УЧЕБНОЕ ПОСОБИЕ

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МЕДИЦИНСКАЯ МИКРОБИОЛОГИЯ, ВИРУСОЛОГИЯ И ИММУНОЛОГИЯ

ЛЕКЦИИ

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Рекомендовано Координационным советом по области образования «Здравоохранение и медицинские науки» в качестве учебного пособия для использования в образовательных учреждениях, реализующих основные профессиональные образовательные программы высшего образования уровня специалитета по направлению подготовки 31.05.01 «Лечебное дело»

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TEXTBOOK

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MEDICAL MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

LECTURE NOTES

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PREFACE

Medical Microbiology, Virology and Immunology is one of the most important sciences studied by medical students. It is the fundamental knowledge that prepares students to learn another medical disciplines.

This textbook “Medical Microbiology, Virology and Immunology. Lecture notes” is recommended for students studying discipline Microbiology, Virology and Immunology in English in medical universities of the Russian Federation. Text is adapted for students studying in English as a foreign language.

The studied material is presented systematically in the form of lectures for study. The textbook consists of 2 parts: General Microbiology, Immunology and Special Microbiology, Mycology and Virology. Each of them includes the main topics of the curriculum in these disciplines. Each topic is presented in a chapter, including the main questions of the topic, answers to them, as well as test questions and study cases that help a student to understand information and check the quality of knowledge. The studied material is illustrated with figures, diagrams and tables.

The textbook “Medical Microbiology, Virology and Immunology. Lecture notes” is written in accordance of educational plan for disciplines Microbiology, Virology and Immunology. Thus the textbook contains of general information required for mastering of these disciplines.

Part 1

GENERAL MICROBIOLOGY, IMMUNOLOGY

Unit 1
GENERAL MICROBIOLOGY

LECTURE 1. SUBJECT AND AIMS OF MEDICAL MICROBIOLOGY. MORPHOLOGY AND TAXONOMY OF MICROORGANISMS. MICROSCOPIC RESEARCH METHOD

Questions for discussion

1. The subject of Medical Microbiology and its importance for practical health care. The history of Microbiology.
2. Taxonomy, system and nomenclature of microorganisms.
3. Microbiology research methods.
4. Simple and complex staining techniques. The mechanism of smear staining. Staining properties of microorganisms.
5. Light microscope, its main characteristics. Types of light microscopy (dark-field, fluorescent). Immersion microscopy principles. Electron microscopy, atomic force microscopy.
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8. Shell of bacteria: cytoplasmic membrane, cell wall, capsule. Structure, functions and methods of detection.
9. Flagella, fimbria (pili). Structure, functions and methods of detection.
10. Bacterial spores. Their role and structural features. Spore formation and methods of detection.

Question 1. The subject of medical microbiology and its importance in practical health care. The history of microbiology

Medical microbiology is the study of causative agents of infectious diseases in humans and their reactions to such infections. It deals with aetiology, pathogenesis, laboratory diagnosis, specific treatment and control of infection (immunisation).

The **subject** of Medical Microbiology is the study of **microorganisms** that cause infectious disease.

Importance of Medical Microbiology for practical health care

Medical Microbiology is essential for:

- diagnosis of human infectious diseases in the laboratories;
- making of treatment (therapy) and prophylaxis (prevention) methods;
- making of vaccines, immunoglobulins and immunomodulators for human immunisation.

Modern Medical Microbiology

- **Bacteriology** is the science of bacteria, the causative agents of numerous infectious diseases.
- **Mycology** is the study of fungi pathogenic to humans.
- **Protozoology** deals with pathogenic unicellular animal organisms.
- **Virology** is the science of viruses, non-cellular living systems, capable of causing infectious diseases in the human body.
- **Immunology** is the science which is concerned with mechanisms of body protection against pathogenic microorganisms.

Periods of Microbiology development

- Morphological.
- Physiological.
- Prophylactic.
- Modern (molecular-genetic).

Morphological period (XVII middle of age)

Anton van Leeuwenhoek: In 1673 he was the first person to observe microorganisms which he called «***animalcules***» (bacteria, protozoa), using single-lens microscopes of his design.

Physiological period in microbiology history

Louis Pasteur is the «Father of Bacteriology»:

- Proved that microorganisms cause the fermentation (the process of destroying the carbohydrates)
- Developed a process that involves heating the liquids (at 65 °C) to kill most bacteria responsible for spoilage **(pasteurisation)**. Production of dairy products, wine, and beer use this method.

- Developed the first vaccines against anthrax; he also ingeniously developed a vaccine against rabies using dried spinal cords of infected rabbits.

Joseph Lister (1859) used disinfectant to treat surgical injuries, significantly reducing infection rates.

Robert Koch (1876):

- the first person to prove that microorganisms cause diseases;
- made methods of the colouring of bacteria;
- developed the methods of pure culture isolation;
- proved that *Bacillus anthracis* causes anthrax in cattle;
- later identified bacterium that causes tuberculosis.

Prophylactic period (after 1914)

Classic Metchnikov's researches defined a prophylactic period in Microbiology history:

- **Ilya Metchnikov** discovered the phagocytosis is the process of microorganisms destroying by human cells (phagocytes). He is one of the **founders** of **immunology**.
- **Paul Ehrlich (1910**) discovered salvarsan that was effective against syphilis.
- **Alexander Fleming (1928)** ascertained that penicillin produced by the mould *Penicillium notatum* was able to prevent microbial growth. He discovered **the first antibiotic**.
- **Rene Dubos (1939):** Discovered two antibiotics (gramicidin and tyrocidine) produced by a bacterium (*Bacillus brevis*).

Modern (molecular-genetic) period (the end of XX century-start of XXI)

- **Discovery of new microorganisms**: Human Immunodeficiency Virus **(HIV)** and infectious proteins — **prions**.
- Studying the **molecular structure of microbes**, **antibodies**.
- Research of the **genes** of almost **all bacteria**.
- Production of new antibiotics.
- Production of **new generation vaccines** - molecular vaccines.
- Creation of **new microbiological** research **methods**: Polymerase Chain Reaction **(PCR)**, **scanning microscopy**.

Question 2. Taxonomy, system and nomenclature of microorganisms

Taxonomy is the science of biological classification, the grouping of organisms according to their mutual similarities.

A specie is a group of similar organisms within a genus, which are designated by biochemical and other phenotypic criteria and by DNA relatedness, which groups strains based on their overall genetic similarity.

A strain is the pure culture of microorganisms that becomes isolated at a particular time and place. The strains of bacteria within the species have different properties.

Microbiological nomenclature

Nomenclature is the assignment of names for purposes of communication and identification.

In microbiology, the accepted system is the **binominal system** (invented by Linnaeus) of nomenclature where **each species** has a **generic** and a **specific name**. The generic name starts with a capital letter, and the specific name — with a small letter (in italics).

For example, ***Escherichia coli*** (**Theodor Escherich** was an Austrian paediatrician who discovered *E. coli* in child faeces with diarrhoea; «**coli**» is a rod in Latin) (table 1).

Table 1. Nomenclature of bacteria

Domain	Domain: Bacteria
Kingdom (*Animalia, Plantae, Fungi, Protista, Archaea, Bacteria*)	**Kingdom: Bacteria**
Phylum	**Phylum:** ***Proteobacteria***
Class	**Class:** ***Gamma Proteobacteria***
Order	**Order:** ***Enterobacteriales***
Family	**Family:** ***Enterobacteriaceae***
Genus (generic name)	**Genus:** ***Escherichia***
Species (specific name)	**Species:** ***Escherichia coli (E. coli)***
Subspecies	

Question 3. Microbiology research methods

Research microbiology methods are used to study microorganisms and diagnose infectious diseases.

1. **Microscopic methods** are used in microbiology for two basic purposes: the initial detection of microbes and the preliminary or definitive identification of microbes. The microscopic examination of clinical specimens is used to detect bacterial cells, fungal elements, parasites and viral inclusions present in infected cells.

2. **Bacteriologic method (cultivation).** It is known that certain metabolic properties are present only in certain groups of bacteria. Specific biochemical tests can be used to eliminate certain groups from the identification process. The more common tests are fermentation of carbohydrates, the use of a specific substrate, and the production of specific products or waste products on culture medium. For identification, the pure culture must be isolated.

3. **Serological tests** are used for the detection of unknown antibodies in patient's serum or unknown antigen with the use of known (commercial) serum. Microorganisms are antigenic, meaning they are capable of triggering the production of antibodies. Solutions of such collected antibodies, called antisera, are commercially available for many medically important pathogens.

4. **Biological method** includes contamination of animals, e.g. hamsters, mice, to cultivate obligate parasitic bacteria and viruses, study pathogenesis of the disease, anatomy of infected organs.

5. **Allergic skin tests** are based on hypersensitivity reaction of human to some pathogens, for example, for tuberculosis.

6. **DNA-technology test** (Polymerase Chain Reaction) allows comparing DNA of isolated microbes with DNA of known microbes.

Question 4. Simple and complex staining techniques. The mechanism of smear staining. Staining properties of microorganisms

Because microbial cytoplasm is usually transparent, it is necessary to stain microorganisms before they can be viewed with the light microscope. There are simple and differential stain techniques.

Simple stain techniques can be performed with basic dyes such as crystal violet or methylene blue. This procedure uses 1 type of dye.

The **differential (complex) stain** technique allows distinguishing two kinds of organisms. An example is the **Gram stain** technique that uses 2 types of dyes.

Gram stain technique

Hans Gram was a Danish bacteriologist. He developed a method for staining bacteria. This differential technique separates bacteria into two groups: **Gram-positive bacteria** and **Gram-negative bacteria**.

- **Crystal violet** is first applied, followed by the mordant *iodine*, which fixes the stain.
- Then the slide is washed with *alcohol*, and the **Gram-positive** bacteria retain the **crystal-violet iodine** stain; however, the **Gram-negative bacteria** aren't stained with that dye.

- The **Gram-negative** bacteria subsequently are stained with the **safranin (or fuchsine)** dye, the counterstain, used next. These bacteria appear **red** under the microscope, while **Gram-positive** bacteria appear **purple**, reflecting the crystal violet retained during the washing step.

Staining properties are the ability of bacteria to **dye** or **hold colour**.

Question 5. Light microscope, its main characteristics. Types of light microscopy (dark-field, fluorescent). Immersion microscopy principles. Electron microscopy, atomic force microscopy

Microorganisms are much too small to be seen with the naked eye; they must be observed with a microscope.

The **total magnification** of a specimen is the power of the objective (4×, 10×, 40×) multiplied by the power of the eyepiece, usually 10×.

Resolution (also called resolving power) is the ability of the lenses to discern fine details and structure.

There are some **types of microscopy** to observe microorganisms.

Oil immersion microscope

- Oil immersion lens are designed to be in direct contact with the oil placed on the coverslip.
- The light passing from the glass through oil does not bend much because the oil has a similar refractive index to that of glass.
- Immersion oil has a refractive index closer to that of glass than to the refractive index of air, so the use of oil increases the cone of light that enters the objective lens (fig. 1).

Dark field microscope

- A dark field microscope is a light microscope in which both blocks direct **light rays and deflect** light off a mirror on the side of the condenser at an **oblique angle**.
- This technique creates a «**dark field**» that contrasts against the highlighted edge of the specimen and results when the oblique rays are reflected from the edge of the specimen upward into the microscope objective.

Phase-contrast microscope

- The specimen is illuminated by light passing through an annular (ring-shaped) diaphragm.
- Direct light rays (unaltered by the specimen) travel a different path from reflected or diffracted light rays as they pass through the specimen. These

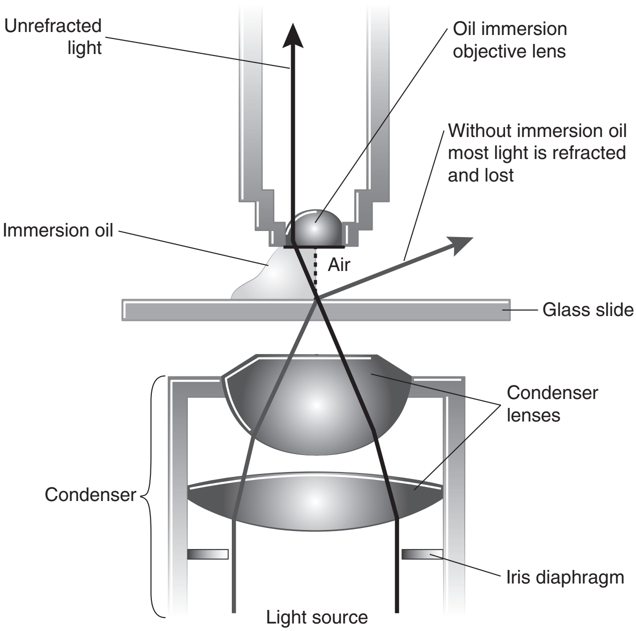

Fig. 1. Refraction in the compound microscope using an oil immersion objective lens (Tortora G.J., 2013)

two sets of rays are combined in the eye. Reflected or diffracted light rays are indicated in blue; direct rays are red (Bottom).

- Phase-contrast microscopy shows more significant differentiation of internal structures and clearly shows the pellicle.

Fluorescence microscope

- A fluorescence microscope is used to visualise specimens that **fluoresce**, which is the ability to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible).
- Microorganisms may be stained with a group of fluorescent dyes called **fluorochromes.**
- The bacterium can be detected by the appearance of **bright yellow** organisms against a dark background.

Electron microscope

- An electron microscope uses a **beam of electrons** that focused on an **electromagnetic condenser** lens onto a thin specimen.

- As the electrons strike the specimen, they are differentially scattered by the number. Some electrons pass through the specimen and get focused by an electromagnetic objective lens, which presents an image of the specimen to the projector lens system for further enlargement.

Atomic force microscope

- In **atomic force microscopy (AFM)**, a probe is gently forced down onto a specimen. As the probe moves along the surface of the specimen, its movements are recorded, anda three-dimensional image is produced.
- AFM provides the ability to measure the physical properties of biological specimens, microorganisms and molecular processes (rigidity).

Question 6. Morphology of bacteria

Bacteria come in a significant variety of sizes and several distinctive shapes. Most bacteria range from 0.2 to 2.0 μm in diameter and from 2 to 8 μm in length.

They have a few basic shapes: spherical **coccus** (fig. 2) (plural: **cocci**, meaning berries), rod-shaped, and **spiral**.

- **Spherical** (cocci).
- **Rod-shaped** (bacteria, bacilli,and clostridia).
- **Spiral-shaped** (vibrios, spirilla, spirochaetes).

Spherical (cocci) bacteria are:

- micrococci (occur singly);
- diplococci (occur in pairs);
- streptococci (occur in chains);
- staphylococci (occur in clusters);
- tetracocci (occur in tetrads);
- sarcinae (occur in packets).

Rod-shaped bacteria:

- bacteria include microorganisms, which, as a rule, do not produce spores (*E. coli*, *Salmonella*, *Shigella*);
- bacilli (*Bacillus anthracis*) produce spores which diameter does not extend the one of a bacterial cell;
- clostridia (*Clostridium tetani*, *Clostridium botulinum*) include organisms which produce spores that's diameter is more extensive than the one of a bacterial cell.

Spiral-shaped bacteria:

1) **vibrios** are cells, which look like a comma in appearance (curved rods). The typical representative of this group is *Vibrio cholerae;*

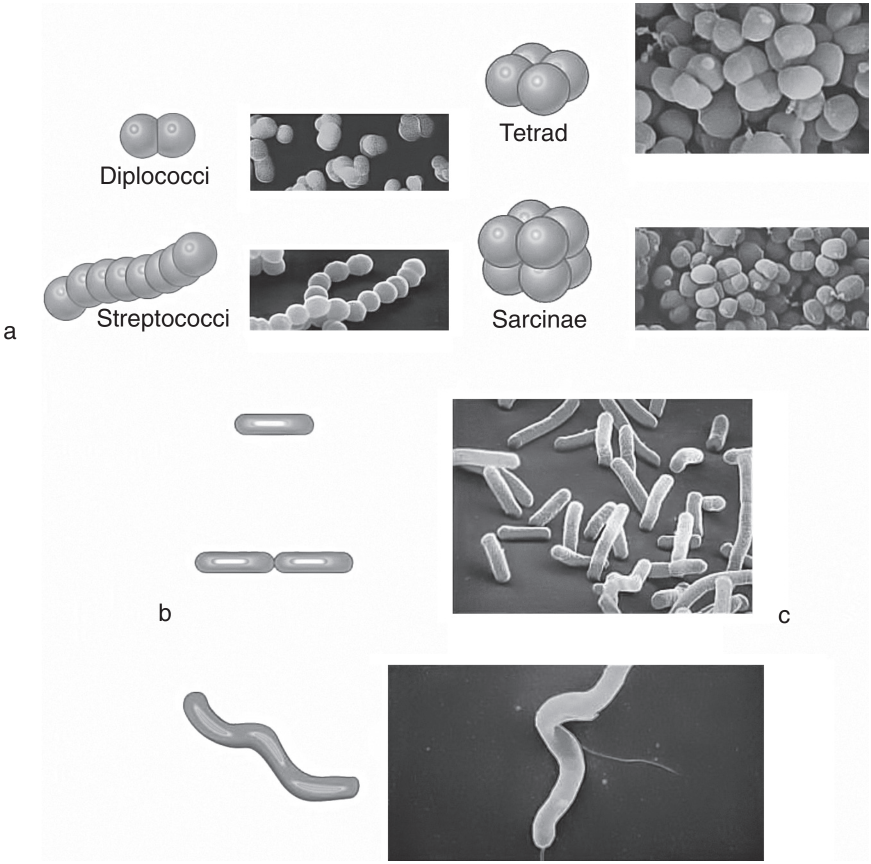

Fig. 2. Shape and arrangements of bacteria: a) cocci; b) rod-shaped; c) spiral (Pommerville J.C., 2011)

2) **spirilla** have a helical shape with a thick, rigid cell wall and flagella that assist movement;

3) **spirochaetes** has a thin, flexible cell wall, but no flagella.

Question 7. Structure of the bacterial cell: genome, cytoplasm, ribosome. Structure, functions and methods of detection

Bacteria are single-celled prokaryotes. The overall structure of the prokaryotic cell is simple (fig. 3). All bacteria contain cytoplasm, ribosomes, a plasma membrane, and a nucleoid. Almost all bacteria have cell walls.

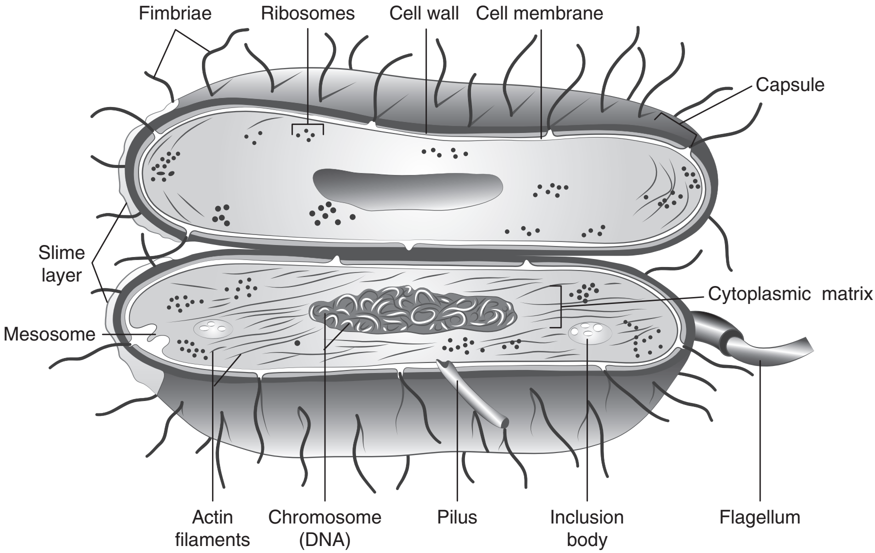

Fig. 3. Bacterial structure (Talaro K.P., 2012)

Cytoplasm

- A gelatinous solution containing water, nutrients, proteins, and genetic material.
- Site for cell metabolism.
- It consists of 70% water, proteins. Approximately 96% of the cell is composed of 6 elements: carbon, hydrogen, oxygen, phosphorus, nitrogen.

Bacterial Genome (nucleoid)

- Contains of a single long, continuous, and frequently circularly arranged thread of double-stranded DNA **(bacterial chromosome)**.
- Bacterial chromosomes are not surrounded by a nuclear envelope (membrane) and do not include histones.
- The nucleoid can be spherical, elongated, or dumbbell-shaped.
- Can be seen with the light microscope in stained material. It is Feulgen-positive, indicating the presence of DNA.
- It carries genetic information.

Prokaryotic ribosome

- Intimately involved in protein synthesis.
- Two subunits, the 30S and 50S, join to form the 70S ribosome, which serves as the structure that facilitates the joining of amino acids.

Question 8. Shell of bacteria: cytoplasmic membrane, cell wall, capsule. Structure, functions and methods of detection

Cytoplasmic membrane

- Thin structure lying inside the cell wall and enclosing the cytoplasm of the cell.
- Consists of phospholipids, which are the most abundant chemicals in the membrane, and proteins.
- Provides a selective ability to different molecules.
- Active transport aided by permease (proteins).
- Plays a role in DNA replication.
- Takes part in cell wall biosynthesis.

Cell wall

Two major groups of bacteria based on the structure of the cell wall are **Gram-positive and Gram-negative** (fig. 4).

Gram positive bacteria have

- Thick peptidoglycan (PG) layer.
- Acidic polysaccharides.
- Teichoic acids and lipoteichoic acids.

Gram negative bacteria have

- Thin peptidoglycan (PG) layer.
- Lipopolysaccharide layer.
- Porins.
- Periplasmic space.

Gram stain is the first step toward bacterial identification.

Functions of bacterial cell wall

- Maintenance of the shape (due to rigidity of peptidoglycan).
- Protection the contents of cytoplasmic membrane cell.