy, the clinician may use other antimicrobial agents.

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