



MICROBIOLOGY WITH VIROLOGY CURRICULUM

The curriculum has been adopted at the meeting of the Faculty Council № 41/08.07.2020

Annotation of the studied subject

Microbiology is the science that studies invisible to the naked eye organisms called microorganisms (microbes). They are widespread in soil, water and air, playing an important role in the circulation of substances. Some microorganisms have adapted to live in other organisms such as humans and animals, becoming parasites. These microorganisms cause infectious diseases and are called pathogenic microbes. Medical microbiology studies: the morphology and structure of microorganisms, their physiology; as well as their interactions with the host and the environment. According to the type of microorganism studied medical microbiology is divided into: bacteriology (studies pathogenic bacteria); virology (viruses); mycology (pathogenic fungi); and parasitology (studying pathogenic protozoa). The subject includes three main sections: general microbiology, the study of infection and immunity, and special microbiology.

General Microbiology section studies the morphology and physiology of microorganisms, bacterial genetics, the antimicrobial drugs used (antibiotics and antiviral drugs), the methods and means for sterilization and disinfection, as well as the principles of applied biotechnologies for the production of biological preparations (vaccines, recombinant biological products, monoclonal antibodies, etc.).

Infection and Immunity section studies the interactions between the host and the pathogenic microorganisms, the defensive mechanisms applied by the host to prevent the disease. It also studies the specific methods for prophylaxis and therapy of infectious diseases.

Special Microbiology section studies the specific pathogenic microorganisms as causative agents of various infectious diseases; their morphology, biology, resistance to physical and chemical factors; mechanisms of infection, pathogenesis and epidemiology of the disease, immunity; methods for microbiological and serological diagnostics, antimicrobial chemotherapy, specific prophylaxis and therapy, prognosis and disease outcome. This section also studies the methods for pathogens detection in the environment: soil, water, air, foods, hospital environment, consumables, drugs etc.

Lectures

WINTER SEMESTER

BASIC MICROBIOLOGY

Lecture 1. Lecture 2. Bacterial morphology. Structure of bacterial cell: capsules, cell wall and cytoplasmic membrane.

Lecture 3. Bacterial morphology. Structure of bacterial cell: flagella, pilli (fimbriae), cytoplasm and cytoplasmic inclusions, ribosomes, nucleoids, spores.

Lecture 4. Bacterial physiology. Chemical composition of bacterial cells. Nutrition. Enzymes. Metabolism – catabolism and anabolism. Bacterial growth and reproduction. Cultivation of bacteria.

Lecture 5. Viruses – main features. Bacteriophages – structure and composition. Major types of interactions between bacterial cells and bacteriophages. Phage infection. Moderate phages, lysogeny, phage conversion. Phage typing.

Lecture 6. Sterilization and disinfection. Impact of physical factors on microorganisms: temperature, desiccation, lyophilization, osmotic pressure, ultraviolet rays, ionization radiation, acoustic energy. Impact of chemical factors on microorganisms. Disinfectants, antiseptics and preservatives; groups, major representatives, mechanism of action.

Lecture 7. Bacterial genetics. Genotype and phenotype at bacteria – bacterial chromosome as a genetic system, non-chromosomal genetic elements (plasmids, moderate phages), mobile genetic elements (Tn and IS). Bacterial changeability: mutations and mechanisms of genetic transfer (transformation, conjugation, and transduction). Recombinant DNA technology (gene engineering).

Lecture 8. Antimicrobial therapy: definition, principles, requirements towards antimicrobial agents, generic and commercial names, spectrum of activity. Mechanisms of action of the major groups of antimicrobial agents.

Lecture 9. Antifungal and antiviral agents. Adverse reactions from the administration of antimicrobial agents – toxic effects, immunological effects, dysbacteriosis. Mechanisms of resistance to antimicrobial agents.
Lecture 10. Modern biotechnologies with medical importance. General characteristics. Recombinant DNA technology. Biosynthesis and biotransformation of bioactive compound. Hybridoma technology. Major groups of products – vaccines, monoclonal antibodies, antibiotics, etc. Methods of microbiology control of medicines and bioproducts – sterility, microbial contamination.

INFECTION AND IMMUNITY

Lecture 11. Infection, infectious process, infectious disease. Relations between micro- and macroorganisms. Features of microorganisms in infectious process – pathogenicity, virulence, infectiousness and contagiousness. Pathogenicity factors. Impact of environment and social conditions on infectious process.
Lecture 12. Immunity – types. Natural Resistance. Acquired Immunity. Antigens. Antigen structure of microorganisms. Immune system. Main steps in immunopoiesis. Immune response. Humoral immunity. Immunoglobulins (antibodies) – structure and biological importance. Monoclonal antibodies.
Lecture 13. Cell-mediated immunity. Mediators of cell-mediated immunity. Local (secretory) immunity and immunosecretory system. Immunity (protection mechanisms) in bacterial infections (caused by toxigenic, extracellular and intracellular bacteria), viral and fungal infections. Immune protection in the oral cavity.
Lecture 14. Immunopathology. Inborn and Acquired Immune Deficiency. Hypersensitivity of Rapid and Delayed Types. Drug-induced (Antibiotics etc.) and Bio-agents (Therapeutic Sera etc.) Induced Allergy.
Lecture 15. Immune prophylaxis and immunotherapy. Vaccines and sera – types, composition, mechanism of action. Immunization calendar of the Republic of Bulgaria. Immunomodulation – immunostimulation and Immunosuppression.

– 4th (summer) SEMESTER

SPECIAL MICROBIOLOGY

Lecture 1. Genus *Staphylococcus*. Genus *Streptococcus* – *S. pyogenes*.
Lecture 2. Genus *Streptococcus* - *S. pneumoniae*, *S. mutans*. Family *Neisseriaceae* - Meningococci and Epidemic Meningitis, Gonococci and Gonorrhoea. Family *Peptococcaceae* – anaerobic cocci. Infections.
Lecture 3. (PM) Family *Enterobacteriaceae*. Genus *Escherichia*. Genera: *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*. Their Significance for Opportunist Hospital Acquired Infections.
Lecture 4. Genus *Salmonella* – Salmonellae, Causing Abdominal Typhus and Food Toxicoinfections; Genus *Shigella* – Dysenterial Bacteria.
Lecture 5. Genus *Yersinia* – *Y. pestis*, *Y. Enterocolitica*. Genus *Vibrio* – Causative Agents of Cholera. Genus *Pseudomonas*.
Lecture 6. Genus *Haemophilus*. Genus *Bordetella*. Genus *Fransisella*.
Lecture 7. Genus *Corynebacterium*. Genus *Mycobacterium*. Genus *Listeria*.
Lecture 8. Genus *Bacillus* - *B. anthracis*. Genus *Clostridium* - Causative Agents of Tetanus and Gas Gangrene and Botulism
Lecture 9. Genus *Clostridium* - *C. botulinum*, *C. difficile*. Spore Non-forming Strict Anaerobic Bacteria.
Lecture 10. Family *Spirochaetaceae*. *Treponema pallidum*. Oral Spirochaetiae. Genus *Borrelia* - *Borrelia burgdorferi*, *Borrelia recurrentis*. Pathogenic Actinomycetes.
Lecture 11. Family *Mycoplasmataceae*. Family *Rickettiaceae*. Family *Chlamydiaceae*. Pathogenic Fungi. Group *Candida*. *Cryptococcus neoformans*.
Lecture 12. Family *Picornaviridae* - Polioviruses, ECHO- and Coxsackieviruses. Family *Ortomyxoviridae* - Influenzaviruses. Family *Paramyxoviridae* – causative agents of parotitis and mumps. Family *Adenoviridae*. Family *Reoviridae* - rotaviruses.
Lecture 13. Family *Rhabdoviridae* – causative agent of rabies. Family *Herpesviridae* - causative agents of herpes simplex, herpes zoster, chicken pox, cytomegaly, infectious mononucleosis. Family *Togaviridae* - *Rubella virus*.
Lecture 14. Causative agents of Hepatitis A, B, C, D and E. Family *Retroviridae* - HIV – causative agent of AIDS.
Lecture 15. Human Normal Flora: Physiological Significance and Etiological Role.

Practical exercises

/ winter semester /

Exercise 1. Microbiological laboratories. Morphology of bacteria. Microscopic methods of examination. Simple methods of staining – Loeffler stain and Pfeiffer stain.

Exercise 2. Morphology of bacteria. Microscopic methods of examination. Differential methods of staining – Gram stain and Neisser stain.

- Exercise 3.** Morphology of bacteria. Differential staining methods: Ziehl-Neelsen stain for acid-fast bacteria and Peshkov stain for spores.
- Exercise 4.** Test examination on bacterial morphology and structure. Bacterial physiology. Nutrition of the bacteria. Culture method.
- Exercise 5.** Bacterial physiology. Metabolism. Bacterial enzymes. Biochemical methods and tests for examination of pure cultures. Bacterial growth and replication. Methods for enumeration of bacteria.
- Exercise 6.** Influence of physical, chemical and biological factors upon microorganisms. Sterilization and disinfection.
- Exercise 7.** Test on bacterial physiology. Bacteriophages. Bacterial genetics. Antimicrobial chemotherapy.
- Exercise 8.** Antibacterial, antiviral, and antifungal chemotherapy. Laboratory methods for antimicrobial susceptibility testing. (part I)
- Exercise 9.** Antibacterial, antiviral, and antifungal chemotherapy. Laboratory methods for antimicrobial susceptibility testing. (part II)
- Exercise 10.** Preliminary test and oral examination on Basic Microbiology.
- Exercise 11.** Infection, infectious process, infectious disease. Natural (nonspecific) defense mechanisms. Experimental infection in laboratory animals.
- Exercise 12.** Immunity. Immune reactions and their use in diagnostics. Agglutination, precipitation, immunoelectrophoresis, immunofluorescence.
- Exercise 13.** Immunity. Immune reactions and their use in the laboratory diagnosis of infectious diseases. Complement fixation. ELISA.
- Exercise 14.** Immunopathology. Immunoprophylaxis and immunotherapy of infectious diseases. Vaccines. Antibody-containing preparations. Immunomodulators. Immunization schedule in Bulgaria.
- Exercise 15.** Microbiological diagnosis of *Mycoplasma spp.* infections. Microbiological diagnosis of *Chlamydia spp.* infections. Microbiological diagnosis of *Candida spp.* infections.

/ summer semester /

- Exercise. 1** General Principles of Microbiological Diagnostics. Microbiological Diagnosis of Staphylococcal Infections.
- Exercise. 2** Microbiological Diagnosis of Streptococcal infections. Microbiological Diagnosis of Diseases Caused by Genus *Neisseria*.
- Exercise. 3** Test on Cocci
Microbiological Diagnosis of infections caused by bacteria of genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Providencia*, *Morganella*
- Exercise. 4** Microbiological Diagnosis of Diseases Caused by Bacteria of Genera *Salmonella*, *Shigella* and *Yersinia*
- Exercise. 5** Microbiological Diagnosis of *Vibrio*, *Pseudomonas*, and *Bordetella*.
- Exercise. 6** Microbiological Diagnosis of Infections Caused by Genera *Haemophilus*, *Corynebacterium*, and *Mycobacterium*.
- Exercise. 7** Microbiological Diagnosis of Anaerobic Infections Caused by Genera *Bacillus* and *Clostridium*. Non-spore anaerobic Infections. *Treponema* and *Borrelia*.
- Exercise. 8** COLLOQUIUM
- Exercise. 9** Viruses – General Characteristics, Taxonomy; Principles of Virological Diagnostics
- Exercise. 10** Family *Picornaviridae*. Family *Reoviridae*.
- Exercise. 11.** Family *Ortomyxoviridae*. Family *Paramyxoviridae*. Family *Adenoviridae*
- Exercise. 12.** Family *Herpesviridae*. Family *Hepadnaviridae*. Family *Retroviridae*.
- Exercise. 13** Microbiological Diagnosis of Actinomycosis. Microbiological Diagnosis of Candidosis.
- Exercise. 14** Microorganisms in the environment. Microorganisms in Drugs and Ready-to use Medicines. Environmental Microbial Flora.
- Exercise. 15** Immunotherapy and Immunoprophylaxis: Vaccines, Immune Sera, and Immunomodulators.

Ongoing assessment

- oral examinations (during seminars) – 3-4 times/semester.
- Test examination – 2 times per semester.
- Colloquium – one per semester.
- Each student prepares also a written paper once per semester – one in immunology and one in clinical microbiology.

Syllabus for practical semester examination

1. Loeffler's stain - preparation, observation and interpretation
2. Gram stain - preparation, observation and interpretation.
3. Neisser stain - preparation, observation and interpretation.

4. Ziehl-Neelsen stain - preparation, observation and interpretation.
5. Peshkov stain - preparation, observation and interpretation.
6. Laboratory Diagnosis of bacterial diseases: principles, specimen collection, maintenance and transport, culture methods for isolation of bacteria, methods for differentiation of processing; expected pathogens; interpretation
7. Simple nutrient media. Types, composition and application. Observation (characteristics) of bacterial growth in broth and agar media. Isolation of pure culture.
8. Special (enriched, differential, selective) nutrient media. Types, composition and application. Characteristics of bacterial growth in broth and agar media. Isolation of pure culture.
9. Methods and nutrient media for cultivation of aerobe and microaerophile bacteria. Isolation of pure culture.
10. Methods and nutrient media for cultivation of anaerobe bacteria. Characteristics of bacterial growth of anaerobe bacteria.
11. Biochemical tests for the identification of bacteria. Tests for determination of carbolytic, proteolytic enzymes and oxidoreductases. Other tests. Kligler's polytrophic medium (Triple Sugar Iron Agar): composition, inoculation and reading.
12. Quantitative methods for the determination of bacterial growth: enumeration with optical standard and determination of the count of live bacteria on a solid medium
13. Methods for microbiological control of drugs for parenteral application. Methods for control of sterility an toxicity.
14. Methods for microbiological control of drugs for oral and local application. Estimation of microbial count.
15. Microbiological examination of drinking water, soil, air, foods. Methods and interpretation of the results
16. Antibiotics and chemotherapeutics - characteristics and usage
17. Serial Dilution Method for Antimicrobial Susceptibility Testing
18. Disk Diffusion Method for Antimicrobial Susceptibility Testing: **Antibiogram** – steps of performing, interpretation
19. Heat sterilization - methods and devices (dry sterilizer and autoclave)
20. Physical and chemical sterilization methods other than heat sterilization methods.
21. Disinfectants. Usage of the particular disinfectants in medical practice
22. Serological methods in laboratory diagnosis: principles, specimen collection, methods and application for diagnosis of infectious diseases
23. Slide agglutination (direct agglutination of Gruber): principle, technique and reading of the results.
24. Widal's serodiagnosis (tube agglutination method) using a patient's serum and *Salmonella* antigens 0:9,12 and H:1,2. Principle, technique and reading of the results.
25. Passive hemagglutination, coagglutination, latex agglutination. Principle, technique and reading of the results.
26. Ring precipitation test (thermoprecipitation). Principle, technique and reading of the results.
27. Titration of hemolytic serum - application, principle, technique and reading of the results.
28. Complement fixation reaction for diagnosis of syphilis: Wassermann's reaction. Application. principle, reading of the results.
29. ELISA (Enzyme-linked immunosorbent assay) - basic principle, reading and interpretation of results. application. Immunofluorescence - direct and indirect immunofluorescence. application.
30. Antibacterial vaccines - characterization, application.
31. Antiviral vaccines - characterization, application.
32. Immune therapeutic sera and human immunoglobulin preparations - production, characterization and application.
33. Immunostimulators - characterization, application.
34. Biochemical identification of the organisms of Family *Enterobacteriaceae*. Tests for identification.
35. Tests for detection and pathogenicity of *Staphylococcus* spp. - reading and interpretation of results.
36. Tests for identification of *Streptococcus* spp. and *Enterococcus* spp.
37. Laboratory Diagnosis of Viral Diseases: principles, specimen collection and methods
38. Viral hemagglutination - basic principle, reading and interpretation of results, application.
39. Hemagglutination-inhibition test (HAI) - principle, reading and interpretation of results, application.
40. Neutralization colour test for estimation of patient's antibodies against polioviruses - principle, reading and interpretation of results, application.

Syllabus for theoretical semester examination

Section I: Basic Microbiology

1. Introduction to Medical Microbiology - The Science of Microbiology. History.

2. The Science of Microbiology - Pathogenic microorganisms. Prokaryotes: Classification.
3. Eukariotes and Prokaryotes. Bacterial Morphology. Structure of the Cell Envelope - Cell Membrane and Cell wall of Gram-positive and Gram negative bacteria.
4. Bacterial Structure: Eukariotes and Prokaryotes. Prokaryotic Cell Structure: Nucleoid, Cytoplasmic Structures.
5. Bacterial Structure: Eukariotes and Prokaryotes. Capsule & Glycocalyx; Flagella and Axial filaments (endoflagella of spirochetes); pili; bacterial spores (endospores).
6. Bacterial Growth and Cell Division: Survival and Death, Measurement of Microbial Concentrations. Generation time, Groth curve - phases of bacterial growth.
7. Microbial (Bacterial) metabolism.
8. Bacterial Genetics: DNA - The Genetic Material - Structure, Replication and Function (Control)
9. Bacterial Genetics: Mutation, Recombination and DNA Exchange.
10. Bacterial Genetics: Genetic Engineering (Recombinant DNA Technology).
11. Modern Biotechnologies in Medicine. Biosynthesis and Biotransformation. Recombinant DNA Technology. Application. –
12. Viruses: Classification, Structure, Replication, and Cultivation.
13. Medical mycology (Fungi): Classification, Structure, Cultivation, Antifungal Agents.
14. Sterilization, Disinfection, and Antisepsis: Definitions, methods for sterilization and disinfections. Disinfectants.
15. Antimicrobial Chemotherapy: Definition; Principle of selective toxicity; Antibacterial Agents - Groups According Molecular Mechanisms of Action, Specter of Activity.
16. Antiviral Chemotherapy: Definition; Principle of selective toxicity; Antiviral Drugs Specific for some viral species and their Molecular Mechanisms of Action.
17. Antimicrobial Chemotherapy: Genetic and Biochemical Mechanisms of Bacterial Resistance. Side effects of antibiotics - toxicity, allergy and disbacteriosis.

Section II: Infection and Immunity

18. Infection and Infectious Disease. Patterns of Infection. The Spread of Infection - Epidemiology.
19. Infection and Infectious Disease. Pathogenicity and Virulence. Pathogenic and Opportunistic bacteria. Mechanisms of Bacterial Pathogenesis. Virulence Factors.
20. Immunity - Natural Resistance and Acquired Immunity. Active and Passive Immunity. Nonspecific Response (Natural Resistance) Against Microbial agents - Physical Barriers, Normal Human Flora (Microbiota).
21. Immunity: Innate Immunity (Natural Resistance) and Adaptive Immunity (Acquired Immunity). Active and Passive Immunity. Mechanisms of Innate Immunity against Microbial agents -Physiologic Barriers, Humoral Defenses and Factors.
22. Immunity: Innate Immunity (Natural Resistance) and Adaptive Immunity (Acquired Immunity). Active and Passive Immunity. - Phagocytic cells and Phagocytosis, Nk cells, Inflammatory Response and Fever.
23. Mechanisms of Specific Host Defense (Adaptive response). Organs and Cells of Immune System and Immune Response. Antigen specific receptors of B- and T-cells. Antigen Processing and Presentation.
24. The humoral immune response. Antigens. Antibodies. Protective functions of antibodies against bacterial and viral infections.
25. Cell-mediated immunity (CMI). T-cells and Macrophages in CMI. Cytokines (lymphokines. monokines, chemokines). Role of the CMI and the local immunity in protection against bacterial, vira and fungal infections. Local (Secretory) immunity.
26. Immunopathology: Hypersensitivity responses (Type 1-4) - allergy and autoimmunity. Immunodefficiency.
27. Immunoprophylaxis and immunotherapy. Vaccines. Hyperimmune sera and immunoglobulin preparations.

Section III: Special Microbiology.

28. Laboratory Diagnosis of Bacterial Diseases. Clinical Specimens. Microscopic and Cultural methods. Methods for Identification of Isolated Bacteria in Pure Culture. Methods for Testing Antibiotic Sensitivity.
29. *Staphylococcus*.
30. *Streptococcus* - group A, B and other β -hemolytic streptococci
31. *Streptococcus* - Viridans streptococci, *Streptococcus mutans*, *Enterococcus* 3 3. *Streptococcus pneumoniae*
32. *Corynebacterium*. *C. diphtheriae*
33. *Listeria*. *L. monocytogenes*

34. *Neisseria* - *N. gonorrhoeae*
35. *Neisseria* - *N. meningitidis*
36. *Enterobacteriaceae*: Common characteristic (**Lec 3**) - *Escherichia coli* and Other opportunistic *Enterobacteriaceae* (*Klebsiella*, *Enterobacter*, *Serratia* -group KES; *Proteus*, *Morganella*, *Citrobacter*)
37. *Enterobacteriaceae* - *Salmonella*
38. *Enterobacteriaceae* - *Shigella*
39. *Enterobacteriaceae* - *Yersinia*. *Y. enterocolitica*, *Y. pestis*
40. *Vibrionaceae* - *Vibrio cholerae*
41. *Campylobacter* and *Helicobacter*. *C. jejuni* and *H. pilory*
42. *Pseudomonas*. *P. aeruginosa*
43. *Bordetella*. *B. pertussis*
44. *Haemophilus*. *H. influenzae*
45. Anaerobic Spore-Forming Bacteria - *Clostridium tetani*. *Clostridium perfringens* and other clostridia causative agents of gas gangrene
46. Anaerobic Spore-Forming Bacteria - *Clostridium botulinum*, *Clostridium difficile*)
47. Aerobic Spore-Forming Bacteria – *Bacillus*.
48. Anaerobic Nonspore-Forming Bacteria.
49. *Mycobacterium*. *M. tuberculosis*
50. Spirochaetes - *Treponema*
51. Spirochaetes – *Borrelia* and *Leptospira*
52. *Mycoplasma* and *Ureaplasma*
53. *Chlamydia*
54. *Rickettsia* and *Coxiella*
55. Pathogenic Fungi - *Candida spp.*, *Cryptococcus neoformans*, *Aspergillus*, *Pneumocystis carinii*
56. Laboratory Diagnosis of Viral Diseases
57. Picomaviruses – Entero and Rhino Viruses
58. Orthomyxoviruses – Influenzaviruses
59. Paramyxoviruses – Parainfluenza, Mumps and Morbilli Viruses
60. Reoviruses – Rotaviruses
61. Togaviruses: Rubella Virus
62. Rhabdoviruses – *Rabies virus*
63. Human Herpesviruses
64. Adenoviruses
65. Retroviruses – HIV and AIDS
66. Papovaviruses - Papillomaviruses
67. Hepatitis Viruses A, B and C
68. Prions
69. Normal human microbial flora in health and diseases.

Academic literature:

1. Medical Microbiology, 25th – 28th editions (Jawetz, Melnick, & Adelberg's Medical Microbiology) by Geo. F. Brooks, Lange, 2010 - 2019
2. Manual for Practical Exercises in Medical Microbiology – part II (editor Prof. Ivan Mitov) 2018. Mitov I., Setchanova L., Boyanova L., Gergova R., Markovska P., Strateva T., Zhelezova G., Baykushev R., Yordanov D., Petrov D. Publishing house ARSO.