Basic Microbiology

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Disclosure

- Jim is employed by Diversey. His expenses to attend this meeting (travel, accommodation, and salary) are paid by this company. Diversey has had no input into this presentation from a commercial interest.

Objectives

- Define: Microbiology
- List the major groups or ‘buckets’ of microorganisms
- Explain the importance of the organism characteristics in our world
Microorganisms

- The study of microorganisms (microbes, pathogens, bugs, germs)
- They are living organisms, mostly invisible
- The majority can only be seen with a microscope
- Make up more than 60% of the Earth’s living matter
- About 2-3 billion species share the planet with us!

Microbiology

- We all have 3–5 POUNDS of bacteria in and on us!
  - Human Microbiome
  - 10x more bacterial cells than tissue or structural cells
The main groups (buckets) of microorganisms

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<tr>
<th>Bacteria</th>
<th>Gram Positive</th>
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Bacteria

- Single cell
- Genetic information is contained in a single loop of DNA
- Some have an extra circle of mobile genetic material called a “plasmid”
  - Plasmids may contain a gene that makes it resistant to certain antibiotics
  - Plasmids can move from one bacterium to another!
Identification of Microorganisms

Staining: To microscopically visualize the microbial structures (bacteria, fungi)

Identification of Microorganisms

Culture: Grow microorganisms on agar plates or in test tubes (bacteria and fungi)

* Culture media encourage growth of microorganisms by providing nutrients
* Swabs can be used, or excretions (urine, feces)
* Environmental testing uses swab or agar to press on surface
* Culture can take 24 – 48 hours

Identification

* PCR (polymerase chain reaction):
  * 1 – 3 hours. Technology now has ‘panels’ of organisms
  * https://www.youtube.com/watch?v=2KoLnlwoZKU
Dr. Gram developed a staining method that allows microscopic differentiation between different types of bacteria:
- The majority of bacteria fall under one of two categories:
  - Gram Positive Bacteria
  - Gram Negative Bacteria
- Based on cell wall composition of bacteria

**Cell Wall**

**GRAM-NEGATIVE**
- Outer membrane
- Porins
- Lipopolysaccharide
- Cytoskeletal membrane

**GRAM-POSITIVE**
- Cell wall

**Gram Stain**
- Gives a quick look at the specimen
- Can interpret quality of specimen
  - Number of “pus” (polymorphonuclear) cells present
    - Infection
  - Number of epithelial cells present
    - Surface, not good
  - Number of bacteria present (and likely Genus)
    - Normal vs. abnormal
**Bacterial Shapes**

- Streptococci
- Staphylococci

**Gram Stain**

- Can help direct antibiotic therapy
- Based on cell wall composition
- Not so helpful if lots of normal flora present
- Throats, stool, pressure injuries
- Quite significant on sterile body sites
- CSF and other fluids
Effect of Disinfectants on Microorganisms

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Adapted from Rutala et al. JICHE 2014;35(7):862

Gram

- **Positive**
  - Aerobic
    - Cocci
    - Neisseria
  - Anaerobic
    - Cocci
    - Veillonella

- **Negative**
  - Aerobic
    - Cocci
    - E. coli, Pseudomonas
  - Anaerobic
    - Bacilli
    - Bacteroides
Growth Characteristics

- Oxygen requirements
- Able to ferment or oxidize sugars to produce acid end products
- Temperature ranges
- Salt tolerance
- Chemical tolerance
- Enzymes
- Motile

Oxygen Requirements

- Bacteria can either grow in the presence of oxygen or not
  - **Aerobic**: Require Oxygen: Pseudomonas, Bacillus
  - **Anaerobic**: Can't grow with Oxygen: Clostridium, Bacteriodes
  - **Facultative Anaerobe**: Can grow either with, or without Oxygen: E. coli, K. pneumonise

Appearance

- Hemolysis – ability to break down red blood cells in agar
  - **Beta**: complete destruction
  - **Alpha**: partial destruction of the cells, leaving a greenish hue to the blood
  - **No hemolysis**
Enzymes

- Catalase
  - Tests the organism’s ability to liberate oxygen from hydrogen peroxide
  - Main distinguishing feature between Staphylococci and Streptococci/Enterococci
  - Pure organism placed into H$_2$O$_2$ – observe!
Coagulase
- The ability of the organism under study to clump, clot, or coagulate rabbit plasma, turning a solution from liquid to semi-solid
- Can use plasma or latex particles
- Used as main identification of Staphylococcus aureus, distinguishing it from other Staph. species (coagulase negative Staph or CNS)

Temperature Ranges
- 37°C (98.6°F)
  - Most human pathogens
- 4°C (39°F)
  - Yersinia, Listeria (food borne organisms)
- 42°C (107.6°F)
  - Campylobacter (enteric organism)
- 56°C (132.8°F)
  - Fecal E. coli – water testing
Growth Quantitation

Biochemical Identification

* Use various sugars and substrates to detect ability to ferment, oxidize or use an enzyme (e.g. gelatinase)
* Most of this is now automated
Agar Plates

- Nutritive
  - Blood agar, chocolate agar
- Selective/Differential
  - MacConkey, Mannitol Salt

Resistance to Antibiotics

- Naturally occurring (genetic)
- Acquired
  - Genetic mutation
  - Transfer of resistance from another bacterium (plasmid)
- Antibiotics are only effective on bacteria
Basic exposure of the organism to antibiotic and see if it kills the bug.

- Antibiotic impregnated discs
- Micro-wells to which an organism suspension is added
- 4-24 hours
  - E-test (determines minimum inhibitory concentration)

Sensitivity Testing

Kirby-Bauer

www.sciencebuddies.org

E-test

MIC - Minimum Inhibitory Concentration
Resistant Organisms

- Antibiotic resistance does NOT confer disinfectant resistance!
  - E. coli is E. coli whether it can produce a beta lactamase or a carbapenemase
- Antibiotics are an elegant “Lock and Key”
- Disinfectants are more “Dynamite” or “Sledgehammer”
  (Weber 2006, Rutala 1997)

Antibiotics

Antibiotic Resistance
**Representative Organisms**

**Gram Negative**
- *Escherichia coli*
- *Burkholderia cepacia*
- *Salmonella enteritidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia*
- *Acinetobacter baumanii*

**Family Enterobacteriaceae**
- *Klebsiella pneumonia*
- *Serratia marcescens*
- *Proteus mirabilis*
- *Salmonella enteritidis*
- *Shigella flexneri*
- *Yersinia enterocolitica* *(Y. pestis – plague)*

**Gram Positive**
- *Staphylococcus aureus* *(MRSA)* *(MDRO)*
- *Coagulase Negative Staph*  
- *Streptococcus pyogenes* *(Group A Strep)*  
- *Enterococcus species* *(VRE)*

**Representative Organisms**

**Gram Positive**
- *Staphylococcus aureus* *(15.6%)*
- *Enterococcus* *(11.9%)*
- *Coagulase-negative Staphylococcus* *(11.4%)*
- *Klebsiella* *(8.0%)*  
- *Pseudomonas aeruginosa* *(7.5%)*  
- *Enterococcus faecalis* *(6.8%)*  
- *Candida albicans* *(5.3%)*  
- *Enterobacter species* *(4.7%)*  
- *Other Candida species* *(4.1%)*  
- *Enterococcus faecium* *(4.1%)*  
- *Enterococcus species* *(3.0%)*  
- *Proteus species* *(2.5%)*  
- *Serratia species* *(2.1%)*  
- *Acinetobacter baumanii* *(1.8%)*

**Table 3.** Most Prevalent Pathogens Causing Healthcare-Associated Infections (HAIs)  

<table>
<thead>
<tr>
<th>Recommended organism (% of HAIs caused)</th>
<th>Why organisms are relevant</th>
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<td><em>Staphylococcus aureus</em> <em>(15.6%)</em></td>
<td>Most prevalent overall contributors to HAIs (NIOSH/CDC)</td>
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Rutala 2014
Outbreak and Ward Closure

- *Clostridium difficile* spores
- Norovirus
- Rotavirus
- Adenovirus
- Aspergillus

Mycobacteria / TB

- *M. tuberculosis*
- Cell wall very different from other bacteria
- “Waxy” in nature, difficult to stain, difficult to penetrate
- Acid Fast Bacilli or AFB
- 24 hours to reproduce
- “Tuberculocidal”

- “Tuberculocidal germicides ... will not interrupt and prevent the transmission of *M. tuberculosis* in health-care settings.”
- “The same cleaning procedures used in other rooms in the health-care setting should be used to clean ALL rooms.”
- Follow Airborne precautions while cleaning if air exchanges have not been adequate.

(Centers for Disease Control and Prevention. Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings. MMWR 2005;54(No. RR-07):79)
Some bacteria can form endospores
Formed in vegetative bacteria in times of stress
These are dormant structures, which are extremely resistant to hostile physical and chemical conditions such as heat, natural UV radiation and most disinfectants
This makes destroying them very difficult
Spore-Forming Bacteria

- Many endospore-producing bacteria are nasty pathogens
- *Clostridium difficile* (*Clostridium difficile* Infection – CDI)
- *C. perfringens* (gas gangrene), *C. botulinum* (botulism)
- *C. tetani* (tetanus)
- *Bacillus anthracis* (anthrax – bioterrorism)
- *B. cereus* (food poisoning)

Viruses

- “Obligate Intracellular Parasites”
  - Need host cell machinery to reproduce
  - Small: diameter 20 – 400 nanometers
  - Shapes: usually geometric
  - Identification
    - PCR
    - Electron microscopy
    - Tissue culture
Viruses

- Enveloped Viruses
  - **E** = Easy to kill
- Non-enveloped Viruses
  - **NE** = Not Easy to kill

Viruses

- Large non-enveloped viruses are easier to kill than small non-enveloped viruses
  - Large
    - Adenovirus, Rotavirus
  - Small
    - Norovirus (FCV), Poliovirus, Rhinovirus, Hepatitis A

Bloodborne Pathogens

- Bloodborne pathogens are infectious microorganisms present in blood that can cause disease in humans
- These pathogens include Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus (HIV), the virus that causes AIDS
- All of these pathogens are quite easy to kill
Bloodborne Pathogen Standard – OSHA

- 1991 – product must be tuberculocidal
- 1997 – product must be effective against HIV, HBV and HCV

Effect of Disinfectants on Microorganisms

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<td>A. niger, P. chrysogenum</td>
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Adapted from Rutala et al. CID 2016 52(15)

Fungi
**Effect of Disinfectants on Microorganisms**

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**Fungi**

- Approximately 100,000 species of fungi are divided into two groups
  - Macroscopic (visible) fungi such as mushrooms and puffballs
  - Microscopic fungi such as molds and yeasts

**Clinical Fungi**

- A small number cause disease in humans
  - Athletes' foot, ringworm, oral or vaginal thrush
- Invasive disease is severe (sterile body site such as blood, lung or CSF)
- *Candida auris* in the news – resistant to common antifungal agents
Common fungal pathogens include:

- *Trichophyton mentagrophytes* (athlete’s foot)
- *Tinia corpus* (ringworm)
- *Aspergillus fumigatus* (issue during construction/renovation)
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- *Aspergillus niger* (black mold)
- *Candida albicans* (mucous membrane thrush)

Fungicidal Test Organisms

- *Trichophyton mentagrophytes*
- *Aspergillus niger/brasiliensis*
- *Microsporum canis*
- *Candida albicans*
Summary

The main groups (buckets) of microorganisms

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### References

- Jarvis WD. Bennett and Brachman’s Hospital Infections, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2013. Chapter 11

### References

- Dr. Prameet Sheth, Medical Microbiologist, Kingston General Hospital

### References

- Weber, DJ, Rutala WA. Use of germicides in the home and the healthcare setting: is there a relationship between germicide use and antibiotic resistance? ICHE 2006;27(10):1107-19
- james.gauthier@diversey.com
- brady.biddle@diversey.com