Basic Microbiology Part 1

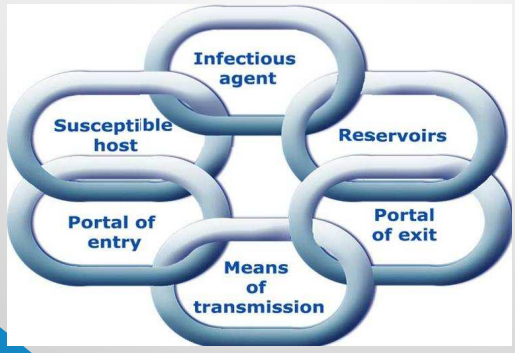
Infection Prevention & Control
Boot Camp For Long-Term Care Facility
Infection Preventionists

Objectives

At the conclusion of this presentation, participants will be able to:

- Demonstrate a basic knowledge of the fundamentals of microbiology
- Describe the microbiota, or normal flora, of a person
- Discuss the common pathogens found in healthcare-acquired infections (HAIs)
- Describe the basic diagnostic tests in used in microbiology

Chain of Infection



Basic Microbiology Part 1

Microbiology has Two Important Functions in Infection Prevention

Clinical

- Identification & diagnosis of infections

Epidemiological

- Understand infectious organisms in residents (and other populations), to find sources and routes of transmission necessary for prevention efforts

Definitions

Aerobe	An organism that requires air or free oxygen for life
Anaerobe	An organism that does not require oxygen for growth
Commensal	Organisms that reside on the skin and derive benefit from this, but not providing any benefit
Indigenous Microbiota	Term for Normal Flora preferred by researchers in the field
Normal Flora	Microorganisms that normally inhabit given sites of the body without causing disease and are sometimes beneficial to the body
Pathogen	An organism that causes disease or infection, the degree of which is determined by its virulence
Symbionts	• Microorganism which is mutually beneficial for the host (human) and the microorganism

Definitions

Fomite	An inanimate object capable of transmitting organisms
Localized infection	An infection that affects only one body part or organ
Opportunistic infection	An infection caused by organisms that take advantage of a weakened immune system
Sensitivity	The ability of a test to correctly identify those with the disease (true positive rate)
Specificity	The ability of a test to correctly identify those without the disease (true negative rate)
Systemic infection	An infection that affects the entire body
Virulence	The ability of an organism to cause disease

Basic Microbiology Part 1

Microorganisms

Microorganisms

Microbes are ubiquitous
- Some are pathogenic
- Some are beneficial

Types of Microorganisms
- Bacteria
- Viruses
- Fungi
- Parasites
- Prions

Bacteria

- Bacteria are unicellular microscopic organisms that lack a true nucleus
- Less than 10% of bacteria cause diseases
- Scientific name includes the genus and the species
- After first use, abbreviated with first letter of genus and then the species:
  - *S. aureus*
  - *E. coli*
- Gram negative
- Gram positive
- Some produce toxins (endotoxin or exotoxin)

Staphylococcus infection
Salmonella
Toxic Shock Syndrome
Diphtheria
Tetanus
Strep throat
Gonorrhea

Basic Microbiology Part 1

Virus

- A virus is not a cell
  - Nucleic acid molecule, either DNA or RNA, enclosed in a protein coat, or capsid
  - Contain genetic/protein material
- Lack many of the attributes of cells, including the ability to replicate
- Known to infect all cells, including microbial cells
- No metabolism
- Will not propagate on their own
  - Replicated only when they are in a living host cell
- Consist of DNA or RNA core (never both)

- Acquired-Immunodeficiency Syndrome (AIDS)
- Poliomyelitis
- Common Cold
- Chickenpox
- Influenza
- Hepatitis

Fungi

- Both multicellular (molds and mushrooms) and unicellular – single cell – (yeasts)
- Well-defined cell wall composed of polysaccharides
- Considerable variation in size
- Use organic materials for energy
- Source of antibiotics, food, bread, alcohol
- Internal molecular system
- Gaining importance in immunosuppressed patients and increased use of antibiotics

- Aspergillosis
- Coccidioidomycosis
- Candidiasis
- Athlete's Foot (Tinea)
- Thrush
- Ringworm
- *Pneumocystis* Pneumonia (PCP)

Parasites

- An organism that lives in or on another organism and benefits by deriving nutrients at the host's expense
- Some parasites cause bacterial diseases
- Protozoa
  - Microscopic, one-celled organisms
  - Multiply in humans
- Helminths
  - Parasitic worms
  - Tape worms, flukes, pinworms
- Ectoparasites (Arthropoda)
  - Possess an external skeleton
  - Attach or burrow into the skin
  - Remain for relatively long periods of time
  - May be vectors of different pathogens

- Scabies
- Lice
- Cysticercosis (Tapeworm)
- Toxoplasmosis
- Trichomoniasis
- Malaria
- Giardia

Prion

- Transmissible spongiform encephalopathies (TSE)
- Rare form of neurodegenerative disorders
  - Affects both humans and animals
- An infectious agent composed entirely of protein material
  - Called PrP for Prion protein
- Found most abundantly in the brain
- Misfolded proteins
- Slow infection (20-30 years)
- No cure

- Creutzfeldt-Jacob Disease (CJD)
- Variant Creutzfeldt-Jacob Disease
- Kuru
- Gerstmann-Straussler-Scheinker Syndrome
- Scrapie
- Chronic Wasting Disease (CWD)
- Bovine Spongiform Encephalopathy (BSE) (Mad Cow)

Identification of Microorganisms

Lab Tests Used to Identify Microorganisms

Gram Stain

- Helpful in guiding initial empiric therapy
- Performed on all sputum specimens
  - Used to determine acceptability of the specimen for culture
- Not routinely performed on urine, stool, gastric aspirate
- Aids the microbiologist in selecting special growth media for culture
- Results should be conveyed to the physician as soon as possible

Basic Microbiology Part 1

Gram Stain

Used to differentiate bacterial species into two groups

Gram Positive	Gram Negative
• Cocci	• Cocci
• Rods	• Rods

- Based on differences in chemical and physical properties of bacteria cell walls
- Usually the first step in preliminary identification of a bacteria

Gram Stain

Gram Negative (Cocci, Rods)

- *Neisseria* sp.
  - *Neisseria meningitides*
  - *Neisseria gonorrhoeae*
- *Moraxella catarrhalis*
- *Escherichia coli*
- *Proteus* sp.
- *Pseudomonas aeruginosa*
- *Enterobacter* sp.
- *Citrobacter* sp.
- *Acinetobacter* sp.
- *Stenotrophomonas maltophilia*
- *Bacteroides* sp.
- *Haemophilus influenzae*

Gram Positive (Cocci, Rods)

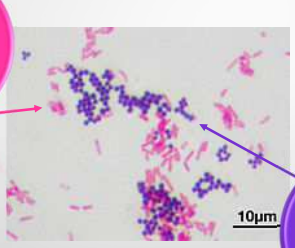
- Clusters: *Staphylococci*
  - *S. Aureus*
  - Coagulase negative *Staph*
  - *S. Saprophyticus*
- Pairs & Chains: *Streptococci*
  - *Strep. Pneumoniae*
  - *Strep. Pyogenes*
  - *Strep. Viridans*
  - *Enterococcus* sp.
- *Corynebacterium diphtheria*
- *Listeria monocytogenes*
- *Clostridium tetani*
- *Bacillus anthracis*

Gram Stain (continued)

- Bacteria are described by
  - Gram stain color color (purple or red/pink)
  - Shape
    - Rod
    - Spherical
    - Spiral
    - Corkscrew
    - Comma
  - Size
  - Quantity
  - Arrangement
  - Presence or absence of spores

Gram Stain (continued)

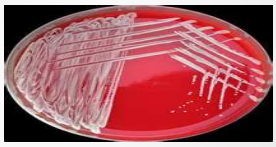
Gram Negative
- Pink rods
- *E. coli*



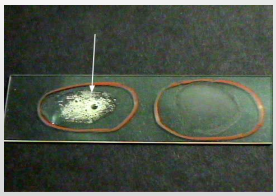
Gram Positive
- Purple cocci clusters
- *S. aureus*

Coagulase Test

Test used to differentiate *Staph aureus* (coagulase positive) from *Staph epidermidis* (coagulase negative)



The next step after incubating the bacteria is to determine which Staph species it is

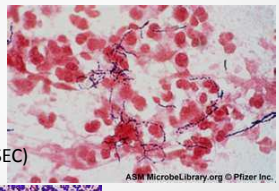


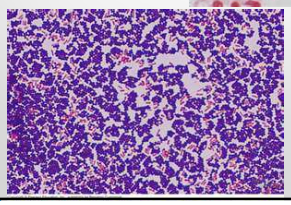
Assessing Accuracy of Lab Findings

- Many factors affect accuracy of lab tests
  - Pre-analysis: Prior to arrival in the lab, how was the specimen
    - Collected
    - Handled
    - Transported
    - Preserved
  - **Analysis: Evaluating lab results consider**
    - Presence of other cells
    - Polymicrobial
    - Quantity

Sputum Gram Stain

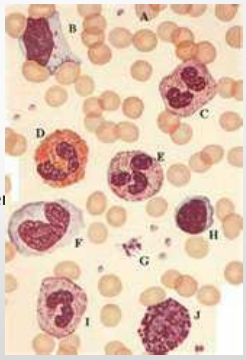
Significance of results
- Bacteria
- White blood cells (WBC)
- Squamous epithelial cells (SEC)





Sputum Gram Stain (continued)

- White Blood Cell (WBC)
  - <10 No infection
  - 10-25 Equivocal
  - >25 Infection is evident (purulen



Urine Gram Stain

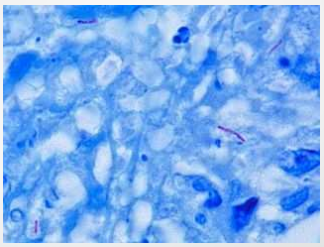
Significance of Results
- Specimen collection
  - Clean-catch voided
  - Sterile
- Colony count
  - Colony counts >100,000 organisms/ml
  - Polymicrobial growth (more than 2 organisms)
- Infection vs. colonization

Other Sites – Gram Stain

Significance of results

- A positive gram stain from normally sterile sites, e.g., blood, tissue, cerebrospinal fluid (CSF), joints is a significant result and should be reported to the physician immediately

Acid Fast Stain



- Differential stain used to identify acid-fast microorganisms
- Most frequently used to test for the genus *Mycobacteria*
  - Used to test sputum when Tuberculosis (*Mycobacteria tuberculosis)* is suspected
  - Also used on blood, tissue, and other specimens

Culture & Sensitivity

Culture

- Test to identify germs in a specimen
  - Specimen is added to a substance to promote the growth of germs

Bacteria	Fungi	Virus
▪ Results usually take 2 days	• Results take longer than bacteria	• Requires specific order • Not routinely done • Results usually take 7 days

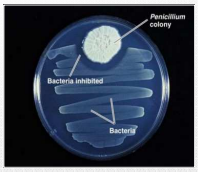
Culture & Sensitivity

Sensitivity Analysis (Susceptibility Testing)

- Performed on pathogens identified from the culture
- Used to determine if an organism is susceptible or resistant to a selection of antimicrobial agents
- Determines the ability of the drug to kill the bacteria

Minimum Inhibitory Concentration (MIC)

Highest dilution (lowest concentration) of a drug effective in inhibiting growth of the organism



Kirby-Bauer Disk Diffusion

Measures the zone of inhibition of growth of bacteria

Disks impregnated with antibiotics placed on culture medium with organism

Culture & Sensitivity

Sensitivity Analysis (Susceptibility Testing)

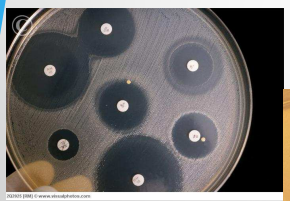
Kirby-Bauer Disk Diffusion Results

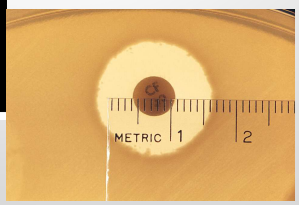
Susceptible	Intermediate	Resistant
Likely to inhibit growth of organism	Has limited effectiveness	Not effective at inhibiting growth of organism
May be appropriate choice for antibiotic treatment	May need higher dose, more frequent dosing, or limited to certain body sites	Not appropriate choice for antibiotic treatment

Culture & Sensitivity

Sensitivity Analysis (Susceptibility Testing)

Kirby-Bauer Disk Diffusion Results





Clostridium difficile Testing

Enzyme Immunoassay (EIA)

- Also Enzyme-Linked Immunosorbent Assay (ELISA)
- Used to detect an antibody or an antigen in a sample
- Tests for Toxin A & B
- Sensitivity – 54%
- Specificity – 87%

Culture

- Not commonly performed
- Takes 3 days or longer for results

Polymerase Chain Reaction (PCR)

- Amplifies DNA
- Bypasses the need to use bacteria to amplify DNA
- Turn around time (TAT) is within an hour
- Sensitivity – 94%
- Specificity – 94%

Clostridium difficile Testing

The best test to detect *Clostridium difficile* infection is the nurse's nose

Understanding Lab Reports

Organism #1: 50,000 COLONIES/ML Proteus mirabilis
Organism #2: 50,000 COLONIES/ML Enterococcus spp

Antibiotics	Proteus mirabilis	Enterococcus spp
Amikacin	<=2 S	
Ampicillin	<=2 S	8 S
Ampicillin/Sulbactam	<=2 S	
Benzylpenicillin		32 R
Cefazolin	32 I	
Cefepime	32 R	
Ceftazidime	32 R	
Ceftriaxone	>=64 R	
Ciprofloxacin	0.5 S	>=8 R
Gentamicin	<=1 S	
Imipenem	8 R	
Levofloxacin	>=8 R	>=8 R

Basic Microbiology Part 1