
Sterilization and disinfection
Sterilization is defined as the process where all the living microorganisms, including bacterial spores, are killed. Sterilization can be achieved by physical, chemical and physicochemical means. Chemicals used as sterilizing agents are called chemisterilants.

Disinfection is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.

Asepsis is the employment of techniques (such as usage of gloves, air filters, uv rays, etc.) to achieve microbe-free environment.

Antisepsis is the use of chemicals (antiseptics) to make skin or mucus membranes devoid of pathogenic microorganisms.

Chemotherapeutic agents
Chemotherapeutic agents can be classified into groups:
- broad spectrum chemotherapeutics
- narrow spectrum chemotherapeutics
- those with bacteriostatic effect
- those with bactericidal effect

Chemotherapeutic agents may have different mechanisms of action on bacterial cell:
1. agents that inhibit cell wall synthesis – penicillins, cephalosporins, vancomycin, bacitracin
2. agents that inhibit protein synthesis - streptomycin, neomycin, erythromycin, doxycycline, gentamycin, chloramphenicol, clindamycin, lincomycin
3. agents that inhibit the cell membrane function : nystatin, polymixin, amphotericin
4. agents that inhibit nucleic acid synthesis : nalidixic acid, quinolones
5. agents that act as antimetabolites: sulfonamides, isoniazid, trimethoprim, nitrofurans.

Proper selection of an antimicrobial agent for the therapy should be based on many criteria, including both microbial and host factors.
Microorganism may manifest different mechanisms of the resistance to antibiotics. Some mechanisms of antibiotic resistance will be discussed during the course. In order choose the most effective antibiotic, susceptibility tests must be performed. There are 2 types of the tests: qualitative tests (e.g., disc diffusion method) and quantitative tests (E-test, MIC, MBC).


*Staphylococcus*
Gram-positive cocci, arranged in grape-like clusters.
The main pathogenic species is *Staphylococcus aureus*. The pathogenicity of *S. aureus* is connected with production of enzymes and toxins: plasmocoagulase, protein A, leukocidins, haemolysins, lipase, hyaluronidase, TSST-1 toxin, exfoliatin, enterotoxins. *S. aureus* may be responsible for many local skin infections (folliculitis, furuncle, “scalded skin syndrome”, impetigo), but also for serious invasive diseases (pneumoniae, gastroenteritis, osteomyelitis, sepsis). The diagnosis is based on culturing a sample on blood agar or Chapman medium. Also coagulase-negative *Staphylococci* (*S. saprophiticus* and *S. epidermidis*) may be pathogenic (biomaterial-associated infections, urinary tract infections in women). In antibiotic therapy of infection caused by *Staphylococcus*, the presence of MRSA and MRCNS strains must be taken into account.

The most important species include: *Streptococcus pyogenes*, *S. pneumoniae*, *S. agalactiae*, *Enterococcus faecalis* and *E. faecium*.

The streptococci are facultatively aerobic, catalase-negative, Gram-positive cocci that grow in pairs or chains. Virtually all pathogenic species can colonize the host without causing an infection. The upper respiratory, gastrointestinal and the female genitourinary tracts are the sites customarily colonized. During periods of immune system dysfunction, streptococci may quickly transform from harmless commensals to life-threatening pathogens. In the microbiological laboratory, streptococci are initially categorized by their manner of growth on 5% sheep blood agar and type of haemolysis. Laboratory tests used for diagnosis of streptococci are, e.g., direct antigen detection (Lancefield grouping), optochin susceptibility test (for *S. pneumoniae*) or bacitracin susceptibility test, and ASO reaction (for *S. pyogenes*). *S. pyogenes* is an important cause of upper respiratory tract (pharyngitis, tonsillitis) and cutaneous infections (e.g. impetigo, erysipelas, cellulitis, myositis). In complications, the influence of protein M is very important.

*S. pneumoniae* is the leading cause of community-acquired bacterial pneumonia and, in children, of bacteraemia, otitis media, sinusitis and meningitis. *S. agalactiae* is an important cause of neonatal sepsis and meningitis. *S. mutans* plays an important role in dental caries formation and endocarditis.
The enterococci are members of the normal gastrointestinal tract flora of humans. They are important aetiologic agents of urinary and biliary tracts infections, wound infections, intra-abdominal abscesses, endocarditis, bacteraemia and a variety of nosocomial infections.

**Pseudomonas aeruginosa**

*Pseudomonas* are Gram-negative rods. They are motile, non-fermentative aerobes that can utilize acetate for carbon and ammonium sulphate for nitrogen. Many species are resistant to high salt, dyes, weak antiseptics and most antibiotics. *P. aeruginosa* can grow at 42° and it produces many exoenzymes including haemolysins, leukocidins and proteases. In addition, a toxin, called toxin A, is the most toxic product produced by *Pseudomonas*. This product causes the ADP-ribosylation of translation factor EF-2, producing ADP-ribosyl-EF-2. The effect of this enzymatic activity is the loss of host cell protein synthesis capability. This mechanism is identical to that produced by diphtheria toxin. *Pseudomonas* can be found in the soil, in water, or on vegetation.

On average, 3% of persons entering the hospital have *Pseudomonas* in their stools. After a hospital stay of just 72 hrs, 20% patients have *Pseudomonas*. The organisms are spread from patient to patient via staff, contaminated reservoirs, respiratory equipment, food, sinks, taps, mops; most moist environments. Pseudomonas produces localized infections following surgery or burns. Localized infections can lead to generalized, and occasionally fatal, bacteraemia. *Pseudomonas* is also responsible for a number of nosocomial infections including urinary tract infections following catheterization, pneumonia resulting from contaminated respirators, and eye and ear infections.

**Haemophilus**

*Haemophilus influenzae* is responsible for producing a variety of infections including meningitis and respiratory infections. Six serological types (a,b,c,d,e,f) are recognized, based on the antigenic structure of the capsular polysaccharides. Non-encapsulated strains are nontypable. Other species of *Haemophilus* include: *H. parainfluenzae* (pneumonia, endocarditis), *H. ducreyi* (venereal chancre) and *H. aegyptius* (conjunctivitis). The genus *Haemophilus* is composed of Gram-negative coccobacilli. These organisms are fastidious and require factors X (haemin) and/or V (NAD). *Haemophilus* contains LPS in the cell wall but produces no apparent extracellular toxins. *Haemophilus* is transmitted from an infected human being to other humans. The organisms colonize the nasopharynx and are spread by direct contact. *Haemophilus* are capable of penetrating the epithelium to produce a bacteraemia that may lead to spread of the organisms to many organs. Its capsule is the major determinant of virulence yet unencapsulated strains produce ear, sinus and respiratory infections. *H. influenzae* type b is the most common cause of bacterial meningitis in children aged 6 months-2 years. It is uncommon in adults because of protective antibody. Cellulitis, conjunctivitis, epiglottitis and arthritis may also result from *Haemophilus* infection. For pneumonia in adult men, the unencapsulated *H. influenzae* may provide the cause.
**Legionella**

*Legionellaceae* are facultative Gram-negative rods, and intracellular parasites. The *Legionellaceae* family includes 34 species, but the most important for human diseases is *Legionella pneumophila*. The organism gains entry to the upper respiratory tract by aspiration of water containing the organism, or by inhalation of a contaminated aerosol. *Legionellaceae* primarily cause respiratory tract infections: Legionnaires’ disease (LD) and Pontiac fever. Legionnaires’ disease (LD) is an atypical, acute lobar pneumonia with multisystem symptoms. Predisposing factors include, for example, immunocompromise, pulmonary compromise. Pontiac fever is an influenza-like illness that characteristically infects otherwise healthy individuals.

### 3. Viruses.

Viruses depend on the host cells that they infect to reproduce. When found outside of host cells, viruses exist as a protein coat or capsid, sometimes enclosed within a membrane. The capsid encloses either DNA or RNA which codes for the virus elements.

Some viruses may remain dormant inside host cells for long periods, causing no obvious change in their host cells (a stage known as the lysogenic phase). But when a dormant virus is stimulated, it enters the lytic phase: new viruses are formed, self-assemble, and burst out of the host cell, killing the cell and going on to infect other cells. The diagram below at right shows a virus that attacks bacteria, known as the lambda bacteriophage, which measures roughly 200 nanometers.

Viruses cause a number of diseases in eukaryotes. In humans, smallpox, the common cold, chickenpox, influenza, shingles, herpes, polio, rabies, Ebola, hanta fever, and AIDS are examples of viral diseases. Even some types of cancer - though definitely not all - have been linked to viruses.

The *Herpesviridae* are a large family of DNA viruses that cause diseases in animals, including humans. The members of this family are also known as herpesviruses. *Herpesviridae* can cause latent or lytic infections. Herpesviruses all share a common structure—all herpesviruses are composed of relatively large double-stranded, linear DNA genomes encoding 100-200 genes encased within an icosahedral protein cage called the capsid, which is itself wrapped in a protein layer called the tegument, containing both viral proteins and viral mRNAs and a lipid bilayer membrane called the envelope. This whole particle is known as a virion.

**Human Papilloma Virus (HPV).** Papillomaviruses are widespread and warts are common in young adults. Humans are the only host for HPV and infections are generally transmitted by direct contact. However, the virus can survive for extended periods (months) outside the host, and this may provide another means of transmission. While there is a strong correlation between HPV infection and certain forms of cancer (e.g. cervical cancer), infection alone does not result in malignancy; rather, additional factors such as radiation, immunosuppression, or tobacco use are involved. **Diagnosis: clinical:** warts of the skin, oral cavity and genital area are generally diagnosed by appearance. **Laboratory:** Microscopy of wart scrapings shows a characteristic histologic appearance.
Herpesviruses: Herpes Simplex (HSV-1, HSV-2) HSV-1 is responsible for a variety of infections. Most commonly, HSV-1 produces the condition known as gingivostomatitis in which oral cavity vesicles or ulcers form. These lesions may recur frequently as "cold sores" (herpes labialis). Another condition produced by HSV-1 is herpetic keratitis, which may be serious if accompanied by conjunctivitis because this can lead to corneal scarring and blindness. Another condition known as "whitlows" appears as lesions on the fingers.

HSV-2 is commonly referred to as genital herpes. This virus produces lesions on the genitals, urethra and bladder. Recurrence may be frequent. In neonates, infection may be local or disseminated and has about 50% mortality if untreated. HSV-2 may also cause meningitis or encephalitis

- Polioviruses: Poliovirus types 1, 2 and 3 are recognized. Their genome contains a 7000 base positive strand of RNA. These viruses adsorb only to intestinal epithelial cells and motor neuron cells of the central nervous system.
- Coxsackie: These viruses are divided into two groups; A and B. There are 23 serotypes of A, 6 serotypes of B. In humans, Coxsackieviruses produce respiratory disease, herpangitis, "hand, foot and mouth" disease, febrile rashes, pleurodynia, pericarditis, myocarditis, aseptic meningitis and paralytic disease.
- Echoviruses: An acronym for "Enteric Cytopathogenic Human Orphan" viruses, the Echoviruses contain 31 serotypes and produce respiratory disease, febrile illness (with or without a rash), aseptic meningitis and paralytic disease.
- Rhinoviruses: This group of viruses are sensitive to acid pH and their optimal growth occurs at 33°C. There are over 100 serotypes of Rhinoviruses and they produce the common cold.
- Clinical: Diagnosis of enteroviral infections is usually not possible based on clinical presentation. However, some symptoms (pleurodynia, myocarditis) or conditions (aseptic meningitis) are suggestive. Diagnosis of rhinoviral infections, in contrast, is usually based on clinical presentation.
- Laboratory: Recovery of Enterovirus from the throat or feces is diagnostic. Recovery of Rhinoviruses is simply not practical.
- Paramyxoviruses: parainfluenza, mumps, measles, respiratory syncytial virus (RSV). Parainfluenza: These viruses generally produce local infections in the upper and lower respiratory tract. The viruses implant in ciliated epithelia of respiratory tract (nose and throat). The virus can be shed over 3-16 days and the main pathologic response is inflammation. The most important (i.e. serious) diseases are croup, bronchiolitis and pneumonia. The severe diseases occur most often with types 1 and 2. RSV: The RS virus initiates a local infection in the upper or lower respiratory tract but illness varies with age and previous experience. The virus infects ciliated epithelia of the nose, eye and mouth and remains generally confined. Virus spreads extracellularly and by fusion. Severe disease may present as bronchiolitis, pneumonia or croup, particularly in infants. Some evidence suggests that there are possible immunopathologic mechanisms involved. Orthomyxoviruses: Influenza. The Orthomyxoviruses are composed of one genus and 3 types; A, B and C.
• The disease caused by these viruses, influenza, is an acute respiratory disease with prominent systemic symptoms despite the fact that the infection rarely extends beyond the respiratory tract mucosa.
• Type A is responsible for periodic worldwide epidemics; types A and B cause regional epidemics during the winter.
• The recurring pattern of the influenza viruses is due to their ability to exhibit variation in surface antigens. Two phenomena account for this variability:
  1. **Antigenic drift** is due to mutations in the RNA that leads to changes in the antigenic character of the H and N molecules. Antigenic drift involves subtle changes that may cause epidemics but not pandemics.
  2. **Antigenic shift** is due to rearrangement of different segments of the viral genome that produces major changes in the antigenic character of the H and N molecules. Antigenic shift usually occurs in animal hosts and is responsible for producing both epidemics and pandemics.
• Orthomyxoviruses contain a single stranded, negative RNA genome divided into 8 segments.
• The viruses have a lipid bilayer envelope with surface glycoproteins (haemagglutinin and neuraminidase)
• There are 3 viral antigens of importance: the nucleoprotein antigen that determines the virus type (A, B or C), the haemagglutinin (H) antigen, and the neuraminidase (N) antigen. The H and N antigens are variable. There are about 13 different H antigens and 9 different N antigens found in birds. This provides a total of 117 (13 x 9) possible combinations, 71 of which have been observed. There are only about 3 combinations that affect humans, however.
• Viral attachment is mediated by the haemagglutinin. The virus enters host cells by pinocytosis and uncoating occurs by fusion of the viral envelope with the membrane of the vacuole. The RNA is capped and replication proceeds in the nucleus. The progeny are released by budding and cell death ensues.
• The segmented genome of the influenza virus allows rearrangements to occur in simultaneously infected cells. This accounts for the periodic appearance of new variants. The new variants are responsible for the process of antigenic shift.

4. Viruses.

* **Cytomegalovirus**
  Several species of cytomegalovirus have been identified and classified for different mammals. The most studied is human CMV (HCMV), which is also known as human herpesvirus-5 (HHV-5).
  CMV is transmitted from person to person via a close contact
  Symptomatic CMV disease in immunocompromised individuals can affect almost every organ of the body, resulting in fever of unknown origin, pneumonia, hepatitis, encephalitis, myelitis, colitis, uveitis, retinitis, and neuropathy.
Individuals at an increased risk for CMV infection include individuals who attend or work at daycare centers, patients who undergo blood transfusions, persons who have multiple sex partners, and recipients of CMV mismatched organ or bone marrow transplants.

*Epstein-Barr virus* (EBV)
The Epstein–Barr virus, also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, which includes herpes simplex virus 1 and 2, and is one of the most common viruses in humans. It is best known as the cause of infectious mononucleosis. It is also associated with particular forms of cancer, particularly Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and central nervous system.

Transmission of this virus through the air or blood does not normally occur. The incubation period, or the time from infection to appearance of symptoms, ranges from 4 to 6 weeks. Symptoms of infectious mononucleosis are fever, sore throat, and swollen lymph glands. Sometimes, a swollen spleen or liver involvement may develop. Heart problems or involvement of the central nervous system occurs only rarely, and infectious mononucleosis is almost never fatal.

**Cell cultures**

Cell culture is the complex process by which cells are grown under controlled conditions. In practice, the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture.

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO₂ for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed. Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from animal blood, such as calf serum.

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and other products of biotechnology. Biological products produced by recombinant DNA technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals. Vaccines for polio, measles, mumps, rubella, and chickenpox are currently made in cell cultures. Cell cultures are used in viral diagnosis, also.

The examples of human cell lines: HeLa (cervical cancer), AGS (gastric cancer), Lncap (prostate cancer), MCF-7 (breast cancer).

*Rubella virus*

Belongs to family of Togaviridae, it is a RNA-virus with spherical capsid, enveloped. Responsible for the disease of rubella, which may develop as a postnatal rubella or congenital rubella.

Postnatal form is a mild disease, observed mainly in children (more severe in adults).
Rubella is very serious infection for pregnant women, the virus may penetrate through the placenta and infect the foetus (congenital form of the disease). Congenital rubella may result in severe abnormalities of the foetus, premature birth or foetal death. Laboratory diagnosis of Rubella infection is based on serological tests or isolation of the virus. The prophylaxis consists in vaccination. Rubella vaccine contains the live, attenuated viruses (MMR vaccine).

**Family of Flaviviridae**

**Genus: Flavivirus**

RNA-virus, with spherical capsid, enveloped, transmitted to human by mosquito. Viruses of medical importance are: Dengue fever virus, West Nile virus, Yellow fever virus and Japanese encephalitis virus.

**Dengue fever virus** is responsible for dengue- the disease which each year attacks 100 million people in tropics. The disease is caused by any one of four viruses: DEN-1, DEN-2, DEN-3 or DEN-4. It may develop in 3 clinical manifestations: classical dengue fever, dengue haemorrhagic fever or dengue shock syndrome. There is no specific medication or vaccination for treatment of the infection. Infection caused by West Nile virus may course as an asymptomatic infection, mild febrile syndrome (West Nile fever) or neuroinvasive disease (West Nile meningitis or West Nile encephalitis). There is no vaccine or specific treatment against the disease.

**Yellow Fever virus** is responsible for the serious infections in tropical and subtropical areas of South America and Africa (90% of all infections). The disease course is an acute haemorrhagic disease. Because there is not specific treatment, the prevention plays an important role (recommendations of WHO: a mass vaccination). Prevention based on vaccination with vaccine containing attenuated, live viruses.

**Japanese encephalitis virus** is responsible for the disease prevalent in Southeast Asia and Far East. May course as asymptomatic infection or acute encephalitis. There is no specific treatment. Prevention based on vaccination with vaccine containing inactivated viruses.

Diagnosis of infections caused by Flavivirus is based on serological tests, PCR or isolation of viruses (rare).

**HIV**

The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV belongs to the family Retroviridae and subfamily Lentivirinae. This virus was first reported, as a lymphadenopathy-associated virus (LAV), in France in 1983 by Luc Montagnier and associates and in the United States (as HTLV-III) by Robert Gallo and colleagues. The core of HIV contains two molecules of positive-stranded
RNA and the associated enzyme systems. There are three major structural genes (\textit{gag}, \textit{pol} and \textit{env}) and numerous regulatory genes.

Infection with HIV may, in some cases, result in an early, acute phase of disease, with influenza-like symptoms. In general, antibodies to HIV develop within a few months of infection. This is followed by a lengthy asymptomatic period of several to many years. During this phase the number of CD4-positive T lymphocytes declines and eventually the onset of AIDS is signalled by the appearance of one or more of the characteristic infections (e.g. \textit{Pneumocystis carinii} pneumonia, CMV disease, toxoplasmosis, cryptosporidiosis), neoplasms (e.g. Kaposi’s sarcoma) or symptoms (e.g. fever, night sweats, weight loss, generalized lymphadenopathy).

Specific diagnosis of HIV infection is usually based upon the demonstration of antibodies to HIV or identification of genetic sequences by an appropriate amplification technology.

\textbf{Hepatitis viruses}

Several diseases of the liver, collectively known as hepatitis, are caused by viruses. The viruses involved, five of which have been reasonably well characterized, come from a wide range of virus families. Hepatitis A virus is a picornavirus, a small single strand RNA virus; hepatitis B virus belongs to the hepadnavirus family of double stranded DNA viruses; hepatitis C virus is a flavivirus, a single strand RNA virus; hepatitis E, also an RNA virus, is similar to a calicivirus. Hepatitis D which is also known as Delta agent is a circular RNA that is more similar to a plantal viroid than a complete virus.


\textbf{Oral fungal infections}

Fungi are eukaryotic and possess a defined nucleus and other cellular inclusions. Fungi grow either as ovoid cells or as thin filamentous hyphal elements. The most common oral fungal infection is candidosis, caused by \textit{Candida spp.}, particularly \textit{C. albicans}.

Oral candidosis is usually a secondary infection superimposed on another medical condition. \textit{C. albicans} possesses a range of virulence factors that can be phenotypically expressed and enhance its pathogenic potential. These virulence factors in \textit{C. albicans} include adhesins and the ability of hyphae to secrete aspartyl proteinases and phospholipases.

About 50\% of the population are symptomless oral carriers of \textit{Candida spp.}, but only a small proportion of individuals have the signs and symptoms of infection. The pathogenesis of oral candidosis involves a complex interaction between host defence mechanisms and fungal virulence factors.

A number of different forms of oral candidosis can be seen in the oral cavity. The common forms of oral candidosis are pseudomembranous, erythematous (including HIV-associated infection and denture-related candidosis), hyperplastic, and angular cheilitis.

The treatment of oral candidosis involves removing or ameliorating the underlying condition, followed by the prescription of antifungal agents. The antifungals used for oral candidosis are
the polyenes and the azoles. Used correctly both the polyenes and the azoles can alleviate and, in many cases, resolve oral candidosis.

The development of resistance in *Candida spp.* to azole drugs, such as fluconazole, may follow prolonged treatment and has been linked to treatment failure. Oral lesions caused by fungi other than *Candida spp.* are rare and are usually secondary to primary infections of the lungs.

6. Resident oral microflora.

The oral microflora

The mouth supports the growth of a wide diversity of microorganisms including bacteria, yeasts, mycoplasmas, viruses and even on occasions protozoa. Bacteria are the predominant components of the resident oral microflora. Bacterial genera found in the oral cavity-cocci: *Abiotrophia, Enterococcus, Peptostreptococcus, Streptococcus, Staphylococcus, Stomatococcus, Moraxella, Neisseria, Veillonella, Rods: Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propionibacterium, Rothia, Actinobacillus, Campylobacter, Capnocytophaga, Desulfovibrio, Eikenella, Fusobacterium, Haemophilus, Porphyromonas, Prevotella, Selenomonas, Treponema, Wolinella.* Many are fastidious in their nutritional requirements and are difficult to grow and identify in the laboratory, many are also anaerobes.

Study of the oral flora is complicated by the large number of species, their fastidious nutritional requirements, and slow growth together with the complexities of species identification. *Streptococci* comprise the largest proportion of the oral flora. There are four main species groups of oral streptococci: *mutans, oralis, milleri,* and *salivarius* groups.

Chemotaxonomic methods and molecular approaches have resolved many long-standing problems with the classification of many oral bacteria. The result benefits in classification included the finding of closer associations of individual species with sites in health and disease.

The high diversity of the oral microflora reflects the wide range of nutrients available endogenously, the varied types of habitat for colonization, and the opportunity provided by biofilms such as plaque for survival on surfaces. Despite this diversity, many microorganism commonly isolated from neighbouring ecosystems, such as the skin and the gut, are not found in the mouth, emphasizing the unique and selective properties of the mouth for microbial colonization.

For successful colonization, organisms must first adhere to a surface. This involves interaction between adhesins on the microbial cell surface and ligands on the host surface. Factors affecting the growth of oral microorganisms include the diet, host defences, salivary components, and oxygen tension.


*Bacteroides*

Are Gram-negative rods, normally representing the most common organisms in the oral cavity, the female genital tract, and the lower gastrointestinal tract.
The major disease-causing bacteroides species is *B. fragilis*. *B. fragilis* is transmitted from the colon to the blood or peritoneum, following abdominal trauma.

**Clostridium difficile**

Such a type of diarrhea is a common complication of anti-microbial and anti-neoplastic drug treatment and can cause a pseudomembrane colitis. *C. difficile* is a component of the normal flora of the large intestine. Pathogenic strains produce two toxic polypeptides, A and B. Toxin A is an enterotoxin that causes fluid secretion. Toxin B is a cytotoxin.

Clinical significance:
- AAD – antibiotic-associated diarrhea
- AAC - antibiotic-associated colitis
- PMC – pseudomembranous colitis

Laboratory identification
- Cultured from stools. ELISA for developed exotoxins A and B.

Treatment:
- Stop antibiotic treatment
- Fluid replacement
- Metronidazol or vancomycin.

**8. Dental plaque. The role of saliva in the maintenance of oral health.**

**Dental plaque**

Dental plaque biofilm is a tenacious microbial community which develops on soft and hard-tissue surfaces of the mouth, comprising living, dead and dying bacteria and their extracellular products, together with host compounds mainly derived from saliva. Plaque biofilm is found on dental surfaces and appliances especially in the absence of oral hygiene.

The organisms in dental plaque are surrounded by an organic matrix which comprises about 30% of the total plaque volume. Plaque samples are described in relation to their site of origin and are categorized as supragingival and subgingival. There are probably some 350 different cultivable species and a further proportion of unculturable flora, currently identified using molecular techniques.

Streptococci are the predominant supragingival bacteria; they belong to four main species groups: mutans, salivarius, anginosus and mitis. The predominant cultivable species in subgingival plaque are *Actinomyces*, *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Veillonella* spp.

**9. Dental caries.**

**Caries**

Dental caries is a disease of human dentition characterized by loss of the mineralized surfaces of the tooth to the extent that the surfaces are permanently damaged and the underlying dentin is at risk or already damaged. The disease has been characterized as an
ecological collision in the mouth, involving infectious bacteria and the ready availability of sugars in the diet, which the microbial population uses to produce destructive organic acids.

The most heavily investigated aetiological agent of dental caries is *Streptococcus mutans*, a Gram-positive coccus, though other bacteria including *S. sobrinus*, certain other acid-tolerant oral streptococci, *Lactobacillus* species, and, in some cases, strains of *Actinomyces* may be involved in human dental caries and have been shown to cause caries in animal models.

*S. mutans* utilizes dietary sucrose to produce polymers of glucan and fructan through the action of glucosyltransferases and a fructosyltransferase, respectively. Insoluble glucan polymers help attach the bacterial cells to the tooth surface. Glucan and fructan can also be used as a food reserve.

10. Microbiology of periodontal diseases.

Periodontal diseases
Plaque-related gingivitis is a non-specific response to plaque which is characterized clinically by gingival redness, bleeding, and oedema. In plaque-associated gingivitis the microflora becomes progressively more diverse, with an increase in plaque mass and a shift from the streptococcal domination of gingival health to one in which *Actinomyces* species, capnophilic organisms, and obligate anaerobic Gram-negative organisms predominate. Gingivitis does not lead inevitably to periodontitis. Periodontitis is defined clinically as inflammation of the supporting tissues of the teeth, commonly presenting as a progressively destructive change leading to loss of bone and periodontal ligament, with an attachment loss greater than 3 mm. Periodontitis may be the result of infection with specific bacteria (the specific plaque hypothesis), non-specific response to plaque bacteria (the non-specific plaque hypothesis), or the establishment of the appropriate ecological conditions for the expression of sufficient virulence factors to result in tissue destruction (the plaque ecology hypothesis).

The predominant microflora found in disease differs from that in health, but there is no single or unique pathogen. Most of the bacteria associated with disease are Gram-negative and obligately anaerobic, except for localized juvenile periodontitis, where the microflora is mainly capnophilic. In periodontitis there is a progressive change in the composition of the microflora from aerobic, non-motile, Gram-positive cocci to anaerobic, motile, Gram-negative bacilli. Some Gram-negative bacteria implicated in the aetiology of periodontal disease include *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Fusobacterium nucleatum*, and *Capnocytophaga spp, Eubacterium spp.*

Many of these species are highly proteolytic and can degrade host tissues and/or components of the host defences including key regulatory proteins of the inflammatory response. Bacterial invasion of tissues is rare except in some acute conditions such as ANUG and localized juvenile periodontitis. Acute forms of periodontal disease may also be due to abnormalities in the functioning of the host defences. Tissue destruction is generally mediated by bacterial cell surface proteases and extracellular cytotoxic compounds. Periodontal diseases involve the
destruction of tissues directly by bacterial enzymes and indirectly as a consequence of the host inflammatory response.

Bacteria present in periodontal pockets may be detected by microbial culture techniques, detection of certain microbial enzymes, immunological methods, and DNA/RNA probes. Clinical outcome in periodontal disease is likely to be a result of the complex interactions between a wide range of individual host and microbial factors. Methods for the treatment of periodontal disease include supragingival plaque control, root surface debridement, periodontal surgery, and the use of antimicrobial agents. Systemic antibiotics should not be used routinely for the management of chronic periodontal disease.


Infections of the pulp, periapical tissues, and bone of the jaw
Dental caries is the commonest cause of pulpal necrosis. Bacterial infections of the pulp are often mixed and largely anaerobic. Dentoalveolar abscesses are endogenous infections usually caused by a mixture of bacteria including obligate anaerobes. The microflora of chronic abscesses is thought to be similar in composition to acute abscesses, with a mixture of facultative and obligate anaerobic bacteria. Drainage of pus is the essential element of treatment for a dentoalveolar or periodontal abscess.

Ludwig’s angina is a life-threatening infection involving the sublingual and submandibular spaces. Maintenance of the airway is paramount in the management of Ludwig’s angina. Osteomyelitis of the jaws is uncommon, but typically occurs in patients with deficient host defences or reduced vascularity of the bone. Osteomyelitis of the jaws is usually a mixed infection, requiring both medical and surgical treatment.

Actinomycosis is endogenous infection, associated with Actinomyces israelii, which presents as a swelling, often at the angle of the mandible. Sulphur granules are particles seen in pus from actinomycotic lesions and which contain aggregates of actinomyces filaments. Actinomycosis is treated by surgical drainage and long term administration of antibiotics, ideally penicillin.