Basic Microbiology
Jim Gauthier, Providence Care
A Webber Training Teleclass

Welcome to the Bug Man's World!
And yes, it's a Small World!

Jim Gauthier
Hosted by Paul Webber
paul@webbertraining.com
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Objectives
- Be at ease with the terminology
- Understand normal vs. abnormal flora
- Demystify all the Latin and Greek (ya, right)!
- See some of the wonders of the microbial world

The Basics
The bugs are small – 2-5 microns (10^-6 meters)
- Viruses are even smaller – nanometers (10^-9)
Classification based on three things:
- Shape
- Gram Reaction
- Growth Requirements

The Basics
Microscopes give a phenotype view
- Phenotype: what you can see
Growth and playing gives the genotype view
- What it can do because of genetics
Staff generally wants the results yesterday!

The Basics
Most human pathogenic bacteria take 18-24 hours
to grow enough on the laboratory media to be
visible and to be able to distinguish single colonies
with the naked eye.
Sensitivity testing from a pure culture can be
anywhere from 4 – 24 hours later.
Full identification can also take up to 24 – 48 hours.

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

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Identification

- PCR, Gene probes
  - In use more and more
  - Chlamydia, GC, Tuberculosis, MRSA, VRE
  - Norovirus
- ELISA
  - Organism is an antigen and reacts with labelled antibody
    • Influenza, RSV, Rotavirus

The Basics- Terms

- Bacteria can either grow or not grow in the presence of oxygen
- Oxygen: **Aerobic** (Pseudomonas, Bacillus)
- No Oxygen: **Anaerobic** (Clostridium, Bacteroides)
- Either: **Facultative Anaerobe** (E. coli)

The Basics - Terms

- Hemolysis
  - Beta: complete destruction of the red blood cells in the (sheep) blood agar plate
  - Alpha: partial destruction of the cells, leaving a greenish hue to the blood
  - Gamma: old term, no hemolysis

The Basics - Terms

- Catalase
  - Tests the organism’s ability to liberate oxygen from hydrogen peroxide
  - Main distinguishing feature between Staphylococci and Streptococci / Enterococci
  - Pure organism placed into H₂O₂ – observe!

The Basics - Terms

- 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.

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- Quantitation of growth based on how many organisms are present in a certain inoculum
- Growth medium first used by Robert Koch

The Gram Stain
- Developed in the late 1800's by Dr. Gram, a pathologist
- Originally noted while staining lung (more trivia)
- Gram positive organisms are purple
- Gram negative organisms are red
- Based on cell wall composition
Cell Wall Composition – Simple!

Gram Stain

- Gives a quick look at the specimen
  - Presumptive identification
- Can interpret quality of specimen
  - Number of “pus” (polymorphonuclear) cells present
    - Infection
  - Number of epithelial cells present
    - Surface
  - Number of bacteria present (and likely Genus)
    - Normal vs. abnormal

Gram Stain

- Can help direct antibiotic therapy
  - Based on cell wall composition
- Not so helpful if lots of normal flora present
  - Throats, stool, decubital ulcers
- Quite significant on sterile body sites
  - CSF and other fluids
  - Aspiration from petechiae
  - Assists in the interpretation of culture results

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Intracellular Gram-negative diplococci

Biochemical Identification

Use various sugars and substrates to detect ability to ferment, oxidize or use an enzyme.
Most of this is now automated.

Sensitivity Testing

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Kirby-Bauer

http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/AntibioticDisk.htm

**VITEK Card Convenience**
- Compact
  - easy to handle
  - space saving
  - low waste volume
- Sealed
  - spill resistant
  - safe disposal
- No reagents to add

**Naming Scheme**

**Gram Positive**
- Aerobic Cocci
  - Staphylococcus, Streptococcus*, Enterococcus* spp.
- Anaerobic Cocci
  - Peptostreptococcus, Peptococcus spp.
- Aerobic Bacilli
  - Bacillus, Listeria, Corynebacterium, Erysipelothrix spp.
- Anaerobic Bacilli
  - Clostridium, Propionibacterium spp.

**Gram Negative**
- Aerobic Cocci
  - Neisseria, Moraxella (Branhamella) spp.
- Aerobic Bacilli
  - Haemophilus*, Pseudomonas, Stenotrophomonas spp.
- Facultative Anaerobic
  - Escherichia, Klebsiella, Enterobacter spp.
- Anaerobic
  - Prevotella, Bacteroides spp.

**Staphylococci**
- Catalase Positive
- Coagulase divides group into Staph. aureus, and coagulase negative Staph.
  - Allows Staph. aureus to be a great pathogen, as it can cover itself in a coagulated shield of plasma, evading treatment
- All are potential pathogens

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**Staphylococci**

- *Staph. aureus* can be normal flora
  - Nose, skin, vagina, rectum, feces, mouth
- All CNS are considered skin flora
  - Presence in blood or sterile body fluid needs to be interpreted carefully
  - Collection is very important
    - antiseptics

**Streptococci, Enterococci**

- Catalase negative
- **Streptococci**
  - Facultative anaerobic
  - Normal flora – alpha haemolytic
    - Oral flora, viridans streptococci, *Str. pneumoniae*  
  - Pathogenic – beta haemolytic
    - Groups A – G potential pathogens

**β-Streptococcus**

**Enterococci**

- Gut flora
  - Over half of the bacteria in feces can be Enterococci
- Not very virulent
  - Third leading cause of urinary tract infections
  - Fecally contaminated abscess
  - Resistance
    - VRE

**Gram Negatives**

- **Neisseria**
  - *N. gonorrhoea, N. meningitidis*
    - Pathogenic
  - *N. lactamica, N. sicca*
    - normal respiratory flora
- **Moraxella catarrhalis**
  - Many name changes, potential pathogen
    - Neisseria, Branhamella

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**Haemophilus**
- Coccobacilli
- Normal flora of throat, nose
- "Satellites" around Staph. aureus
- Finicky growth requirements
- Was leading cause of meningitis in children until HIB vaccine developed

**Enterobacteriaceae**
- Gram negative, facultative ArO$_2$ rods
- All ferment glucose
- Catalase positive
- Many are gut flora
- Many cause nosocomial infections
- Many are referred to as "coliforms"—From the gut
- Grow on MacConkey Agar—selective-differential

**Other Gram Negatives**
- Pseudomonas species
  - Environmental bugs
  - Think "water"
- Stenotrophomonas maltophilia
  - Opportunistic
  - Think "sink drain"

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Other Gram Negatives

- *Acinetobacter calcoaceticus*
  - anitratus, lwofli
  - Think oral contamination
  - Many are very resistant to antibiotics

Clostridia

- Anaerobic Gram positive bacilli
- Spore bearing
- *C. perfringens*
  - Gas gangrene
- *C. difficile*
  - Antibiotic associated diarrhea
- *C. tetani*
  - tetanus

Yeasts

- Single cell organisms
- Numerous species
  - Candida albicans
  - Germ tube test
- Opportunistic
  - Normal respiratory flora
- Urinary, vaginal, systemic

Germ Tubes

Mycobacteria

- Do not stain with Gram’s stain
- Use carbol fuchsin, heated, then decolorize with HCl and alcohol for 5 minutes
  - Acid fast (AFB)
  - Retain red color
- *M. tuberculosis (MTb) [human pathogen]*
- *M. avium-intracellulareae (MAI) [HIV]*
Mycobacteria

- Divide once every 24 hours
  - 2-8 weeks for visible colonies
- Some environmental species
  - M. gordonae, M. marinum
- MOTT: Mycobacterium other than TB

Unusual Organisms?

- “Atypical” respiratory and genital pathogens
- Mycoplasma
  - No cell wall, just cell membrane
  - Very fastidious to grow and stain
    - Not Gram!
- Ureaplasma ureolyticum
- Chlamydia
  - pneumonia, trachomatis

What is a virus?

- Viruses are NOT like bacteria!
- Viruses are NOT little bacteria
- Viruses DO NOT “grow” or divide
- Viruses make copies of themselves using:
  - Tools (enzymes, proteins) they code for
  - Cell machinery

What is a Virus?

- Obligate intracellular parasite
  - “Pirate of the cell”
- NOT a cellular organism
  - No organelles or ribosomes, energy-less
- NOT FREE-LIVING
  - Completely dependent on host cells

Viruses

- Enveloped
  - Easier to kill, less hardy
- Non-enveloped
  - Hardy, resistant to lower concentrations of alcohol
- Both DNA and RNA viruses

Normal Flora

- Positive culture doesn’t necessarily mean infection or clinical significance
- Many organisms are part of the “normal flora” of that site
- Most surface and mucosal surfaces are not “sterile” and are loaded with bacteria

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Vaginal Flora of Normal Women

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>5 - 10</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>50</td>
</tr>
<tr>
<td>Group B Strep</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Group A Strep</td>
<td>3</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>15</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>15 - 20</td>
</tr>
<tr>
<td>Gardnerella</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Peptococcus</td>
<td>80</td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>30</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>15 - 35</td>
</tr>
<tr>
<td>Fusobacteria</td>
<td>10</td>
</tr>
<tr>
<td>Clostridia</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Yeast</td>
<td>15 – 30 (30-40 if pregnant)</td>
</tr>
</tbody>
</table>

Normal Respiratory Flora

- Oral anaerobes
  - Fusobacterium, Bacteroides, Peptostreptococcus
- Streptococci esp. viridans group
- Neisseria spp. (incl. meningococcus)
- Corynebacterium spp.
- Haemophilus spp.

Normal Respiratory Flora

- S. pneumoniae
- H. influenzae
- S. pyogenes (Group A)
- M. catarrhalis
- Enterobacteriaceae
- Yeast

But these are also important & recognized causes of pneumonia

Never Normal Flora

- Mycobacterium tuberculosis
- Legionella spp.
- Brucella spp.
- etc.

Not Normal But May Still Be Asymptomatic

- Neisseria gonorrhoeae
- Salmonella spp.
- Bacillus anthracis
- etc.

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#### What we Won’t Get To!
- Other Anaerobes
- Actinomycetes
  - Norcardia, Rhodococcus, Streptomyces
- Gardnerella
- Brucella, Francisella, Bordetella
- Parasites
- Fungus

#### Summary
- The names may change but the bugs stay the same
  - Please don’t get mad at the lab!
- Not as rapid a science as we would like
- Take a good swab to get good results!

#### Thanks!
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