

# Basic Microbiology



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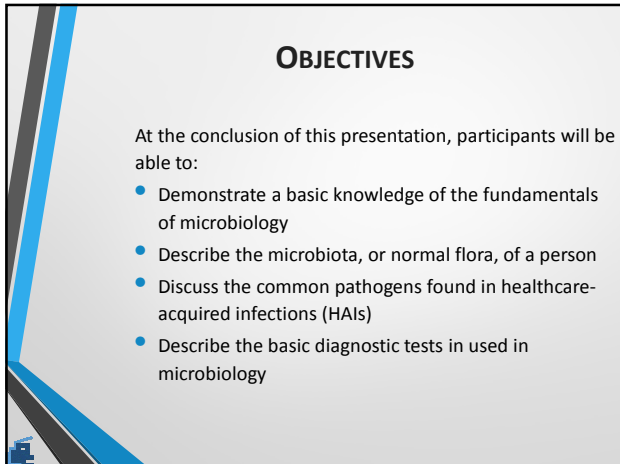
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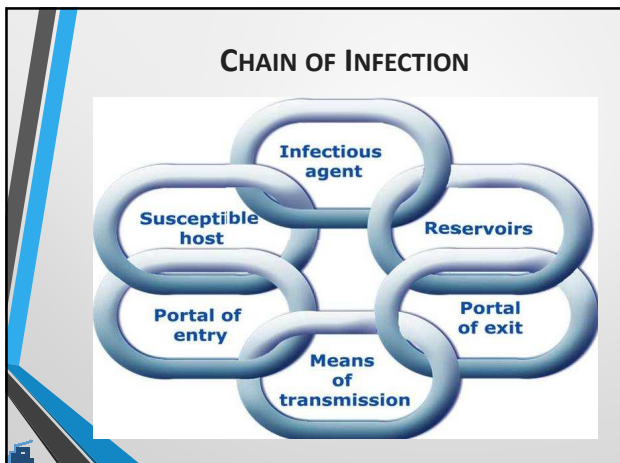
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# Basic Microbiology

## 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

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## 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

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## 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

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# Basic Microbiology



**Microorganisms**

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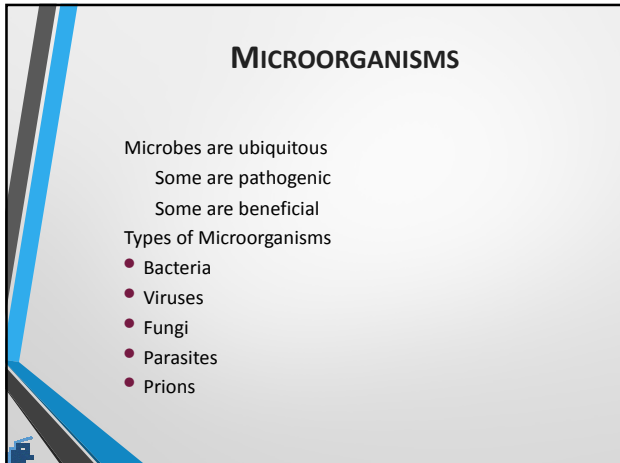
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**MICROORGANISMS**

Microbes are ubiquitous  
Some are pathogenic  
Some are beneficial

Types of Microorganisms

- Bacteria
- Viruses
- Fungi
- Parasites
- Prions

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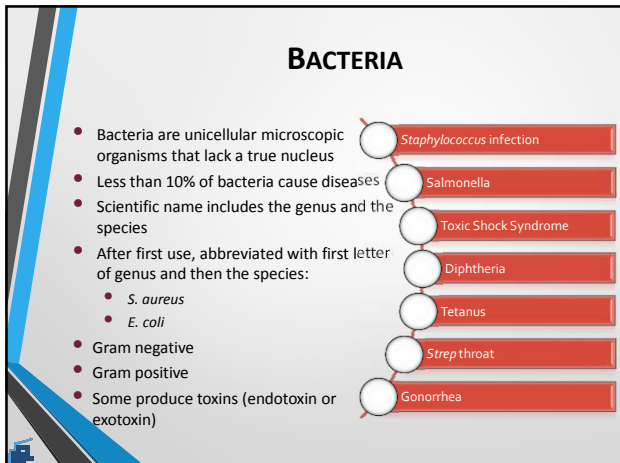
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**BACTERIA**

- 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

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# Basic Microbiology

## 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

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## 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)

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## 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

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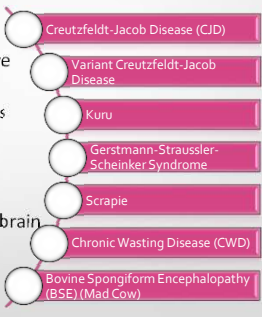




# Basic Microbiology

### 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)

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### IDENTIFICATION OF MICROORGANISMS

## Lab Tests Used to Identify Microorganisms

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### 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

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# Basic Microbiology

## GRAM STAIN

Used to differentiate bacterial species into two groups

Gram Positive	Gram Negative
<ul style="list-style-type: none"> <li>• Cocci </li> <li>• Rods </li> </ul>	<ul style="list-style-type: none"> <li>• Cocci </li> <li>• Rods </li> </ul>

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

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## GRAM STAIN

<b>Gram Negative</b> • Cocci • Rods	<i>Neisseria sp.</i> <small><i>Neisseria meningitidis</i> <i>Neisseria gonorrhoeae</i></small>	<i>Moraxella catarrhalis</i>
	<i>Escherichia coli</i> <i>Proteus sp.</i> <i>Pseudomonas aeruginosa</i> <i>Enterobacter sp.</i> <i>Citrobacter sp.</i>	<i>Acinetobacter sp.</i> <i>Stenotrophomonas maltophilia</i> <i>Bacteroides sp.</i> <i>Haemophilus influenzae</i>
<b>Gram Positive</b> • Cocci • Rods	<b>Clusters:</b> <i>Staphylococci</i> <small><i>S. Aureus</i> Coagulase negative Staph <i>S. Saprophyticus</i></small>	<b>Pairs &amp; Chains:</b> <i>Streptococci</i> <small><i>Strep. Pneumoniae</i> <i>Strep. Pyogenes</i> <i>Strep. Viridans</i> <i>Enterococcus sp.</i></small>
	<i>Corynebacterium diphtheria</i> <i>Listeria monocytogenes</i>	<i>Clostridium tetani</i> <i>Bacillus anthracis</i>

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## 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

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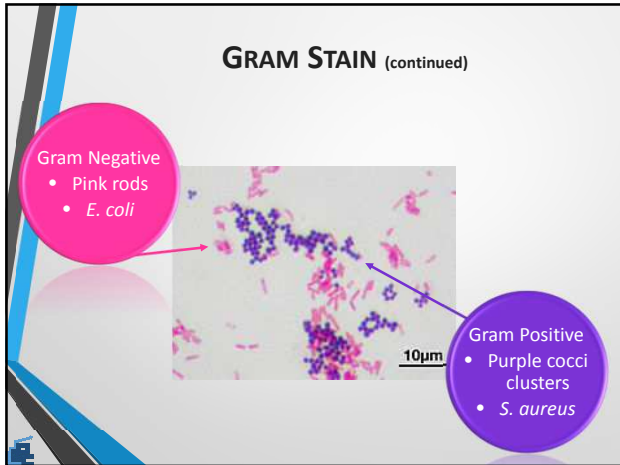
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# Basic Microbiology



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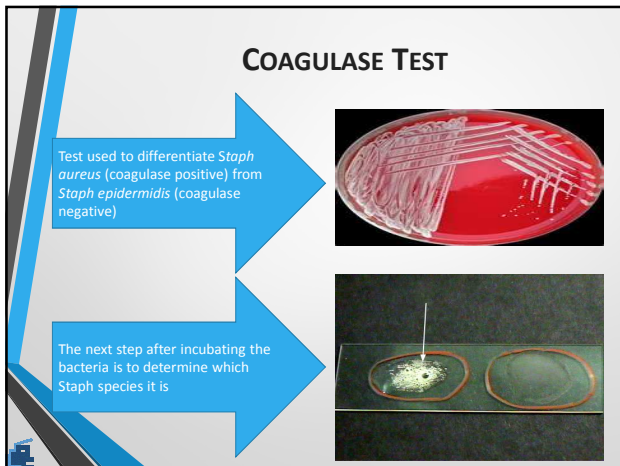
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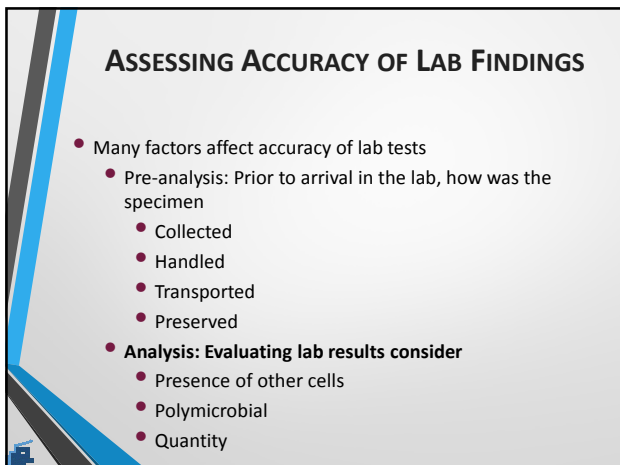
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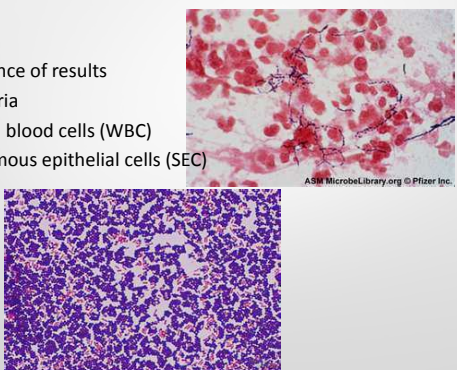


# Basic Microbiology

### SPUTUM GRAM STAIN

Significance of results

- Bacteria
- White blood cells (WBC)
- Squamous epithelial cells (SEC)



ASW MicrobiLibrary.org © Pfizer Inc.

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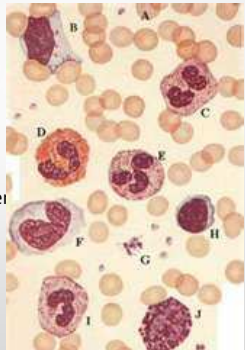
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### SPUTUM GRAM STAIN (continued)

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



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### 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

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# Basic Microbiology

## 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

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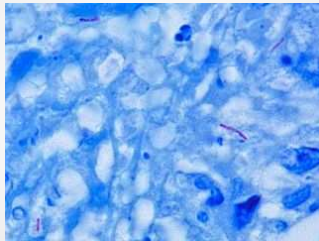
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## ACID FAST STAIN



- 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

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## CULTURE & SENSITIVITY

### Culture

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

Results usually take 2 days

Bacteria



Results take longer than bacteria

Fungi



- Requires specific order
- Not routinely done
- Results usually take 7 days

Virus



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# Basic Microbiology

## CULTURE & SENSITIVITY

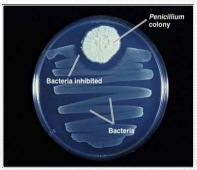
Sensitivity Analysis (Susceptibility Testing)

- 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.

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## 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

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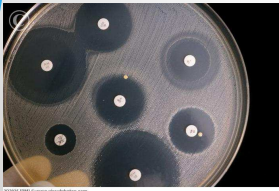
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## CULTURE & SENSITIVITY

Sensitivity Analysis (Susceptibility Testing)

Kirby-Bauer Disk Diffusion Results





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# Basic Microbiology

### 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%

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### CLOSTRIDIUM DIFFICILE TESTING



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

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### UNDERSTANDING LAB REPORTS

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

Antibiotics	<i>Proteus mirabilis</i>	<i>Enterococcus</i> spp
Amikacin	<=2 S	
Ampicillin	<=2 S	8 S
Ampicillin/Subactam	<=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

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# Basic Microbiology

**GALLERY OF ORGANISMS**



Match the photo with the type of organism

Fungi Parasites Bacteria Prions Helminths Viruses

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**Normal Flora/  
Microbiota**

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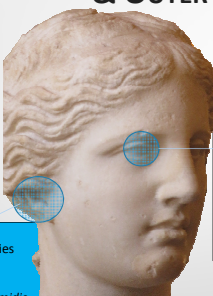
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**NORMAL MICROBIOTA OF THE CONJUNCTIVA  
& OUTER EAR**



**Outer Ear**

- *Corynebacterium* species
- Diphtheroids
- *Pseudomonas* species
- *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*)

**Conjunctiva**

- *Haemophilus* species
- *Moraxella* species
- *Neisseria* species
- *Staphylococcus aureus*
- *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*)
- *Streptococcus* species

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# Basic Microbiology

## NORMAL MICROBIOTA OF THE MOUTH, NOSE, OROPHARYNX

- *Acinetobacter* species
- *Actinomyces* species
- *Bacteroides* species
- *Candida albicans*
- *Corynebacterium* species
- *Fusobacterium* species
- *Haemophilus* species
- *Lactobacillus* species
- *Leptotrichia* species
- *Moraxella* species
- *Mycoplasma* species
- *Neisseria* species
- *Peptostreptococcus* species
- *Staphylococcus aureus*
- *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*)
- *Streptococcus pneumoniae*
- *Streptococcus* species
- *Veillonella* species



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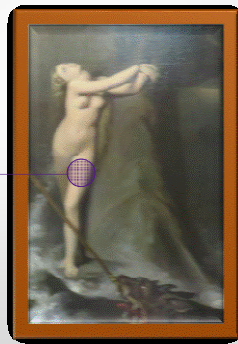
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## NORMAL MICROBIOTA OF THE SKIN

- *Bacillus* species
- *Candida* species
- *Corynebacterium* species
- Diphtheroids
- *Micrococcus* species
- *Mycobacterium* species
- *Pityrosporum ovale*
- *Propionibacterium acnes*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*)
- *Streptococcus pyogenes*
- *Streptococcus* species



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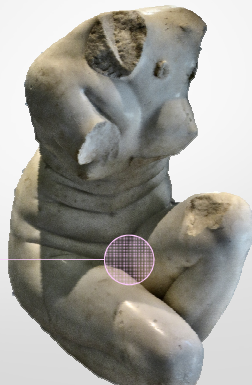
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## NORMAL MICROBIOTA OF THE VAGINA

- Alpha-hemolytic *Streptococcus*
- *Bacteroides* species
- *Streptococcus* species
- Diphtheroids
- *Candida albicans*
- *Candida* species
- *Clostridium* species
- *Loctobacillus* species
- *Gardnerella Vaginalis*
- *Peptostreptococcus* species
- *Staphylococcus epidermidis* (Coagulase-negative *Staphylococcus*)
- Yeast



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# Basic Microbiology

### NORMAL MICROBIOTA OF THE GI TRACT



**Large and Small Intestine**

- *Achromobacter* species
- *Acinetobacter calcoaceticus*
- *Actinomyces* species
- *Aeromonas* species
- *Bacillus* species
- *Bacteroides* species
- *Clostridium* species
- *Enterococcus* species
- *Enterobacteriaceae*
- *Escherichia coli*
- *Flavobacterium* species
- *Fusobacterium* species
- *Klebsiella* species
- *Lactobacillus* species
- *Mycobacterium* species
- *Peptostreptococcus* species
- *Proteus* species
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Streptococcus viridans*
- *Vibrio* species
- *Yersinia enterocolitica*

**Stomach**

- *Clostridium sordellii*
- *Lactobacillus* species
- *Peptostreptococcus* species
- *Streptococcus* species
- *Staphylococcus aureus*

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### NORMAL MICROBIOTA OF THE URETHRA



**Urethra**

- *Bacillus* species
- *Bacteroides* species
- *Candida albicans*
- *Corynebacterium* species
- Diphtheroids
- *Fusobacterium* species
- *Mycobacterium* species
- *Peptostreptococcus* species
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*  
(Coagulase-negative *Staphylococcus*)
- *Streptococcus* species

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### Pathogens



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# Basic Microbiology

## NO NORMAL FLORA – STERILE SITES



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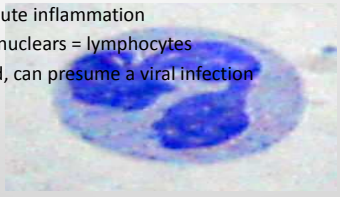
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## DEFINITIONS

WBC = white blood cells = leukocytes

- Major types of WBC
  - PMN, Polys = polymorphonuclear leukocytes
    - If elevated, likely to indicate bacterial infection
  - Segs, Neuts = segmented neutrophils – are the landmark of acute inflammation
  - Lymphs/mononuclears = lymphocytes
    - If increased, can presume a viral infection



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## COMMON LOWER RESPIRATORY PATHOGENS

*Streptococcus pneumoniae*

- Primarily community acquired infection (CAP)
- Uncommon as HAI pneumonia

*Haemophilus influenzae*

- Primarily CAP

*Pseudomonas aeruginosa*

- Often ventilator or ICU related

*Moraxella catarrhalis*

- Most often CAP, but can be HAI

*Staphylococcus aureus*

- CAP and HAI
- High mortality, must be recognized quickly



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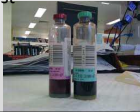
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# Basic Microbiology

## BLOOD CULTURES

- A single blood culture consists of two bottles:
  - Bottles are designed to recover aerobes (blue) and anaerobes (red)
- If ordered "blood cultures x2" they should be drawn 15-30 minutes apart
  - Irrelevant which bottle has growth or if both or only one bottle has growth
  - If other lab tests ordered for same time, make certain the blood cultures are drawn first



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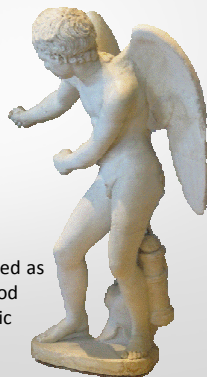
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## BLOOD CULTURE CONTAMINANTS

- Coagulase negative staph
- Diphtheroids
- Bacillus
- Propionibacteria
- Viridans strep
- Aerococcus
- Micrococcus



For these bacteria to be interpreted as causing infection, two sets of blood cultures are required PLUS specific signs and symptoms (fever)

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## COMMON UTI PATHOGENS



- Gram negatives
  - *E. coli*: causes 80% of all UTIs
  - *Proteus*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Gardnerella*, 5-10%
- Gram Positives
  - MRSA, *Enterococcus*, *Staph saprophyticus*, 10-20%
- Positive leukocyte esterase and/or nitrite positive on UA can be helpful in determining infection status
- Remember, presence of an organism in the urine may not be infection, may be colonization

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# Basic Microbiology

## URINE COLONIZATION IN LTC RESIDENTS

Up to 38% of male residents

Up to 57% of female residents

Simao L, Bahr GT. Urinary tract infections in older adults residing in Long-term care facilities. Ann Longterm Care. 2012 Apr; 20(4): 33-38

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## CLINICAL MICROBIOLOGY

**Physician's perspective:**

- What's growing?
- What antibiotic can be used?
- Determine either by predictive value of the organism type (i.e., gram negative or positive bacillus) or by completed result with sensitivities

**IP or Epidemiologist's perspective:**

- Conduct Surveillance
  - Need both the organism's genus and species (i.e., *Pseudomonas aeruginosa*) and sensitivity pattern
  - Microbiology results used to detect clusters/outbreaks and assessing for trends

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## RULE OF THUMB #1

Keep in mind that no lab test is 100% accurate 100% of the time!

- Interpret all results carefully
- Compare results to clinical condition of the resident
- Diagnosis of infection is based on clinical condition of resident and laboratory findings

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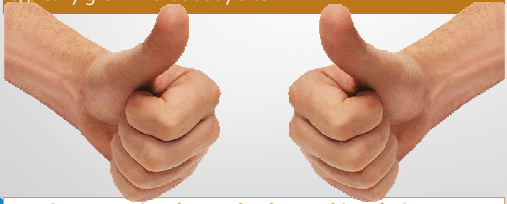
# Basic Microbiology

## RULE OF THUMB #2

For normally sterile body sites, growth on cultures may indeed be an infection

Interpret all cultures knowing what pathogens would typically grow in that body site

*Just because a bug is growing in a resident it does not mean it's causing disease – it could mean colonization*



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## COMMON BOWEL FLORA

- Normal mix of bacterial flora keeps numbers of yeast, *C. difficile* and other potential pathogens in the gut in check
  - With altered flora (which can happen with antibiotic treatment)
    - Yeast can proliferate
    - *Clostridium difficile* can proliferate
    - *Pseudomonas* can proliferate
    - VRE can proliferate
    - Etc., etc., etc.

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## COMMON PATHOGENS OF SUPERFICIAL SURGICAL SITE INFECTIONS (SSI)



- Not usually caused by anaerobes
- Generally skin flora, but not necessarily
- Can be gram negative rods (GNR)

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# Basic Microbiology

## DID YOU ALSO KNOW?

- Studies have found that 25-75% of all antibiotics used, both systemic and topical, were unnecessary or used inappropriately<sup>1</sup>
- 50-70% of residents in LTC will receive an ATB during the year<sup>1</sup>



<sup>1</sup>Nimelle Stone, MD, MS, "Managing MOROs in LTCF: Practical Tips for Preventing Transmission," December, 15, 2011

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## ROLE OF MICROBIOLOGY IN HAI PREVENTION

Critical for:

- Infection surveillance
- Interpretation of microbiological results
- Knowledge of new microbes or unusual resistance patterns
- Create antibiograms for design of antibiotic formulary
- Education of health care staff
- Outbreak management and investigation
- Performing additional tests for epidemiologic analyses

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## ROLE OF MICROBIOLOGY IN HAI PREVENTION



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# Basic Microbiology



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