Course name …… Microbiology ……..

Faculty PharmD

Curriculum

I. General microbiology
1. Classification of microorganisms
2. Morphology of prokaryotic and eucaryotic cells
3. Microbial metabolism and growth.
4. Microbial genetics

II. Interaction between microbe and host
1. Principles of infectious disease and epidemiology
2. Microbial mechanisms of pathogenicity
3. Nonspecific and specific defences of the host
4. Introduction to immunology, vaccines, immune serum and immunoglobins

III. Pharmaceutical microbiology
1. Sterilisation, disinfection, antisepsis, aseptics
2. Antimicrobial drugs.
3. Microbiological control of pharmaceuticals, medical materials and their production process
4. Preservation of pharmaceutical products
5. Hospital infection

IV. Detailed microbiology
1. Classification and pathogenicity of chosen bacteria
2. Elements of mycology
3. Elements of virology

Course source materials

1. Hugo W.B. Russell AD. (Eds).: Pharmaceutical Microbiology, Blackwell Sci., London, 2001
2. Tartora G.J., Fanke B. R., Case C. L.: Microbiology: an introduction; Benjamin Cummings; San Francisco, 2001
3. Collins C.H.: Lyne P.M., Grange J.M.: Microbiological Methods, Butterworths, London 1989
4. Block S.S. (Ed.): Disinfection, Sterilization and Preservation. Lippincott Williams and Wilkins, Philadelphia, 1999
5. Benson H.J.: Microbiological Applications, WCB-MeGraw-Hill, Boston 1998
6. Ayliffe GA.J., Lowbury E.J.L. (Eds), Control of hospital infection. A practical handbook, Chapman and Hall Med., London 1996
7. Wenzel R.P. (Ed) Prevention and Control of Nosocomial Infections, Williams and Wilkins, Baltimore and London 1997

Detailed curriculum

Lectures – 15 hrs

I. General microbiology

1. Classification of microorganisms
   - Scientific nomenclature
   - The taxonomic hierarchy
2. Morphology of prokaryotic and eucaryotic cells
   - The size, shape and arrangement of bacterial cells
   - The procaryotic cell
   - Bacterial cell structures: slime and capsules, pili, fimbria, cell wall, cytoplasmic membrane, cytoplasm, mezosomes, ribosomes, cytoplasm inclusion bodies, nukleoid, extrachromosomal inheritance factors
   - The eukaryotic cell
3. Microbial metabolism and growth
   - Nutritious requirements
   - Environment influence on bacteria development: gaseous phase, oxidation-reduction potential, temperature, desiccation, osmotic pressure, surface tension, hydroxyl ions
   - Bacterial division
   - Generation time
   - Phases of growth
4. Microbial genetics
   - Bacterium genome
   - DNA replication
   - RNA and protein synthesis
   - Mutations
   - Extrachromosomal inheritance: plazmids, genetic translocational elements,

II. Interaction between microbe and host

1. Principles of infectious disease and epidemiology
   - Predisposing factors
   - Reservoirs of infection
   - The transmission of infection
   - Infectious disease; characteristics, registration, treatment
   - Introduction to epidemiology, epidemiologic investigation
2. Microbial mechanisms of pathogenicity
   - Pathogenic properties of microorganisms: adherence, extracellular factors of pathogenicity, components of the cell wall,
   - Production of enzymes and toxins

3. Nonspecific and specific defences of the host
   - Skin and mucous membrane
   - Phagocytosis
   - Inflammatory responses to infectious agents
   - Humoral immunity
   - Cell-mediated immunity
4. Introduction to immunology, vaccines, immune serum and immunoglobins
   - Antigens and antibodies
   - Vaccines
   - Vaccination timetable

III. Pharmaceutical microbiology

1. Sterilisation, disinfection, antisepsis, aseptics
   - Sterilization: sterilization by steam under pressure, dry heat sterilization, radiosterilization, gas sterilization, filtration, other methods of sterilization, sterilization process control
   - Disinfection and antisepsis: factors determining the effectiveness of disinfectants, major chemical groups of disinfectants; hand skin antisepsis, operating surface antysepsis, mucous membrane antisepsis, wound and burn antisepsis
   - Methods of evaluating the activity and effectiveness of disinfectants
   - Aseptics, HEPA filters
2. Antimicrobial drugs
   - A review of chemical groups of antibiotics: penicillin, betalactamase inhibitor combinations, cephalosporins, carbapenems, monobactams, macrolides, tetracyklines, aminoglycosides. glikopeptides, lincosamides, quinolones, sulphonamide, nitroimidazoles, nitrofurane, antituberculotic drugs, antimycotic drugs, antiviral chemotherapeutics
   - Means, extent and mechanisms of activity of antibiotics
   - Mechanisms of bacterial resistance to antibiotics: place of action modification, inactivation of antibiotics (modifying and degrading enzymes), distortion of permeability barriers, omission of a link blocked by an antibiotic
   - Clinical importance of resistance to antibiotics
3. Microbiological control of pharmaceuticals, medical materials and their production process
   - Microbiological quality of pharmaceutical preparations
   - Factory and hospital hygiene and good manufacturing practice (GMP)
4. Microbial spoilage and preservation of pharmaceutical products
5. Hospital infection
   - Criteria of defining and hospital infections definitions
   - Sources, reservoirs and ways of spreading hospital infections

- Etiological factors; microorganisms most frequently causing hospital infections;
- Clinical forms and frequency of occurrence of hospital infections
- Changes in the process of hospital infections: infection profile, resistence phenotypes to antibacterial factors
- Risk factors: dependant on the microorganism, on the patient, connected with procedures and treatment
- Preventive measures and action against hospital infections- the role of the pharmaceutical chemist

IV. Detailed microbiology

1. Classification and pathogenicity of chosen bacteria
   - Gram-positive cocci: *Staphylococcus, Streptococcus, Enterococcus*
   - The family *Enterobacteriaceae*
   - Non-fermentative gram-negative rods: *Pseudomonas, Acinetobacter, Stenotrophomonas, Burkholderia,*)
   - *Vibrio*
   - *Aeromonas*
   - *Campylobacter, Helicobacter,*
   - *Francisella, Brucella, Bordetella,*
   - *Gardnella, Legionella, Haemophilus*
   - Gram-positive bacilli: *Bacillus, Corynebacterium, Listeria*
   - Anaerobic bacteria
   - *Treponema, Borrelia, Leptospira*
   - *Rickettsia, Chlamydia, Mycoplasmas*
   - *Mycobacteria*
   - *Spirochetes*
2. Elements of mycology
   - Fungi characteristics; cell cytology; reproduction
   - Taxonomy of medically important fungi
   - Fungal diseases: systemic mycoses (systemic mycoses due to primary pathogens, systemic mycoses due to opportunistic pathogens), subcutaneous mycoses, skin mycosis (superficial fungal infections, dermatophytosis)
   - Some pathogenic fungi: *Candida albicans, Cryptococcus neoformans, Torulopsis, Pityrosporum, Blastomyces, Coccidiodes immitis, Histoplasma capsulatum, Sporotrichum schoenckii, Geotrichum candidum, Aspergillus, Dermatophytes*
3. Elements of virology
   - Classification of DNA and RNA viruses; RNA viruses (Pikornaviridae, Orthomyxoviridae, Paramyxoviridae, RSV, Flaviridae, Togaviridae, Retroviridae and others); DNA viruses (Herpesviridae, Adenoviridae, Poxviridae, Papavaviridae, Parvoviridae); hepatitis viruses

Classes – 75 hrs

I. Microscopic observation of microorganisms
Staining bacteria (part I)

1. Rules and regulations
2. Safety in the microbiological laboratory
3. Microscopes
4. Microscopic examination of stained cell preparation
5. Microscopic examination of living bacterial preparations
6. Preparation of bacterial smears
7. Morphological stain: simple stain, negative stain

II. Staining bacteria (part II)

1. Differential stain
    - Gram stain
    - acid-fast staining – Ziehl-Neelsen method

2. Staining for visualisation of bacterial cell structures:
    - spore stain – Schaeffer-Fulton method
    - capsule stain
    - metachromatic granules – Loeffler method

3. Preparation of a smear and Gram stain of mixed bacteria

III. Growth of microorganisms: nutritional requirements, influence of environment factors

1. The requirements for growth
    - chemical requirements (macro and microelements, oxygen)
    - physical requirements (temperature, pH)

2. Culture media

3. Biochemical activities of microorganisms
    - carbohydrate fermentation (oxidation – fermentation test)
    - tryptophan hydrolysis (indole production)

- urea hydrolysis
- hydrogen sulfide production
- citrate utilization

4. Cultural characteristics of microorganisms Nutrient agar plate culture:
    - nutrient agar plate culture - colony morphology (size, surface, texture, color, elevation, margin)
    - nutrient broth culture

5. Isolation of pure cultures by the streak-plate method

IV. Enumeration of microbial populations

1. Enumeration the total number of microbial cells (living and dead bacteria)

2. Methods of enumeration living microbial cells – viable count
    - pour plate method
    - surface spread method
    - membrane filter method
    - most probable number (MPN)

3. Indirect viable counts – rapid methods

4. Quantitation of microorganisms in urine specimen - the application of measuring bacterial population in clinical microbiology
    - calibrated loop method
    - dipslide method

5. Expression of results according to the European Standard ISO 7218

V. Sterilization , disinfection, antisepsis

1. Sterilization methods
    - steam sterilization (autoclave)
    - dry heat
    - ionizing radiation
    - gas sterilization
    - plasma sterilization
    - filtration
    - chemical sterilization

2. Sterilization control
    - physical indicators
    - chemical indicators
    - biological indicators

3. Disinfection and antisepsis
    - methods of disinfection
    - evaluation of chemical disinfectants and antiseptics

VI. Antibiotics and antimicrobial susceptibility testing

1. Chemistry, mode of action of antimicrobial agents

2. The major groups of antimicrobial agents

3. In vitro antimicrobial susceptibility testing
    - qualitative susceptibility tests:
        - disk diffusion tests
    - quantitative dilution susceptibility tests :
        - agar dilution MIC tests;
        - macrobroth dilution MIC tests;
        - microbroth dilution MIC tests;
        - tests for bactericidal activity – MBC test
    - quantitative diffusion susceptibility tests – E-test

4. Automated antimicrobial susceptibility tests

VII. Detecting of selected mechanisms of bacterial resistance

1. The major mechanisms of bacterial resistance to antimicrobial agents

2. Tests for detecting of selected mechanisms of bacterial resistance
    - modification of target site: the appearance of a new PBP - methicillin-resistant staphylococci (MRSA, MRSE, MRCNS)
    - betalactameses: staphylococcal penicillinases, extended spectrum batalactamases of gram-negative roods

VIII. The basics of identification of Gram-positive cocci

1. Differentation of Staphylococi from Micrococci
    - catalase test

- oxidation- fermentation test
- furazolidone test

2. Tests for differentiation of Staphylococcal species
    - mannitol salt agar: growth, fermentation
    - Baird- Parker agar: growth, pigmentation, precipitation
    - coagulase test
    - haemolysis
    - novobiocin test

3. Test for differentiation of Streptococci from Enterococci
    - haemolysis
    - bacitracin test
    - optochin test
    - bile esculin hydrolysis
    - salt tolerance (6,5% NaCI)

4. Commercial biochemical identification systems
    - nomautomated system API Staph

IX. The basics of identification of Enterobacteriaceae

1. Growth requirements and cultural characteristics
    - media: TSA, Mac Concey's, SS

2. Biochemical identyfication
    - catalase production (catalase test)
    - oxidation-fermentation test (Hugh-Leifson medium)
    - reduction of nitrate to nitrite or nitrogen gas (Nitrate medium)
    - oxidase production (oxidase test)
    - H_2S, gas production (Kligler medium)
    - Urease production (urea broth / Christinsen medium)
    - lactose fermentation (10% lactose broth)
    - indole production (indole broth)
    - production of acetoin, methyl red test (Methyl Red-Voges Proskauer medium)

3. Commercial biochemical identification systems:
    - nonautomated systems - API 20E test
    - semiautomated systems - ATB
    - automated systems - VITEK

X. The basics of identification of nonfermentative Gram-negative roods and anaerobic bacteria

1. Growth requirements and cultural characteristic
    - media: TSA, Mac Conkey agar

2. Biochemical identifications
    - catalase test
    - oxidation- fermentation test Hugh- Leifson medium)
    - oxidase test

3. Commercial biochemical identification systems
    - nonautometed systems: API 20 NE, API 20 E

4. Cultivation of anaerobes
    - media: fluid thioglycollate
    - anaerobic system: Genbag anaer

XI. The basics of identification of fungi

1. Characteristics of fungi
2. Vegetative structures of fungi
3. Reproductive structures of fungi
4. General approaches to the isolation and identification of clinically significant fungi
    - specimen collection and transport
    - direct microscopic examination of clinical specimens
    - culture procedures
    - identification of fungi
    - observation of colonial morphology
    - microscopic Examination
    - physiologic tests: germ tube test, chlamydospore production test, assimilation test, fermentation test
    - commercial systems

XII. Microbiological examination of pharmaceutical products

1. Microbiological quality of pharmaceutical preparation

2. Microbiological examination of sterile products (sterility)

3. Microbiological examination of non-sterile products
   - total viable aerobic count (membrane filtration, plate-count methods, most probable number method)
   - test for specified microorganisms (Enterobacteria and certain other gram-negative bacteria, Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus)

XIII. Microbiological evaluation of clean rooms and other controlled environment

1. Manufacture of sterile and nonsterile products
   - clean areas for the manufacture of sterile products
   - personnel

2. Methodology and instrumentation for quantitative estimation of viable airborne microorganisms
   - settling plates method
   - quantitative estimation of the microbial contamination

3. Methodology and equipment for sampling of surfaces for quantitative estimation of viable microbial contaminants in controlled environments
   - contact plates or RODAC (Replicate Organism Detection and Counting)
   - the swabbing method

4. Quantitative estimation of viable microbial contaminants on hands