**LESSON 4.**

**Ultrastructure of bacteria. Flagella and capsule. Investigation of bacterial motility ("crushed and hanging" drop methods, vital staining). Detection of the capsule by Gins-Burry stain**

**LESSON PLAN:**

* The bacterial cell structure (capsule, glicocalix, flagella, pili)
* The motile bacteria. The structure, function and location of flagella.
* To movement study of microbes prepeared by «crushed and hanging» drop methods.
* Vital staining method.
* The encapsulated bacteria, chemical composition, structure and importance of the capsule
* The detection of capsule by Gins-Burry method

**Flagella**

* Flagella is a movement organ, composed of the flagellin protein
* It is mainly a movement (reptile, floating) organelles of rod- and spiral bacteria shapes.
* It connects to the cytoplasmic membrane with the basal body (blepharoplast).
* The basal body joins the layers of the cell membrane with a pair of helical rings. Gram-positive organisms have two of these basal body rings, one in the peptidoglycan layer and one in the plasma membrane. Gram-negative organisms have four such rings
* Flagellin contains several thousand protein molecules (H antigen)
* Spirochetes contain poles called axial filaments instead of flagella (endoflagella)

**Flagella has 3 parts:**

1. Basal body
2. Hook
3. Flagellar filament

|  |  |
| --- | --- |
| **Monotrichous** | **Shigella, Klebsiella, Acinetobacter** |
| **Lophotrichous** | **Campylobacter, V.cholera, Pseudomonas** |
| **Amphitrichous** | **Helicobacter** |
| **Peritrichous** | **E.coli, Proteus, Salmonella** |

**Pili (Fimbriae)**

* Pili is composed of pilin protein
* Pili begins from the cytoplasmic membrane
* adhesion
  + Mannose sensitive (E.coli-D mannose)
  + Mannose resistant
* Transfer of genetic material
  + Sex-fertile pili
  + Transfer of genetic material in conjugation

**Determination of bacterial motion**

* With naked eye → “collective motion”

“crushed and hanged” drop preparations

Vital staining

Stains - working with tannins - can be detected by the Lefler method

* Flagella staining

**“Crushed” drop preparations**

Microbial motion is studied with the “crushed” drop preparations

1 drop of microbial suspension is placed in the center of the slide and covered with cover glass

Microscopy is performed on a dark-field microscope

**“Hanging” drop preparation**

1-2. The microbe suspension is put on the cover glass.

3. The slide with hole in the middle is placed over the cover glass and immediately returned in the opposite direction. At this point, the drop is attached to the center of the hole

**Vital Staining**

* Vital staining is used to study bacteria while alive.
* Reproduction of microorganisms
* Spore formation
* Influence of physical and chemical factors
* This method uses 100,000 times dilution of methylene sucker, neutral red solution

**Capsule**

* The bacterial cell is coated with a mucous membrane (viscous layer) from the outside – glycocalyx
* Capsule protects bacteria from environmental damage – dryness
* The capsules that bacteria produce in the human and animal organism protect them from the effects of phagocytes (antibodies)
* Since the capsule is antigenic in nature (K-antigens), antibodies are formed in the body against it
* Polysaccharide – Sreptococcus pneumoniae, Klebsiella
* Protein - Basillus antracis
* Hyaluronic acid - Streptococcus pyogenes

**Gins-Burry staining for detection capsule**

* Due to the poor perception of color solutions, the capsule can be distinguished by a special staining method - Gins-Burry  
  A drop of 1:9 drops of india ink and bacterial suspension are added to one side of the slide. Take another clean slide, and holding at an angle of about 45 deg., touch the smear with one end of the slide so the smear runs along the edge of the slide  
  Allow the slide to dry in air and fixated using a chemical method (Nikifirov solution)
* Then add the Pfeiffer fuchsin, wait 3-5 minutes, wash, dry and view smear under the microscope