

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
COURSE IN MICROBIOLOGY FOR STUDENTS OF FACULTY OF MEDICINE

The name of Unit in which the subject is realized
Department of Microbiology and Immunology

Head
Prof. dr hab. Stefania Giedrys-Kalemba

Total hours:
75 hours include:
22 h of seminars
53 h classes

Tutor:
dr n. med. Ludmiła Szymaniak

ECTS: 8

Aims of teaching

1. To familiarize with the positive and negative role of microorganisms for humans and their environment
2. To know the most important biological features of commensal and pathogenic bacteria, viruses and fungi, also mechanisms of microorganism-host relationship
3. To recognize and detect infections: collection and transport of specimens, isolation and identification of infective agents, interpretation of microbiological and serological results
4. To know the methods of prevention and control of infections (disinfection, sterilization, antimicrobial chemotherapy, vaccinations, hospital infection control)

FORMS OF ACTIVITIES

Microbiology is carried out in V and VI terms in forms classes, seminars and examination. There are no lectures.

1. **Seminars** are conducted during 22 h (11 in V and 11 in VI terms). During the seminars the theory is presented and discussed with using slides and sometimes films.
2. **Practical classes** include 53 h ((11 in V and 11 in VI terms). Seminars and classes are lasting together 3 h 15 min. During the classes students are controlled by tutor with acquaintance of subject theory (short tests: 10 questions, 10 min), watch microorganisms under light and fluorescent microscopes, prepare and analyze different tests etc. The classes are carried out in 10-11 group of students per tutor. Students are obliged to use only those protective coats, which are available in our department. Lessons are obligatory. The topics are given prior to the lessons on the notice board. Every absence has to be justified and the lesson has to be made up in the microbiological laboratory and passed theoretically.
3. **The examination** consists of two parts: practical and theoretical exam. The final examination in microbiology is conducted in a written form (the test) in July (1st and 2nd attempt) at the time scheduled by the Dean. All lessons have to be completed before the exam.

Program of microbiology

1. The basics of the bacteria and fungi differentiation. Part I

Morphology of bacteria and fungi: dimensions, shape, arrangement, structure of bacterial cell, external (flagella, fimbriae, capsule, slime) and internal (cytoplasmic membrane, mesosomes, chromosomal DNA, plasmids, transposones, ribosomes, cytoplasmic inclusions, spores) structures, differences between Gram-positive and Gram-negative bacterial cell wall.

Bacteria with deficient cell wall: mycoplasmas, L-forms, spheroplasts, protoplasts. Basic differences between bacteria and fungi.

Direct examination of morphology - microscopic examination, stained and fresh (wet) smears, application of different types of microscopes in microbiology.

Methods of staining: positive, negative, positive-negative, practical application of Gram, Ziehl-Neelsen, Neisser staining.

General criteria for classification of microorganisms – family, genus, species, strain, biotype, serotype.

The major groups of Gram-positive bacteria – cocci: *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Peptostreptococcus*; bacilli: *Bacillus*, *Clostridium*, *Corynebacterium*, *Listeria*, *Propionibacterium*, *Lactobacillus*, *Actinomyces*, *Nocardia*, *Mycobacterium*.

The major groups of Gram-negative bacteria: cocci: *Neisseria*, *Veilonella*; rods: *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella*, *Salmonella*, etc.), nonfermentative rods: *Pseudomonas*, *Acinetobacter*, other bacilli: *Vibrio*, *Campylobacter*, *Helicobacter*, *Haemophilus*, *Bordetella*, *Gardnerella*, *Legionella*, etc.; anaerobes: *Bacteroides*, *Fusobacterium*; the spirochetes: *Treponema*, *Borrelia*, etc.; *Rickettsia*, *Chlamydia*, *Mycoplasma*.

Practice:

Oil immersion microscopy. Evaluation of microorganisms, dimension and shape – earlier prepared smears.

Preparation and Gram staining of smears from solid and broth media, microscopic evaluation.

Detection of bacterial motility on agar medium, semi-solid medium and in wet smears.

2. The basics of the differentiation of bacteria and fungi. Part II

Physiology of microorganisms – basic growth requirements (chemical composition of bacterial cell, growth requirements of different groups of microorganisms); metabolism – source of carbon and energy (autotrophs, heterotrophs, chemolithotrophs, chemoorganotrophs); atmosphere (obligate aerobes, facultative and obligate anaerobes, microaerophils); temperature (psychrophils, mesophils, thermophils); pH, pressure. Differences in culture requirements (most bacteria – artificial media, rickettsiae and chlamydiae – cell cultures).

Growth and division of bacteria and fungi – life cycle, phases of growth, rapidity of growth on artificial media.

Cultivation methods, categories of bacteriologic media (fluid, solid, semisolid, nutrient, enriched, selective, differential, transport media, media with chromogens), practical application. Microbial differentiation on the basis of type of growth in liquid (turbidity) and solid media (morphology of bacterial colonies). Use of metabolism (biochemical features) of bacteria for their differentiation.

Bacterial identification: colonial and microscopic morphology, biochemical tests.

Variability of bacteria – genotype, phenotype, mutation, recombination (conjugation, transduction, transformation).

Biological and medical importance of genotype changes: change of morphologic and biochemical features, pathogenicity, antibiotic susceptibility.

Practice:

Examination of different media: nutrient agar, blood agar, MacConkey agar, Sabouraud agar, chocolate agar, Chapman agar, Löwenstein-Jensen, nutrient broth, biphasic agar-broth medium. Evaluation of the growth bacteria and fungi – colonial morphology. Demonstration of jars for anaerobic (GasPak system) and microaerophilic (candle jar) bacteria.

Biochemical tests – API and ATB system. Visit in culture medium centre and bacteriology lab. – glass and media preparing. Application of media in a daily diagnostics, visual and automatic reading of biochemical features of bacterial strain.

3. The basics of virology

General features of viruses differentiating them from other microorganisms. Size and structure of viruses. Properties and participation of individual structural elements of viruses in the pathomechanism of infection, in diagnostics, vaccines production. Stages of virus replication, the influence of type of replication on the viral infection course. Prions.

Methods of cultivation of viruses (tissue cultures, chick embryos, sensitive laboratory animals).

Methods of detection of virus-infected cells: cytopathic effect, plaque assay, hemagglutination, hemadsorption, neutralization test, microscopic methods.

Bacteriophages, mycophages and their application in medicine. Lytic phages.

The major groups of RNA viruses: (*Orthomyxoviridae* (Influenza); *Paramyxoviridae* (Parainfluenza, Mumps, Measles, RSV); *Rabdoviridae* (Rabies); *Filoviridae* (Marburg, Ebola virus); *Bunyaviridae* (Hantavirus); *Picornaviridae* (rhinoviruses, HAV, enteroviruses: Polio, Coxsackie, Echo); *Reoviridae* (Rotavirus); *Retroviridae* (HIV, HTLV); *Togaviridae* (Rubella); *Coronaviridae*; *Calciviridae* (Norwalk virus); *Flaviviridae* (Yellow fever, Dengue virus, HCV); the groups of viruses causing: haemorrhagic fevers, neuroinfections (*Toga-*, *Flavi-*, *Bunya-*, *Arenaviridae*); arboviruses.

The major groups of DNA viruses: *Herpesviridae* (Herpes simplex, Varicella-zoster, CMV, EBV, HHV6, HHV7); *Adenoviridae*; *Papovaviridae* (HPV, JC virus); *Parvoviridae* (B19); *Hepadnaviridae* (HBV); *Poxviridae*: (Variola, Vaccinia).

Practice:

Inoculation of embryonated eggs, examination of amniotic and allantoic fluids. Demonstration of cytopathic effect and hemadsorption in cell culture. Detection of viral hemagglutinins – qualitative and quantitative tests. Examination of Negri inclusion bodies (rabies) in brain tissue. Inoculation of swabs from nose, throat, skin.

4. Host - parasite interactions

Forms of relationships between microorganisms: synergy, antagonism, indifference – examples.

Relationships between microorganisms and host: symbiosis, commensalism, parasitism, opportunism, carrier state, antibiosis.

Normal microbial flora of the human body (skin, respiratory, intestinal and urogenital tracts. Role of the resident flora.

Pathogenicity (virulence) of microorganisms – infectivity, invasiveness, toxigenicity, toxicity.

Microbial virulence factors: superficial structures (fimbriae, capsules, mucoid substances, adhesive proteins), toxins (exo-, endo-, entero-, neurotoxins, mode of action), enzymes (e.g. coagulase, hyaluronidase, other).

Some epidemiological terms connected with infection and the infective diseases epidemiology: adhesion, colonisation, contamination, invasion, evasion, infection (acute, chronic, opportunistic, local, systemic, generalized, asymptomatic, symptomatic, latent, mixed, primary, reinfection, superinfection, nosocomial community-acquired, endo- and exogenous, congenital), anthroponosis, anthroozoonosis, zoonosis, sapronosis, bacteraemia, sepsis, intoxication, contagion, reservoir, entry, source and routes of infection, incubation period, prevalence, outbreak, endemia, pandemia, morbidity and morbidity rate, mortality and mortality rate, fatality, measurements, surveillance.

Practice:

Some examples of relationship between microorganisms – plates with aerobic and anaerobic bacteria, culture with „wet nurse”.

Examination of cultures from different parts of the body.

Preparation of cultures from fingers and surfaces (e.g. table, floor, window sill, etc.)

Preparation of air culture (sedimentation method).

5. Sanitization, disinfection, sterilisation

Sanitization, disinfection and sterilisation - definition, practical application.

Disinfection –

- physical: thermal (pasteurisation, tyndallization, decoction – boiling), UV irradiation;
- chemical: acids, alkali, aldehydes, compounds containing active chlorine or iodine, phenolic derivatives, detergents and soaps, oxidizing and heavy metal compounds, others. The principles of disinfectants selection.

Sterilisation –

- high – temperature (dry heat - hot-air oven; moist heat (steam under pressure): autoclave; incineration; flaming – bacteriological loop);
- low – temperature (gas –ethylene oxide, formaldehyde; fumigation);
- penetrating radiation;
- chemical: disinfectants – aldehydes, halogens, potassium perborate;
- mechanical – filtration;
- plasmic;

Sterilisation control: physical, chemical and biological indices. Control of air, surface and equipment contamination.

Physical and chemical antiviral agents.

Practice:

Demonstration of apparatus for sterilisation. Sterilisation control – different indices. Examination of plates with action of UV and disinfecting agents. The most used disinfectants – prospects. Examination of own fingers, air and surfaces cultures.

6. Chemotherapy of bacterial infections. Part I.

General characteristics and division of antimicrobial agents - chemotherapeutics, antibiotics: beta-lactams (penicillins, cephalosporins, monobactams, carbapenems, beta-lactams inhibitors), aminoglycosides, quinolones, tetracyclines, macrolides, lincosamides, glycopeptides, metronidazole, other.

Mode of action (bacteriostatic, bactericidal); spectrum of activity (narrow and broad, extended); mechanism of action of particular antibiotic groups (inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein or nucleic acid synthesis, antimetabolic action).

Side effects: hypersensitivity, toxicity, biological effects, postantibiotic effect.

Antimicrobial susceptibility testing in vitro: disk diffusion, tube and plate dilution test, E-tests.

Clinical importance of MIC and MBC.

Practice:

Discussion about standardisation of antimicrobial susceptibility tests according to NCCLS guideline (appropriate inoculation, medium, selection of antibiotic discs). Antibiogram form.

Reading and interpretation of disk diffusion (sensitive, intermediate, resistant), broth dilution and E-test (MIC determination) for different kinds/groups of microorganisms.

7. Chemotherapy of bacterial infections. Part II.

Topical issues of antimicrobial chemotherapy – the increase of drug resistance, changeability of etiological agents of infections.

Mechanisms of antimicrobial drug resistance – intrinsic (inherent) and acquired: chromosomal (mutations) and extrachromosomal (plasmids, transposons) – transduction, transformation, conjugation. Resistant strains selection. Phenotypic expression of resistance to antimicrobial agents – synthesis of inactivating enzymes, modification of target site, altered penetration barrier (permeability), altered metabolic pathway, active antibiotic efflux.

Mechanisms of antibiotic resistance exhibited by clinically important pathogens: *Staphylococcus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus*, *Haemophilus influenzae*, *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Acinetobacter*.

Practice:

Detection of different antibiotic resistance mechanisms: beta-lactamases ESBL and AmpC, MLS mechanism, MRSA, VISA, HLAR, VRE, GISA strains.

Clinical interpretation of antibiogram.

8. Chemotherapy of bacterial infections. Part III.

Chemotherapy of viral infections: groups of antiviral drugs, mechanisms of action.

Chemotherapy of fungal infections: groups of antifungal drugs, mechanisms of action, susceptibility testing.

Drugs applied in infections caused by mycobacteria, anaerobes, atypical bacteria.

Indications and principles of rational antimicrobial therapy: empiric, guided, deescalation therapy (choice of drug according to clinical and laboratory diagnosis).

Practice:

Examination of antifungal susceptibility tests: Fungitest, Candifast, ATB – Fungus, E - test.

9. Specimen collection and processing

The purpose and importance of bacteriological examination.

General principles of specimen collection and transportation: time, kind of specimen, containers, transport media, clinical diagnosis, requirements for microbiology.

Specimen processing:

- o direct examination – Gram-stained (or other) smears, detection of antigen; molecular methods;
- o media selection, inoculation and isolation;
- o culture examination (colonies morphology), culture smears,
- o identification: biochemical, serological (antigen typing), bacteriophages typing, bacteriocin typing;
- o tests of antimicrobial drugs, molecular methods.

Clinical interpretation of the result.

Detection of antibody in serum.

Practice:

Discussion about application form. Examination of pus – smear, inoculation. Examination of direct smears and cultures from different specimens. Biochemical and serological differentiation.

10. Gram-positive and Gram-negative cocci aerobic and facultatively anaerobic

Gram-positive, catalase-positive: *Staphylococcus*, *Micrococcus*, *Stomatococcus*

Gram-positive, catalase-negative: *Streptococcus*, *Enterococcus*, *Aerococcus*, *Gemella*

Gram-negative cocci: *Neisseria*, *Moraxella*

Occurrence, the pathogenicity determining factors, clinical manifestation and significance, the host defence mechanisms – type of inflammatory reaction, epidemiology, microbiological diagnostics and therapy of staphylococcal infections (*S. aureus*, CNS group (*S. epidermidis*, *S. saprophyticus*), *Streptococcus* (serological groups: A – *S. pyogenes*, B – *S. agalactiae*, C – *S. equisimilis*, G, *S. pneumoniae*, „viridans group”), *Enterococcus*. (*E. faecalis*, *E. faecium*), *Neisseria* (*N. meningitidis*, commensal neisserias occurring physiologically in the oral cavity), *Moraxella catarrhalis*.

Practice:

Direct smear from *carbunculus* – staphylococcal infection. Evaluation of different colonies of staphylococci on nutrient, blood and mannitol agar, catalase test. Differentiation of staphylococci: slide and tube coagulase tests, DNA-ase production. Evaluation of disk diffusion tests for staphylococci: PSSA, MSSA, MRSA. Differentiation of streptococci: colonies and haemolysis on blood agar, serological diagnostic (Streptokit), optochin test, ASO test. Evaluation of disk diffusion tests for streptococci and pneumococci. Differentiation of enterococci: colonies on nutrient, blood and D-coccosel agar, PYR test, HLAR test. Differentiation of meningococci and *Moraxella catarrhalis*.

11. Gram-negative rods aerobic and facultatively anaerobic

Nonfermentative Gram-negative aerobic: *Pseudomonas*, *Stenotrophomonas*, *Burkholderia*, *Acinetobacter*, *Alcaligenes*, *Moraxella*, *Flavobacterium*.

Enterobacteriaceae: *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*).

Oxidase-positive, fermentative: *Vibrio*, *Aeromonas*, *Plesiomonas*, *Campylobacter*, *Helicobacter*, Small rods (cocci/bacilli): *Francisella*, *Pasteurella*, *Brucella*, *Bordetella*, *Gardnerella*, *Haemophilus*, *Legionella*. Occurrence, the pathogenicity determining factors, clinical manifestation and significance, the host defence mechanisms, epidemiology, microbiological diagnostics and therapy of infections caused by: *Escherichia coli* (ETEC, EPEC, EIEC, EHEC strains), *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*), *Salmonella* (serotypes: *S. typhi* (D) – dur brzuszny, *S. paratyphi* (A, B, C), *S. enteritidis*, *S. agona*, *S. typhimurium*, *S. heidelberg*..., *Klebsiella* (*K. pneumoniae*, *K. oxytoca*, *K. rhinoscleromatis*, *K. ozonae*), *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *Acinetobacter baumannii*, *Vibrio cholerae*, *Campylobacter jejuni*, *Helicobacter pylori*.

Practice:

Evaluation of different rods on MacConkey agar: lactose-positive, lactose-negative, mucoid. Evaluation of *Salmonella* colonies on SS agar and *Pseudomonas* on Pyocyanosel. Oxidase test. Biochemical differentiation of Gram-negative rods - API tests, ATB, other. Serological differentiation of *Salmonella*, *Shigella*, *E. coli*. Evaluation of Widal test. Phages typing. Disc diffusion tests from rods. ESBL test. *Helicobacter* smears and identification: urease reaction.

12. Gram-positive aerobic bacilli

Spore-forming: *Bacillus*.

Non-sporing: *Corynebacterium*, *Listeria*, *Erysipelothrix*, *Mycobacterium*.

Branching: *Nocardia*, *Streptomyces*, *Rhodococcus*, *Actinomyces*.

Mycobacterium – classification, morphology and physiology, pathogenicity, immunity and allergy, clinical significance, specimen collection, smears, cultivation, molecular methods, Bactec system, antimicrobial susceptibility. Epidemiology and prophylaxis of tuberculosis.

Corynebacterium diphtheriae and *Nocardia asteroides* infections: pathogenicity, clinical significance, diagnostic, therapy.

Practice:

Evaluation of Gram and Neisser smears and cultures: *Corynebacterium diphtheriae*, diphtheroids.

Nocardia Gram-stained smears and culture on nutrient agar.

Mycobacterium: Ziehl-Neelsen and fluorescent smears, Löwenstein culture, antimicrobial test.

Tuberculin test at guinea pig.

13. Anaerobic bacteria

Gram-positive cocci: *Peptococcus*, *Peptostreptococcus*, *Sarcina*.

Gram-negative cocci: *Veillonella*.

Gram-negative, non-sporing rods: *Bacteroides*, *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Leptotrichia*.

Gram-positive, non-sporing bacilli: *Actinomyces*, *Propionibacterium*, *Lactobacillus*, *Bifidobacterium*, *Mobiluncus*

Gram-positive, spore-forming bacilli: *Clostridium*.

Anaerobes as a part of physiological flora. Factors favourable for anaerobic infections, the pathogenicity

determining factors, clinical manifestation and significance of anaerobic infections.

Basic principles of specimen processing for materials with anaerobic bacteria: collection and transport (transport media), direct examination, culture (media, atmosphere), identification (simultaneous aerobic control of growth), susceptibility to antibiotics.

Infections caused by *Actinomyces* and *Clostridium* – pathogenicity, diagnostics, therapy.

Immunological aspects of anaerobic infections.

Practice:

Anaerobic growth system: jars, liquid media.

Preparing and examination of smears from dental plaque, stool.

Evaluation of smears and anaerobic cultures: *Actinomyces*, *Clostridium*, *Propionibacterium*.

Biochemical tests for anaerobes.

Evaluation disc diffusion tests.

14. Antropozoonoses

Aetiological agents: bacteria, viruses, protozoa, fungi.

Gram-negative rods: brucellosis (*Brucella abortus*, *B. melitensis*, *B. suis*), tularaemia (*Francisella tularensis*), plague (*Yersinia pestis*), yersiniosis (*Yersinia enterocolitica* and *Yersinia pseudotuberculosis*), septic animal bite (*Pasteurella multocida*)

Gram-positive, non-sporing bacilli: listeriosis (*Listeria monocytogenes*), erysipeloid (*Erysipelothrix rhusiopathiae*)

Spirochetes: leptospirosis (*Leptospira interrogans* – serotypes: *L. icterohaemorrhagiae*,

L. grippityphosa, *L. canicola*), Lyme disease (*Borrelia burgdorferi*), recurrent fever (*Borrelia recurrentis*), cat-scratch disease (*Bartonella henselae*)

Rickettsiae – epidemic typhus (*Rickettsia prowazeki*) and spotted fever group, Q fever (*Coxiella burnetii*), ehrlichiosis (*Ehrlichia*), psittacosis (*Chlamydia psittaci*).

Gram-positive, spore-forming bacilli: anthrax (*Bacillus anthracis*).

Pathogenicity, diagnostics, epidemiology.

Practice:

Evaluation of Gram-stained smears and cultures of bacilli. Evaluation of Gram-stained smears with brucellae. Examination of Wright test. Detection of *Borrelia burgdorferi* by IF method.

15. Viral infections

Reminder of: viral structure, methods of isolation, cultivation and identification viral pathogenesis and host response. General indications and principles of viral diagnostics – virus isolation:

- specimens (kind of material, optimal period of sampling, storage, transportation);
- specimen processing in the virological laboratory – direct detection of virus or viral antigens, isolation of viruses in culture, presumptive and definitive virus identification (microscopic, serologic and molecular methods);

Application of serologic reactions in the viral infections diagnostics (complement fixation, neutralisation, hemagglutination and haemadsorption inhibition tests, immunofluorescence, enzyme immunoassays, latex tests) to:

- identification of viral isolates;
- determination of antibody titer in patient serum (CFS, stool);
- detection of viral antigens in patient sample;

Application of molecular methods in the viral infections diagnostics.

Epidemiology, basic clinical aspects of some viral infections: influenza, rubella, viral hepatitis, HIV, rabies.

Antiviral chemotherapy – antiviral drugs, their mode of action. Prion diseases.

Practice:

Evaluation of inclusion bodies in rabies. Detection of antigens or specific antibodies in viral infections – IF, Elisa tests.

16. Fungal infections

Reminder of fungi morphology and reproduction.

Practical classification of fungi: dermatophytes, yeasts, filamentous fungi, dimorphic fungi.

Occurrence of fungi in the environment and in the normal human flora.

Fungal diseases: infections, mycotoxicoses, allergic reactions, clinical features and relationships. Host response in fungal infections. Factors influencing the occurrence of mycoses.

Infections caused by *Candida sp.*, *Cryptococcus neoformans*, *Geotrichum candidum*, *Malassezia furfur*, *Aspergillus sp.*, *Penicillium sp.* and dimorphic fungi.

General scheme of mycologic investigation: direct smears, detection of antigen, cultures, colonial morphology, biochemical activity, detection of antibody in serum, skin tests.

Antifungal agents and antifungal susceptibility tests.

Practice:

Evaluation of fungal morphology in KOH, Gram – stained smears.

Evaluation of colonial morphology on Sabouraud agar.

Evaluation of direct smears from sputum and vagina.

Examination of germ tube test – *Candida albicans*.

Examination of biochemical tests.

Examination of antifungal susceptibility tests.

17. Respiratory tract and the eye infections

Reminder of the normal flora and immunity of respiratory tract.

Most common URT (upper respiratory tract) and LRT (lower respiratory tract) infections, clinical forms, aetiological agents (viruses, bacteria – streptococci, staphylococci, *Haemophilus influenzae*, *Bordetella pertussis*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydia pneumoniae* and *Chlamydia trachomatis*, *Coxiella burnetii*, *Corynebacterium diphtheriae*, mycobacteria, Gram-negative rods, fungi). Community and hospital - acquired infections.

Principles of diagnosis (swabs, sputum, BAL, aspirates, microscopic examination, cultures, identification of pathogens, serological tests) and treatment.

Infections of the eye: causative organisms, specimens, diagnostics, treatment.

Practice:

Evaluation of direct smears from sputum.

Evaluation of different cultures from throat, ear, sinuses, eye, sputum, discussion about identification of bacteria.

Examination and interpretation of antimicrobial susceptibility tests.

18. Diarrhoeal diseases, food poisonings.

Reminder of the gastrointestinal normal flora and immunity.

Epidemiology, clinical features (vomiting, diarrhoea, enteric fever, toxic food poisoning), causative organisms (bacteria, viruses, protozoa), diagnosis, identification, treatment.

Microbiological investigations in gastrointestinal tract infectious diseases:

- examination of faeces and rectal swabs: isolation on MacConkey and selective media, biochemical and serological tests, phage typing;
- cultures of blood, faeces, urine, bile, serological tests (Widal test) – typhoid and paratyphoid fever;
- detection of toxins – *Clostridium botulinum*, *Clostridium difficile*, *Staphylococcus aureus*;
- detection of *Rotavirus* antigen in stool;

Prophylaxis of enteric infections, carrier state detection, control of water.

Practice:

Application of selective media in bacteriological stool examination.

Evaluation of positive cultures for salmonellae.

Evaluation of biochemical tests.

Serological typing of salmonellae, shigellae, *E. coli*.

Examination of Widal test.

Examination of water contamination.

Detection of rotaviruses.

Isolation of *C. difficile* and detection of toxins.

19. Urinary tract infections

Reminder of normal flora and immunity of urinary and genital tract.

Factors predisposing urinary tract infections, clinical forms, causative organisms.

Bacteriological diagnostics of urine: sampling, transport, cultures (quantitative and qualitative evaluation), biochemical identification, antibacterial susceptibility tests.

Different pictures of vagina smears – physiology and pathology.

The most common infections of female genital tract: candidiasis, trichomoniasis, bacterial vaginosis (*Gardnerella vaginalis*), chlamydiosis (*Chlamydia trachomatis*), genital herpes (*Herpes simplex virus 2*) – examination of vaginal discharge, diagnosis, treatment.

Intrauterine and perinatal infections: TORCH (*Toxoplasma gondii*, *Rubella virus*, CMV, HSV), *Treponema pallidum*, *Streptococcus agalactiae*.

Practice:

Inoculation of urine.

Quantitative and qualitative evaluation of urine cultures.

Examination of antibacterial susceptibility tests from urine (record and interpretation).

Examination of different direct vaginal smears and cultures (*Lactobacillus*, *Gardnerella vaginalis*, *Streptococcus agalactiae*).

20. Sexually transmitted diseases (STD)

Currently important aetiological agents of sexually transmitted diseases: *Treponema pallidum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Candida albicans*, *Gardnerella vaginalis*, *Papilloma virus*, *Herpes simplex virus 2*, *HIV*, *HBV*, *HCV*, *Haemophilus ducreyi*.

Syphilis (lues):

- morphology and physiology of treponemas (*Treponema pallidum*, *T. pertenue*, *T. carateum*, saprophytic treponemas);
- immunity and clinical forms of treponemas infections;
- diagnostics depending on disease period: direct smear, nonspecific (flocculation, complement fixation) and specific serologic tests (FTA–ABS, TPHA, TPI);

Gonorrhoea:

- morphology and physiology of
- immunity and clinical forms of *Neisseria gonorrhoeae* infection;
- diagnostics of acute and chronic gonorrhoea (direct smear, culture, identification, antigen detection);

Nongonococcal urethritis (NGU): *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*.
Chemotherapy of STD-s.

Practice:

Evaluation of direct smears with gonococci. Culture and identification of *Neisseria gonorrhoeae* (oxidase test).

Preparing of VDRL test. Evaluation of FTA-ABS test. Detection of mycoplasmas. Detection of *Chlamydia trachomatis* by IF methods.

21. Meningitis, sepsis, endocarditis, infections of skin, bones and joints

Factors predisposing for CNS infections, porta of infection.

Aetiological agents of meningitidies and cerebrites:

- bacterial purulent: *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus sp.*, *Streptococcus agalactiae*, gram-negative rods;
- bacterial nonpurulent: *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Borrelia burgdorferi*, *Treponema pallidum*;
- fungal: *Cryptococcus neoformans*, *Candida sp.*;
- parasitic: *Toxoplasma gondii*;
- viral (lymphocytic): neurotropic viruses – enteroviruses: *Polio*, *Coxsackie*, *Echo*, arboviruses, rabies virus, non-neurotropic viruses giving cerebral complications – measles, mumps, rubella, herpes, adenoviruses-, latent infection of CNS;

Neuroinfection diagnostics: CSF examination (collection and transport, direct examination, culture and identification, detection of bacterial antigen), examination of other specimens (e. g. stool, swab from ear), serological tests.

Bacteraemia, septicaemia, infective endocarditis - predisposing factors, clinical sequela, causative agents, bacteriological diagnostics: general principles of blood collection for culture (timing, volume, number of blood specimens), blood culture methods, culture estimation, interpretation of results.

Skin and subcutaneous tissue infections, bone and joint infections – causative agents, diagnostics.

Antimicrobial therapy of meningites and septicemias.

Practice:

Demonstration of devices and media for CSF and blood collection.

Evaluation of smears from CSF and blood.

Evaluation of most common bacteria causing meningitis, skin and blood infections.

Interpretation of blood culture results.

22. Hospital –acquired (nosocomial) infections

Definition of hospital - acquired infections

Sources (endogenous, exogenous), spread, prevention of hospital infections. Colonisation, carrier – state, infection.

Clinical forms of hospital acquired infections.

Aetiological agents - bacteria, viruses, fungi, parasites. Characteristic of hospital pathogens – variability, antibiotic and disinfectant resistance.

Immunocompromised host (transplant recipients, neoplastic, AIDS, dialysed patients) infections.

important in hospital infections, differences between wards, characteristics of hospital bacteria, role of microbiology laboratory in control of hospital infection, antibiotic policy, epidemiological investigations: antibiogram, biotyping, serotyping, molecular methods.

Infections in immunocompromised patients.

Practice:

Evaluation of different bacteria and antibiograms from hospital infections.

Textbooks:

Medical Microbiology – Jawetz, Melnik, Adelberg's, 1998, ISBN 0 8385 6316 3

Notes on Medical Bacteriology – J. Douglas Sleight, Morag C. Timbury, 1998, ISBN 0 443 05848 2

Notes on Medical Virology - Morag C. Timbury

Clinical and pathogenic microbiology – Barbara J. Howard, 1997, ISBN 0 8016 6426 8