

## 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 intracellular structures (nucleoid, mesosomes, chromosomal DNA, plasmids, transposons, ribosomes, cytoplasmic inclusions, spores). Differences in cell wall structure: Gram-positive bacteria (peptidoglycan, teichoic acids), Gram-negative (peptidoglycan, lipopolysaccharide, porin proteins), mycobacteria (**mycolic acids**, **lipoarabinomannan**, **waxes**). Microorganisms with defective cell wall: mycoplasmas, rickettsiae, chlamydiae, protoplasts, spheroplasts, L forms. Key features differentiating bacteria (Prokaryota) and fungi (Eukaryota).

Methods of microbial morphology examination - microscopic investigations: observation of microorganisms in the living state, stained preparations, the use of different types of microscopes in microbiology. Staining methods: types, practical application (the method of Gram, Ziehl-Neelsen, Neisser, Giemsa, Löffler).

General principles of classification of microorganisms: family, genus, species, strain, biotype, serotype, serovar.

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 bacterial cells morphology – exemplary smears.

Preparation and Gram staining of smears from solid and liquid cultures, microscopic evaluation of smears.

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

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

Microbial physiology - nutritional requirements (chemical composition of the bacterial cell, nutritional requirements for different groups of microorganisms), metabolism - a sources of carbon and energy (autotrophs, heterotrophs, chemolithotrophs, chemoorganotrophs), atmosphere (obligate aerobes, facultative and obligate anaerobes, microaerophils, capnophiles), the influence of temperature (psychrophils, mesophils, thermophils), pH, pressure, redox potential on the growth of bacteria. Differences in growth requirements of different groups of microorganisms (some bacteria - an artificial media, rickettsiae, chlamydiae - propagation in a living cell).

The growth and multiplication of bacteria and fungi - development cycles, bacterial growth phases, growth rate on artificial media (typical bacteria, mycobacteria, anaerobes, fungi, yeast, molds, dermatophytes).

Media for cultivation of microorganisms – classification of bacteriological media (liquid, solid, semisolid, nutrient, simple, enriched, selective, differential, transport media, chromogenic), application of various media in the microbiological diagnostics. Differentiation of microorganisms based on the growth type in liquid media (turbidity) and solid media (colony morphology). The usage of metabolic features (enzyme activity, biochemical characteristics) for the identification and differentiation of microorganisms.

Bacterial identification: colony 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:

Observation 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, smell. Demonstration of equipment for culture of anaerobic (GasPak system) and microaerophilic (candle jar) bacteria.

Biochemical tests – API and ATB system, VITEK 2 Compact, Mycoplasma Duo, Candifast. A visit to the media preparation room and bacteriology lab – glassware and media preparing. Application of media in a routine 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 propagation, 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.

Basic virus taxa:

dsDNA: **Herpesviridae** (Human herpesvirus - HHV-1, HHV-2, HHV-3 (VZV), HHV-4 (EBV), HHV-5 (CMV), HHV-6, HHV-7, HHV-8; **Adenoviridae** (Human adenovirus -HAdV-A, -B, -C, -D, -E, -F); **Polyomaviridae** (BK polyomavirus - BKPyV, JCPyV); **Papillomaviridae** (Human papillomavirus - HPV); **Poxviridae**: (Vaccinia virus - VACV, Variola Variola virus (VARV),

ssDNA: **Parvoviridae** (B19 virus - B19V)

używające odwrotnej transkryptazy: **Hepadnaviridae** (Hepatitis B virus HBV); **Retroviridae** (Human immunodeficiency virus - HIV-1, HIV-2, Primate T-lymphotropic virus - PTLV-1, PTLV-2 (HTLV)

dsRNA: **Reoviridae** (Rotavirus A – RV-A, Rotavirus B – RV-B, Colorado tick fever virus- (CTFV)

ssRNA(-): **Orthomyxoviridae** (Influenza A- FLUAV, Influenza B - FLUBV, Influenza C - FLUCV);

**Paramyxoviridae** (Human parainfluenza virus - HPIV-1, HPIV-3, Measles virus – MEV, Mumps virus – MuV, Human respiratory syncytial virus – HRSV); **Rabdoviridae** (Vesicular stomatitis New Jersey virus – VSNJV, Rabies virus – RABV); **Bornaviridae** (Borna disease virus – BDV); **Filoviridae** (Za Seoul virus –

SEO; Zaire Ebola Virus, Marburg virus); **Bunyaviridae** (Hantaan virus – HTNV, Dobrava-Belgrad virus –

DOBV, Puumala virus – PUUV, Sin Nombre virus – SNV, Rift Valley fever virus – RVFV); **Arenaviridae**

(Lassa virus - LASV, Junin virus – JUNV, Machupo virus – MACV, Guanarito virus – GTOV, Sabia virus –

SABV), **Hepatitis delta virus** - HDV; **Picornaviridae** (enterovirusy: Coxackie, Echo Polio; rinowirusy: Human

rhinovirus - HRV-A, HRV-B; **Hepatitis A virus** – HAV, **Foot-and-mouth disease virus** – FMD); **Calciviridae**

(Norovirus – (Norwalk virus) - NV, **Sapporo virus** – SV); **Astroviridae** Human astrovirus – HastV);

ssRNA(+): **Coronaviridae** (Coronavirus, SARS, Torovirus); **Togaviridae** (Rubella virus - RUBV); **Flaviviridae**

(Tick-borne encephalitis virus –TBEV, Yellow fever virus - YFV, Dengue virus - DENV, West Nile virus- WNV, **Hepatitis C virus** – HCV); **Hepeviridae**: Hepatitis E virus;

### **Practice:**

Inoculation of embryonated eggs, examination of amniotic and allantoic fluids. Demonstration of cytopathic effect and hemadsorption in cell culture.

Detection of virus by viral hemagglutination - slide reaction (rapid, qualitative) and tube test (to determine viral titre).

Detection of rabies virus antigen in brain tissue by DIF.

Demonstration of plaques (bacteriophages) on bacterial lawn.

## **4. Chemotherapy of bacterial infections. Part I.**

General characteristics of the substances acting on microorganisms: bacteria, viruses, fungi, chemotherapeutics, antibiotics. Classification: groups, main representatives.

Antibacterial drugs: 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).

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

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 and EUCAST guidelines (appropriate inoculation, medium, selection of antibiotic discs, incubation time and the principles of reading and interpretation of results). Antibiogram form.

Students make antibiograms with disc diffusion method (*S. aureus* - pus, carrier-state, *S. pyogenes*, *E. faecalis*, *E. coli* - pus, urine, Pseudomonas), read and interpret disk diffusion test (sensitive, intermediate, resistant), MIC based on the broth dilution method and E-test, calculate BMQ index for beta-lactam antibiotics and discuss its importance in clinical practice.

## **5. Chemotherapy of bacterial infections. Part II.**

Current problems of antimicrobial chemotherapy: increase of resistance, high diversity and changeability of infective agents, indications and principles of rational therapy: Empirical therapy, targeted therapy, therapy deeskalacyjna.

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, AmpC, KPC, MBL; MLS mechanism, MRSA, VISA, HLAR, VRE, GISA strains.

Clinical interpretation of antimicrobial susceptibility tests.

**6. Normal human flora (microbiota). Fundamentals of infections detection.**

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. The purpose and importance of bacteriological examination.

The rules (procedures) of collection the material for microbiological examination (bacteriological, virological, mycological): the period of collection, types of materials, methods of collection, storage and transportation, request form to microbiological laboratory; pre-analytical laboratory errors.

Specimen processing - implementation and practical significance of the various stages, laboratory error.

Specimen processing:

M examination - preparation of a Gram-stained direct or possibly another demonstration of the antigen directly in the material or genetic serological methods; - culture

- o direct microscopic examination – Gram-stained (or other) smears, detection of antigen; molecular methods;
- o media selection, inoculation on appropriate bacteriological media, culture under aerobic / anaerobic / other;
- o identification of cultured microorganisms - morphological characteristics (smears from culture, colony morphology), biochemical characteristics, antigenic characteristics (determination of the serotype/ serovar), other (eg, phage typing, genotyping)
- o antimicrobial susceptibility testing and detection the mechanism of resistance (phenotypic and genetic methods)
- o the study of microorganism virulence ( *in vivo* and *in vitro* methods);
- o clinical interpretation of the result of bacteriological examination - normal flora, carrier-state, colonization, etiological agent; post-analytical error;
- o

Indirect diagnostics - determination of serum antibody titer with serological reactions.

**Practice:**

Performance of cultures: swabs from different parts of the body (nose, ear, eye, throat), fingerprints (before washing, after washing with soap, after disinfection), inanimate surfaces (Count-Tact method, swabs), and a study of air pollution by sedimentation method.

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

Watching the kits for sample collection (swabs, transport media, growth media used for transport, etc.). Discussion of the bacteriological examination of pus/ other specimen - a direct preparation, cultures on media: blood agar, MacConkey, Chapman, thioglycolate broth; differentiation grown colonies (staphylococcus - coagulase, *E.coli* - a biochemical identification kit ), antibiogram. Watching the reference sera for the differentiation of bacteria.

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

## **7. The methods of destroying microorganisms outside the human body (sanitization, disinfection, sterilisation). Healthcare-associated infections (HAI).**

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.

Methods of test for bacterial contamination of air and surfaces/ equipment: a method of spontaneous sedimentation and with forced circulation, swabs - usefulness in practice (the pros and cons).

Healthcare-associated infection: definition, clinical forms, sources, reservoir, transmission routes, portals of entry.

Exogenous and endogenous HAI. Etiologic agents of HAI - bacterial, viral, fungal, alarm pathogens. Surveillance, control, prevention of hospital infections - passive and active registration. Principles of HAI chemotherapy.

### **Practice:**

The demonstration of different types of equipment for sterilization. Watching chemical indicators controlling sterilization process. Reading of sporotest A culture.

Watching the plate with an example of activity of UV radiation and disinfectants. Overview of brochures, leaflets with the most commonly used chemical disinfectants and sterilizing compounds.

Viewing and interpretation of cultures from body sites, fingers, surfaces and air.

Watching and reading of antibiogram in HAI cases. Principles of the epidemiological investigation HAI: phenotypic and genotypic differentiation of HAI strains (MRSA, Gram-negative rods). Detection of MRSA in the specimen (eg, nasal swab) by Real-Time PCR in Gene-Expert technology - demonstration and application of the test in daily clinical work.

Recommended reading - the newest editions of:

1. Jawetz, Melnick & Adelberg's Medical Microbiology – Geo. F. Brooks (red.), Karen C. Carroll (red.), Janet S. Butel (red.), Stephen A. Morse (red.), Timothy A. Mietzner (red.) ISBN: 0071624961, ISBN13: 9780071624961.; McGraw-Hill, rok wydania: 2010
2. Notes on Medical Microbiology, 2nd Edition Including Virology, Mycology and Parasitology, autorzy: Bishan Thakker, A. Christine McCartney, Katherine N. Ward, nr: cl01480, ISBN: 0443102848, ISBN13: 9780443102844, Churchill Livingstone,: 2008
3. Clinical and pathogenic microbiology – Barbara J. Howard, 1997, ISBN 0 8016 64268
4. Microbiology and Infectious Diseases – Gabriel Virella, 1997, ISBN 0683062352
5. Clinical Microbiology made ridiculously simple Ed. 4, M. Gladwin, B. Trattler
6. Medical Microbiology - Patrick R., Ph.D. Murray, Ken S., Ph.D. Rosenthal, George S., Ph.D. Kobayashi, Michael A. Pfaller
7. Oxford Handbook of Infectious Diseases and Microbiology - Estee Torok, Ed Moran, and Fiona Cooke
8. Antimicrobial Chemotherapy.D. Greenwood, R. Finch, P. Davey, Ebrary of PMU