PROGRAMME OF MICROBIOLOGY AND IMMUNOLOGY COURSE
FOR 2nd YEAR ENGLISH SPEAKING STUDENTS
FACULTY OF MEDICINE & DENTISTRY, MAJOR: DENTISTRY
POMERANIAN MEDICAL UNIVERSITY, ACADEMIC YEAR 2023/2024

Winter semester of 2nd year
Course duration: - total 40hrs / seminars 20hrs (including 3hrs e-learning); practical classes: 20hrs.

Seminar 1: The basics of bacteria and fungi differentiation – 4h

Bacterial/fungal cell morphology: structure, dimensions, shapes, arrangements, external and internal structures; differences in bacterial cell wall structure between Gram-positive, Gram-negative bacteria and mycobacteria; types of bacteria with deficient cell wall. Differential characteristics of Procaryota and Eucaryota.

Bacterial/fungal physiology - basic growth requirements: source of carbon energy (autotrophs, heterotrophs, chemolitotrophs, chemoorganotrophs); O_2/CO_2 ratio (obligate aerobes, facultative and obligate anaerobes, microaerophils); temperature (psychrophils, mesophils, thermophils), pH and pressure.

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

Detection of microorganisms: microscopy: unstained (wet mount) slide preparation, stained smear; direct and culture smear; types of microscope. Staining techniques: simple/differential staining, positive/negative staining; staining types: Gram, Ziehl-Neelsen (acid –fast), Neisser and Giemsy staining.

Culture of microorganisms: types of culture media (consistency and composition), transport media, chromogenic media, application of culture media in microbiological diagnosis. Differentiation of microorganisms in broth media (turbidity) and on solid media (colony morphology)

Presumptive identification of bacteria/fungi: cell morphology (microscopy), colony morphology (media).

Full identification of bacteria/fungi: biochemical tests/kits (API tests, Vitek Compact); mass spectrometry

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

Biological and medical importance of genotype changes- changes of morphological and biochemical properties, pathogenicity, antibiotic susceptibility

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

The major groups of Gram positive and Gram negative cocci and rods. Anaerobes; the spirochetes;

Detection of infectious diseases: the purpose and importance of bacteriological examination.

Specimen collection and transportation: general principles; time of collection, kind of specimens, containers, transport media, shelf-life, clinical diagnosis, requisition form for microbiological examination.

Specimen processing: direct examination by Gram-stained (or other) smears, detection of antigen; molecular methods; media selection, inoculation and isolation; culture examination, culture smears; Identification: biochemical, serological (antigen typing), bacteriophages typing, bacteriocin typing; antimicrobial susceptibility testing, molecular methods. Clinical interpretation of bacteriological report. Detection of antibody in serum.

Practical part 1: Laboratory detection of bacteria and fungi – 4h

Oil immersion microscopy. Ready smears - evaluation of bacterial morphology: size, shape, arrangement
Preparation and Gram- stained smears.
Microscopic evaluation of direct smears from clinical specimens
Detection of bacterial motility on agar medium and semi-solid medium.
Examination of different media for bacterial growth before and after culture.
Evaluation of the bacterial and fungal growth- colonial morphology (“appearance”)
Demonstration of jars/sets for culture of anaerobic (Gas Pak system) and microaerophilic (candle jar) bacteria.
Demonstration of biochemical kits
Demonstration of specimen collection tools.

Sources:

1. L Samaranyake. Essential Microbiology for Dentistry- 2018, 5th ISBN**:** 9780702074356
2. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768
3. R.A.Harvey, C.N.Cornelissen, B.D. Fisher. Lippincott’s Illustrated Reviews: Microbiology, 2012, ISBN: 9781608317332

E-learning 1: Basics of virology - 1h

General features of viruses- size and structure. Stages of viral replication, the influence of type of replication on the viral infection course. Prions.

Classification of viruses. The major groups of RNA virus families and their representatives:

dsRNA: *Reoviridae;* **ssRNA (-)**: *Orthomyxoviridae*; *Paramyxoviridae), Rabdoviridae, Bornaviridae, Filoviridae, Bunyaviridae, Arenaviridae, Picornaviridae* (enteroviruses, rinoviruses); *Calciviridae, Astroviridae*

ssRNA (+): *Coronaviridae, Togaviridae , Flaviridae*

The major groups of DNA virus families and their representatives

dsDNA: *Herpesviridae*; *Adenoviridae, Polymoaviridae, Papillomaviridae*; *Poxviridae*

ssDNA: *Parvoviridae*

with reverse transcriptase*: Hepadnaviridae (Hepatitis B virus*- HBV), *Retroviridae (Humanimmunodeficiency virus*- HIV-1, HIV-2, *Primate T- lymphotropic virus*- PTLV-1, PTLV-2 (HTLV)

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

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

Bacteriophages, mycophages and their application in medicine. Lysis and lysogenia.

General indications and principles of viral diagnostic- the purpose of virusological examination, sampling, storage and transportation, diagnostic methods (virus isolation, microscopy, serology, genetics)

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1. L Samaranyake. Essential Microbiology for Dentistry- 2018, 5th ISBN**:** 9780702074356
2. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768

Seminar 2: Basics of chemotherapy of infections - 2h

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;

Mechanism of action of particular antibiotic groups (inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, inhibition of nucleic acid synthesis, postantibiotic effect (PAE).

Side/adverse effects: hypersensitivity, toxicity, biological effects, mechanisms of action.

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

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

Mechanisms of antimicrobial drug resistance- intrinsic (inherent, natural) and acquired: chromosomal (mutations) and extrachromosomal (plasmids, transposons)

Phenotypic expression of resistance to antimicrobial agents- synthesis of inactivating enzymes, modification of target site, altered permeability, altered metabolic pathway, active antibiotic efflux.

Indications and principles of rational antimicrobial therapy: empiric, quided, combination therapy.

Antimicrobial susceptibility testing in vitro (AST; antibiogram):qualitative methods- disk diffusion, quantitative methods- tube and plate dilution test, E-test; screening tests- detection of MRSA, HLAR

Practical part 2: Antimicrobial susceptibility testing – 2h

Rules of AST according to EUCAST recommendations (inoculation, medium, selection of antibiotic discs).

Reading and interpretation of disc diffusion results (susceptible, resistant) and E-test results (MIC determination).

Detection of antibiotic resistance mechanisms for rods (ESBL, AmpC, KPC, MBL)

Detection of antibiotic resistance mechanisms for cocci (MLSb, MRSA, HLAR, VRE)

Demonstration of antifungal susceptibility testing (Candifast and E-test)

Clinical interpretation of antibiogram

Making a culture from clinical samples: nose, throat, skin swabs.

Sources:

1. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768

E-learning 2: Human microbiota – 1h

Interactions between microorganisms: synergy, antagonism, indifference- examples

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

Human microbiota of skin and respiratory, intestinal and urogenital tracts. Importance of human microbiota.

Pathogenicity (virulence) of microorganisms- infectively, invasiveness, toxicity. Microbial virulence factors: superficial structures (fimbriae, capsules, mucoid substances, adhesive proteins), toxins (exo-, endo-, enterotoxins, mode of their action), enzymes (coagulase, hyaluronidase, other).

Epidemiology of infective diseases – basic definitions: adhesion, colonization, 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, antropozoonosis, zoonosis, apronosis, bacteriaemia, sepsis, intoxication, contagion, reservoir, entry, source and routes of infection, incubation period, prevalence, outbreak, endemia, pandemia.

Seminar 3: Cocci - Gram-positive, Gram-negative, aerobes and anaerobes – 1h

Gram- positive, catalase- positive: *Staphylococcus(S.aureus, CNS groups: S.epidermidis, S.saprophyticus*) *Micrococcus.*

Gram- positive, catalase- negative: *Streptococcus* (serological groups: A – *S.pyogenes*, B- *S.agalactiae*, C- *S.equisimilis*; *S.pneumoniae*, „viridans group"), *Enterococcus(E. faecalis, E.faecium)*. Gram- negative cocci: *Neisseria (N. meningitides, N.gonorrhoeae, oral commensal neisserias), Moraxella* (*M. catarrhalis).*

Characteristics of staphylococci, streptococci, enterococci , neisserias and moraxella by the following scheme: 1) occurrence, 2) the pathogenicity factors, 3) clinical manifestations of infection, 3) epidemiology, 4) microbiological diagnosis and 5) therapy of infections.

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

Gram- negative anaerobic cocci: *Veilonella*

Practical part 3: Human microbiota. Cocci - Gram-positive, Gram-negative, aerobes and anaerobes -3h

Examination of culture from different body sites

Bacteriological examination of pus from carbunculus (staphylococcal infection)

Differentation of staphylococci: blood and mannitol agar result, catalase test, slide coagulase test (CF- clumping factor) and tube coagulase test. Antibiograms for PSSA, MSSA, MRSA.

Differentation of streptococci: haemolysis on blood agar, catalase test, serological diagnostic (Streptokit), optochin test, ASO test. Antibiograms for streptococci and pneumococci.

Differentation of enterococci: blood and D- coccosel agar. Antibiogam for HGLAR

Differentation of menigococci and *Moraxella catarrhalis*.

Sources:

1. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768.
2. Notes on Medical Microbiology – K.N. Ward, K.C. McCartney, B. Thakker, 2008, ISBN 9780443102844

Seminar 4: Rods - Gram-negative, Gram-negative, aerobes and anaerobes – 2h

Non- fermentative Gram- negative aerobic rods: *Pseudomonas, Stenotrophomonas, Burkholderia, Acinetobacter,*

Gram- negative rods (enteric):*Enterobacteriaceae: Escherichia coli* (ETEC, EPEC, EIEC, EHEC), *Salmonella, Shigella, Klebsiella, Enterobacter, Citrobacter, Serratia, Proteus, Morganella, Providencia, Yersinia.*

Gram- positive fermentative: *Vibro, Aeromonas, Plesiomonas, Campylobacter, Helicobacter*.

Small rods (coccobacilli): *Francisella, Pasteurella, Brucella, Bordatella, Gardnerella, Haemophilus, Legionella.*

Diagnostics rules of gastrointestinal disease with diarrhea.

Spore- forming bacilli: *Bacillus*

Non- sporing bacilli: *Corynebacterium, Listeria, Erysipelotrix, Mycobacterium.*

Branching bacilli*: Nocardia, Streptomyces, Rodococcus, Actinomadura*.

Mycobacteria infections- specimen collection, specimen classification, morphology and physiology, pathogenicity, clinical significance, diagnostics- specimen collection, specimen decontamination, smears, cultivation (Loewenstein- Jensen medium, BACTEC system), molecular methods, antimicrobial susceptibility. Epidemiology and prophyloxis of tuberculosis.
Nocardia asteroids infections: diagnostics and therapy
Corynebacterium diphteriae infections: diagnostics, therapy, prophylaxis.
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 in physiological flora of human. Predisposing factors for anaerobic infections, virulence factors, clinical manifestation of anaerobic infections.

Practical part 4: Rods - Gram-positive, Gram-negative, aerobes and anaerobes -2h

Evaluation of different rods on McConkey agar; lactose- positive (*E.coli, Klebsiella*), lactose- negative (*Salmonella, Shigella, Proteus*), mucoid. Evaluation of *Salmonella* colonies on SS agar and *Pseudomonas* on Pyocyanosel.
Biochemical differetation of Gram- negative rods- API tests, ATB, other. Serological differentiation of *Salmonella, E.coli*. Evaluation of Widal test. Disc diffusion tests from rods. ESBL test. Helicobacter culture smears and its identification with urease test.
Evaluation of Gram and Neisser smears and cultures: *Corynebacterium diphtheria*, diphtheroides.
Nocardia Gram- stained smears and culture on nutrient agar.
Mycobacterium: Ziehl- Neelsen and fluorescent smears, Lowenstein- Jensen culture, antimicrobial test.
Anaerobic growth system: jars with Gas- Pak, liguid medium.
Evaluation of smears and anaerobic cultures: *Actinomyces, Clostridium*, *Propionibacterium*.
Biochemical test for identification of anaerobes- API
Evaluation of antibiogram results for anaerobes.
Making a culture from own fingerprint, surfaces of inanimate objects and control of air contamination in class room.

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1. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768.
2. Notes on Medical Microbiology – K.N. Ward, K.C. McCartney, B. Thakker, 2008, ISBN 9780443102844

Seminar 5. Rules of infection control program. Resistance mechanisms of microorganisms. Hygiene procedures –1h

Infections associated with dental clinic environment, cross- infections, hospital-acquired infections. Sources (endogenous, exogenous), reservoirs of infection, spread, route of infection (mode of entry). Endogenous and exogenous infections. Colonization, carrier- state, infection. Clinical forms of dental clinic- acquired and hospital-acquired infections. Surveillance and control of infections in ambulant (dental clinic) and in hospital conditions. Specificity of infection control in dentistry. Aetiological agents of dental clinic acquired infections: bacteria, viruses, fungi, parasites.
Characteristic of hospital pathogens- variability, antibiotic and disinfectant resistance- alert pathogens (MRSA, HLAR, ESBL, KPC, MBL).
Antibiotic policy and preventive measures of hospital infections (role of microbiological laboratory in control of infection).
Methods of effective elimination of microorganisms- sanitization (hand- washing), disinfection, sterilization (definition, classification, practical application)
Disinfection (classification): physical (thermal- pasteuristation, tyndallization, decoctation- boiling), UV irradiation, chemical (acids, alkali, aldehydes, chlorine or iodine, phenolic derivatives, detergents and soaps, oxidizing and heave metal compounds, dyes, others), gaseous (chambers with ethylene oxide and formaldehyde). The principles of disinfectans selection. Disinfectants and antiseptic agents with application in dentistry.

Sterilization (classification): high temperature (dry heat- hot-air oven, moist heat- autoclave; incineration; flaming – bacteriological loop), low- temperature (gas- ethylene oxide, formaldehyde; fumigation), chemical (disifectants- alcohols, aldehydes, halogens, potassium perborate), mechanical: (filtration), plasmic
Sterilization control: physical, chemical and biological indicators.
Control of air, surface and equipment contamination: sedimentation method, induced air method, environmental swabs.

Practical part 5:Rules of infection control program. Resistance mechanisms of microorganisms. Hygiene procedures-3h

Evaulation of antibiogram for alert- pathogens causing hospital infections.
Disinfection and sterilization in dental office in your country.
Demonstration of machines for sterilization.
Sterilization control- different indicators.
Examination of plates with action of UV and disinfecting agents.
The most used disinfectants- prospects.
Examination of own fingers, air and surfaces cultures.

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1. Murray P.R. Basic Medical Microbiology 2017, ISBN: 9780323476768.
2. Notes on Medical Microbiology – K.N. Ward, K.C. McCartney, B. Thakker, 2008, ISBN 9780443102844

Seminar 6: Basics of immune system function. Nonspecific immunity -1h

The lymphoid system: primary (central) and secondary (peripheral) lymphoid organs. Cells of the immune system and their functions: stem cells, B, T, NK lymphocytes, macrophages, granulocytes, dendritic cells, mast cells, platelets. Soluble mediators: complement, antibodies, cytokines, interferons, inflammatory mediators.
Immunity: innate and adaptive (acquired), active and passive, specific and nonspecific, natural and artificial, cellular and humoral. Immunity and immune response.
Nonspecific immunity: exterior defenses and physical and biochemical barriers, the role of normal flora, nonspecific factors humoral (complement, interferons, lysozyme, lactoferrin, C- reactive protein, heat shock proteins) and cellular (mononuclear and polymorphonuclear phagocytes, NK cells). Pathological barrier-inflammation.
Complement: classical and alternative pathways, biological effects (increase of vascular permeability, chemotaxis, neutrophils activation, opsonization, lysis). Complement receptors.
Phagocytosis: migration and chemotaxis of phagocytes, adhesins molecules (integrins, selectins), chemotactic factors (complement proteins, chemokines), phagocytes receptors, opsonization, ingestion, digestion (killing), oxygen- dependent and oxygen – independent microbicidal activity.
Natural cytotoxicity- NK cells- mechanisms of recognition and action (perforins).

Practical part 6: Methods of studying of nospecific immuno response – 3h

Testing of nonspecific immune system function (complement and phagocytosis)
Phagocytosis testing – phagocytosis (engulfment) index, killing index -NBT (nitrobluetetrazolium test)
Complement testing - measurement of components' concentration - C3, C4, C1q inhibitor (radial immunodiffusion assay), hemolytic activity (CH50 haemagglutination assay).

*Sources:*Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller. Medical Microbiology , 2015; ISBN: 0323299563

Seminar 7: Basics of immune system function. Specific immunity -3h

Antigens: types, immunogenicity, specificity.
Lymphocytes: subpopulation: B (B1,B2), T (Th1, Th2, Ts, Tc), NK, NC, CD markers, receptors for antigen (B-Ig, T- TCR), circulation of lymphocytes.
Specific response: processing and presentation of antigen, induction (recognition of antigen), central phase (activation, clonal selection and proliferation of T and B lymphocytes, interactions between cells of immune system), effector phase- types of immune response. Cooperation of specific humoral and cellular response. Primary and secondary immune response, immunological memory.

Antibodies: structure, types of immunoglobulins, biological functions, monoclonal antibodies.
Antigen- antibody interactions: in vivo- neutralization, immunological complexes, opsonization; in vitro- agglutination, precipitation, passive haemagglutination, complement fixation, direct and indirect immunofluorescence, ELISA, RIA, immunoblotting.

Practical part 6: Methods of studying of nospecific immuno response – 1h

Testing of specific immune system function (humoral and cellular immune response).
Specific humoral testing - antigen/antibodies detection (slide/tube agglutination assay, precipitation assay, complement- mediated lysis test), autoantibodies detection (IF, ELISA, immunoblotting), detection of specific CD markers of B lymphocytes (IF, flow cytometry)
Specific cellular response -blood separation of lymphocytes (cell density gradient), detection of specific CD markers and relevant cytokines of T lymphocytes (IF, flow cytometry).
Discussion about importance and application of different methods for studying of immune response

Seminar 8: Biological effects of immune response – immunodeficiencies, hypersensitivity, graft rejection-2h

Primary and secondary immunodeficiences, infections typical for different types of immunodeficiency.
Immunoproliferative disorders
Hypersensitivity (allergy): types of humoral and cellular reactions: anaphylaxis, cytotoxic and cytolytic reactions, immune- complex, DTH, contact hypersensitivity). Diseases associated with hypersensitivity reactions.
Regulation of immune response, autoimmunity and tolerance.
Transplantation immunology: transplantation antigens- HLA class I and II, the rules of transplantation of different tissues.
Sources: Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller. Medical Microbiology , 2015; ISBN: 0323299563

Practical part 8: Human Leucocyte Antigens (HLA) typing and matching – 2h

Lymphocytotoxic test (LCT)
HLA antigens class I and II typing: serological methods, molecular methods (PCR-SSP, PCR-SSO).
Immunological donor- recipient maching – rules, crosswords
Importance of HLA matching and cross-match in grafts rejection.

E-learning: Infection immunity, immunoprophylaxis, immunotherapy – 1h

Infection immunity: the role of particular mechanisms of specific and nonspecific defense in infections caused by: bacteria (immune response to extracellular and intracellular bacteria, bacterial evasion of host- defense mechanisms, toxins, fungi, viruses (viral neutralization by antibody, cell-mediated and humoral antivirial mechanisms), protozoa and worms.
Immunoprophylaxis: vaccination, types of vaccines, obligatory and recommended vaccinations
Immunotherapy: indications, non-specific vaccines, autovaccines, nonspecific immunomodulation.

Sources: Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller. Medical Microbiology , 2015; ISBN: 0323299563

Seminar 9: Make-ups and repetitions -1h