**LESSON 5.
Microbiology diagnosis of diseases, caused by anaerobes (clostridium and bacteroides)**

**LESSON PLAN:**

• The causative agent of tetanus, morpho-biological characteristics, pathogenesis of the disease, microbiological diagnosis, principles of specific treatment and prevention.

• The causative agent of botulism, morpho-biological characteristics, pathogenesis of the disease, microbiological diagnosis, specific treatment and prevention principles.

• Causative agents of gaseous anaerobic infections (C.perfringens, C.novyi, C.septicum, C.histolyticum, C.sordellii), morpho-biological characteristics, pathogenesis of the disease. Other diseases caused by C. perfringens (food poisoning, necrotic enteritis)

• Microbiological diagnosis of gaseous anaerobic infections, principles of specific prevention and treatment.

• Clostridium difficile, morpho-biological characteristics, pathogenesis of pseudomembranous colitis, microbiological diagnosis, prevention and treatment problems

• Morpho-biological characteristics of bacteria from the genus Bacteroides, principles of microbiological diagnosis of diseases.

**Clostridium**

***CLOSTRIDIUM DIFFICILE***

**Trigger Words**

Spore former, fecal carriage, toxins A and B, antibiotic-associated diarrhea, pseudomembranous colitis

**Biology and Virulence**

Large anaerobic rod characterized by abundant spore formation, rapid growth, and production of volatile fatty acids

Most strains produce two toxins: an enterotoxin that attracts neutrophils and stimulates their release of cytokines, and a cytotoxin that increases permeability of the intestinal wall and subsequent diarrhea

Spore formation allows the organism to persist in the hospital environment and resist decontamination efforts

Resistance to antibiotics such as clindamycin, cephalosporins, and fluoroquinolones allows *C. difficile* to overgrow the normal intestinal bacteria in patients exposed to these antibiotics and produce disease

**Epidemiology**

Colonizes the intestines of a small proportion of healthy individuals (<5%)

Exposure to antibiotics is associated with overgrowth of *C. difficile* and subsequent disease (endogenous infection)

**Diseases**

Antibiotic-associated diarrhea: acute diarrhea generally developing 5 to 10 days after initiation of antibiotic treatment; may be brief and self-limited or more protracted with recurrent bouts of diarrhea

Pseudomembranous colitis: most severe form of *C. difficile* disease, with profuse diarrhea, abdominal cramping, and fever; whitish plaques (pseudomembranes) form over intact colonic tissue; can progress to death

**Diagnosis**

ᑏᑏ*C. difficile* disease is confirmed by detecting cytotoxin or enterotoxin or the toxin genes in the patient’s feces

**Treatment, Prevention, and Control**

The implicated antibiotic should be discontinued

Treatment with metronidazole or vancomycin should be used in severe disease; fecal transplants of colonic bacteria from healthy individuals can be used to treat recurrent disease

Relapse is common because antibiotics do not kill spores; a second course of therapy with the same antibiotic is usually successful, although multiple courses may be necessary

The hospital room should be carefully cleaned after the infected patient is discharged

***CLOSTRIDIUM PERFRINGENS***

**Trigger Words**

Spore former, myonecrosis, sepsis, food poisoning

**Biology and Virulence**

Large gram-positive rods with spores rarely observed

Distinct colony morphology and rapid growth

Produces many toxins and enzymes that lyse blood cells and destroy tissues, leading to diseases such as overwhelming sepsis, massive hemolysis, and myonecrosis

Produces a heat-sensitive enterotoxin that binds to receptors on the epithelium of the small intestine leading to loss of fluids and ions (watery diarrhea)

**Epidemiology**

Ubiquitous; present in soil, water, and intestinal tract of humans and animals

Type A strains are responsible for most human infections

**Diseases**

Food poisoning associated with contaminated meat products (beef, poultry, gravy) held at temperatures between 5° C and 60° C, which allows the organisms to grow to large numbers

Soft-tissue infections typically associated with bacterial contamination of wounds or localized trauma

**Diagnosis**

ᑏᑏ Reliably recognized in Gram-stained tissue specimens (large, rectangular, gram-positive rods)

ᑏᑏGrows rapidly in culture with characteristic colony morphology and hemolytic pattern

**Treatment, Prevention, and Control**

Rapid treatment is essential for serious infections

Severe infections require surgical debridement and high-dose penicillin therapy

Symptomatic treatment for food poisoning

Proper wound care and judicious use of prophylactic antibiotics will prevent most infections

***CLOSTRIDIUM TETANI***

**Trigger Words**

Spore former, environmental, neurotoxin, contaminated wounds, tetanus, vaccine

**Biology and Virulence**

Organism extremely oxygen sensitive, which makes detection by culture difficult

The primary virulence factor is tetanospasmin, which is a heat-labile neurotoxin that blocks release of neurotransmitters for inhibitory synapses (i.e., gamma-aminobutyric acid, glycine)

**Epidemiology**

Ubiquitous; spores are found in most soils and can colonize the gastrointestinal tract of humans and animals

Exposure to spores is common, but disease is uncommon, except in developing countries in which there is poor access to vaccine and medical care

Risk is greatest for people with inadequate vaccine-induced immunity

Disease does not induce immunity

**Diseases**

Disease is characterized by unrelenting

muscle spasms and involvement of the autonomic nervous system

**Diagnosis**

ᑏᑏDiagnosis is based on clinical presentation and not laboratory tests

ᑏᑏMicroscopy and culture are insensitive, and neither tetanus toxin nor antibodies are typically detected

**Treatment, Prevention, and Control**

Treatment requires the combination of wound debridement, antibiotic therapy (penicillin, metronidazole), passive immunization with antitoxin globulin, and vaccination with tetanus toxoid

Prevention through use of vaccination, consisting of three doses of tetanus toxoid followed by booster doses every 10 years

***CLOSTRIDIUM BOTULINUM***

**Trigger Words**

Spore former, environmental, neurotoxin, foodborne and infant botulism, no vaccine

**Biology and Virulence**

Multiple distinct botulinum toxins are produced, with human disease caused most commonly by types A and B; types E and F are also associated with human disease

Botulinum toxin prevents release of the neurotransmitter acetylcholine, blocking neurotransmission at peripheral cholinergic synapses, leading to a flaccid paralysis

**Epidemiology**

*C. botulinum* spores are found in soil worldwide Relatively few cases of botulism in prevalent in developing countries Infant botulism more common; associated with ingestion of contaminated soil or contaminated foods (particularly honey)

**Diseases**

Foodborne botulism is characterized by blurred vision, dry mouth, constipation, and abdominal pain, with progressive weakness of the peripheral muscles and flaccid paralysis

Infant botulism begins with nonspecific symptoms but progresses to flaccid paralysis

Other forms of botulism include wound botulism and inhalation botulism

**Diagnosis**

ᑏᑏDiagnosis of foodborne botulism is confirmed if toxin activity is demonstrated in the implicated food or in the patient’s serum, feces, or gastric fluid

ᑏᑏ Infant botulism is confirmed if toxin is detected in the infant’s feces or serum, or the organism cultured from feces

ᑏᑏWound botulism is confirmed if toxin is detected in the patient’s serum or wound, or the organism cultured from the wound

**Treatment, Prevention, and Control**

Treatment involves the combination of administration of metronidazole or penicillin, trivalent botulinum antitoxin, and ventilatory support Spore germination in foods prevented by maintaining food at an acid pH, by high sugar content (e.g., fruit preserves), or by storing the foods at 4° C or colder

Toxin is heat labile; therefore, it can be destroyed by heating of food for 10

minutes at 60° C to 100° C

**Important Clostridia**

*Clostridium closter,* a spindle

*C. botulinum botulus,* sausage (the first major outbreak was associated with insufficiently smoked sausage)

*C. difficile difficile,* difficult (difficult to isolate and grow; refers to the extreme oxygen sensitivity of this organism)

*C. perfringens perfringens,* breaking through (associated with highly invasive tissue necrosis)

*C. septicum septicum,* putrefactive (associated with sepsis and a high mortality)

*C. tertium tertium,* third (historically, the third most commonly isolated anaerobe from war wounds)

*C. tetani tetani,* related to tension (disease caused by this organism characterized by muscle spasms)

**Pathogenic Clostridia and Their Associated Human Diseases**

***Clostridium difficile***

**Antibiotic-associated diarrhea:** acute diarrhea generally developing 5 to 10 days after initiation of antibiotic treatment (particularly clindamycin, penicillins, cephalosporins, fluoroquinolones); may be brief and self-limited or more protracted

**Pseudomembranous colitis:** most severe form of *C. difficile* disease, with profuse diarrhea, abdominal cramping, and fever; whitish plaques (pseudomembranes) over intact colonic tissue seen on colonoscopy

***Clostridium perfringens***

***Soft-Tissue Infections***

**Cellulitis:** localized edema and erythema with gas formation in the soft tissue; generally nonpainful

**Suppurative myositis:** accumulation of pus (suppuration) in the muscle planes, without muscle necrosis or systemic symptoms

**Myonecrosis:** painful, rapid destruction of muscle tissue; systemic spread with high mortality

***Gastroenteritis***

**Food poisoning:** rapid onset of abdominal cramps and watery diarrhea with no fever, nausea, or vomiting; short duration and self-limited

**Necrotizing enteritis:** acute, necrotizing destruction of jejunum, with abdominal pain, vomiting, bloody diarrhea, and peritonitis

***Clostridium tetani***

**Generalized tetanus:** generalized musculature spasms and involvement of the autonomic nervous system in severe disease (e.g., cardiac arrhythmias, fluctuations in blood pressure, profound sweating, dehydration)

**Localized tetanus:** musculature spasms restricted to localized area of primary infection

**Neonatal tetanus:** neonatal infection primarily involving the umbilical stump; very high mortality

***Clostridium botulinum***

**Foodborne botulism:** initial presentation of blurred vision, dry mouth, constipation, and abdominal pain; progresses to bilateral descending weakness of the peripheral muscles, with flaccid paralysis

**Infant botulism:** initially nonspecific symptoms (e.g., constipation, weak cry, failure to thrive) that progress to flaccid paralysis and respiratory arrest

**Wound botulism:** clinical presentation same as with foodborne disease, although the incubation period is longer and fewer gastrointestinal symptoms are reported

**Inhalation botulism:** rapid onset of symptoms (flaccid paralysis, pulmonary failure) and high mortality from inhalation exposure to botulinum toxin

***Non–Spore-Forming Anaerobic Bacteria***

***BACTEROIDES FRAGILIS***

**Trigger Words**

Pleomorphic gram-negative rod, capsule, abscess formation, drug resistance

**Biology and Virulence**

Anaerobic, pleomorphic, gram-negative rod

Surrounded by polysaccharide capsule

Lipopolysaccharide major cell wall component but without endotoxin activity

Polysaccharide capsule major virulence factor

Heat-labile metalloprotease toxin responsible for diarrheal disease

**Epidemiology**

Colonizes the gastrointestinal tract of animals and humans as a minor member of the microbiome; rare or absent from the oropharynx or genital tract of healthy individuals

Endogenous infections

**Diseases**

Associated with pleuropulmonary, intraabdominal, genital, and skin and soft-tissue infections characterized by abscess formation; bacteremia

**Diagnosis**

ᑏᑏCharacteristic Gram stain from clinical specimens

ᑏᑏGrows rapidly in cultures incubated anaerobically

ᑏᑏ Identified by biochemical tests, gene sequencing, or matrix-assisted laser desorption ionization mass spectrometry

**Treatment, Prevention, and Control**

 Resistant to penicillin and 25% of isolates resistant to clindamycin; uniformly susceptible to metronidazole and most strains to carbapenems and piperacillin tazobactam

**ANAEROBIC GRAM-POSITIVE COCCI**

*Anaerococcus - an,* without; *aer,* air; *coccus,* berry or coccus (anaerobic coccus)

*Atopobium - atopos,* uncommon; *bios,* life

*Finegoldia -* Named after the American microbiologist Sid Finegold

*Micromonas - micro,* tiny; *monas,* cell (tiny cell)

*Peptoniphilus - peptonum,* peptone; *philus,* loving (loving peptones, major source of energy)

*Peptostreptococcus - pepto,* cook or digest (the digesting streptococcus)

*Schleiferella -*Named after the German microbiologist K.H. Schleifer

**ANAEROBIC GRAM-POSITIVE RODS**

*Actinomyces - aktinos,* ray; *mykes,* fungus (ray fungus, referring to the radial arrangement of filaments in granules)

*Bifidobacterium - bifidus,* cleft; *bakterion,* small rod (a small clefted or bifurcated rod)

*Cutibacterium - cutis*, skin (skin bacteria)

*Eubacterium- eu,* good or beneficial (a beneficial rod, that is, a rod normally present)

*Lactobacillus - lacto,* milk (milk bacillus; organism originally recovered in milk; also, lactic acid is the primary metabolic product of fermentation)

*Mobiluncus - mobilis,* capable of movement or being active; *uncus,* hook (motile curved rod)

*Propionibacterium - propionicum,* propionic acid (propionic acid is the primary metabolic product of fermentation)

**ANAEROBIC GRAM-NEGATIVE COCCI**

*Veillonella -* Named after A. Veillon, the French bacteriologist who isolated the type species

**ANAEROBIC GRAM-NEGATIVE RODS**

*Bacteroides - bacter,* staff or rod; *idus,* shape (rod-shaped)

*Fusobacterium - fusus,* a spindle; *bakterion,* a small rod (a small, spindle-shaped rod)

*Porphyromonas - porphyreos,* purple; *monas,* unit (pigmented rods)

*Prevotella -* Named after the French microbiologist A.R.Prevot, a pioneer in anaerobic microbiology

**Anaerobic, Non–Spore-Forming, Gram-Positive Rods**

*Actinomyces* spp. Localized oral infections, actinomycosis (cervicofacial, thoracic, abdominal, pelvic, central nervous system)

*Cutibacterium (Propionibacterium)* spp. Acne, lacrimal canaliculitis, opportunistic infections

*Mobiluncus* spp. Bacterial vaginosis, opportunistic infections

*Lactobacillus* spp. Endocarditis, opportunistic infections

*Eubacterium* spp. Opportunistic infections

*Bifidobacterium* spp. Opportunistic infections

**Predominant Anaerobic Gram-Negative Bacteria Responsible for Human Disease**

****