

Revised: Fall 2016

MDL 237 Clinical Bacteriology

COURSE OUTLINE

Prerequisites:

BIO 101 or equivalent

Course Description:

This course will introduce the student to the basic concepts of clinical microbiology with an emphasis on bacterial identification of clinically significant pathogenic bacteria and fungi. Topics covered include biochemical identification and molecular biology identification techniques of pathogenic bacteria. Proper specimen collection and plating of bacteria from human body sites will be emphasized also. Susceptibility testing and antibiotic resistance will also be discussed.

Semester Credits: 4

Lecture Hours: 2

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MDL 237 Clinical Bacteriology

Course Outcomes:

At the completion of this course, the student should be able to:

- Perform a gram stain of samples containing bacteria to aid in a presumptive identification
- Plate biological samples containing bacteria onto the proper media and be able to identify the purpose of various selective media
- Identify bacteria by their colony characteristics and growth on selective media
- Identify bacteria by using biochemical testing methods and molecular testing methods such as PCR and DNA fingerprinting
- Understand the mechanism of action of common antibiotics and the basis of antibiotic resistance
- Describe and differentiate the characteristics of the various species of pathogenic bacteria including the staphylococci, streptococci, and enterococci, and be able to differentiate one species from another such as *Staphylococcus aureus* and *epidermidis*
- Learn proper isolation techniques for aerobic vs. anaerobic and facultative anaerobic bacteria
- Isolate and successfully identify pathogenic fungi and various non-bacterial pathogens such as *Mycoplasma* and *Ureaplasma*
- Understand the theory behind automated techniques of bacterial identification

Internet access required

Textbook: Textbook of Diagnostic Microbiology 5th edition by C.R. Mahon, et al.
ISBN: 978-0-323-08989-0

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Course Outline

I. Bacterial Cell Structure, Physiology, Metabolism, and Genetics

- A. Significance
- B. Overview of the Microbial World
- C. Classification/Taxonomy
 - Nomenclature
 - Classification by Phenotypic and Genotypic Characteristics
 - Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria
- D. Comparison of Prokaryotic and Eukaryotic Cell Structure
- E. Bacterial Morphology
 - Microscopic Shapes
 - Common Stains Used for Microscopic Visualization
- F. Microbial Growth and Nutrition
 - Environmental Factors Influencing Growth
 - Bacterial Growth
 - Fermentation and Respiration
 - Biochemical Pathways from Glucose to Pyruvic Acid
 - Anaerobic Utilization of Pyruvic Acid (Fermentation)
 - Aerobic Utilization of Pyruvate (Oxidation)
 - Carbohydrate Utilization and Lactose Fermentation
- G. Bacterial Genetics
 - Genetic Elements and Alterations
 - Mechanisms of Gene Transfer

II. The Laboratory Role in Infection Control

- A. General Concepts in Infection Prevention and Control Practice
 - Frequently Identified Microbes
 - Outbreak Investigation
 - Steps of an Outbreak Investigation
 - Investigation Support from the Laboratory
 - Environmental Culturing
 - Infection Prevention and Control Education
- B. Emerging and Reemerging Pathogens
 - Examples of Emerging and Reemerging Pathogens
 - Response Plans



III. Performance Improvement in the Microbiology Laboratory

A. General Guidelines for Establishing Quality Control

- Temperature
- Thermometer Calibration
- Media Quality Control
- Reagent Quality Control
- Antimicrobial Susceptibility Control
- Personnel Competency
- Use of Stock Cultures
- Quality Control Manual

B. Performance Improvement

- Vision and Mission Statements
- Indicators of Performance Improvement: Process vs. Outcome
- Establishing Performance Monitors
- Problem-Action Form
- Fixing the Process
- Benchmarking
- Commercially Purchased Monitors

C. Analytic Analysis of Tests

- Analytic Sensitivity and Specificity
- Accuracy

D. Clinical Analysis of Tests

- Clinical or Diagnostic Sensitivity

E. Operational Analysis of Tests

- Predictive Values of Tests
- Efficiency of Tests

F. Choosing a Laboratory Method

G. Test Validation

IV. Specimen Collection and Processing

A. Basic Principles of Specimen Collection

- Collection Procedures
- Patient-Collected Specimens
- Labeling and Requisitions
- Safety

B. Preservation, Storage and Transport of Specimens

- Specimen Storage
- Preservatives and Anticoagulants
- Holding of Transport Media
- Shipping Infectious Substances



C. Specimen Receipt and Processing

- Specimen Priority
- Rejection of Unacceptable Specimens
- Macroscopic and Microscopic Evaluation
- Primary Inoculation
- Specimen Preparation
- Isolation Techniques
- Incubation

D. Culture Workup

- Nonroutine Specimens

E. Communication of Laboratory Findings

V. Microscopic Examination of Materials from Infected Sites

A. Preparation of Samples

- Smears from Swabs
- Smears from Thick Liquids or Semisolids
- Smears from Thick, Granular or Mucoïd Materials
- Smears from Thin Fluids
- Cytocentrifuge Preparations

B. Stains

C. Microscopes

D. Examination of Prepared Material

- Characterization of Background Materials
- Search for Microorganisms
- Evaluation of Choice of Antibiotic
- Initiation of Special Handling for Unsuspected or Special Pathogens

E. Grading or Classifying Materials

- Contaminating Materials
- Local Materials
- Purulence
- Mixed Materials

F. Reports of Direct Examinations

G. Examples of Sample Observations and Reports

- Quality Control in Direct Microscopic Interpretation



VI. Use of Colony Morphology for the Presumptive Identification of Microorganisms

- A. Importance of Colonial Morphology as a Diagnostic Tool
- B. Initial Observation and Interpretation of Cultures
- C. Gross Colony Characteristics Used to Differentiate and Identify Microorganisms
 - Hemolysis
 - Size
 - Form or Margin
 - Elevation
 - Density Color
 - Consistency
 - Pigment
 - Odor
- D. Colonies with Multiple characteristics
- E. Growth of Organisms in Liquid Media

VII. Biochemical Identification of Gram Negative Bacteria

- A. Carbohydrate Utilization
 - Oxidation –Fermentation Tests
 - Triple Sugar Iron Agar
 - Ortho-Nitrophenyl- β -D-Galactopyranoside Test
- B. Glucose Metabolism and Its Metabolic Products
 - Methyl Red Test
 - Voges-Proskauer Test
- C. Amino Acid Utilization
 - Decarboxylase and Dihydrolase Tests
 - Deaminase Test
 - D. Miscellaneous Tests
 - Citrate Utilization
 - DNase
 - Gelatin Liquefaction
 - Indole Production
 - Malonate Utilization
 - Motility
 - Nitrate and Nitrate reduction
 - Oxidase
 - Urease
 - Lysine Iron Agar Slant
 - Motility-Indole-Ornithine Agar
 - Sulfide-Indole-Motility Agar



D. Manual Multitest Systems

- Principles of Identification
- Analytical Profile Index

E. Rapid and Automated Identification Systems

- Rapid Biochemical Tests Performed on Isolated Colonies
- Identification Systems Relying on Carbohydrate Utilization or Chromogenic Substrates
- Automated Identification Systems
- Evaluation of Identification Systems

VIII. Applications of Molecular Diagnostics

A. Nucleic Acid Hybridization Techniques

- Hybridization Reaction Variables
- Hybridization Formats
- Applications of Nucleic Acid Hybridization Techniques

B. Nucleic Acid Amplification Procedures

- Polymerase Chain Reaction
- Other Nucleic Acid Amplification Reactions

C. Strain Typing and Identification

- Nonamplified Typing Methods
- Amplified Typing Methods

D. Future of Molecular Diagnostics Testing in the Clinical Microbiology Laboratory

- Sequencing
- Pyrosequencing
- DNA Microarrays and Nanoarrays
- Proteomics
- MALDI-TOF Mass Spectrometry

E. Nanomedicine

IX. Antimicrobial Agent Mechanisms of Action and Resistance

A. Antibiotic Targets and Mechanisms of Action

- Inhibition of Bacterial Cell Wall Biosynthesis
- Inhibition of Folate Synthesis
- Interference with DNA Replication
- Interference with DNA Transcription
- Interference with mRNA Translation
- Origins of Antibiotic Resistance
- Acquired Mechanisms of Resistance

B. Dissemination

C. Nanotechnology to Deliver Therapeutic Agents



X. Antimicrobial Susceptibility Testing

- A. Reasons and Indications for Performing Antimicrobial Susceptibility Tests
 - Factors to Consider When Determining Whether Testing is Warranted
- B. Selecting Antimicrobial Agents for Testing and Reporting
 - Selection of Test Batteries
 - Reporting of Susceptibility Test Results
- C. Traditional Antimicrobial Susceptibility Testing
 - Inoculum Preparation and Use of McFarland Standards
 - Dilution Susceptibility Testing Methods
 - Disk Diffusion Testing
 - Modified Methods for Testing Slow-Growing or Fastidious Bacteria
 - Additional Organism and Antimicrobial Agent Testing Concerns
- D. Automated Antimicrobial Susceptibility Test Methods
 - Principles of Technologies Used
 - Currently Available Automated Systems
 - Nonautomated Antimicrobial Susceptibility Test Methods: Etest
- E. Interpretation of In Vitro Antimicrobial Susceptibility Test Results
- F. Methods of Detecting Antimicrobial-Inactivating Enzymes
 - β -Lactamase Tests
- G. Quality Control of Antimicrobial Susceptibility Tests
- H. Selecting an Antimicrobial Susceptibility Test Method
- I. Susceptibility Testing Challenges
 - Rapid Susceptibility Determination
- J. Special Antimicrobial Susceptibility Tests
 - Minimum Bactericidal Concentration Test
 - Controlling Test Variables
 - Time-Kill Assays
 - Synergy Tests
 - Serum Bactericidal Test
 - Molecular Probes for Identifying Determinants of Antimicrobial Resistance
 - Measurement of Antimicrobial Agents in Serum and Body Fluids

XI. The Staphylococci

- A. General Characteristics
- B. Clinically Significant Species
 - *Staphylococcus epidermidis*
 - *Staphylococcus saprophyticus*
 - *Staphylococcus lugdunensis*
 - Other Coagulase Negative Staphylococcus



- C. Laboratory Diagnosis
 - Specimen Collection and Handling
 - Microscopic Examination
 - Isolation and Identification
 - Rapid Methods of Identification
- D. Antimicrobial Susceptibility
 - Methicillin-Resistant Staphylococci
 - Vancomycin-Resistant Staphylococci
 - Macrolide Resistance

XII. *Streptococcus*, *Enterococcus*, and Other Catalase-Negative Gram-Positive Cocci

A. General Characteristics

Cell Wall Structure

Hemolysis

B. Clinically significant Streptococci and Streptococcus-like Organisms

- *Streptococcus pyogenes*
- *Streptococcus agalactiae*
- Groups C and G Streptococci
- *Streptococcus pneumoniae*
- Viridans Streptococci
- Streptococcus-like Organisms

C. Laboratory Diagnosis

- Classification Schemes

D. Noncultural Identification

E. Susceptibility Testing

XIII. Aerobic Gram-Positive Bacilli

A. Non-Spore-Forming, Nonbranching Catalase Positive Bacilli

- *Corynebacterium*
- *Rothia*
- Related Genera and Undesignated CDC *Corynebacterium* Groups

B. Non-Spore-Forming, Nonbranching Catalase Negative Bacilli

- *Erysipelothrix rhusiopathiae*
- *Arcanobacterium*
- *Gardinerella vaginalis*

C. Non-Spore-Forming, Branching Aerobic Actinomycetes

- *Nocardia*
- Other Actinomycetes



D. Spore-Forming, Nonbranching Catalase-Positive Bacilli

- *Bacillus anthracis*
- *Bacillus cereus*
- Other *Bacillus* species

XIV. *Neisseria* Species and *Moraxella catarrhalis*

A. Pathogenic *Neisseria* Species

- Virulence Factors
- *Neisseria gonorrhoeae*
- *Neisseria meningitides*

B. Commensal *Neisseria* Species

- *Neisseria cinerea*
- *Neisseria flavescens*
- *Neisseria lactamica*
- *Neisseria mucosa*
- *Neisseria polysaccharea*
- *Neisseria sicca*
- *Neisseria subflava*
- *Neisseria elongata*
- *Neisseria weaver*

XV. *Haemophilus* and Other Fastidious Gram-Negative Bacilli

A. *Haemophilus*

General Characteristics

Haemophilus influenzae

Infections Associated with Other *Haemophilus* Species

Laboratory Diagnosis

B. HACEK Group

Aggregatibacter aphrophilus

Aggregatibacter actinomycetemcomitans

Cardiobacterium hominus

Eikenella corrodens

Kingella

C. *Capnocytophaga*

D. *Pasteurella*

E. *Brucella*

F. *Franciella*



Legionella

A. Clinical Significance

Virulence Factors

Infections Caused by *Legionella*

Epidemiology

B. Laboratory Diagnosis

Specimen Collection and Handling

Microscopic Examination

Isolation and Identification

Serologic Testing

Bordetella

A. Clinically Significant Species

- *Bordetella pertussis*
- *Bordetella parapertussis*
- Miscellaneous species

B. Laboratory Diagnosis

- Specimen Collection and Handling
- Nucleic Acid Detection
- Microscopic Examination
- Isolation and Identification
- Serologic Testing

C. Antimicrobial Susceptibility

XVI. Enterobacteriaceae

A. General Characteristics

Microscopic and Colony Morphology

Classification

Virulence and Antigenic Factors

Clinical Significance

B. Opportunistic Members of the Family Enterobacteriaceae and Associated Infections

- *Escherichia coli*
- *Klebsiella* and *Raoutella*
- *Enterobacter*, *Cronobacter*, and *Pantoea*
- *Serratia*
- *Proteus*
- *Morganella*
- *Providencia*
- *Edwardsiella*

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- *Erwinia and Pectobacterium*
- *Citrobacter*
- C. Primary Intestinal Pathogens of the Family Enterobacteriaceae
 - *Salmonella*
 - *Shigella*
 - *Yersinia*
- D. Other Genera of the Family Enterobacteriaceae
 - *Budivicia*
 - *Buttiauxella*
 - *Cedecea*
 - *Ewingella*
 - *Kluyvera*
 - *Leclercia*
 - *Leminorella*
 - *Moellerella*
 - *Obesumbacterium*
 - *Photorhabdus*
 - *Rahnella*
 - *Tatumella*
 - *Trabulsiella*
 - *Yokenella*
- E. Laboratory Diagnosis of Enterobacteriaceae
 - Specimen Collection and Transport
 - Isolation and Identification
 - Screening Stool Cultures for Pathogens
 - Serologic Grouping

XVII. *Vibrio, Aeromonas, Plesiomonas, and Campylobacter* Species

A. *Vibrio*

- General Characteristics
- *Vibrio cholera*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Vibrio alginolyticus*

Laboratory Diagnosis

B. *Aeromonas*

- General Characteristics
- Clinical Manifestations
- Laboratory Diagnosis
- Antimicrobial Susceptibility



C. *Plesiomonas*

- Epidemiology
- Clinical Manifestations
- General characteristics
- Laboratory Diagnosis
- Antimicrobial Susceptibility

D. *Campylobacter* and *Campylobacter*-Like Species

- Epidemiology
- Clinical Manifestations
- Laboratory Diagnosis
- Antimicrobial Susceptibility

XVIII. Nonfermenting and Miscellaneous Gram-Negative Bacilli

A. General Characteristics of Nonfermenters

- Clinical Infections
- Biochemical Characteristics and Identification
- *Pseudomonas* Fluorescent Group
- *Acinetobacter*
- *Stenotrophomonas maltophilia*
- *Burkholderia*
- *Moraxella*, *Oligella*, and *Psychrobacter*

B. Less Commonly Encountered Nonfermentative, Gram-Negative Bacilli

- *Alcaligenes* and *Achromobacter*
- *Brevundimonas*
- CDC Groups EO-3, EO-4, and *Paracoccus*
- *Chromobacterium*
- *Comamonas* and *Delftia*
- *Flavobacteriaceae*
- *Methylobacterium* and *Roseomonas*
- *Ralstonia* and *Cupriavidus*
- *Shewanella*
- *Sphingomonas*

XIX. Anaerobes of Clinical Importance

A. Important Concepts in Anaerobic Bacteriology

- Anaerobes Defined
- Why Some Organisms Are Anaerobes
- Where Anaerobes Are Found
- Anaerobes at Specific Anatomic Sites
- Factors That Predispose Patients to Anaerobic Infections



- Indications of Anaerobe Involvement in Human Disease
- B. Frequently Encountered Anaerobes and Their Associated Diseases
 - Gram-Positive Spore-Forming Anaerobic Bacilli
 - Gram-Positive Non-Spore Forming Bacteria
 - Anaerobic Gram-Negative Bacilli
- C. Specimen Selection, Collection, Transport, and Processing
 - Specimen Quality
 - Processing Clinical Samples for Recovery of Anaerobic Pathogens
- D. Procedures for Identifying Anaerobic Isolates
 - Preliminary Procedures
 - Indications of the Presence of Anaerobes in Cultures
 - Presumptive Identification of Clinically Significant Anaerobes
 - Definitive Identification of Anaerobic Isolates
 - Identification of Clostridium Species
 - Identification of Anaerobic Non-Spore-Forming, Gram-Positive Bacilli
 - Identification of Anaerobic Gram-Negative Bacilli
 - Identification of Anaerobic Cocci
- E. Antimicrobial Susceptibility Testing
 - Problems in Susceptibility Testing of Anaerobic Isolates
- F. Treatment of Anaerobe-Associated Diseases
 - Surgical Therapy
 - Hyperbaric Oxygen
 - Antimicrobial Therapy
 - Antitoxins

XX. The Spirochetes

- A. Leptospire
 - General Characteristics
 - Virulence Factors and Pathogenicity
 - Infections Caused by Leptospire
 - Epidemiology
 - Laboratory Diagnosis
 - Antimicrobial Susceptibility
 - Clinically Significant Species
 - *Borrelia recurrentis* and Similar *Borreliae*
 - *Borrelia burgdorferi*



B. Treponemes

- General Characteristics
- Clinically Significant Species
- *Treponema pallidum* Subsp. *pallidum*
- Other Treponemal Diseases

XXI. *Mycoplasma* and *Ureaplasma*

A. General Characteristics

B. Clinical Infections

- *Mycoplasma pneumoniae*
- *Mycoplasma hominus* and *Ureaplasma* Species

C. Laboratory Diagnosis

- Specimen Collection and Transport
- Direct Examination
- Culture

D. Antimicrobial Susceptibility

E. Interpretation of Laboratory Results

XXII. *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria

A. Clinical Significance of the *Mycobacterium tuberculosis* Complex

- *Mycobacterium tuberculosis*
- *Mycobacterium bovis*
- Rapidly Growing Species

B. *Mycobacterium leprae*

C. Isolation and Identification of the Mycobacteria

- Laboratory Safety Considerations
- Specimen Collection
- Digestion and Decontamination of Specimens
- Staining for Acid-Fast Bacilli
- Culture Media and Isolation Methods
- Laboratory Identification

D. Susceptibility Testing of *Mycobacterium tuberculosis*

E. Immunodiagnosis of *Mycobacterium tuberculosis* Infection

- Skin Testing
- Serology



XXIII. Medically Significant Fungi

A. General Characteristics

- Yeast vs. Molds
- Hyaline vs. Phaeoid
- Dimorphism and Polymorphism
- Reproduction

B. Taxonomy

- Mucorales
- Ascomycota
- Basidiomycota
- Fungi Imperfecti

C. Mycoses

- Superficial mycosis
- Cutaneous mycosis
- Subcutaneous mycosis
- Systemic Mycosis

D. Clinically Significant Species

- Agents of Superficial Mycosis
- Agents of Cutaneous Mycosis
- Agents of Subcutaneous Mycosis
- Agents of Systemic Mycosis
- Agents of Opportunistic Mycosis
- Agents of Yeast Infections
- Pneumocystis Infection

E. Laboratory Diagnosis of Fungi

- Specimen Collection, Handling, and Transport
- Direct Microscopic Examination of Specimens
- Isolation Methods
- Identification of Fungi

F. Immunodiagnosis of Fungal Infections

G. Antifungal Susceptibility

- Antifungal Agents
- Antifungal Susceptibility Testing



Laboratory Topics

1. Laboratory Safety, Equipment, and Materials; Basic Techniques
2. Survey of the Microbial World
3. Staining Techniques
4. Media: Enriched, Differential and Selective
5. Growth
6. Control of Microbial Growth
7. DNA Fingerprinting
8. Microbes and Disease
9. Diagnostic Immunology
10. Identifying Microbes
11. Hydrolytic Enzymes
12. Biochemical Testing
13. Identification of Unknown Bacteria by Biochemical and Molecular Methods

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