What’s Bugging You?
The IP’s Guide to Microbiology

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Objectives

1. Discuss the significance of the microbiology lab in infection control and prevention
2. Review the basics of lab testing and result reporting
3. Correlate important organisms with their associated sources and infections

No disclosures
Infection Prevention is a Team Sport

• Microbiology lab key player and essential component

• Lab helps to identify:
  • Clinical diagnosis and treatment
  • Surveillance
  • Outbreaks
  • Exposures

Infection Prevention is a Team Sport

• Antimicrobial Stewardship
• Infection Prevention and Control Committee
• Infection Prevention Risk Assessment and Plan

• Communication BOTH ways
• Develop relationship
  • Micro rounds

Whenever you feel sad just remember that there are billions of cells in your body and all they care about is you.

4/4/13, 9:00 PM
The Human Microbiome

Your body houses 10x the bacteria than cells!!!

SKIN = $10^{12}$ bacteria
MOUTH = $10^{10}$ bacteria
GI TRACT = $10^{14}$ bacteria

The New York Times 2013

Types of Bugs

- **Bacteria**
  - Free, living single-celled organisms
  - Reproduce on their own without a host
- **Virus**
  - Replicates only when within cell of a living host
- **Fungi**
  - Lack chlorophyll
  - Yeast, molds, mushrooms
Good Bugs vs. Bad Bugs

- Normal Flora
  - mix of bacteria found on skin and mucosa
- Transient flora
  - “temporary” bacteria
- Pathogens
  - cause disease

Normal Flora

- Skin
  - Staphylococcus species
  - Diptheroids
  - Yeast
- Mouth
  - Streptococcus species
  - Staphylococcus
- GI tract
  - E. coli
  - Enterococcus

Don’t let normal flora fool you!

Normal flora can:
- Cause disease, especially in immunocompromised patients
- Easily overgrow and interfere with ID of pathogens
- Turn into a pathogen!
Sterile Sites

Normal flora NOT in these sterile sites:

- CSF
- ORGANS
- BILE
- BLOOD
- URINE
- MUSCLE AND CONNECTIVE TISSUE
- JOINTS

Poll the Audience - #1

Sources of Bugs:

- EXOGENOUS: outside the host
- ENDOGENOUS: host own flora
Sick with a Bug

Disease risk factors
- Virulence or bacterial strain
- Inoculum size

Infectious disease process
- Colonization precedes infection
- Two studies in 2008 showed MRSA colonization was associated with a strong increase risk of infection

Fundamentals of Specimen Collection

Garbage in = Garbage out!
1. Appropriate culture ordered
2. Collection
   - Device, aseptic technique, container
   - Quantity and quality
3. Optimal timing/transport
4. Antibiotic interference
5. Appropriate labeling

Poll the Audience - #2
Specimen Quality

Aspirate Swab

What happens after collection?
1. Gram stain
2. Set up culture
3. Incubate
4. Identify
5. Antimicrobial testing

1. Gram Stain
• Most useful stain in Microbiology
• Based on cell wall composition
1. Gram Stain Basics

Distinguishes morphology (shape, color, and grouping)

3 Major Shapes
• Cocci
• Bacilli
• Spiral

1. Gram Stain: What does it tell us?

• Indicates the quality of the specimen
• Can guide further lab testing
• Give presumptive diagnosis
• Guide empiric treatment
• Aid in infection prevention strategies

Poll the Audience - #3
1. Gram Stain: Sputum

Know your lab reporting format for quantifying bacteria and thresholds/rejection criteria for sputums.

重点：VAE 监测！

1. Gram Stain Examples

- **Gram positive cocci in clusters:**
  - *Staphylococcus* species

- **Gram positive cocci in pairs and chains:**
  - *Streptococcus* species
  - *Enterococcus* species

1. Gram Stain Examples, cont.

- **Gram positive rods:**
  - *Bacillus* species
  - *Clostridium* species

- **Gram negative rods:**
  - *E. coli*
  - *Klebsiella pneumoniae*
1. Gram Stain Examples, cont.

**Gram negative diplococci:**  
• Neisseria species

**Gram negative coccobacilli:**  
• *Haemophilus influenza*

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1. Other direct specimen stains

*Acid fast stain*  
• Guides criteria for airborne isolation for TB

*Direct fluorescent stain*  
• Calcofluor (fungus)  
• Legionella  
• Viruses  
• PNA-FISH

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2. Set up culture

For Optimal growth,  
**Bacteria need:**

*Proper media*  
• Nutrient  
• Enrichment  
• Selective  
• Differential
2. Set up culture

For Optimal growth, bacteria need:

- Specific temperatures
  - Body temp 35-37°C
- Proper atmosphere
  - Aerobic
  - Anaerobic
  - Microaerophilic

3. Incubate (wait)

TIME: Bacterial cultures require 18-24 hours to 'grow' before preliminary results can be available

- Cannot 'grow' STAT

4. Identify bacteria

- Colony morphology
- Stain characteristics
- Oxygen utilization
- Enzymatic tests and sugar fermentations
- Automated methods
4. Identify bacteria
Technology geared to more rapid results
• MALDI-TOF – molecular fingerprint

5. Antimicrobial Susceptibility Testing
GOAL: predict in-vivo success or failure of antibiotic
• Tests are performed in-vitro
• Results guide antibiotic therapy
• Combined with clinical information and experience
• Raw data is either zone size or an MIC (Minimum inhibitory concentration)
• Not ALL identified organisms warrant testing

GOOD
Bug not growing around antibiotic disk

BAD!
Bug growing up to antibiotic disk
5. Antimicrobial Susceptibility Testing

- Most testing is automated
- MDROs, fastidious organisms or unusual (mucoid, dry, chunky) may need alternative testing performed
- Results reported as:
  - S – Susceptible
  - I – Intermediate
  - R – Resistant

Poll the Audience - #4

5. Other: Antibiograms

- Information on antimicrobial classes
  - Most used – guide empiric treatment
  - Most misused – evaluate effectiveness of ASP
- Monitors trends in drug resistance
- Pharmacy supply
- Published annually
**Bugs of Interest to IP**

<table>
<thead>
<tr>
<th>Gram Positive</th>
<th>Gram Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus species</strong></td>
<td><strong>Enterics</strong></td>
</tr>
<tr>
<td><strong>Streptococcus species</strong></td>
<td><strong>E coli</strong></td>
</tr>
<tr>
<td><strong>Enterococcus species</strong></td>
<td><strong>Klebsiella pneumoniae</strong></td>
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<tr>
<td><strong>Listeria species</strong></td>
<td><strong>Proteus species</strong></td>
</tr>
<tr>
<td><strong>Clostridium species</strong></td>
<td><strong>Serratia marcescens</strong></td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td><strong>Pseudomonas aeruginosa</strong></td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td><strong>Acinetobacter baumanii</strong></td>
</tr>
<tr>
<td><strong>Proteus species</strong></td>
<td><strong>Neisseria meningitidis</strong></td>
</tr>
<tr>
<td><strong>Serratia marcescens</strong></td>
<td><strong>Legionella pneumophila</strong></td>
</tr>
<tr>
<td><strong>Non-fermenters</strong></td>
<td><strong>Haemophilus influenzae</strong></td>
</tr>
</tbody>
</table>

**Bugs of Interest: Gram Positive**

**Staphylococcus**

(gram positive cocci in clusters)

Two major species

- **S. aureus** — coagulase positive, can be normal flora or pathogenic, commonly associated with bloodstream and skin and soft tissue infections, MRSA
- **S. epidermidis** or coagulase negative staph — normal flora, common skin contaminant, but most common pathogen for CLABSI

**Bugs of Interest: Gram Positive**

**Streptococcus**

(gram positive cocci in pairs or chains)

- **S. pneumoniae** — "pneumococcus", cause pneumonia, sepsis, or meningitis,
- **S. pyogenes** — "Group A", cause pharyngitis, necrotizing fasciitis, sepsis
- **S. agalactiae** — "Group B", cause neonatal sepsis, meningitis, pneumonia, urogenital infections
**Bugs of Interest: Gram Positive**

- **Enterococcus species** – gram positive coccii in pairs or chains, in GI tract, cause UTIs, BSIs, and intra-abd infections, VRE

- **Listeria monocytogenes** – gram positive rod, in soil, cause perinatal listeriosis, meningitis

- **Clostridium difficile** – gram positive spore-forming rod, intestinal tract, pseudomembranous colitis, exotoxins

**Bugs of Interest: Gram Negative**

- **Escherichia coli** – enteric, gram negative rod, normal flora in GI tract, cause UTI, septicemia, neonatal meningitis, GI infections

- **Klebsiella pneumoniae** – enteric, gram negative rod, normal flora in GI tract, cause pneumonia, UTI, wound infections

- **Proteus species** – enteric, gram negative rod, normal flora in GI tract, cause respiratory or urinary tract, sepsis, wound infections

**Bugs of Interest: Gram Negative**

- **Serratia marcescens** – enteric, gram negative rod, normal flora in GI tract, cause pneumonia, UTI, and multidose vials outbreaks

- **Pseudomonas aeruginosa** – enteric, gram negative rod, in environment/water, variety of infections, antibiotic resistance

- **Acinetobacter baumanii** – gram negative rod, causes pneumonia, UTI, wounds, and septicemia, may be highly resistant
Bugs of Interest: Gram Negative

- *Neisseria meningitidis* — gram negative diplococcus, normal flora of oro/nasal pharynx, causes meningitis, pneumonia
- *Legionella pneumophila* — gram negative rod, associated with water, cause pneumonia, hard to grow!
- *Haemophilus influenzae* — gram negative cocccobacilli, normal flora of upper respiratory tract, cause meningitis, otitis media, usually found in pediatrics

Resources

http://labproject.site.apic.org/
http://www.asm.org/

Case Study #1
In Summary

1. Develop relationship with microbiology lab
2. Find out how your lab tests, reports, and communicates results
3. Know your resources – references or call the lab!!

Questions? Thank you!!