Introduction to Microbiology

Infection Preventionist Training for Skilled Nursing Facilities
Healthcare-Associated Infections Program
Center for Health Care Quality
California Department of Public Health

California Department of PublicHealth

Objectives

- Describe role of the laboratory in infection prevention
- Describe basic laboratory tests for infectious pathogens
- Discuss common Healthcare Associated Infection (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

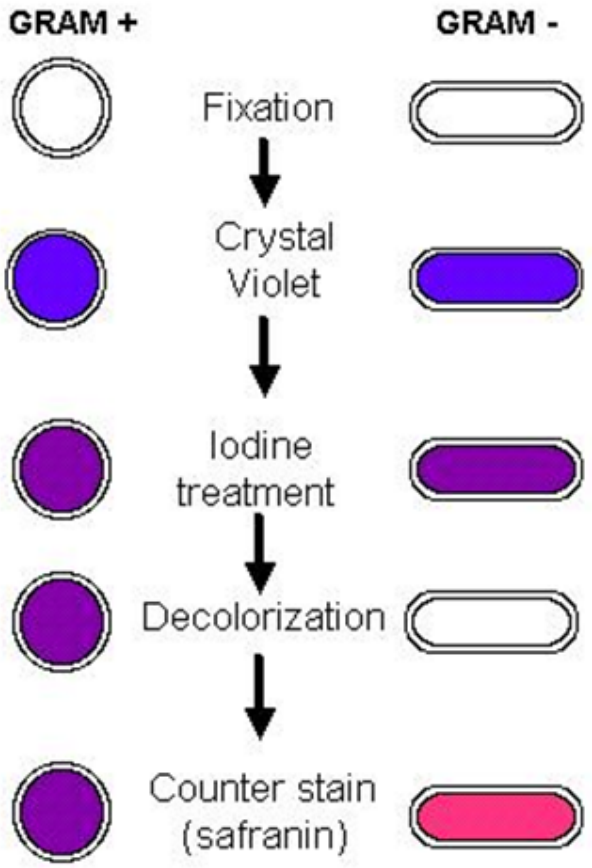
- **Lymphocytes** mature in the lymphatic portion of the immune system
  - Include pathogen-specific immune response (B cells, T cells)
  - Increase may be indicative of viral infection
- **Monocytes** (or macrophages) have phagocytic function and eat cellular debris and foreign pathogens in the immune system

Serology

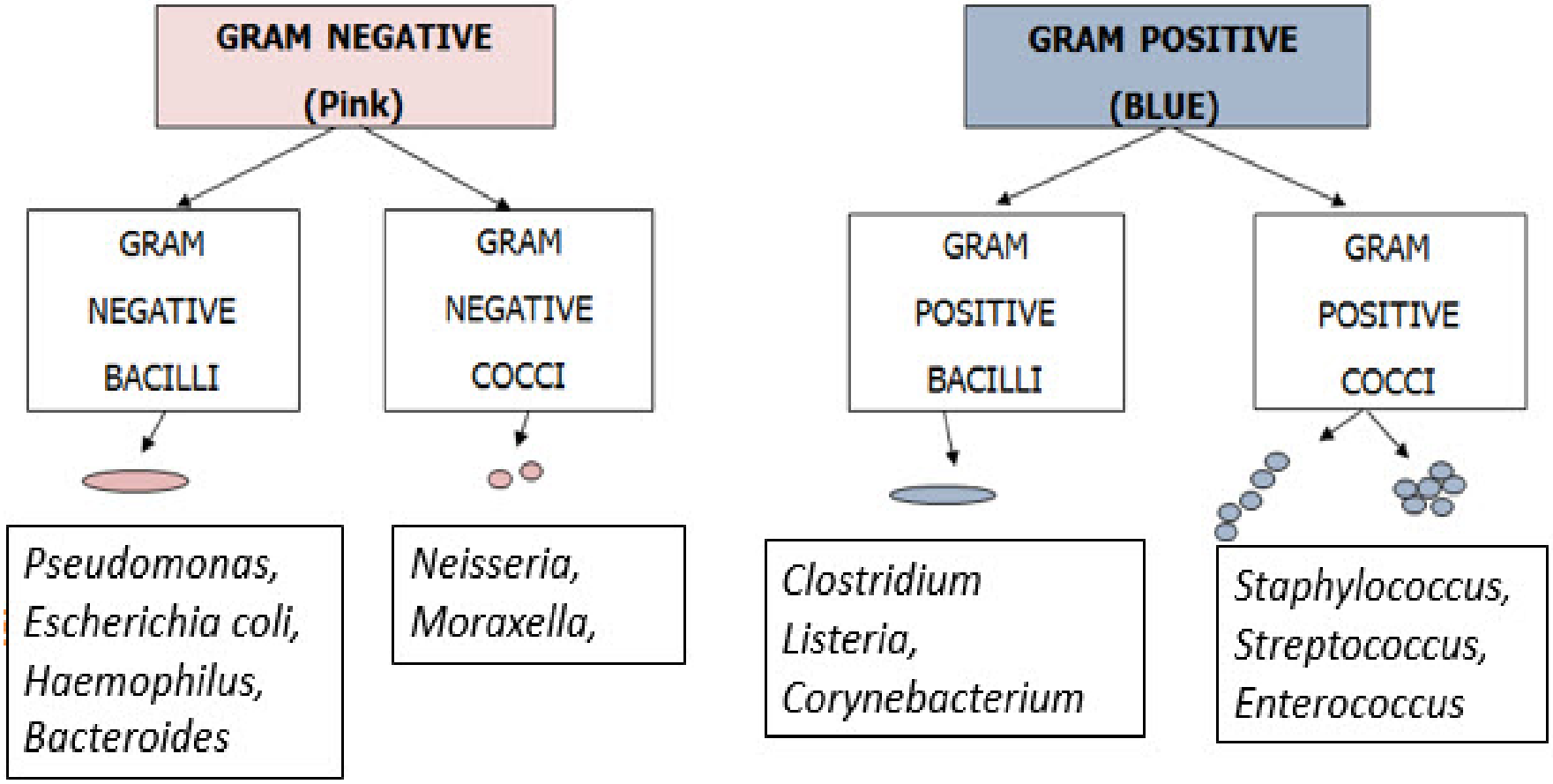
- Diagnostic test that identifies immunoglobulins (antibodies) in blood serum
- Immunoglobulins (Ig) are proteins that bind to viruses and bacteria
- Types
  - IgM: produced immediately after exposure (acute phase of disease)
  - IgG: most abundant; long term response to disease (chronic disease)
  - 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



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 catarhallis* (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

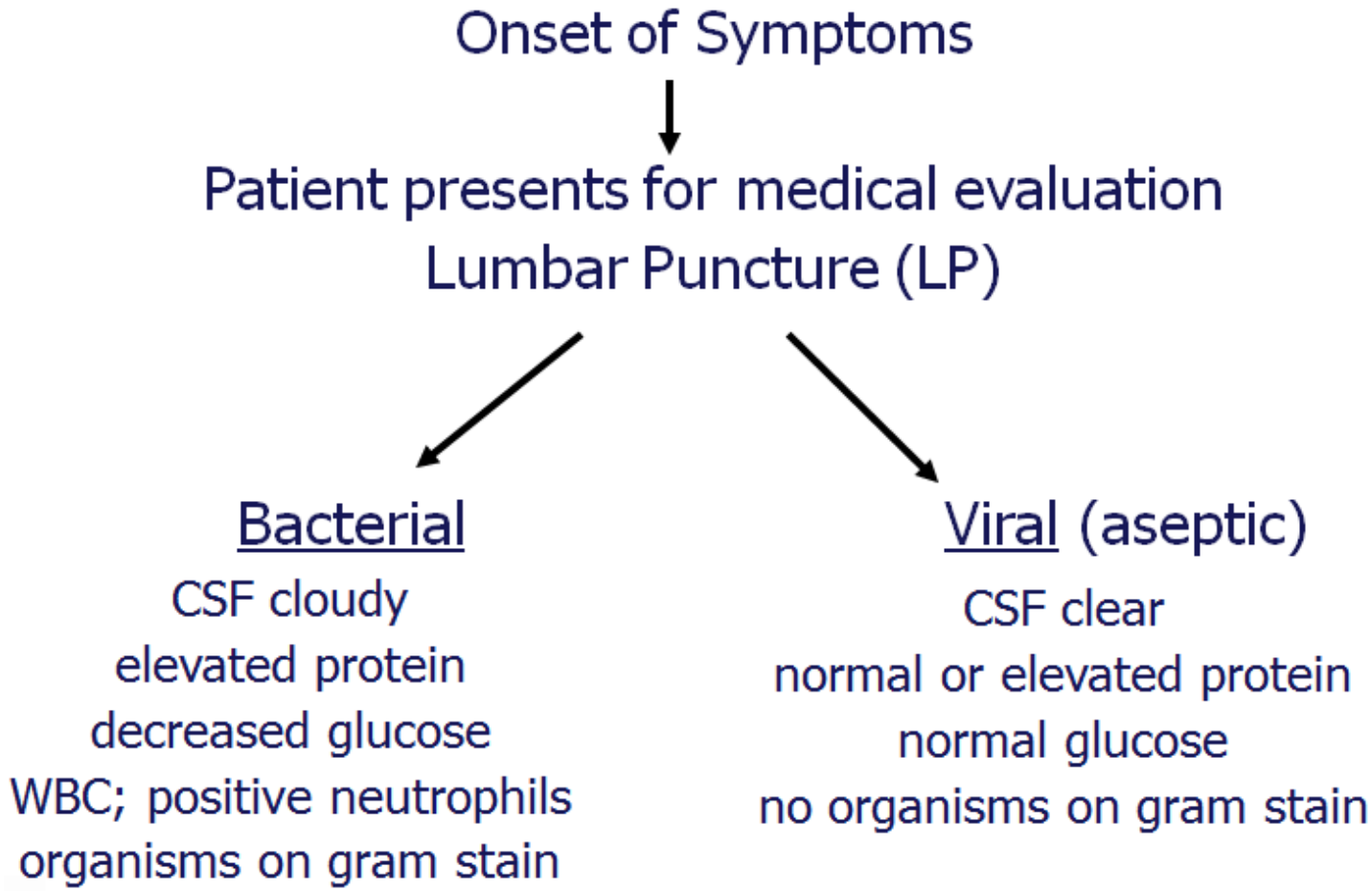
Interpreting Results from Sputum Specimens

- 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 (≤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

Resistance: Extended Spectrum Beta-Lactamase (ESBL) Producing Gram-Negative Rods (GNR)

- 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
(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

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

Test / Term	Definition
antigen	Foreign microbe causing an immune response
antibody	Immune (proteins) response to an antigen
IgM	**I**mmune **g**lobulin **M**, 1st antibody to appear after exposure to an antigen
HB	**h**epatitis **B** virus
HBsAG	**s**urface **a**nti**g**en test; detects a current infection
anti-HBc	**c**ore **anti**body test; detects if ever been infected
anti-HBs	**s**urface **anti**body test; past infection or vaccination (immune)
IgM anti-HBc	**anti**body response due to initial exposure to **HB c**ore antigen
HbeAG	**HB e anti**gen; acute HB infection marker indicates highly infectious

CDC Interpretation of Hepatitis B Serologic Test Results (PDF)
(www.cdc.gov/hepatitis/HBV/PDFs/SerologicChartv8.pdf)

Hepatitis B Virus Test Results

#	Interpretation	HBsAG	anti-HBc	anti-HBs	IgM anti-HBc	HbeAG
1	Susceptible to HBV infection	neg	neg	neg		
2	Immune due to prior HBV infection	neg	pos	pos		
3	Immune due to hepatitis B vaccination	neg	neg	pos		
4	Acutely infected with HBV	pos	pos	neg	pos	
5	Chronically infected with HBV	pos	pos	neg	neg	
6	***Highly Infectious***					***pos***

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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*, 4rd Ed., 2018

Questions?

For more information, please contact

HAIProgram@cdph.ca.gov

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