

# Isolation, identification and antibiotic susceptibility of Gram-negative anaerobic bacteria in oral cavity

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## SUMMARY

Gram-negative bacteria include more than 20 genera. The most commonly isolated genera are: *Bacteriodes* spp., *Porphyromonas* spp., *Fusobacterium* spp. and *Prevotella* spp. The following genera were isolated somewhat less frequently: *Tanerella* spp., *Leptotricha* spp., *Veilonella* spp., *Wollinella* spp., *Selenomonas* spp. and *Treponema* spp. Anaerobic bacteria have an anaerobic type of metabolism and therefore their incubation is significantly longer and more demanding than aerobic bacteria. The genera *Prevotella* spp., *Porphyromonas* spp. and *Fusobacterium* spp. are part of the resident flora of the oral cavity and in unfavorable conditions cause periodontal diseases, and sometimes dentogenic infections and systemic diseases, such as Alzheimer's disease, cardiovascular diseases, metabolic diseases and inflammatory bowel diseases. The three most important steps for the successful diagnosis of anaerobic bacteria are: proper sampling with avoiding sample contamination, rapid transport of samples to the microbiological laboratory and proper handling of samples. A combination of beta-lactam with the addition of beta-lactamase inhibitors, metronidazole, clindamycin and moxifloxacin is used for treatment for infections caused by anaerobic Gram-negative bacteria. It is important to note that antibiotics should be used only with a clear indication and to choose the right antibiotic in the optimal dose. The aim of this review is to point out the role of Gram-negative anaerobic bacteria in periodontal diseases, and its isolation, identification and antibiotic susceptibility.

**Keywords:** Gram-negative anaerobic bacteria; systemic diseases; isolation, identification; antibiotics; resistance

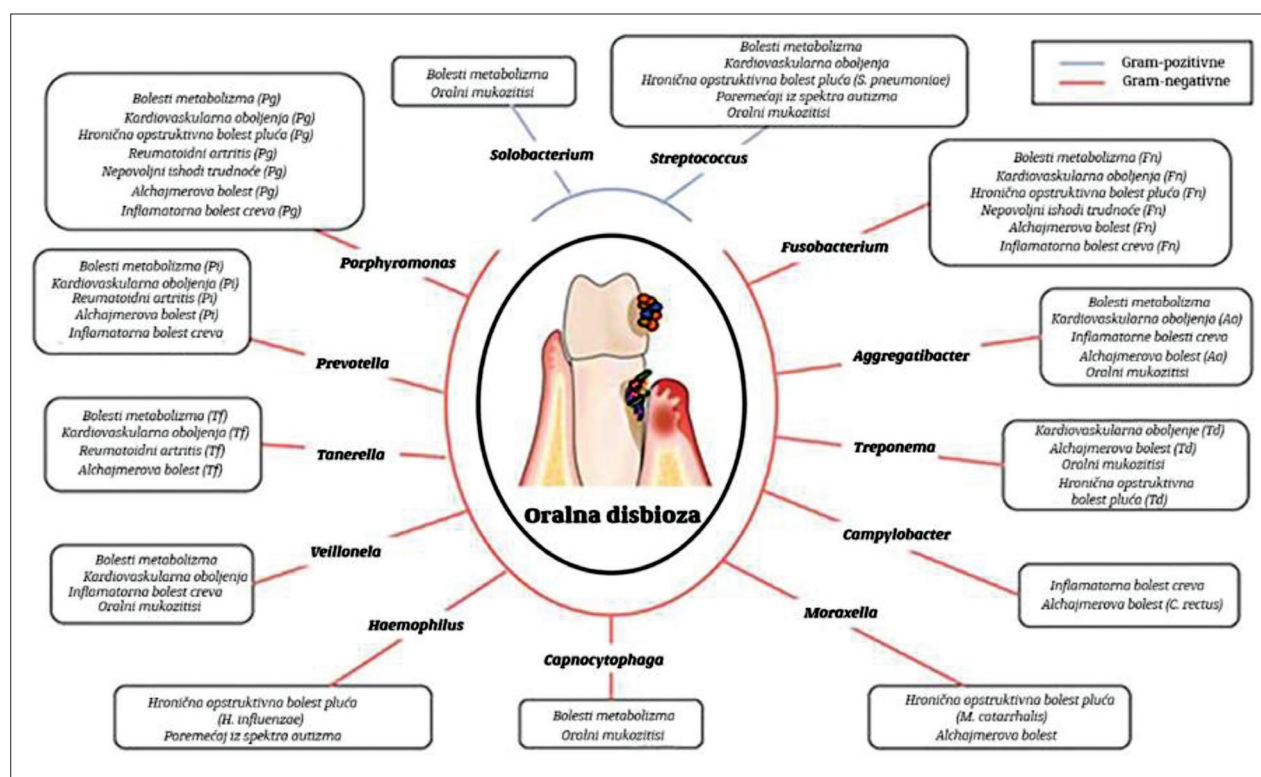
## INTRODUCTION

The most important infectious diseases of the oral cavity are dental caries, periodontal diseases, dentoalveolar infections and others. The oral cavity is naturally inhabited by microorganisms. It is considered that the oral cavity is sterile at birth and that oral colonization of microorganisms takes place after birth. At the end of 19<sup>th</sup> century, Gram-negative anaerobic bacteria were discovered and more than 20 genera have been described so far. The following genera are most commonly isolated from oral cavity: *Bacteriodes* spp., *Porphyromonas* spp., *Tanerella* spp., *Fusobacterium* spp. and *Prevotella* spp. In addition, the following genera *Leptotricha* spp., *Wollinella* spp., *Veilonella* spp., *Selenomonas* spp., and *Treponema* spp are detected [1]. Anaerobic bacteria have an anaerobic type of metabolism and therefore their incubation is significantly longer and more demanding than aerobic bacteria. Mutualism and opportunism are important characteristics of the symbiotic relationship between the host and colonizing species from the genera *Bacteriodes*, *Prevotella*, *Porphyromonas* and *Fusobacterium*. *Fusobacterium necroforum* is found as part of the normal flora of the oral cavity and can cause severe infection of the head and neck. Anaerobic bacteria can participate in the development of systemic disease, such as Alzheimer's disease, cardiovascular diseases, metabolic diseases and inflammatory bowel diseases. *Fusobacterium* spp. and *Prevotella* spp.

can cause respiratory tract infections [1, 2]. They have a tendency to form abscesses and the most common localization of abscesses is oropharynx, abdominal cavity, lungs and genital tract of women. Several species of the genus *Fusobacterium* have been found to be associated with colon cancer [3]. A combination of beta-lactam with the addition of beta-lactamase inhibitors, metronidazole, clindamycin and moxifloxacin is used for treatment for infections caused by anaerobic Gram-negative bacteria.

## Main characteristics of Gram-negative anaerobic bacteria

It has been described 49 species within the genus *Prevotella*. *Prevotella* spp are moderately saccharolytic bacteria (ferment carbohydrates), nonmotile bacilli and strict anaerobes. The main species are: *P. intermedia*, *P. nigrescens*, *P. corporis*, *P. oralis*, *P. oris*, *P. dentalis*. Some strains *P. denticola*, *P. intermedia* and *P. nigrescens* are difficult to differentiate using simple physiological tests. Some species occur in increased numbers in periodontal diseases and are isolated from abscesses [1]. Within the genus *Porphyromonas*, 15 species have been isolated so far. They are mainly composed of asaccharolytic bacteria that use proteins and peptides for their growth. They are nonmotile bacilli and strict anaerobes. The main species are: *P. gingivalis*, *P. endodontalis*, *P. catoniae*. They are



**Figure 1.** Some major periodontal diseases are associated with other systematic infections [4].

**Slika 1.** Neke glavne parodontalne bolesti povezane su s drugim sistemskim infekcijama [4].

associated with chronic periodontitis and dentoalveolar abscesses. *P. gingivalis* is mostly isolated from subgingival locations, from advanced periodontal lesions, but also from the tongue and tonsils. *P. endodontalis* is mainly isolated from the root canal of the tooth and a *P. catoni* is mainly found in healthy localities and in shallow periodontal pockets. The genus *Fusobacterium* currently has 14 species, which 10 of them have been isolated from humans. They represent thin, pleomorphic bacilli and for their cultivation they require enriched media. *F. nucleatum* produces  $\text{NH}_3$  and  $\text{H}_2\text{S}$  from cysteine and methionine, so it is considered to cause halitosis. These bacteria have a characteristic shape of long filaments, similar to a Cuban cigarette. The colonial appearance is variable. They are isolated in root canal infections, dentoalveolar abscesses and are present in the spread of odontogenic infections. In order to get the necessary energy, fusobacteria metabolize amino acids (aspartate, glutamate, lysine). If there are no amino acids, peptides are used for this purpose. Within the genus *Treponema*, the main species are: *T. denticola*, *T. socranskii*, *T. vincenti*, *T. maltophilum*, *T. amylovarum*, *T. parvum*, *T. pectinorum*, *T. putidum*, *T. medium*. *T. denticola* is asacharolytic. These bacteria are found in the gingival sulcus and are associated with periodontal disease. They are Gram-negative spiral bacilli in three sizes. They are strict anaerobes and are difficult to cultivate *in vitro*. Within the genus *Tannerella*, the main species is *T. forsythia*. They are non-motile, pleomorphic, spindle-shaped Gram-negative bacilli. Sometimes it takes 14 days for visible growth *in vitro*. It grows better with co-cultured with *Fusobacterium nucleatum* and when

*N*-acetylmuramic acid is added to the media. It is found in supragingival and more often in subgingival plaque. The frequency of isolation is closely related to the depth of the periodontal pocket, it is more often isolated from the place where periodontal disease develops, so it is a generally accepted as periodontal pathogen. *Tannerella forsythia*, *Treponema denticola* and *Porphyromonas gingivalis* are designated as three agents of the “red complex” of bacteria, which is always associated with periodontal disease. The main species in the genus *Wolinella* is *W. succinogenes* are strict anaerobes that move using polar flagella. They can be found in the gingival sulcus as well as in aggressive periodontal disease.

Bacteria from the genus *Veillonella* are strictly anaerobic Gram-negative cocci, they don't break down carbohydrates, but use lactate and thus raise pH value and have an anticariogenic effect. They are isolated from most of the surfaces in oral cavity [2, 3, 4]. Lactic acid is the strongest acid produced by bacteria in oral cavity and breaks down tooth enamel. *Veillonella* spp. can convert lactic acids to weaker acids, mostly propionic carboxylic acid.

### Laboratory diagnostics

The three most important steps for successful diagnosis of anaerobic bacteria are: proper sampling with avoiding sample contamination, rapid transport of samples to the microbiological laboratory, proper handling of samples. During the examination, it is the best to take sample from the base of periodontal socket at the right time, taking

care not to cause contamination. Only sterile equipment should be used for sampling. Samples should be inoculated immediately on media for anaerobic bacteria, cultured for different times up to 14 days, under anaerobic conditions. If we transport samples to the laboratory, we must use transport systems. Samples must not be refrigerated. Whenever possible, the sample should be taken before starting antibiotic therapy [5].

#### Genus *Prevotella*

Gram staining shows pale-stained Gram-negative pleomorphic bacilli or coccobacilli. Microscopically, they cannot be distinguished from *Bacteriodes* spp. They are isolated from periodontal pockets, dental plaque, chronic periodontitis and dentoalveolar abscesses. The procedure for isolating bacteria of the genus *Prevotella* includes the following steps: preparation of sterile medium for growing bacteria, taking a sample from the environment where their presence is expected (oral cavity or gastrointestinal tract), inoculation of the sample onto a sterile substrate using a sterile loop or pipette, incubation of the substrate at the optimal temperature and conditions, identification of colonies of bacteria of the genus *Prevotella*. In the genus *Prevotella*, the colonies are similar to *Bacteriodes* spp., except that some species are pigmented (it can be pale brown to black). Cultivation on anaerobic blood agar produces brilliant colonies. *Prevotella* spp. are moderately saccharolytic, while *Porphyromonas* spp. non-saccharolytic. After incubation on blood anaerobic agar for 7-14 days, irregularly shaped colonies with a diameter of about 1mm are formed [6, 7]. Virulence factors are capsular polysaccharide in genus *Prevotella* and inhibit opsonization and therefore phagocytosis, as well as fimbriae and enzymes. They cause skin abscesses and soft tissue infections. They also cause lung abscess, empyema, brain abscess, mastoiditis and inflammation of the middle ear, osteomyelitis of facial bones. The basic approach is abscess drainage and surgical treatment. They are sensitive to metronidazole, clindamycin, rifampicin and resistant to vancomycin and erythromycin.

#### Genus *Porphyromonas*

They are associated with chronic periodontitis and dentoalveolar abscesses. *P. endodontalis* is mainly isolated from infected root canals. Isolation of *Porphyromonas* bacteria can be done as follows: a sample can be collected from mouth, teeth, gingiva or other oral surfaces using a sterile spatula or swab. The sample must be transported to the laboratory within 30 minutes after sampling. The sample is inoculated onto *Porphyromonas* media, such as Brucella agar with hemin (10–15%) and vitamin K (0,5%) or tryptone soy agar supplemented with blood (TSBA) with hemin and vitamin K added. Incubation at a temperature of 37 °C for 7-14 days. Colonies are <1.0 mm in diameter after 48 hours of incubation, smooth, shiny and gray. Dark brown or black pigments develop only after 3-7 days [6, 7] and therefore together with bacteria of the genus *Prevotella*, they are classified as black-pigmented anaerobes [1, 5].

#### Genus *Fusobacterium*

It is isolated from tonsils, normal gingival sulcus and periodontal infections. They require enriched substrates for cultivation. Isolation requires several steps: taking a sample bacteria of the genus *Fusobacterium* can be grown on culture media containing large amounts of blood (blood agar) or similar substances. Confirmation of identification: antibiotic susceptibility and PCR. The appearance of the colonies is variable, but most are 1-3 mm in diameter, with an irregular or jagged margin. They vary from transparent to grainy and opaque. They grow on media with 20% bile and on blood agar for anaerobic bacteria, they cause hemolysis [8]. Virulence factors are lipopolysaccharide endotoxins (LPS) that are endotoxic. *F. necrophorum* causes Lemierre's disease (causes jugular vein thrombosis). The infection can spread to the lungs, brain, liver and bones. In the most severe case, a fatal outcome can occur. It also causes gingivitis and periodontal disease, skin and soft tissue infections, osteomyelitis [9]. Severe invasive diseases, such as Lemierre's disease, are usually treated with a combination of penicillin and metronidazole for 2-6 weeks [10]. It is important to point out that the isolation of these bacteria is an extremely difficult process due to their sensitivity to oxygen.

### Diseases caused by Gram-negative anaerobic bacteria

#### Periodontitis

Periodontitis is a disease that affects general population and leads to tooth loss, most often in people over 40 years old. It is considered to be the most common global disease in the world. The clinical picture is characterized by: inflammation, gingival recession, bleeding, loss of alveolar bone around the teeth, sometimes halitosis. In certain studies, in deep periodontal pockets there is the presence of "red complex" bacteria consisting of *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola* [11, 12].

#### Necrotic diseases of the periodontium

Necrotic diseases of the periodontium are diseases characterized by the presence of bacteria such as *Fusobacterium*, *Veillonella*, anaerobic streptococci. They occur in some people with HIV infection [11, 12].

#### Phlegmon of the mouth floor - Ludwig's angina (LA)

It is characterized by massive solid swelling of the entire floor of the mouth (both sides). Complications including airway obstruction is possible. Bacteria associated with Ludwig's angina are: *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp. and anaerobic streptococci. Broad-spectrum antibiotics are used in therapy. Penicillin and metronidazole are combined. It is important to ensure patency of the airways, fluid replacement, taking a sample for microbiological examination and removing the possibility of a source of infection [11, 12].

## Periodontal abscess

A periodontal abscess is localized pus collection that is usually located at the apex of the root of the tooth. The cause of an abscess is a bacterial infection that then spreads to the top of the tooth root. Bacteria are not naturally found in periodontium, but they can get there if there is damage to the enamel and dentin, such as caries. The causative agents include bacteria from subgingival plaque, *Porphyromonas*, *Prevotella*, *Fusobacterium*, spirochetes, *Capnocytophaga*, *Actinomyces* spp. The clinical picture is characterized by: sudden onset, swelling, redness, pain is continuous or is related to the bite. The treatment consists in draining the abscess with periodontal procedures. A poor prognosis results in tooth extraction [11, 12].

## Antibacterial drugs

Antibiotics are drugs that are toxic for bacteria, but non-toxic or slightly toxic to the human body. The choice of antibiotics is based on the “best guess” principle.

When to include antibiotics in dental practice?

Antibiotics are the most commonly prescribed drugs by dentists, after analgesics. However, they are often inadequately and unnecessarily prescribed. Some of the most commonly prescribed antibiotics are amoxicillin, alone or in combination with clavulanic acid. Antibiotics should be used in case of: the spread of infection in the deep spaces of the head and neck, high body temperature and appearance of general signs of infection, immunocompromised patients (uncontrolled diabetes, patients on long-term corticosteroid and other immunosuppressive therapy, HIV-positive hemodialysis patients), occurrence of canine cavity infection.

In addition to eliminating the cause of infection, antibiotics are also used in prophylaxis. Preventive prescription of antibiotics occurs only in patients with weakened immune system, in whom the occurrence of infection can lead to severe complications. The most commonly prescribed broad-spectrum antibiotics belong to the group of penicillin and cephalosporins. The duration of action of antibiotics is very subjective and depends on the patient. For successful healing, it is necessary to follow the dentist's instructions and complete prescribed therapy. Independent discontinuation of therapy or improper use of antibiotics can lead to relapse [13].

Some of the typical drugs used to treat Gram-negative bacteria are: metronidazole, amoxicillin, cephalosporins, moxifloxacin, clindamycin

## Bacterial resistance to antibiotics

Resistance is protection of bacteria to the action of antibiotics, so that the bacteria survive or even multiply. Bacterial resistance can be innate or acquired. In each susceptible population, bacteria mutate and resistant bacteria emerge. Acquired resistance is induced due to long-term use of antibiotics. There is a noticeable trend

of increasing resistant strains of Gram-negative anaerobic bacteria [1, 13–17].

There are several methods to slow down the development of resistance: optimal application of antibiotics, synthesis of new antibacterial drugs, synthesis of resistance plasmid propagation inhibitors, use of combinations of antibacterial drugs. It is important to note that antibiotics should be used only with a clear indication and to choose the right antibiotic in the optimal dose. Irrational and uncritical prescribing of antibiotics represents a huge global and national problem. A special problem is multi-resistant bacteria, especially hospital strains bacteria, resistant to at least three classes of antibiotics. Only “reserved antibiotics” still work on them. The body is not given enough time to fight the infection on its own. The choice of appropriate antibiotic is based on the antibiogram, the state of the organism, the site of infection, as well as the side effects of the drug [1, 13–17].

## CONCLUSION

Gram-negative anaerobic bacteria are the cause of periodontal and sometimes systemic diseases, and due to their slow growth, suitable media and anaerobic atmosphere, their isolation, identification and antibiotic susceptibility are demanding and time-consuming. In the case of diseases caused by Gram-negative anaerobic bacteria, in the case of need for antibiotic therapy, special attention should be paid to the rational use of appropriate antibiotics due to global trend of increasing bacterial resistance to antibiotics.

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# Izolacija, identifikacija i ispitivanje osetljivosti na antibiotike Gram-negativnih anaerobnih bakterija usne duplje

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## KRATAK SADRŽAJ

U Gram-negativne bakterije spada više od 20 rodova. Najčešće su izolovani sledeći rodovi: *Bacteriodes* spp., *Porphyromonas* spp., *Fusobacterium* spp. i *Prevotella* spp. Nešto ređe se izoluju sledeći rodovi: *Tanarella* spp., *Leptotricha* spp., *Veilonella* spp., *Wollinella* spp., *Selenomonas* spp. i *Treponema* spp. Anaerobne bakterije imaju anaerobni tip metabolizma i zbog toga im je inkubacija znatno duža i zahtevnija u odnosu na aerobne bakterije. Rodovi *Prevotella* spp., *Porphyromonas* spp. i *Fusobacterium* spp. deo su rezidentne flore usne duplje, a u nepovoljnim uslovima izazivaju oboljenja parodonticijuma, a ponekad dentogene infekcije i sistemska oboljenja, kao što su Alchajmerova bolest, kardiovaskularna oboljenja, bolesti metabolizma i inflamatorne bolesti creva. Tri najvažnija koraka za uspešnu dijagnostiku anaerobnih bakterija su: pravilno uzimanje uzoraka, uz izbegavanje kontaminacije uzorka, brzi transport uzoraka u mikrobiološku laboratoriju i pravilno rukovanje uzorcima. Za lečenje infekcija uzrokovanih anaerobnim Gram-negativnim bakterijama upotrebljava se kombinacija beta-laktamskih antibiotika uz dodatak inhibitora beta-laktamaza, metronidazol, klindamicin i moksifloksacin. Važno je napomenuti da antibiotike treba koristiti samo uz jasnu indikaciju i da treba odabrati pravi antibiotik u optimalnoj dozi.

Cilj ovog rada je da se ukaže na ulogu Gram-negativnih anaerobnih bakterija u oboljenjima parodonticijuma, njihovu izolaciju, identifikaciju i osetljivost na antibiotike.

**Ključne reči:** Gram-negativne anaerobne bakterije; sistemska oboljenja; izolacija, identifikacija; antibiotici; rezistencija

## UVOD

Najvažnija infektivna oboljenja usne duplje su zubni karijes, oboljenja parodonticijuma, dentoalveolarne i druge infekcije. Usna duplja je prirodno naseljena mikroorganizmima. Smatra se da je po rođenju sterilna i da se oralna kolonizacija mikroorganizama odvija nakon rođenja. Krajem 19. veka otkrivene su Gram-negativne anaerobne bakterije i do sada je opisano više od 20 rodova. Najčešće se iz usne duplje izoluju sledeći rodovi: *Bacteriodes* spp., *Porphyromonas* spp., *Tanarella* spp., *Fusobacterium* spp. i *Prevotella* spp. Opisano je prisustvo i sledećih rodova: *Leptotricha* spp., *Wollinella* spp., *Veilonella* spp., *Selenomonas* spp., *Treponema* spp. Anaerobne bakterije imaju anaerobni tip metabolizma, te im je inkubacija zahtevnija u odnosu na aerobne bakterije. Mutualizam i oportunitizam su važne karakteristike simbiotskog odnosa domaćina i kolonizujućih vrsta iz rodova *Bacteriodes*, *Prevotella*, *Porphyromonas* i *Fusobacterium*. *Fusobacterium necroforum* se nalazi kao deo normalne flore usne duplje, a može uzrokovati tešku infekciju glave i vrata. Anaerobne bakterije mogu učestvovati u nastanku sistemskih oboljenja, kao što su Alchajmerova bolest, kardiovaskularna oboljenja, bolesti metabolizma i inflamatorne bolesti creva. *Fusobacterium* spp. i *Prevotella* spp. mogu uzrokovati infekcije disajnih puteva [1, 2]. Imaju sklonost za formiranje apscesa, a najčešća lokalizacija apscesa su orofarinks, trbušna duplja, pluća i genitalni trakt žena. Otkriveno je da je nekoliko vrsta iz roda *Fusobacterium* povezano i sa karcinomom debelog creva [3]. Za lečenje infekcija uzrokovanih anaerobnim Gram-negativnim bakterijama koristi se kombinacija beta-laktamskih antibiotika i inhibitora beta-laktamaza, metronidazol, klindamicin i moksifloksacin.

## Glavne karakteristike Gram-negativnih anaerobnih bakterija

Do sada je opisano 49 vrsta u okviru roda *Prevotella*. *Prevotella* spp. su umereno saharolitične bakterije (fermentuju ugljene

hidrate), nepokretni bacili i striktni anaerobi. Glavne vrste su: *P. intermedia*, *P. nigrescens*, *P. corporis*, *P. oralis*, *P. oris*, *P. dentalis*. Neki sojevi *P. denticola*, *P. intermedia* i *P. nigrescens* teško se diferenciraju ako se koriste jednostavni fiziološki testovi. Neke vrste se javljaju u povećanom broju u parodontalnim bolestima, a izoluju se iz apscesa [1]. U sklopu roda *Porphyromonas* do sada je izolovano 15 vrsta. Uglavnom ih čine asaharolitične bakterije koje za svoj rast koriste proteine i peptide. Nepokretni su bacili i striktni anaerobi. Glavne vrste su: *P. gingivalis*, *P. endodontalis*, *P. catoniae*. Povezuju se sa hroničnom parodontopatijom i dentoalveolarnim apscesima. *P. gingivalis* se uglavnom izoluje iz subgingivalnih lokaliteta, iz uznapredovalih parodontalnih lezija, ali i sa jezika i tonzila. *P. endodontalis* uglavnom se izoluje iz kanala korena zuba, a *P. catoniae* se uglavnom nalazi u zdravim lokalitetima i u plitkim parodontalnim džepovima. Rod *Fusobacterium* trenutno broji 14 vrsta, od kojih je 10 izolovano kod ljudi. Predstavljaju tanke pleomorfne bacile za čiju kultivaciju zahtevaju obogaćene podloge. *F. nucleatum* produkuje  $\text{NH}_3$  i  $\text{H}_2\text{S}$  iz cisteina i metionina, pa se smatra uzročnikom halitoze. Ove bakterije su karakterističnog oblika dugih filamenata, nalik kubanskoj cigareti. Kolonijalni izgled je promenljiv. Izolovani su kod infekcija kanala korena, dentoalveolarnih apscesa i zastupljeni su kod širenja odontogenih infekcija. Da bi došlo do potrebne energije, fuzobakterije metabolišu amino-kiseline (aspartat, glutamat, lizin). Ako nema amino-kiselina u tu svrhu koriste peptide. U okviru roda *Treponema* glavne vrste su: *T. denticola*, *T. socranskii*, *T. vincenti*, *T. maltophilum*, *T. amylovarum*, *T. parvum*, *T. pectorum*, *T. putidum*, *T. medium*. *T. denticola* je asaharolitična. Ove bakterije se nalaze u gingivalnom sulkusu i povezane su sa parodontalnim bolestima. To su Gram-negativni spiralni bacili u tri veličine. Striktni su anaerobi i teško se kultiviraju *in vitro*. U sklopu roda *Tannerella* glavna vrsta je *T. forsythia*. To su nepokretni, pleomorfni, vretenasti Gram-negativni bacili. Za vidljiv porast *in vitro* potrebno je nekada i 14 dana. Bolje raste kada se kultiviraju zajedno sa *Fusobacterium nucleatum* i kada se podlogama doda *N-acetilmuraminska kiselina*. Nalazi se u

supragingivalnom, a češće u subgingivalnom plaku. Učestalost izolacije je u uskoj vezi sa dubinom parodontalnog džepa, češće se izoluje sa mesta gde se razvija oboljenje parodonticijuma, pa je opšteprihvaćen parodontalni patogen. *Tannerella forsythia*, *Treponema denticola* i *Porphyromonas gingivalis* označavaju se kao tri agensa „crvenog kompleksa“ bakterija, koji je uvek udružen sa parodontalnom bolešću. Glavna vrsta u rodu *Wolinella* je *W. succinogenes*. To su striktni anaerobi koji se kreću pomoću polarnih flagela. Mogu se pronaći u gingivalnom sulkusu, kao i kod agresivne parodontalne bolesti. Bakterije iz roda *Veillonella* su striktno anaerobni Gram-negativni koki, ne razgrađuju ugljene hidrate, već koriste laktat i na taj način podižu pH vrednost i imaju antikariogeni efekat. Izoluju se sa većine površina u usnoj duplji [2, 3, 4]. Mlečna kiselina je najjača kiselina koju produkuju bakterije usne duplje i razgrađuje zubnu gleđ. Veilonele mogu da je konvertuju u slabije kiseline, najvećim delom u propionsku karboksilnu kiselinu.

### Laboratorijska dijagnostika, glavna mesta izolacije iz usne duplje

Tri najvažnija koraka za uspešnu dijagnostiku anaerobnih bakterija su: pravilno uzimanje uzoraka, uz izbegavanje kontaminacije uzorka, brzi transport uzoraka u mikrobiološku laboratoriju, pravilno rukovanje uzorcima. Prilikom ispitivanja, uzorak je najbolje uzeti sa baze parodontalnog džepa u pravom trenutku, vodeći računa da ne dođe do kontaminacije. Za uzimanje uzorka treba koristiti samo sterilnu opremu. Uzorke treba odmah zasejati na podloge za anaerobne bakterije, kultivisati tokom različitih vremenskih perioda, ponekad i do 14 dana, u anaerobnim uslovima. Ako uzorke transportujemo u laboratoriju, obavezno treba koristiti transportne sisteme. Uzorci se nikako ne smeju stavljati u frižider. Kada god je moguće, uzorak treba uzeti pre početka antibiotske terapije [5].

#### Rod *Prevotella*

Bojenjem po Gramu vide se blede obojeni Gram-negativni pleomorfni bacili ili kokobacili. Mikroskopski se ne mogu razlikovati od *Bacteriodes* spp. Izoluju se iz parodontalnih džepova, zubnog plaka, kod hronične parodontopatije i dentoalveolarnih apscesa. Postupak izolacije bakterija roda *Prevotella* uključuje sledeće korake: priprema sterilne podloge za uzgajanje bakterija, uzimanje uzorka iz okoline gde se očekuje njihova prisutnost (usna šupljina ili gastrointestinalni trakt), inokulacija uzorka na sterilnu podlogu pomoću sterilne petlje ili pipete,

inkubacija podloge na optimalnoj temperaturi i pod optimalnim uslovima, identifikacija kolonija bakterija roda *Prevotella*, ispitivanje osetljivosti na antibiotike. Kada je reč o rodu *Prevotella*, kolonije su slične kao kod *Bacteriodes* spp, osim što su neke vrste pigmentovane (mogu biti bledosmeđe do crne). Kultivacijom na anaerobnom krvnom agaru stvaraju sjajne kolonije. *Prevotella* spp. su umereno saharolitične, dok je *Porphyromonas* spp. nesaharolitičan. Nakon inkubacije na krvnom anaerobnom agaru u trajanju od 7 do 14 dana stvaraju se kolonije nepravilnog oblika, promera oko 1 mm [6, 7].

Faktori virulencije su kapsularni polisaharid kod prevotela i inhibiraju opsonizaciju, a samim tim i fagocitozu, te fimbrije i enzimi. Stvaraju kožne apscese i infekcije mekih tkiva. Uzrokuju

plućni apsces, empijem, moždani apsces, mastoiditis i upalu srednjeg uha, osteomijelitis kostiju lica. Osnovni pristup je drenaža apscesa i hirurška obrada. Osetljivi su na metronidazol, klindamicin, rifampicin, a otporni na vankomicin i eritromicin.

#### Rod *Porphyromonas*

Povezuje se sa hroničnom parodontopatijom i dentoalveolarnim apscesima. *P. endodontalis* se uglavnom izoluje iz inficiranih kanala korena zuba. Izolacija bakterija roda *Porphyromonas* može se izvesti na sledeći način: uzorak se može prikupiti iz usta, zuba, gingive ili drugih oralnih površina pomoću sterilne špatule ili brisa i mora se transportovati u laboratoriju u roku od 30 minuta nakon uzorkovanja.

Uzorak se zasejava na podloge za *Porphyromonas*, kao što su Brucella agar sa heminom (5–10%) i vitaminom K (0,5%) ili tripton soja agar sa dodatkom krvi (TSBA), sa dodatkom hemina i vitamina K. Inkubacija je na temperaturi od 37°C tokom 7–14 dana. Kolonije su < 1,0 mm u prečniku nakon 48 sati inkubacije, glatke, sjajne i sive. Tamnosmeđi ili crni pigmenti se razvijaju tek nakon 3–7 dana [6, 7] i zato se zajedno sa bakterijama roda *Prevotella* ubrajaju u crno pigmentovane anaerobe [1, 5].

#### Rod *Fusobacterium*

Izoluje se iz tonzila, normalnog gingivalnog sulkusa i parodontalnih infekcija. Za kultivaciju zahteva obogaćene podloge. Izolacija zahteva uzimanje uzoraka bakterije roda *Fusobacterium*, koji mogu da se uzgajaju na hranljivim podlogama koje sadrže velike količine krvi (krvni agar) ili slične supstance. Potvrda identifikacije je test osetljivosti na antibiotike i PCR. Izgled kolonija je promenljiv, ali većina je prečnika 1–3 mm, sa nepravilnom ili nazubljenom ivicom. Variraju od providnih do zrnastih i neprozirnih. Rastu na podlogama sa 20% žuči, a na krvnom agaru za anaerobne bakterije stvaraju hemolizu [8]. Faktor virulencije je lipopolisaharidni endotoksin, koji deluje kao endotoksin. *F. necrophorum* uzrokuje Lemiereovu bolest (uzrokuje trombozu jugularne vene). Infekcija se može proširiti na pluća, mozak, jetru i kosti. U najtežem slučaju može doći do letalnog ishoda. Uzrokuje takođe i gingivitis i parodontalnu bolest, infekcije kože i mekih tkiva, osteomijelitis [9]. Teške invazivne bolesti, kao što je Lemierova bolest, obično se leče kombinacijom penicilina i metronidazola tokom 2–6 nedelja [10]. Važno je istaći da je izolacija ovih bakterija izuzetno težak proces zbog osetljivosti na kiseonik.

### Oboljenja koja uzrokuju Gram-negativne anaerobne bakterije

#### Parodontitis

Parodontitis predstavlja oboljenje koje zahvata opštu populaciju i dovodi do gubitka zuba najčešće kod osoba starijih od 40 godina. Smatra se da je najčešće oboljenje na svetu. Kliničku sliku karakterišu inflamacija, recesija gingive, krvarenje, gubitak alveolarne kosti oko zuba, ponekad halitoza. Kod određenih studija u dubokim parodontalnim džepovima identifikovano je prisustvo bakterija „crvenog kompleksa“, koji čine *Tannerella forsythia*, *Porphyromonas gingivalis* i *Treponema denticola* [11, 12].

## Nekrozna oboljenja parodonticijuma

Nekrozna oboljenja parodonticijuma su oboljenja koja odlikuje prisustvo bakterija kao što su *Fusobacterium*, *Veillonella*, anaerobni streptokoki. Javljaju se kod nekih osoba sa HIV infekcijom [11, 12].

## Flegmona poda usta – Ludvigova angina

Karakteriše se masivnim čvrstim otokom kompletnog poda usta (obostrano). Moguće su komplikacije koje uključuju opstrukciju disajnih puteva. Bakterije koje se povezuju sa Ludvigovom anginom su: *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp. i anaerobni streptokoki. U terapiji se koriste antibiotici širokog spektra dejstva. Kombinuju se penicilin i metronidazol. Važno je obezbediti prohodnost disajnih puteva, nadoknadu tečnosti, uzimanje uzorka za mikrobiološki pregled i ukloniti mogući izvor infekcije [11, 12].

## Periodontalni apsces

Periodontalni apsces je lokalizovana gnojna kolekcija koja se uglavnom nalazi na samom vrhu korena zuba. Uzrok apscesa je bakterijska infekcija koja se potom širi na vrh korena zuba. Bakterije se prirodno ne nalaze u periodoncijumu, ali tamo mogu dospeti ako na gleđi i dentinu postoje oštećenja, kao što je karijes. Uzročnicima pripadaju bakterije iz subgingivalnog plaka, *Porphyromonas*, *Prevotella*, *Fusobacterium*, spirohete, *Capnocytophaga*, *Actinomyces* spp. Kliničku sliku karakterišu nagli početak, otok, crvenilo, bol je kontinuiran ili je u vezi sa zagrižajem. Lečenje se sastoji u dreniranju apscesa parodontološkim postupcima. Loša prognoza rezultuje vađenju zuba [11, 12].

## Antibakterijski lekovi

Antibiotici su lekovi koji su toksični za bakterije, a netoksični ili slabo toksični za čovekov organizam. Izbor antibiotika zasniva se na principu „najbolje pretpostavke“ (*best guess*).

Kada uključiti antibiotike u stomatološku praksu?

Antibiotici su, od strane stomatologa, najčešće propisivani lekovi, posle analgetika. Međutim, često se neadekvatno i nepotrebno propisuju. Neki od najčešće propisivanih antibiotika su amoksicilin, samostalno, ili u kombinaciji sa klavulanskom kiselinom. Antibiotici bi trebalo da se koriste u slučaju širenja infekcije u duboke prostore glave i vrata, visoke telesne temperature i pojave opštih znakova infekcije, kod imunokompromitovanih pacijenata (nekontrolisani dijabetes, pacijenti na dugotrajnoj kortikosteroidnoj i drugoj imunosupresivnoj terapiji, HIV pozitivni pacijenti na hemodijalizi), pojave infekcije očnjačke jame. Osim eliminacije uzročnika infekcije, antibiotici se koriste i u

profilaksi. Preventivno propisivanje antibiotika događa se samo kod pacijenata sa oslabljenim imunološkim sistemom, kod kojih pojava infekcije može dovesti do teških komplikacija. Najčešće propisivani antibiotici širokog spektra delovanja spadaju u grupu penicilina i cefalosporina. Vreme delovanja antibiotika je veoma subjektivno i zavisi od pacijenta do pacijenta. Za uspešno izlečenje potrebno je slediti uputstvo stomatologa i dovršiti propisanu terapiju. Samostalno prekidanje terapije ili nepravilna upotreba antibiotika mogu dovesti do recidiva [13].

## Antimikrobni lekovi koji se najčešće koriste u stomatologiji

Najčešće propisivani antibiotici širokog spektra spadaju u grupu penicilina i cefalosporina.

## Rezistencija bakterija na antibiotike

Rezistencija je otpornost bakterija na dejstvo nekog antibiotika, tako da bakterije opstaju ili se čak umnožavaju. Bakterijska rezistencija može biti urođena i stečena. U svakoj osetljivoj populaciji bakterije mutiraju i nastaju rezistentne bakterije. Stečena rezistencija je indukovana usled dugotrajne primene antibiotika. Primetan je trend porasta rezistentnih sojeva Gram-negativnih anaerobnih bakterija [1, 13–17]. Postoji nekoliko metoda za uspostavljanje rezistencije: optimalna primena antibiotika, sinteza novih antibakterijskih lekova, sinteza inhibitora razmnožavanja plazmida rezistencije, upotreba kombinacija antibakterijskih lekova. Važno je napomenuti da antibiotike treba koristiti samo uz jasnu indikaciju i odabrati pravi antibiotik u optimalnoj dozi. Neracionalno i nekritičko propisivanje antibiotika predstavlja ogroman globalni i nacionalni problem. Poseban problem predstavljaju multirezistentne bakterije, posebno bolnički sojevi bakterija, rezistentne na najmanje tri klase antibiotika. Na njih jedino još deluju „rezervni antibiotici“. Ne ostavlja se dovoljno vremena organizmu da se sam izbori sa infekcijom. Izbor odgovarajućeg antibiotika vrši se na osnovu antibiograma, stanja organizma, mesta infekcije, kao i neželjenih dejstava leka [1, 13–17].

## ZAKLJUČAK

Gram-negativne anaerobne bakterije su izazivači parodontalnih, a nekada i sistemskih oboljenja, a zbog sporog rasta, odgovarajućih podloga i anaerobne atmosfere njihova izolacija, identifikacija i ispitivanje osetljivosti na antibiotike zahtevni su i dugotrajni. U slučaju oboljenja izazvanih Gram-negativnim anaerobnim bakterijama, u slučaju potrebe za antibiotskom terapijom, treba obratiti posebnu pažnju na racionalnu upotrebu odgovarajućih antibiotika zbog globalnog trenda porasta rezistencije bakterija na antibiotike.