**LESSON 7.
Microbiology diagnosis of diseases, caused *by*** [***Corynebacteria, Bordetella, Haemophilus, Gardnerella and Legionella***](https://www.google.com/search?newwindow=1&rlz=1C1OKWM_ruAZ837AZ837&q=corynebacterium,+bordetella,+haemophilus+bacteria+and+legionella&spell=1&sa=X&ved=0ahUKEwjZgs2_tcPjAhXikYsKHUHGAqsQkeECCCsoAA) **genus**

**LESSON PLAN:**

• Bacteria from the genus Corynebacterium. Morpho-biological characteristics of the causative agent of diphtheria, distinguishing features from diphtheroids, pathogenicity factors, mechanism of action of C.diphtheriae toxin, pathogenesis of diphtheria.

• Microbiological diagnostic methods of diphtheria

• Principles of specific treatment and prevention of diphtheria

• Diphtheroids and their role in human pathology

• Bordetellas, classification, morpho-biological characteristics. Morpho-biological features, distinguishing features, pathogenicity factors, pathogenesis of the causative agents of pertussis and pertussis-like diseases.

• Methods of microbiological diagnosis of pertussis and pertussis-like disease

• Principles of specific treatment and prevention of whooping cough

• Hemophilic bacteria. Haemophilus influenzae, morpho-biological characteristics, pathogenicity factors. Pathogenesis, microbiological diagnosis of diseases caused by it

• H. ducreyi, morpho-biological characteristics and microbiological diagnosis

• Gardnerella vaginalis, morpho-biological characteristics, pathogenetic characteristics, microbiological diagnosis

• Legionella, morpho-biological characteristics, pathogenicity factors. Legionellosis pathogenesis, clinical forms, microbiological diagnosis

***CORYNEBACTERIUM DIPHTHERIAE***

**Trigger Words**

Diphtheria toxin, pharyngitis, vaccine

**Biology and Virulence**

ᑏᑏGram-positive pleomorphic rods

ᑏᑏ The major virulence factor is the diphtheria toxin, an A-B exotoxin; inhibits protein synthesis

**Epidemiology**

ᑏᑏ Worldwide distribution maintained in asymptomatic carriers and infected patients

ᑏᑏ Humans are the only known reservoir, with carriage in oropharynx or on skin surface

ᑏᑏ Spread person to person by exposure to respiratory droplets or skin contact

ᑏᑏ Disease observed in unvaccinated or partially immune children or adults traveling to countries with endemic disease

ᑏᑏ Diphtheria is very uncommon in the United States and other countries with active vaccination programs

**Diseases**

ᑏᑏ Etiologic agent of diphtheria: respiratory and cutaneous forms

**Diagnosis**

ᑏᑏ Microscopy is nonspecific; metachromatic granules observed in *C. diphtheriae* and other corynebacteria

ᑏᑏ Culture should be performed on nonselective (blood agar) and selective (cysteine-tellurite agar, Tinsdale medium, colistin-nalidixic agar) media

ᑏᑏ Presumptive identification of *C. diphtheriae* can be based on the presence ofcystinase and absence of pyrazinamidase;definitive identification by biochemicaltests or species-specific genesequencing

ᑏᑏ Demonstration of exotoxin is performed by Elek test or polymerase chain reaction assay

**Treatment, Prevention, and Control**

ᑏᑏ Infections treated with diphtheria antitoxin to neutralize exotoxin, penicillin or erythromycin to eliminate *C. diphtheriae* and terminate toxin production, and immunization of convalescing patients with diphtheria toxoid to stimulate protective antibodies

ᑏᑏ Administration of diphtheria vaccine and booster shots to susceptible population

*Corynebacterium coryne-,* a club; *bakterion*, a small rod (a small,club-shaped rod)

*C. diphtheriae-diphthera,* leather or skin (reference to theleathery membrane that forms initially on the pharynx)

*C. jeikeium- jeikeium* (species originally classified as group JK)

*C. urealyticum- urea*, urea; *lyticum*, lyse (capable of lysing urea; species rapidly hydrolyzes urea)

*Corynebacterium diphtheriae-* Diphtheria (respiratory, cutaneous); pharyngitis and endocarditis (nontoxigenic strains)

*C. jeikeium* (group JK) Septicemia, endocarditis, wound infections, foreign body (catheter, shunt, prosthesis) infections

*C. urealyticum* Urinary tract infections (including pyelonephritis and alkaline-encrusted cystitis), septicemia, endocarditis, wound infections

***BORDETELLA PERTUSSIS***

**Trigger Words**

Slow growing, whooping cough, pertussis toxin, person to person, vaccination

**Biology and Virulence**

ᑏ Very small gram-negative coccobacilli

ᑏᑏ Non-fermentative but can oxidize amino acids as an energy source

ᑏᑏ Strict aerobe

ᑏᑏ Growth in vitro requires prolonged incubation in media supplemented with

charcoal, starch, blood, or albumin

ᑏᑏ Adherence to eukaryotic cells mediated by pertactin, filamentous hemagglutinin, and fimbria; localized tissue destruction mediated by dermonecrotic toxin and tracheal cytotoxin; systemic toxicity produced by pertussis toxin

**Epidemiology**

ᑏᑏ Pertussis is a human disease with no known animal or environmental reservoir

ᑏᑏWorldwide distribution with a high prevalence in unvaccinated populations

ᑏᑏChildren younger than 1 year are at greatest risk for infection and mortality

ᑏᑏ In vaccinated populations, disease is observed in older children and young adults

ᑏᑏUnvaccinated individuals are at greatest risk for disease

ᑏᑏDisease spread person to person by infectious aerosols

**Diseases**

ᑏᑏ Pertussis characterized by three stages: catarrhal, paroxysmal, and convalescent

ᑏᑏ Most severe disease is in unvaccinated individuals, particularly children

**Diagnosis**

ᑏᑏMicroscopy is insensitive and nonspecific

ᑏᑏCulture is specific but insensitive

ᑏᑏNucleic acid amplification tests are the most sensitive and specific tests

ᑏᑏDetection of immunoglobulin (Ig)G or IgA can be used as a confirmatory test

**Treatment, Prevention, and Control**

ᑏᑏ Treatment with macrolide (i.e., azithromycin, clarithromycin) is effective in eradicating organisms and reducing length of infectious stage

ᑏᑏ Azithromycin is used for prophylaxis

ᑏᑏ Vaccines containing inactivated pertussis toxin, filamentous hemagglutinin,

and pertactin are effective

ᑏᑏ Pediatric vaccine administered in five doses (at ages 2, 4, 6, and 15 to 18 months, and between ages 4 and 6 years); adult vaccine administered at ages 11 to 12 years and between 19 to 65 years

***LEGIONELLA PNEUMOPHILA***

**Trigger Words**

Poor-staining slender rods, legionnaires disease, Pontiac fever, contaminated water, BCYE agar

**Biology and Virulence**

ᑏᑏ Slender, pleomorphic, non-fermentative, gram-negative rods

ᑏᑏ Stains poorly with common reagents

ᑏᑏNutritionally fastidious, with requirement for L-cysteine and enhanced growth with iron salts

ᑏᑏCapable of replication in alveolar macrophages (and in amebae in nature)

ᑏᑏ Prevents phagolysosome fusion

**Epidemiology**

ᑏᑏ Capable of sporadic, epidemic, and nosocomial infections

ᑏᑏ Commonly found in natural bodies of water, cooling towers, condensers, and water systems (including hospital systems)

ᑏᑏ Estimated to be as many as 18,000 cases of infection in United States annually

ᑏᑏ Patients at high risk for symptomatic disease include patients with compromised pulmonary function and patients with decreased cellular immunity (particularly transplant patients)

**Diseases**

ᑏᑏ Responsible for legionnaires disease and Pontiac fever

**Diagnosis**

ᑏᑏMicroscopy is insensitive

ᑏᑏAntigen tests are sensitive for *L. pneumophila* serogroup 1 but have poor sensitivityfor other serogroups and species

ᑏᑏCulture on buffered charcoal yeast extract agar is the diagnostic test of choice

ᑏᑏ Seroconversion must be demonstrated; this can take as long as 6 months to develop; positive serology may persist for months

ᑏᑏNucleic acid amplification assays are as sensitive and specific as culture

**Treatment, Control, and Prevention**

ᑏᑏ Macrolides (e.g., azithromycin, clarithromycin) or fluoroquinolones (e.g., ciprofloxacin, levofloxacin) are the treatment of choice

ᑏᑏ Decrease environmental exposure to reduce risk of disease

ᑏᑏ For environmental sources associated with disease, treat with hyperchlorination, superheating, or copper-silver ionization

**Important Miscellaneous Gram-Negative Rods**

*Bordetella -* Named after Jules Bordet, who first isolated the organism responsible for pertussis

*B. pertussis - per,* very or severe; *tussis,* cough (a severe cough)

*B. parapertussis - para,* resembling (resembling pertussis)

*B. bronchiseptica - bronchus,* the trachea; *septicus,* septic (an infected bronchus)

*B. holmesii -* Named after the microbiologist Barry Holmes

*Brucella -* Named after Sir David Bruce, who first recognized the organism as a cause of “undulant fever”

*B. abortus - abortus,* abortion or miscarriage (this organism is responsible for abortion in infected animals)

*B. melitensis - melitensis,* pertaining to the Island of Malta (Melita), on which the first outbreak was recognized by Bruce

*B. suis - suis,* of the pig (a swine pathogen)

*B. canis - canis,* of the dog (a dog pathogen)

*Cardiobacterium hominis - cardia,* heart; *bakterion,* small rod; *hominis,* of man (small rod of the hearts of men; refers to the predilectionof this bacterium to cause endocarditis in humans)

*Francisella -* Named after the American microbiologist Edward Francis, who first described tularemia

*F. tularensis* subsp. *tularensis* (type A) - *tularensis,* pertaining to Tulare County, California, in which the disease was first described

*F. tularensis* subsp. *holarctica* (type B) - *holos,* whole; *arctos,* northern regions (reference to distribution in the arctic or northern regions)

*F. tularensis* subsp*. mediaasiatica - media,* middle; *asiatica,* Asian (pertaining to middle Asia)

*F. tularensis* subsp. *novicida - novus,* new; *cida,* to cut (a “new killer”)

*Legionella pneumophila - Legionella,* first recognized outbreak was at an American Legion convention; *pneumôn,* lung; *phila,* loving; *pneumophila,* lung-loving.

*Streptobacillus moniliformis - streptos,* twisted or curved; *bacillus,* rod; *monile,* necklace; *forma,* shape (twisted, necklace-shaped bacillus; refers to the pleomorphic morphology of the bacteria)

**Clinical Summaries**

***Bordetella pertussis***

**Pertussis:** after a 7- to 10-day incubation period, disease is characterized by the catarrhal stage (resembles the common cold), progressing to the paroxysmal stage (repetitive coughs followed by inspiratory whoops), then the convalescence stage (diminishing paroxysms and secondary complication)

***Bordetella parapertussis:*** produces a milder form of pertussis

***Bordetella bronchiseptica:*** primarily a respiratory disease of animals but can cause bronchopneumonia in humans

***Bordetella holmesii:*** uncommon cause of sepsis

***Legionella pneumophila***

**Pontiac fever:** self-limited febrile disease with chills, myalgias, malaise, and headache but no evidence of pneumonia

**Legionnaires disease:** severe pneumonia with acute onset of fever, chills, nonproductive cough, and headache progressing to multilobar consolidation of the lungs and multiorgan failure

**Clinical presentation of *Bordetella pertussis* disease.**



**Haemophilus bacteria**

• They usually develop in nutritious environments enriched with blood (the name of the genus is related to this: Greek, haima - blood, philos - to love). For their development, growth factors called X and V contained in erythrocytes are required.

• Factor X is a thermostable tetrapyrrole included in hematin and hemin in erythrocytes. Species requiring this factor are unable to synthesize protoporphyrin from delta-aminolevulinic acid. This characteristic is used in the identification of hemophilic bacteria.

• Factor V is a thermolabile substance containing nicotinamide adenine nucleotide (NAD) or nicotinamide adenine nucleotide phosphate (NADF). This factor is a component of group B vitamins involved in oxidation-reduction reactions in bacterial cells.

• These factors are released after the breakdown of erythrocytes due to high temperature. The optimal nutrient medium is the medium with added heated blood - "Chocolate agar".

• The genus Haemophilus belongs to the family Pasteurellaseae and includes about 20 species.

• Haemophilus influenzae, the typical species of the genus, is more important in human pathology.

***Haemophilus* - TAXONOMY**

* (Domain): Bakteriyalar
* (Kingdom): Pseudomonadota
* (Class): Gammaproteobacteria
* (Order): Pasteurellales
* (Family): Pasteurellaceae
* (Genus): Haemophilus
* (Species): ***H.influenzae****, H.ducreyi, H.aegypticus*

**Haemophilus influenzae - (morpho-biological characteristics)**

• Gram-negative, small, polymorphous bacteria measuring 0.3-0.4x1-1.5 µm. The morphology depends on the period of acquisition of the pure culture or the type of nutrient media. They are mostly coccobacilli, or tube-shaped, sometimes they form pairs, short chains or long threads.

• They are immobile, do not form spores, have piles (fimbriae). Some strains form a polysaccharide capsule.

• It is a facultative anaerobic, it grows better under aerobic conditions. Factors X and V are required for their development. The optimal nutrient medium for cultivation is "chocolate agar". It forms R- and S-colonies in this medium at 35-370C for 1-2 days. Highly virulent encapsulated strains produce slimy, relatively large (3-4 mm) S-colonies, while weakly virulent non-encapsulated strains produce small (1 mm), granular, wrinkled R-colonies.

• The phenomenon of satelliteism is used in the identification of hemophilic bacteria.

• Hemophilic bacteria that cannot develop in blood agar can develop in the zone of hemolysis formed by staphylococci or other bacteria: As a result of hemolysis, factors X and V are released and accelerate the development of hemophilic bacteria - the phenomenon of satelliteism is observed.

• H. influenzae has a somatic O-antigen, and capsular variants also have a polysaccharide-containing K-antigen.

• H. influenzae is divided into 6 serotypes (a, b, c, d, e, f) depending on the structural features of the capsule antigen.

• The capsule antigen of serotype b is a polymer (polyribophosphate) consisting of ribose and ribitol combined with phosphoric acid.

• Most strains of H. influenzae, representative of the normal microflora of the respiratory tract, do not form a capsule.

Pathogenicity factors:

• H. influenzae does not produce exotoxin. LPS of the outer membrane plays an important role in the process of adhesion and invasion of hemophilic bacteria as an endotoxin.

• Endotoxin also paralyzes the cilia of the protective epithelium of the human respiratory tract, leading to the colonization of microbes in the upper respiratory tract.

• The main pathogenicity factor of H. influenzae is the capsule, which protects it from phagocytosis and ensures its survival in the body. It is no coincidence that capsular strains of type b cause more severe infections.

• H. influenzae secretes IgA-protease that can inactivate antibodies. The cells of pathogens and IgA-protease play a leading role in their adhesion and settlement to the epithelium of the respiratory tract.

**Source of infection and ways of infection:**

• H. influenzae is a bacterium that is only pathogenic for humans.

• The source of infection is patients and carriers of bacteria.

• Infection occurs mainly through airborne droplets.

**Pathogenesis and clinic**

• H. influenzae entered the upper respiratory tract adheres to the epithelial epithelium and settles there. In people with weak immunity, they pass into the submucous layer and with the help of their endotoxins, they cause local purulent-inflammatory processes such as otitis, sinusitis (inflammation of the sinuses), bronchitis, and pneumonia.

• H.influenzae capsular strains, especially serotype b, spread either locally in the upper respiratory tract or hematogenously, causing generalized infections such as septicemia, meningitis, etc. causes.

• According to world statistics, Haemophilus influenzae is one of the main causes of child death. This bacterium is especially dangerous for children under 5 years old

• H. influenzae, along with pneumococci and meningococci, are the leading etiological factors of meningitis in young children.

• It is one of the most serious diseases caused by H. influenzae b serotype. Epiglottitis, a phlegmon-type progressive infection of the larynx and surrounding tissues, is most common in children aged 2-5 years. Epiglottitis can quickly lead to death by asphyxiation.

**Microbiological diagnostics:**

Examination materials: nasopharyngeal mucus, blood, sputum, cerebrospinal fluid (in case of meningitis)

• In purulent meningitis, a smear made from cerebrospinal fluid can be stained with Gram's method and subjected to microscopy.

• IFR can be applied to detect capsular strains in cerebrospinal fluid.

• In other cases, the microscopic method is less informative.

• Obtaining and identification of the causative agent from pathological materials is carried out by the bacteriological method.

• The material is inoculated on chocolate or blood agar.

• Differentiation of H. influenzae from other gram-negative bacteria is carried out based on their requirement for X- and V-factors, absence of hemolysis in blood agar, satellism phenomenon and other tests.

• Small Gram-negative, immobile, catalase-positive polymorphic bacilli that do not grow in normal nutrient media, grow well in chocolate agar, and poorly in blood agar, do not produce hemolysis, are identified as Haemophilus influenzae.

* **Treatment**

• Third generation cephalosporins, such as ceftriaxone, cefotaxime, etc., are the drugs of choice. It is used.

• Some strains of H. influenzae serotype b are sensitive to ampicillin and chloramphenicol, but many strains synthesize beta-lactamase.

**Prevention**

• The vaccine used for the specific prevention of diseases caused by H. influenzae b type includes a purified capsule antigen.

• Since this vaccine has poor immunogenicity in infants, it has been conjugated with a carrier protein to increase its effect. As a carrier protein, diphtheria or tetanus anatoxin, as well as outer membrane proteins of group B meningococci are used.

**Haemophilus ducreyi (the causative agent of soft chancre)**

• It is a 0.2-2 μm, ovoid-shaped, non-motile rod-shaped bacterium, parallel chains ("railway lines"), groups or pairs are observed under the microscope.

• H. ducreyi requires X-factor for growth, not V-factor.

• Forms small grayish-yellow shiny colonies on solid nutrient medium reminiscent of streptococci colonies on blood nutrient mediums.

• Does not cause turbidity in liquid nutrient media.

• The source of infection is sick people. Infection occurs sexually.

• A red spot formed at the site of the pathogen's entry turns into an ulcerated papule after a few days. At first, these ulcers, which are the size of a lentil, grow up to 2 cm in size in a few weeks, the edges are indented-protruding, bitten, uneven, and the bottom is covered with a yellowish-fatty crust.

• The consistency of the ulcer is soft. It differs from the solid chancre seen in syphilis by being painful and prone to bleeding during palpation. Chancres can be multiple or coalesce to form large, crawling sores. Regional lymph nodes become enlarged and painful.

• Immunity is not formed after the disease.

**Microbiological diagnostics:**

• In smears prepared from chancre contents, characterization is based on the microscopic detection of small, Gram-negative bacilli with morphology.

• It is possible to obtain a culture of the causative agent and identify it by inoculating the pathological material into appropriate nutrient media.

• In some cases, PCR is used to identify the causative agent.

**Gardnerella - TAXONOMY**

* (Domain): Bakteriyalar
* (Kingdom): Actinomycetota
* (Class): Actinomycetia
* (Order): Bifidobacteriales
* (Family): Bifidobacteriaceae
* (Genus): Gardnerella
* (Species): ***G.vaginalis***

**Gardnerella vaginalis (morpho-biological characteristics)**

• 1-2x0.3-0.6 µm small bacilli or coccobacilli, they do not have capsules and flagella, they do not form spores. In smears, cells are located singly or in pairs (end-to-end).

• Sometimes a wall or V-shaped arrangement is observed, such as Corynebacterium vaginalis (previously called Corynebacterium vaginalis).

• Neisser stained smears reveal metachromatic granules.

• Stains variably by Gram method.

• Facultative anaerobic, capnophilic.

• It is demanding on nutrient mediums, it does not develop in normal nutrient mediums, in mediums supplemented with casein, yeast extract, serum and blood at 35-370C, as well as in V ("vaginalis") agar after 24-48 hours, small-sized, raised, homogeneous, forms smooth, colorless colonies.

• On blood agar, they form very small (0.25-0.5 mm diameter) colonies with - and -hemolysis zone.

**• Antigen structure** - 7 serogroups of gardnerella are distinguished in the precipitation reaction. All serogroups have a common antigen of glycopeptide nature. It has common antigens with Candida albicans.

• **Pathogenicity factors** - some strains of gardnerella synthesize mucinase, which breaks down the glycopeptides of the uterine mucosa.

• **Source of infection** and ways of infection – the source of infection is sick people. Infection occurs sexually. Women of reproductive age are more likely to get sick.

**Clinic**

• G. vaginalis is a conditionally pathogenic bacterium whose ecological stage is the uterus. Like other opportunistic microorganisms, Gardnerella also causes diseases under certain conditions. The main clinical manifestation of gardnerellosis in women is bacterial vaginosis.

• Bacterial vaginosis (formerly called nonspecific vaginitis) accounts for approximately 40% of all vaginitis. In most women, uterine bleeding is accompanied by a watery homogeneous discharge with a sharp unpleasant "fishy" smell, caused by the sensation of itching and the formation of abnormal amines. Sometimes it has an asymptomatic course.

• In some cases, the entry of bacteria into the uterine cavity, fallopian tubes and ovaries causes acute, as well as chronic and recurrent infection in the indicated organs - endometritis and salpingophoritis.

• Entry of the pathogen from the uterus into the urethra can result in gardnerellosis of the urinary tract with hemorrhagic cystitis, pyelonephritis and symptomatic bacteriuria.

• G. vaginalis rarely causes disease in men due to factors that prevent colonization in men's urinary tract. In some cases, it causes non-specific urethritis in association with other pathogens.

**Microbiological diagnosis**

• It is based on the microscopy of native or Gram-stained smears made from the material taken from the cervix.

• In the Gram-stained smear, the surface of the epithelial cells is covered with small, variably Gram-stained coccobacilli. Such epithelial cells are called "clue cells".

• Gram-stained smear microscopy is very informative in the diagnosis of bacterial vaginosis.

• The bacteriological method based on obtaining and identifying the culture of the causative agent is rarely used due to the difficulty of cultivation.

• The pH of the material taken from the uterine tract is higher than 4.5 can also be considered a diagnostic sign. Adding a few drops of 10% KOH to the material produces a "fishy" smell.

**Treatment**

• It is done with metronidazole.

• In addition to metronidazole, local vaginal eubiotics (lactobacteria) are prescribed to restore the destroyed normal flora.

• It is not advisable to treat men who are sexual partners of sick women.