Introduction to Microbiology
Objectives

• Describe role of the laboratory in infection prevention
• Describe basic laboratory test for infectious pathogens
• Discuss common HAI pathogens
Microbiology and Infection Prevention

Microbiology has two important functions related to the prevention and control of infections:

• **Clinical**: identify pathogens and their susceptibility to treatment

• **Epidemiological**: identify pathogens causing disease or outbreak in a population and potential sources for these pathogens
Assessing Accuracy of Lab Results

- No lab test is 100% accurate 100% of the time
- Many factors can affect accuracy of laboratory tests
  1. **Pre-testing**: specimen collection, handling, transportation, and preservation prior to arrival in the lab
  2. **During testing**: specimen processing, skill of the laboratory technician, accuracy of biochemicals and instrument system
  3. **Post-testing**: Accuracy of result transcription, results communicated accurately
Interpreting Microbiology Test Results

• Presence of an organism does not mean it is causing disease
  • For sterile body sites, bacterial growth may confirm an infection
• Interpret all cultures in the context of what pathogens are normally found in that body site
• Contamination of samples can result in inaccurate results and pseudo-outbreaks
• To interpret microbiology test results, use in conjunction with blood cell counts
Complete Blood Cell Count (CBC)

• Blood test used to evaluate overall health
  • Including the detection or absence of infection
• Measures blood components
  • Including white blood cells (WBC)
White Blood Cell (WBC) Types

• **Polymorphonuclear leukocytes** (PMN): provide general response to threat
  • **Neutrophils** (50-60% of WBC); the first line of response to infection; also be called ‘segs’
  • Eosinophils (1-7% of WBC); seen with allergic reactions and parasites
  • Basophils (<1% of WBC); seen with allergic reactions, help mediate strength of immune response
• Left shift = presence of **immature neutrophils** (called ‘bands’ or ‘stabs’) indicating acute infection or inflammatory process
White Blood Cell (WBC) Types - 2

• **Lymphocytes** mature in the lymphatic portion of the immune system
  • Include pathogen-specific immune response (B cells, T cells)
  • Increase may be indicative of viral infection

• **Monocytes** (or macrophages) have phagocytic function and eat cellular debris and foreign pathogens in the immune system
Serology

• Diagnostic test that identifies immunoglobulins (antibodies) in blood serum
• Immunoglobulins (Ig) are proteins that bind to viruses and bacteria
• Types
  • IgM: produced immediately after exposure (acute phase of disease)
  • IgG: most abundant; long term response to disease (chronic disease)
  • IgA: secretory, present in mucosal linings
  • IgE: plays a role in hypersensitivity reactions
Gram Stain

• Microbiology lab method of classifying bacteria into 2 large groups: positive (+) and negative (-)
• Differentiates bacteria by the chemical and physical properties of their cell walls
• Helpful in guiding initial empiric therapy
Gram Stain Identifies Four Basic Bacteria Groups

- **Gram Negative (Pink)**
  - **Gram Negative Bacilli**: Pseudomonas, Escherichia coli, Haemophilus, Bacteroides
  - **Gram Negative Cocci**: Neisseria, Moraxella

- **Gram Positive (Blue)**
  - **Gram Positive Bacilli**: Clostridium, Listeria, Corynebacterium
  - **Gram Positive Cocci**: Staphylococcus, Streptococcus, Enterococcus
Common Lower Respiratory Tract Pathogens

- Community-acquired pneumonia (CAP)
  - *S. pneumoniae, H. influenzae, Mycoplasma*
- Hospital-associated pneumonia; most often ICU or ventilator-associated
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*
- CAP or hospital-associated pneumonia
  - *Staphylococcus aureus* (MRSA or MSSA)
  - *Moraxella catarrhalis* (most often CAP)
Testing for Lower Respiratory Bacterial Pathogens

- Sputum and bronchial wash are often contaminated with oral flora
- Tracheal aspirates and protected brush specimens not contaminated with oral flora
Interpreting Results from Sputum Specimens

• Results are affected by **quality of sputum** specimen
  • Squamous epithelial cells (SEC) shed from the lining of the mouth and pharynx; presence indicates saliva and oral flora
    - <10 - excellent specimen, no appreciable contamination
    - 10-25 - equivocal but acceptable
    - >25 - reject due to unacceptable levels of oral contamination
  • Assess number of WBC
    - < 10 - no infection or poor immune response
    - 10-25 - equivocal
    - >25 - purulence indicates presence of infection
Cerebrospinal Fluid (CSF) Pathogens

• Meningitis often from viruses or upper respiratory flora
• Meningitis due to gram-negative rods or staphylococcus usually associated with predisposing factors such as trauma
• Most common meningitis in an adult, *Streptococcus pneumoniae* (gram-positive cocci in pairs)
  • Generates increased WBC response
• Meningococcus (gram-negative cocci in pairs) is diagnostic of Neisseria
  • A single case is a true infection emergency
Meningitis

Onset of Symptoms

Patient presents for medical evaluation
Lumbar Puncture (LP)

Bacterial
- CSF cloudy
- elevated protein
- decreased glucose
- WBC; positive neutrophils
- organisms on gram stain

Viral (aseptic)
- CSF clear
- normal or elevated protein
- normal glucose
- no organisms on gram stain
Blood Cultures

- A single blood culture specimen is collected in two bottles
  - Bottles are designed to recover either aerobes or anaerobes
  - Growth may occur in one or both bottles
- In adults, low numbers of bacteria in blood ($\leq 30$/mL) can lead to negative-gram staining and false negatives
- Collecting the appropriate volume of blood (40cc blood for 4 bottles) is important
- Poor specimen collection technique can introduce contaminants to the specimen which are often common skin commensal flora
Common Urinary Tract Infection (UTI) Pathogens

- **Gram-negative organisms:**
  - *E. coli*: Causes 80% of all UTI
  - Proteus, Klebsiella, Enterobacter, Pseudomonas, and Gardnerella

- **Gram-positive organisms:**
  - Staph, Enterococcus, *Staphylococcus saprophyticus*
Urinalysis (UA)

- Positive leukocyte esterase or nitrite found on a UA can be helpful in determining presence of WBC
- Increased WBC in urine with negative cultures may indicate infection with chlamydia or gonorrhea
Common Pathogens of Deep Incisional and Organ/Space SSI

- **Anaerobic** – do not require oxygen to grow
  - *B. fragilis*
  - Clostridium
  - Peptostreptococcus
  - Propionibacterium (septic arthritis, endocarditis, suture sites for craniotomy)

- **Aerobic**
  - Staphylococcus
  - Streptococcus
  - Gram-negative rods (GNR)
Common Bowel Flora

• A normal mix of bacterial flora maintain gut health
• With altered conditions, yeast, *C. difficile*, pseudomonas species, VRE, and others can pathogenically dominate the flora
• Enterobacter, Enterococcus, Proteus, Morganella, Peptostreptococcus, Bacteroides, Clostridium and Bifidobacterium species constitute 95-99% of the more than 400 species in the bowel
Antibiotic Resistance (AR)

- AR emerges when some or all of a species or subspecies of bacteria survive exposure to an antibiotic
  - Can be intrinsic or transferred
  - Multi-drug resistance organisms (MDRO) are resistant to multiple antibiotic agents
- An antibiogram shows the proportion of bacteria resistant to specific antibiotics in a hospital or region
  - Used for clinical decision-making

- Each new generation of Cephalosporins have greater activity on GNR through new forms of beta-lactam
  - Resistance develops to new beta-lactams by new forms of beta-lactamases
- GNR are now resistant to 3rd generation Cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone) and Monobactams (e.g., aztreonam) by ESBLs
- ESBL producing GNR remain susceptible to cephamycins (e.g., cefoxitin, cefotetan, cefmetazole) and carbapenems (e.g., meropenem, imipenem)
Resistance: Carbapenem Resistant Enterobacteriaceae (CRE)

- Carbapenems are becoming the last β-Lactam antibiotic class for treatment of ESBL infections
- New Delhi metallo-beta-lactamase 1 (NDM-1) carbapenemase-resistant Enterobacteriaceae (CRE) was detected in 2008; susceptible only to polymyxins and tigecycline.
- Few treatment options are available

CDC guidance for management of CRE infected patients, 2015
https://www.cdc.gov/hai/organisms/cre
Laboratory Tests for Tuberculosis AFB

• Acid Fast Bacillus (AFB)
  • Distinguishes bacteria that retain stain in the presence of an acid decolorizer.
    • Present with Mycobacterium species (tuberculosis, avium and others)
  • Very few structures are acid-fast; which makes acid-fastness particularly useful in diagnosis
• Direct fluorescent antibody (DFA) tests for identification of respiratory viruses
Laboratory Test for Respiratory Viruses

• Direct fluorescent antibody (DFA) tests identify respiratory viruses

• Detected from nasal wash samples of patient/residents with suspected infection
Hepatitis A Virus Test Results

- Hepatitis A Virus (HAV)
  - Hepatitis A Total: current or past HAV
  - Hepatitis A, IgM: acute HAV infection
## Hepatitis B Virus Test Terminology

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>antigen</td>
<td>Foreign microbe causing an immune response</td>
</tr>
<tr>
<td>antibody</td>
<td>Immune (proteins) response to an antigen</td>
</tr>
<tr>
<td>IgM</td>
<td>Immune globulin M, 1st antibody to appear after exposure to an antigen</td>
</tr>
<tr>
<td>HB</td>
<td>hepatitis B virus</td>
</tr>
<tr>
<td>HBsAG</td>
<td>surface antigen test; detects a current infection</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>core antibody test; detects if ever been infected</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>surface antibody test; past infection or vaccination (immune)</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>antibody response due to initial exposure to HB core antigen</td>
</tr>
<tr>
<td>HbeAG</td>
<td>HB e antigen; acute HB infection marker indicates highly infectious</td>
</tr>
</tbody>
</table>

**CDC Interpretation of Hepatitis B Serologic Test Results**

# Hepatitis B Virus Test Results

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>HBsAG</th>
<th>anti-HBc</th>
<th>anti-HBs</th>
<th>IgM anti-HBc</th>
<th>HbeAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible to HBV infection</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune due to prior HBV infection</td>
<td>neg</td>
<td>pos</td>
<td>pos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune due to hepatitis B vaccination</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acutely infected with HBV</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>Chronically infected with HBV</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td><strong>Highly Infectious</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>pos</strong></td>
</tr>
</tbody>
</table>

CDC Interpretation of Hepatitis B Serologic Test Results

Hepatitis C Viral Testing

Hepatitis C Virus (HCV)

- Hepatitis C antibody (Anti-HCV)
  - Exposure to hepatitis C
  - Active, chronic, or resolved
- Hepatitis C Qualitative (RNA PCR)
  - Identifies genetic material of the virus, detectable earlier than antibody tests
  - Used to screen after exposure
  - Confirmatory test of antibodies to the virus
Rapid Diagnostic Laboratory Tests

• Rapid human immunodeficiency virus (HIV) test detects antibodies with high sensitivity and specificity
  • Use confirmatory testing to verify false positives

• Fast antigen detection for influenza but 44-60% false positives
  • Use confirmatory testing to verify

• Rapid Group A Streptococci antigen detection with 95% specificity
  • Will also detect carriers
Rapid Laboratory Tests - 2

• Polymerase chain reaction (PCR) assays
  • Makes thousands of copies of a DNA segment specific to an organism so it can be detected by identifying tests
  • Available for a number of bacterial and viral pathogens
  • Highly sensitive; may not indicate viability of organism
  • Expensive, but getting less so
Many Laboratory Test Methods for Infectious Pathogens and Disease

• Serology testing looks for antibodies that demonstrate exposure/infection
• Cultures identify causative pathogens
• Antibiotic susceptibility tests of bacterial cultures identify the susceptibility or resistance to specific antimicrobial agents
• Microscopic evaluation performed for fungal infections
  • Wet mounts for vaginal organisms, CSF, skin
Summary

• Microbiology laboratory is important for HAI Prevention
  • Managing outbreaks
  • Performing additional screening and confirmatory tests for epidemiologic investigations
  • Infection surveillance
  • Alerts to unusual pathogens or changes in antibiotic susceptibility in the population
  • Local antibiogram development
  • Assistance with interpretation of test results
Additional Resource

• Brooks, K. *Ready Reference for Microbes*, 3rd Ed., 2012
Questions?

For more information, please contact any HAI Liaison IP Team member

Or email 
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