Introduction to Microbiology
Objectives

• Describe role of the laboratory in infection prevention
• Describe factors that can adversely affect reliable laboratory results
• Interpret Gram stains
• Discuss common HAI pathogens
• Discuss laboratory testing methods to confirm infections
Microbiology and Infection Prevention

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

• **Clinical**: identification of pathogens and their susceptibility to treatment

• **Epidemiological**: identification of pathogens causing disease or outbreak in a population, and the 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-analysis: specimen collection, handling, transportation, and preservation prior to arrival in the lab
2. Analysis: specimen processing, skill of the laboratory technician, accuracy of biochemicals and instrument system
3. Post Analysis: Accuracy of result transcription, results communicated accurately
Presence of an Organism Does Not mean there is Disease

- For sterile body sites, growth may confirm an infection
  - Interpret all cultures in the context of what pathogens are normally found in that body site
  - If an organism is found it does not mean it is causing disease
- Pseudo-outbreaks due to contamination of samples can occur
White Blood Cell (WBC) Types

- **Polymorphonuclear leukocytes (PMN):** provide general response to threat
  - Neutrophils (50-60% of WBC) are the first line of response to infection; may 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
B-Lymphocytes

- Immunoglobulins (antibodies) are proteins that bind to viruses and bacteria:
  - IgM: produced immediately after exposure
  - IgG: most abundant, is long term response to disease
  - IgA: secretory, present in mucosal linings
  - IgE: plays a role in hypersensitivity reactions
Gram Stain

- 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 Groups of Bacteria

**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
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, no appreciable contamination
- 10-25  equivocal but acceptable
- >25  reject due to unacceptable levels of oral contamination
Quality of Sputum Specimen

Number of WBC:

- < 10  no infection or poor immune response
- 10-25  equivocal
- > 25  purulence indicates presence of infection
Lower Respiratory Cultures

- Sputum and bronchial wash are often contaminated with oral flora
- Protected brush specimen is not contaminated with oral flora
- Tracheal aspirates often identify the colonizers for ventilator-associated pneumonia
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)
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 Pathogens of Deep and Organ Space SSI

- **Anaerobic** – does not require $O_2$ for growth
  - *B. fragilis*
  - Clostridium
  - Peptostreptococcus
  - Propionibacterium (septic arthritis, endocarditis, suture sites for craniotomy)

- **Aerobic**
  - Staphylococcus
  - Streptococcus
  - Gram-negative rods (GNR)
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

• 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 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](https://www.cdc.gov/hai/organisms/cre)
Hepatitis A Virus Testing

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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td>Immune due to hepatitis B vaccination**</td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>positive</td>
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</tr>
<tr>
<td>anti-HBs</td>
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<td></td>
</tr>
<tr>
<td>HBsAg</td>
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<tr>
<td>anti-HBc</td>
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</tr>
<tr>
<td>IgM anti-HBc</td>
<td>negative</td>
<td></td>
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<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HbeAG</td>
<td>positive</td>
<td>Highly infectious</td>
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</tbody>
</table>

Ag = antigen  c = core  Ab = antibody  s = surface

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
Laboratory Tests – AFB and DFA

- **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 Tests – Rapid Diagnostic Testing

- Human immunodeficiency virus (HIV): detects antibodies, has high sensitivity and specificity, use confirmatory testing to verify false positives
- Influenza: fast antigen detection; 44-60% false positives, use confirmatory testing
- Group A Streptococci: antigen detection with 95% specificity; will also detect carriers
Laboratory Tests - Rapid

- Loop-mediated isothermal amplification (LAMP) uses a constant temperature and fewer primers
  - Newer, faster, expensive, less versatile, best for use with a single target
- Polymerase chain reaction (PCR) assays
  - Makes thousands of copies of a DNA segment specific to the 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
Laboratory Tests

• Serology testing to look for antibodies that demonstrate exposure/infection
  • Indicates patient immunity
  • Testing can also look for antigens
• Antibiotic susceptibility testing performed on bacterial cultures to test the susceptibility or resistance to specific antimicrobial agents
• Viral load testing for HIV, HCV
• Microscopic evaluation for fungal infections such as wet mounts for vaginal organisms, CSF, skin
• Antigen tests for Cryptococcal meningitis
Role of Microbiology in HAI Prevention

Microbiology can support the Infection Prevention Program with:

• Managing outbreaks
• Performing additional tests for epidemiologic analyses
• Infection surveillance
• Alerts to unusual pathogens or changes in antibiotic susceptibility in the population
• Development of a local Antibiogram
• Assistance with interpretation of microbiological results
Additional Resources

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

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

Or email

HAIProgram@cdph.ca.gov