МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ УКРАИНЫ
Харьковский национальный медицинский университет

NON-SPORE FORMING OBLIGATE ANAEROBIC BACTERIA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

НЕСПОРООБРАЗУЮЩИЕ ОБЛИГАТНЫЕ АНАЭРОБНЫЕ БАКТЕРИИ

Методические указания по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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Составитель Н. И. Коваленко

Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III-IV level accreditation.

Learning guide includes sections of taxonomy, morphology and ultrastructure of arbo- and roboviruses that cause encephalitis and hemorrhagic fevers. The most modern information on pathogenesis, epidemiology, methods of laboratory diagnosis and specific prophylaxis is represented.

Theme: Microbiological diagnosis of anaerobic infections

Actuality of the theme.

Goal: Studying of laboratory diagnosis of anaerobic infections.

Concrete goals:

1. Study of biological properties and classification of obligate anaerobic bacteria.
2. Study pathogenesis and clinical manifestations of anaerobic infections.
3. Study of the methods of laboratory diagnosis of anaerobic infections.
4. Study of therapy of anaerobic infections.

Students should be able to:

1. Differentiate of pathogenic anaerobic bacteria on biochemical and antigenic properties.
2. Isolate pure cultures of Bacteroides, Peptococcus and Peptostreptococcus and examine growth on Kitt-Tarrozzi medium and blood agar.
3. Identify of isolated pure culture of anaerobic bacteria for morphology, culture and biochemical properties, antigenic structure.

Equipment: slides, immersion microscope, culture of Bacteroides on Kitt-Tarrozzi medium and blood agar, basic dyes, inoculating loops, biological preparations for laboratory diagnosis, tables, atlas.

Pathogenic Anaerobes. Anaerobic bacteria are widely distributed in nature in oxygen-free habitats. Many members of the indigenous human flora are anaerobic bacteria, including spirochetes and Gram-positive and Gram-negative cocci and rods. For example, the human colon, where oxygen tension is low, contains large populations of anaerobic bacteria, exceeding 10^{11} organisms/g of colon content. Anaerobes in this region frequently outnumber facultative organisms by a factor of at least 100. Oxygen-sensitive organisms also are numerous in other areas of the body, such as the gingival crevices, tonsillar crypts, nasal folds, hair follicles, the urethra and vagina, and tooth surfaces.

Anaerobic indigenous flora components are potentially pathogenic if displaced from their normal habitat. Most anaerobic infections are endogenously acquired from members of the microflora, although Clostridium, found principally in the soil, also produces infections in humans. Proliferation of anaerobic bacteria in tissue depends on the absence of oxygen. Oxygen is excluded from the tissue when the local blood supply is impaired by trauma, obstruction, or surgical manipulation. Anaerobes multiply well in dead tissue. Multiplication of aerobic or facultative organisms in association with anaerobes in infected tissue also diminishes oxygen concentration and develops a habitat that supports growth of anaerobic bacteria.

Infections produced by anaerobic bacteria occur in all parts of the human body (*Table 1*). The infected tissues usually contain a mixture of several kinds of anaerobes and frequently also contain aerobic and facultative bacteria.

Table 1

Principal Medically Important Non-sporeforming Anaerobes

Morphology	Gram stain	Genus	Infections
Bacilli	Gram-positive	Actinomycetes	Head, neck, aspiration pneumonia
		Propionibacterium	Intra-abdominal
		Eubacterium	
	Gram-negative	Bacteroides	Intra-abdominal, soft tissue, genital, liver
		Fusobacterium	Abscesses, wound, pulmonary, genital, intracranial
		Prevotella	Intra-abdominal, genital, soft tissue
		Porphyromonas	Periodontitis, pneumonia
Cocci	Gram-positive	Peptococcus	Oral, respiratory, intra-abdomonal
		Peptostreptococcus	
		Propionibacterium	Schunt infections
		Bifidumbacterium	Ear, abdominal
	Gram-negative	Veillonella	Periodontitis

The types of infections commonly produced by anaerobic bacteria are as follows:

1. **Intra-abdominal infections.** Abscesses, postoperative wound infections, and generalized peritonitis produced by anaerobes occur as a consequence of bowel perforation during surgery or injury.

2. **Pulmonary infections.** Anaerobic lung infections may originate in the bronchi or the blood. Aspirations from the upper respiratory tract, which contain large numbers of anaerobic bacteria, are responsible for initiating infection in the bronchi.

3. **Pelvic infections.** Anaerobic infections of the vagina and uterus sometimes occur after gynecologic surgery or in association with malignancy of pelvic organs.

4. **Brain abscesses.** Anaerobes infrequently produce meningitis, but are a common cause of brain abscesses. The infecting organisms usually originate in the upper respiratory tract.

5. **Skin and soft tissue infections.** Combinations of anaerobes, aerobes, and facultative organisms often act synergistically to produce these infections.

6. **Oral and dental infections.** These local infections frequently extend to the face and neck and sometimes to other areas of the body such as the brain.

7. **Bacteremia and endocarditis.** Anaerobic bacteremia may follow disturbance in an area of the body where an established flora or an infection exists. Endocarditis,

an inflammation of the endothelial lining of the heart cavities, is occasionally caused by anaerobic bacteria, especially anaerobic streptococci.

Culturing of anaerobes needs special skills. It is extremely difficult due to the need to exclude oxygen, slow growth and complex growth requirements. Specimens for anaerobic cultures are ideally biopsy samples of needle aspirates. Anaerobic swabs are discouraged but sometimes cannot be avoided. Swabs are the least desirable because of the small amount of the specimen and effect of drying. There is a greater chance of contamination with normal microflora. For culture of strict anaerobes all traces of oxygen must be removed from medium and for many organisms sample must be kept entirely anaerobic during manipulations.

Anaerobic culture methods: production of vacuum, displacement of oxygen with inert gases like hydrogen, nitrogen, carbon, absorption of oxygen by chemical or biological methods, by using reducing agents.

Deep culture tubes can be used to test whether an unknown organism is anaerobic. Medium usually boiled during preparation and reducing agent added, stored under oxygen-free atmosphere. Most common adaptation of media is the addition of a reducing agent, e.g. Thiglycolate, cysteine (*Fig. 1*).

Pyrogallic acid-sodium hydroxide method can be used, again relies on a chemical reaction to generate an anaerobic environment, but a catalyst rather than a reducing agent. Anaerobic jars (GasPak System) are used to incubate plates in an anaerobic atmosphere, useful if brief exposure to oxygen is not lethal (*Fig. 2*).

Fig. 1. Kitt-Tarozzi medium contains nutrient broth with pieces of fat-free minced cooked meat of ox heart

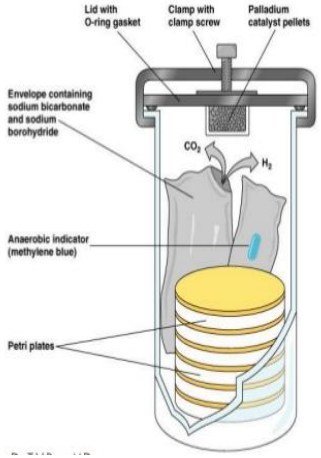


Fig. 2. Anaerobic jar with palladium aluminum coated pellets: catalyst, chemically reduces O_2, reacts with residual O_2 in the presence of H_2 to form H_2O

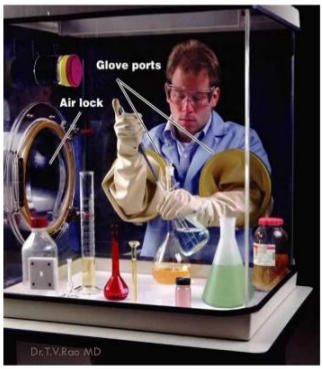


Fig 3. Anaerobic chamber

Obligate anaerobes can be culture in special reducing media or in anaerobic chambers and handled in anaerobic hoods (*Fig. 3*).

The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and digestion, catalase, lipase, lecihinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography, and susceptibility to antibiotics. The antibiotic susceptibility profile is determined by the micro tube broth dilution method.

ANAEROBIC COCCI

Structure, Classification and Antigenic Types. The anaerobic cocci are a physiologically diverse group that has recently undergone significant taxonomic changes. Anaerobic cocci of clinical significance are found in Gram-positive genera (Peptostreptococcus, Peptococcus, Bifidumbacterium, and Propionibacterium) and one Gram-negative genus (Veillonella). There are other genera of anaerobic cocci, but they are rarely isolated from clinical specimens. Not all anaerobic cocci require stringent anaerobic conditions; for example, strains of Streptococcus intermedius are quite aerotolerant and may grow under reduced oxygen tension. Anaerobic cocci may be proteolytic or saccharolytic or both. They produce a variety of short-chain volatile fatty acids (i.e., acetic, propionic, butyric, caproic, and lactic acids) from the fermentation of simple sugars and amino acids. Both *P magnus* and *P anaerobius* possess species-specific cell wall antigens; in other anaerobic cocci, species-specific antigens have not yet been identified. Peptostreptococcus and Streptococcus are the most clinically important genera, with *P magnus* as the most frequent clinical isolate.

The anaerobic Gram-positive cocci are difficult to specifate, but a few biochemical tests can be helpful. *P anaerobius* is the only species susceptible to sodium polyanethol sulfonate (SPS). *P asaccharolyticus* and *P hydrogenalis* are both indole positive, but alkaline phosphatase negative and positive, respectively. Of the indole-negative butyric acid producers, *P tetradius* is strongly saccharolytic and urease-positive, while *P prevotii* is weakly saccharolytic and usually urease-negative. *P magnus* and *P micros* are similar biochemically and are distinguished primarily on the basis of cell size and alkaline phosphatase reaction. The three prominent species of anaerobic cocci that are strongly saccharolytic and

produce large amounts of lactic acid include *S intermedius*, *S constellatus*, *G morbillorum*. These latter species are either aerotolerant or become aerotolerant upon passage on laboratory media. Obligate anaerobic species in the genus Streptococcus are only rarely isolated from clinical specimens, but may be found in human feces, as can other genera of anaerobic Gram-positive cocci.

Three genera of anaerobic Gram-negative cocci can be found in human fecal flora: Veillonella, Acidominococcus, and Megosphora. Veillonella is considered the only clinically significant genus and V parvula is the species most frequently isolated from clinical specimens. Veillonella can be presumptively identified by the red fluorescence of colonies under ultraviolet light. This fluorescence is lost rapidly on exposure to oxygen.

Epidemiology. Anaerobic cocci are part of the normal flora of the skin, the mouth, and the intestinal and genitourinary tracts of healthy individuals. Recently, with increasing study of the anaerobic cocci as pathogens, certain species are being associated with specific types of infection. *P prevotii* and *P anaerobius* are associated with female genital tract and intraabdominal infections. *P magnus*, the most frequently isolated anaerobic coccus, is associated most often with chronic bone and joint infections and ankle ulcers. Pure cultures of this organism are not rare; they account for 15 % of all *P magnus* isolates. The presence of foreign bodies, such as prosthetic joints, seems to be particularly significant in *P magnus* infections. Veillonella and the anaerobic/aerotolerant Streptococcus are the anaerobic cocci isolated most frequently from infected human bites. These organisms are part of the normal oral flora.

Pathogenesis. Anaerobic cocci are opportunistic pathogens that cause a multitude of infections. They are part of the normal microbial flora of a healthy individual, but they can and do cause infections involving traumatized tissue or infections in the compromised host. They are isolated most often from a wide variety of polymicrobic infections, indicating a synergistic role in these infections. Approximately 10–15 % of all isolates of anaerobic cocci come from pure culture infections, thus indicating that these organisms can be significant pathogens rather than innocuous commensals. The anaerobic cocci represent 25–30 % of all anaerobic clinical isolates. Among anaerobes, they are second only to the Gram-negative anaerobic bacilli in frequency of isolation from clinical specimens.

Clinical Manifestations. Anaerobic cocci are not involved in any single specific disease process; rather, they may be present in a great variety of infections involving all areas of the human body. These infections may range in severity from mild skin abscesses, which disappear spontaneously after incision and drainage, to more serious and life-threatening infections such as brain abscess, bacteremia, necrotizing pneumonia, and septic abortion. Infection by anaerobic cocci (and by anaerobes in general) usually involves invasion of devitalized tissue by organisms that are part of the normal flora of the affected tissue or of the surrounding areas.

Anaerobic infections generally occur in the compromised host; that is, in patients who have impaired host defense mechanisms. The primary host defense deficiency in these infections is the disruption of natural barriers (such as the skin and mucous membranes). Diabetes mellitus, connective tissue disorders, atherosclerotic disease, cancer (especially of the colon, uterus, and lung), irradiation damage, immunosuppressive treatment, and alcoholism are conditions that may disrupt these natural barriers.

Brain abscess, with a mortality rate of 40 %, is one of the more serious infections involving anaerobic cocci. Anaerobes, rather than facultative or aerobic organisms, are a major cause; anaerobic cocci, Bacteroides and Fusobacterium, respectively, are the predominant groups isolated. Anaerobic cocci often have been isolated in pure culture from brain abscesses. Chronic otitis media or mastoiditis frequently is the primary source of the organisms and may result as a direct extension of the infection into the brain. Pleuropulmonary infection, sinusitis, congenital heart defects, and bacterial endocarditis are other conditions predisposing individuals to brain abscess by blood-borne metastases.

Pleuropulmonary infections in which anaerobic cocci may be etiologic agents are lung abscesses, necrotizing pneumonia, aspiration pneumonitis, and empyema. The incidence of anaerobes in these infections is 50–90 %; anaerobic cocci account for about 40 % of the anaerobic isolates. *F nucleatum* and *P melaninogenica* are often isolated concomitantly. These organisms are part of the normal microbial flora of the mouth and enter the lower respiratory tract as the result of aspiration, usually in association with altered consciousness.

Anaerobic cocci are involved in several skin and soft tissue infections that may be confused with clostridial myonecrosis (gas gangrene). These infections are anaerobic streptococcal myonecrosis, progressive bacterial synergistic gangrene, necrotizing fasciitis, crepitant cellulitis, chronic burrowing ulcer, and synergistic necrotizing cellulitis. These are severe infections, and the mortality rates may be as high as 75 %. These conditions may be characterized by a purulent exudate, by varying degrees of tissue necrosis involving the skin, fascia, and/or underlying muscles, and sometimes by systemic toxicity. The infecting organisms often produce gas. Anaerobic cocci often are isolated with other organisms in these infections. They are characteristically found with *Staphylococcus aureus* and *Streptococcus pyogenes* in progressive bacterial synergistic gangrene, and also are found with Gram-negative aerobic or facultative bacilli or Bacteroides or both in synergistic nonclostridial myonecrosis and synergistic necrotizing cellulitis. Diabetes mellitus and vascular insufficiency (often associated with trauma) are predisposing factors. Decubitus ulcers and postoperative wound infections are other soft-tissue infections from which anaerobic cocci have been isolated.

Anaerobic cocci have been recognized as significant pathogens in puerperal fever and septic abortion since the early 1900s. Other infections of the female genital tract in which anaerobic cocci have been implicated are pyometra, tuboovarian abscesses, postoperative wound infections following gynecologic

surgery, and pelvic inflammatory disease, often in association with gonococci. Anaerobic cocci (*P prevotii, P anaerobius* and *S intermedius*) and rods *B fragilis* are the most frequently isolated anaerobes from these infections. Like the anaerobic cocci in other infections, these organisms are part of the normal flora of the affected area or of the surrounding tissues in this case, the vagina.

Periodontal disease, peritonitis, intra-abdominal abscesses, and abscesses of the liver, spleen, and pancreas are types of intra-abdominal infections from which anaerobic cocci have been isolated. Again, these are polymicrobic infections; concomitant isolates may be *Bacteroides sp., E. coli* and *Streptococcus* sp.

Host Defenses. In most cases, specific immune responses to anaerobic cocci have not been investigated.

Diagnosis. To establish a definite role for anaerobic cocci in infections, the causative organism must be isolated from the affected tissue or the bloodstream. Because anaerobic cocci are a significant part of the normal flora, the proper choice of specimen is critical. For example, coughed sputum, feces, and vaginal swabs, all of which could be contaminated with normal microbial flora, are unacceptable.

Control. Treatment of infections caused by anaerobic cocci consists of antibiotic therapy and drainage, debridement, or both of necrotic tissue. In general, penicillins (ampicillin) and cephalosporins are drugs of choice, and clindamycin or metronidazole can be used for the patient allergic to penicillin. The clinician should be aware that in vitro antimicrobial susceptibility tests have shown that some strains of anaerobic cocci are resistant to penicillin or to clindamycin. Metronidazole is typically active against most strains of anaerobic cocci; however, aerotolerant species, such as Streptococcus spp. are uniformly resistant. Brain abscesses must be treated with an antimicrobial agent such as chloramphenicol or penicillin or metronidazole, sufficient doses of which can cross the blood barrier. Frequently B fragilis, an anaerobic gram-negative rod, is present in infections containing anaerobic cocci; this organism produces a ß-lactamase that can protect other organisms in the infection from the action of penicillin.

Anaerobic Gram-Negative Bacilli

At present there are over two dozen genera of Gram-negative anaerobic bacilli. In most clinical infections, only the genera Bacteroides, Prevotella, and Fusobacterium need be considered. These genera are prevalent in the body as members of the normal flora (*Fig. 4*), constituting one-third of the total anaerobic isolates from clinical specimens, and may become involved in infections throughout the body. Within the Bacteroides group, *B fragilis* is the most common pathogen. Among the bile-sensitive Prevotella species, the ones most commonly encountered clinically are *P melaninogenica, P oris,* and *P buccae*. *Fusobacterium nucleatum* is the Fusobacterium species most often found as a pathogen, but *F necrophorum* occasionally produces serious disease. These genera contain numerous other species that rarely or never infect humans.

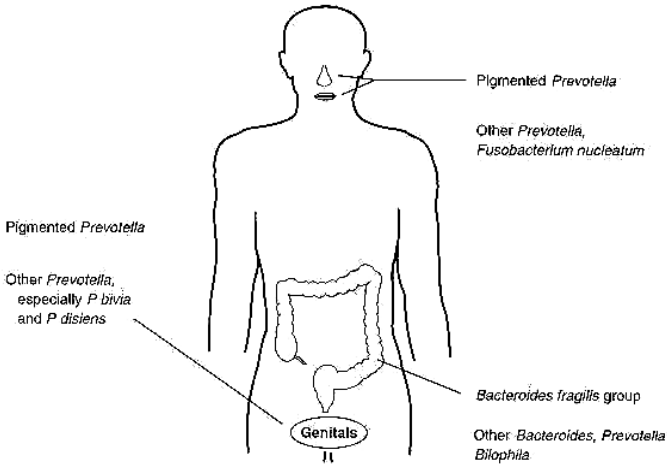
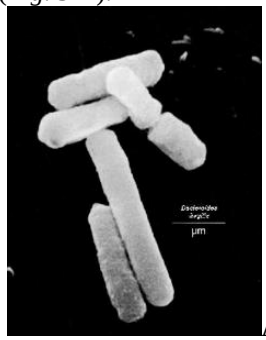
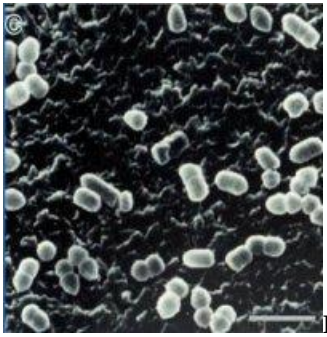


Fig. 4. Predominant sites colonized by Gram-negative anaerobic bacilli

Structure, Classification, and Antigenic Types. *Bacteroides fragilis* (*Fig. 5-A*), the most important of all anaerobes because of its frequency of occurrence in clinical infection and its resistance to antimicrobial agents, is a Gram-negative non-motile bacillus with rounded ends 0.5 to 0.8 µm in diameter and 1.5 to 4.5 µm long. Most strains are encapsulated. Vacuolization or irregular staining is common, particularly in broth media. Some pleomorphism also may be seen. *Prevotella melaninogenica* and *Porphyromonas asaccharolytica* are short to coccoid Gram-negative rods; they produce a distinctive pigment (brown to black), which is a heme derivative that colors the colony (*Fig. 5-B*).



A



B

Fig. 5. Electron micrograph of *Bacteroides fragilis* (A) and *Prevotella melaninogenica* (B)

Members of the genus Fusobacterium (*Fig. 6*) may be spindle shaped or may have parallel sides and rounded ends. Cells of *Fusobacterium necrophorum* often are elongated or filamentous, are curved, and possess spherical enlargements and large, free, round bodies. *F nucleatum*, although not producing infections as serious as those caused by *F necrophorum*, is a virulent organism and is much more common clinically. The cells of this species are usually spindle shaped, are 5 to 10 μm long, and are often seen in pairs, end to end.

Fig. 6. Microscopic morphology of *F nucleatum* from broth culture. Note thin, delicate bacilli with tapered ends

Certain Bacteroides species possess distinguishing enzymes. Superoxide dismutase has been found in *B fragilis, B thetaiotaomicron, B vulgatus*, and *B ovatus*. In general, a good correlation exists between superoxide dismutase activity and oxygen tolerance. ß-Lactamase activity has been demonstrated in several Bacteroides species, some Prevotella, and Bilophila; it accounts for most of the resistance to various ß-lactam antibiotics, such as penicillins and cephalosporins, although other mechanisms are responsible occasionally.

Epidemiology. All infections involving anaerobic Gram-negative bacilli arise endogenously when mucosal damage related to surgery, trauma, or disease permits tissue penetration by members of the indigenous flora. Knowledge of the composition of the indigenous flora at various sites under different circumstances permits the clinician to anticipate the likely infecting species in acute infections at different locations. The pathogenicity of various species also must be taken into account. Ecologic determinants include the oxygen sensitivity of various organisms, the ability of organisms to adhere, and microbial interrelationships. These interrelationships permit one organism to supply growth factors needed by the other, to provide assistance with adherence or motility to another organism, and to facilitate the production of inhibitory substances.

At birth, an infant's oral cavity usually is sterile; but by 12 months of age, Fusobacterium species can be cultured from 50 percent of infants and other Gram-negative anaerobic bacilli species from a smaller percentage. In the

human gingival crevice area, Gram-negative anaerobic rods account for 16 to 20 percent of the total cultivable flora. *Prevotella melaninogenica* is seldom isolated before the age of 6 years, but by the early teens this organism can be isolated from the gingival crevice area of most individuals. Gram-negative anaerobic rods usually constitute 8 to 17 percent of the cultivable flora of human dental plaque. Selective localization is illustrated by the fact that *P melaninogenica* is found routinely in the gingival crevice but is not found, or is only rarely found, on the tongue, cheek, or coronal tooth surface.

The stomach normally has few organisms and, as a rule, no anaerobic bacteria; however, in the presence of pathologic conditions such as duodenal ulcer with bleeding or obstruction, abnormal colonization with *B fragilis* may occur in the stomach. In the terminal ileum, approximately equal numbers of facultative aerobes and anaerobes are present, with Bacteroides being one of the major anaerobes. Bacteroides species are almost invariably found in the feces of adult subjects; the mean count is 10^{11}/g. Fusobacterium species are found in the feces of 18 percent of adults; the mean count is 10^{8}/g. *B thetaiotaomicron* and *B vulgatus* are the dominant species of Bacteroides encountered, followed by *B distasonis, B ovatus*, and *B fragilis*.

Bacteroides, Prevotella, and Fusobacterium species are common in the vaginal flora. In one quantitative study of the vaginal and cervical flora, Bacteroides and Prevotella species were recovered from half of the patients, with mean concentrations of 10^{6}/g of material. Species recovered from the normal cervical flora of healthy women include *B fragilis, B capillosus, P oralis, P bivia, P disiens, P oris, P buccae*, and *B ureolyticus*.

Studies of the normal urethral flora are relatively limited, but Fusobacterium and other Gram-negative anaerobic bacilli have been isolated.

Pathogenesis. Bacteroides, Prevotella, Porphyromonas, and Fusobacterium species are prevalent in the indigenous flora on all mucosal surfaces. They may have an opportunity to penetrate tissues and then to set up infection under certain circumstances such as surgical or other trauma or when tumors arise at the mucosal surface. In certain cases, such as aspiration pneumonia, anaerobic bacteria from a site of normal carriage may move into another area that is normally free of organisms and infect that site. Tissue necrosis and poor blood supply lower the oxidation-reduction potential, thus favoring the growth of anaerobes. Accordingly, vascular disease, cold, shock, trauma, surgery, foreign bodies, cancer, edema, and gas production by bacteria may significantly predispose individuals to infection with anaerobes, as may prior infection with aerobic or facultative bacteria. Antimicrobial agents such as aminoglycosides, trimethoprim/sulfamethoxazole, and quinolones, to which anaerobes are notably resistant, may facilitate anaerobic infection. The more aerotolerant anaerobes are more likely to survive after the normally protective mucosal

barrier is broken and until conditions are satisfactory for their multiplication and invasion. Once anaerobes begin to multiply, they can maintain their own reduced environment by excreting end products of fermentative metabolism. Infections involving Gram-negative anaerobic bacilli often are characterized by abscess formation and tissue destruction.

Bacteroides, Prevotella, Porphyromonas, and Fusobacterium species produce enzymes that may play a role in pathogenesis (*Table 2*). Prevotella melaninogenica is one of the few bacteria that produce collagenase, an enzyme of considerable importance. Porphyromonas gingivalis also produces collagenase and has trypsin-like activity. Neuraminidase may be important in the pathogenesis of Bacteroides infection. This enzyme alters neuraminic acid-containing glycoproteins of human plasma. Hyaluronidase is produced by many strains of the *B fragilis* group and pigmented anaerobic Gram-negative rods. DNase is also produced by *B fragilis* and may be an important factor in infection. Many Gram-negative anaerobic bacilli produce phosphatase. A heparinase produced by *B fragilis* strains may contribute to intravascular clotting and hence increase the dosage of heparin needed to treat septic thrombophlebitis in infections caused by this organism. Fibrinolysin is produced by many *P melaninogenica*group strains and by a few *B fragilis* group strains. *Porphyromonas asaccharolytica* produces proteinases that render it capable of hydrolyzing gelatin, casein, coagulated protein, plasma protein, azacol, and collagen. Strains of Bacteroides and *P gingivalis* degrade complement factors and immunoglobulins G and M. A strain of *P melaninogenica* produces phospholipase A.

Table 2

Factors responsible for virulence of anaerobic bacteria

Virulence factor	Action
Lipopolysaccharide	Promotes abscess formation, enhanced coagulation
Polysaccharide capsule	Correlated with abscess production
Enzymes: – collagenase – heparinase	 Develop thrombophlebitis and septic emboli
– leukocidin – phospholipase – hemolysin	Lysis of erythrocytes
Short chained fatty acids: – butyrate – succinic acid	 Seen in dental plaque Reduces phagocytic killing

F necrophorum produces a leukocidin and hemolyses erythrocytes of humans, horses, rabbits, and, much less extensively, sheep and cattle. Certain *F necrophorum* cells hemagglutinate the erythrocytes of humans, chickens, and pigeons. A bovine isolate of F necrophorum demonstrates phospholipase A and lysophospholipase activity.

Constituents of the cell envelope and cell surface may contribute to pathogenicity. The capsule of organisms such as *B fragilis* is an important virulence factor. Pili (fimbriae) and lectinlike adhesins may also be important in the adherence of Bacteroides cells to epithelial surfaces. Butyrate and succinate produced by Bacteroides show a cytotoxic effect.

Clinical Manifestations. Gram-negative anaerobic bacilli may cause infections anywhere in the body; the most common types are oral and dental, pleuropulmonary, intra-abdominal, female genital tract and skin, soft tissue and bone infections (*Fig. 7*). They may play a role in such diverse pathologic processes as periodontal disease and colon cancer. Bacteroides, Prevotella, Porphyromonas, and Fusobacterium produce enzymes (collagenase, neuraminidase, deoxyribonuclease, deoxyribonuclease [DNase], heparinase, and proteinases) that may play a role in pathogenesis by helping the organisms to penetrate tissues and to set up infection after surgery or other trauma. The incidence of infection by these organisms can best be reduced or eliminated by avoiding conditions that decrease the redox potential of tissues and by preventing introduction of the anaerobes into compromised host tissues.

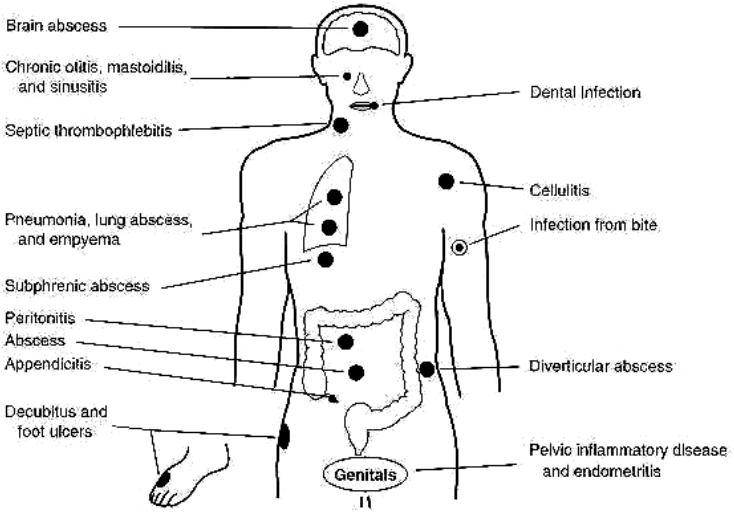


Fig. 7. Sites of anaerobic infections

Host Defenses. Polymorphonuclear leukocytes have oxygen-dependent and oxygen-independent microbicidal systems. Components of both systems might be important in phagocytic killing of anaerobes under conditions of varying oxygen tension. Specifically, polymorphonuclear leukocytes normally kill *B fragilis* under anaerobic and aerobic conditions. Random migration of polymorphonuclear leukocytes does not differ significantly under aerobic and anaerobic conditions. The same holds true for chemotaxis in response to factors generated by immune complexes in plasma; however, chemotaxis in response to factors generated by bacteria in plasma is markedly depressed under anaerobic conditions, and products of Gram-negative anaerobic bacilli may suppress neutrophil chemotaxis and phagocytic killing.

Immunoglobulin and components of the classic and alternative complement pathways participate in chemotaxis, bacteriolysis, and opsonophagocytic killing of various Gram-negative anaerobic bacilli. A study of women with acute pelvic inflammatory disease demonstrated antibody to the capsular antigen of *B fragilis* in women whose infecting flora contained *B fragilis*.

T cells are involved in immunity of humans to *B fragilis*, specifically linked to early stages of abscess formation.

Diagnosis. A definitive diagnosis requires demonstration or isolation of the organisms responsible for the infection. Even direct Gram stain may be helpful because of the frequently unique morphology of Gram-negative anaerobic bacilli. In general, these organisms are pale staining and they may stain erratically. Fusobacterium cells may exhibit classic tapered ends and filamentous forms, with or without swollen areas and large round bodies. Direct gas-liquid chromatography of clinical specimens occasionally provides important clues to the presence of certain Gram-negative anaerobic bacilli. A large amount of butyric acid in the absence of isobutyric or isovaleric acid indicates the presence of Fusobacterium. The presence of succinic acid and only Gram-negative rods seen on Gram stain, or of both succinic and isobutyric acid in the specimen, indicates that Bacteroides is present.

Collection of clinical specimens should avoid the mucosal flora, and transport must be anaerobic. Use of selective and differential media may facilitate isolation and identification of different Gram-negative anaerobic bacilli.

Both direct and indirect fluorescent antibody techniques may be useful for rapid detection of Bacteroides, Prevotella, and Fusobacterium in clinical material. Tests for antibody development in response to the infection are not practical.

Control. There are two primary guidelines in preventing anaerobic infections: avoiding conditions that reduce the redox potential of the tissues and preventing the introduction of anaerobes of the normal flora to wounds, closed cavities, or other sites prone to infection. Prophylactic antimicrobial therapy is effective in selected situations before certain types of surgery. When infection already is

established but surgery is indicated (appendectomy, cholecystectomy), antimicrobial therapy just before surgery again may be helpful.

Drugs active against essentially all Gram-negative (and other) anaerobes are metronidazole, imipenem, chloramphenicol, and combinations of ß-lactam drugs plus a ß-lactamase inhibitor.

Practical tasks, being carried out during practical classes:

1. Studying morphology of anaerobic bacteria (in atlas and microslides).
2. Studying of cultural properties of anaerobic bacteria (in atlas).
3. Studying biological preparations for serological methods (antigens and diagnostic sera).
4. Studying the scheme of laboratory diagnosis of anaerobic infections.
5. Prepare a microslide from Kitt-Tarrozzi medium. Stain the smear after Gram. Examination of the smear with immersion microscope.
6. Studying of the scheme of laboratory diagnosis of anaerobic infections.

Therminology: Peptoctreptococci, Peptococci, Eubacteria, Propionibacteria, Veillonella, Bacteroides, Fusobacteria, Prevotella, Bifidumbacteria, Kitt-Tarozzi medium, anaerobic jar.

Theoretical questions for control:

1. Genera Peptoctreptococci, Streptococci, Bacteroides, Fusobacteria, major characteristics, antigenic structure.
2. Culture properties of anaerobic bacteria.
3. Routes of transmission and pathogenesis of anaerobic infections.
4. Laboratory diagnosis of anaerobic infections.
5. Treatment and control of anaerobic infections.

Test tasks for control:

1. An isolate from an anaerobic blood culture bottle is a Gram-negative rod. It is resistant to both gentamicin and penicillin. Confirmation of this isolate as Bacteroides fragilis can be accomplished by:

A. Proof of its anaerobic nature
B. A study of its biochemical reactivity
C. A test for clindamycin resistance
D. Gas chromatographic analysis of metabolic by-products
E. All of the above

2. Bacteria that can subsist on CO_2 but cannot tolerate oxygen are called:

A. Chemoorganotropic, facultative anaerobes
B. Chemolithotropic, obligate anaerobes
C. Organolithotropic, obligate anaerobes
D. Chemolithotropic, aerotolerant anaerobes
E. None of above

3. Fermentation reactions extract energy by:

A. Oxidative phosphorylation
B. Substrate phosphorylation
C. Cytochrome oxidase
D. Proton motive force
E. None of the above

4. Anaerobic respiration:

A. Involves cytochrome oxidase
B. Involves an obligate anaerobe
C. Does not involve a proton motive force
D. Does not involve an electron transport
E. None of above

5. Which of the following medium is available for cultivation of anaerobes?

A. Endo agar
B. Kitt-Tarozzi
C. Mac Conkey
D. Meat peptone agar
E. Bile broth

6. Obligate anaerobes undergo lethal oxidations due to lack of the enzymes:

A. Catalase, peroxidase
B. Protease, lipase
C. Hemolysin
D. Carbohydrase
E. All of the above

7. An isolate from a wound culture is a Gram-negative rod identified as Bacteroides fragilis. Anaerobic infection with B. fragilisis characterized by:

A. A foul-smelling discharge
B. A black exudate in the wound
C. An exquisite susceptibility to penicillin
D. A heme-pigmented colony formation
E. Severe neurologic symptoms

8. A bacterium is examined and is found to lack superoxide dismutase, catalase, and peroxidase. Which of the following statements best describes this bacterium?

A. This bacterium is an anaerobe
B. This bacterium will survive in an O_2 environment
C. This bacterium is more virulent than one containing the three enzymes
D. This bacterium does not produce superoxide
E. This bacterium does not produce peroxide

9. Microbiologic analysis revealed no growth in oxygen environment, many Gram-negative, rod-shaped bacteria in the wound exudate. The most likely cause of this outbreak is:

A. Staphylococcus aureus
B. Clostridium perfringens
C. Clostridium botulinum
D. Bacteroide fragilis
E. Escherichia coli

10. A patient complained to his dentist about a draining lesion in his mouth. A Gram's stain of the pus showed a few Gram-positive cocci, leukocytes, and many branched Gram-positive rods. The most likely cause of the disease is:

A. Staphylococcus aureus
B. Clostridium perfringens
C. Clostridium botulinum
D. Bacteroide fragilis
E. Actinomyces israelii

Correct answers: 1 – E, **2** – B, **3** – A, **4** – B, **5** – B, **6** – A, **7** – A, **8** – A, **9** – D, **10** – E.

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Учебное издание

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Методические указания по дисциплине «Микробиология, вирусология и иммунология» для студентов II и III курсов медицинского и стоматологического факультетов с английским языком преподавания

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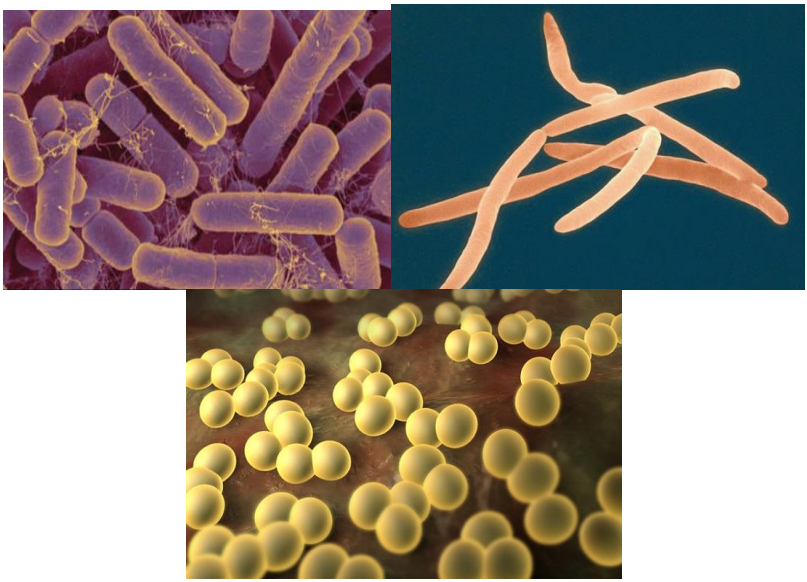

NON-SPORE FORMING OBLIGATE ANAEROBIC BACTERIA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)