Introduction to the Clinical Microbiology Laboratory

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At the conclusion of this program, you will be able to:

• Describe the **primary role** of a clinical microbiology laboratory with a focus on bacteriology.

• Explain how **improperly collected** specimens can contribute to misleading results.

• List examples where **bacteria reported** may **NOT** be contributing to an infection.

• Discuss tests used to determine if a bacterium is **susceptible or resistant to an antimicrobial agent**.

• Describe a **cumulative antibiogram** and how this report can be used to guide empiric therapy and monitor % of bacteria susceptible (%S) to specific antimicrobial agents.
**Scenario:** Sick patient in the hospital

**Physician**
- Physician sends a specimen to the clinical microbiology lab
- What does he/she want to know?
  - Does the specimen contain pathogens?
    - What type?
    - How many?
  - What antimicrobials can I use to treat this patient?

**Infection Prevention**
- Reviews microbiology laboratory reports
- What does he/she want to know?
- Could the pathogens isolated have been acquired while the patient was in this facility?
- What can be done to prevent the spread of the pathogens?
What is Clinical Microbiology?

• **Function** of the clinical microbiology laboratory:

• **Clinical**: diagnosis and management of infections

• **Epidemiological**: understand infectious microbes in patients and populations, and to find sources and routes of transmission necessary for prevention efforts

• **General rules in clinical microbiology:**
  • **#1**: Positive cultures do not make an infection
  • **#2**: No lab test is 100% accurate
Who are clinical microbiologists?

- Bachelor’s degree that includes 2 years of specialized training in clinical laboratory sciences
- **CLS**: clinical laboratory scientist
- **MLT**: medical laboratory technician
- **Ph.D.**: perform research and development
Where are Clinical Microbiologists?

- Huntington Memorial Hospital
- **Pasadena, CA**
- Tertiary care, non-academic
- 619 beds
- 6 outpatient clinics
- 1 urgent care
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  - **#2**: No lab test is 100% accurate
Specimen collection

• Proper specimen collection is one of the most important factors in diagnosing infection

• Follow instructions for collecting and transporting specimens for microbiology tests

• The best specimens are tissue, aspirate, pus, body fluid

• Swabs are low yield and prone to contamination
Challenge: Microbiome

- Microorganisms that live on and within our bodies
- AKA “normal flora”
- Trillions of cells!
- 1000s of species!
- Both beneficial and potentially harmful

**Challenge:** differentiating normal flora from pathogens
Specimen processing

Biosafety Cabinet (BSC)

Direct Gram stain

Culture
Gram stain

• Han Christian Gram: 1884
• Method of classifying bacteria into 2 large groups: Gram positive (+) and Gram negative (-)
• Differentiates bacteria by the chemical and physical properties of their cell walls
• Helpful in guiding initial empiric therapy
• Reported to physicians ASAP
Perform **Direct Gram stain** for bacteria

- Report results within a few hours
- Quick insight into possible causes of an infection
Gram stain reactions for select bacteria

**Gram positive**
- Staphylococcus
- Streptococcus

Cocci = spheres
Cocci in clusters
Cocci in chains

**Gram negative**
- E. coli
- Klebsiella
- Pseudomonas
- Neisseria
- Rods

Also detects markers of infection: White Blood Cells
Direct Gram stain of pus from a wound

Gram-positive cocci in clusters
White blood cells

Staphylococci
Direct Gram stain of urethral discharge

Gram-negative cocci within white blood cells

Gonorrhoeae
Culturing bacteria

Incubator: human body temperature

Should I identify these bacteria and perform antimicrobial susceptibility tests?
Criteria for identification

• Should I identify these bacteria and perform antimicrobial susceptibility tests?
• Body site
• Pure versus mixed culture
• Pathogen versus normal flora
Methods Used to Identify Bacteria

Traditional methods:
• Gram stain and microscopic exam
• Growth rate and colony appearance on various types of agar media
• Reactivity with various chemicals/reagents

Modern methods:
• DNA/RNA content of microorganisms
• Protein profile (MALDI-TOF MS) of microorganisms
Case 1

• 85 year old male
• He has been sick for 3 days
• Getting progressively worse
  • Shortness of breath
  • Fever, chills, sweats, productive cough
• Temperature of 102°F
• Pneumonia
  • Sputum cultures
  • Blood cultures

10 ml sputum in Luken’s trap
Avoid collecting saliva

Blood culture
Common Lower Respiratory Tract Pathogens

- **Community-acquired pneumonia (CAP)**
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Moraxella catarrhalis*
  - “Atypicals” – *Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila*
    - Often more difficult to recover / identify
- **Hospital-acquired pneumonia (HAP):** Most often ICU or ventilator-associated
  - *Klebsiella pneumoniae*
  - *Pseudomonas aeruginosa*
- Either CAP or HAP
  - *Staphylococcus aureus*
    - MRSA or MSSA
Assessing Sputum Specimen Quality

- If saliva instead of sputum is collected, we may not recover the “true pathogens”
- Prepare a direct Gram stain
- Count the number of squamous epithelial cells (SEC)

<table>
<thead>
<tr>
<th># SEC/low power field</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>No significant oral contamination</td>
</tr>
<tr>
<td>≥ 10</td>
<td>Indicates poorly collected specimen</td>
</tr>
</tbody>
</table>
Direct Gram Stain Results

• Report:
  • Many WBCs
  • Many **Gram-positive** cocci in clusters
  • Moderate normal oral flora

• Physicians:
  • Staphylococcus!
When *Staphylococcus* is suspected...

- Many different species of Staphylococci
  - Pathogenicity
  - Antimicrobial susceptibility profiles
- Questions:
  - Is this *Staphylococcus aureus*?
    - If yes, is this methicillin-resistant *S. aureus* (MRSA) or methicillin-susceptible *S. aureus* (MSSA)?
  - Is this another species of *Staphylococcus*?
    - Typically lumped into “*coagulase-negative Staphylococci*” (CoNS)
    - Often contaminant; less clinically significant than MRSA or MSSA
Treatment of *S. aureus* infections

MSSA

Oxacillin* or Nafcillin*

MRSA

Vancomycin

*Methicillin very similar but no longer available*
Blood Culture

- Blood culture bottles
  - **Blue**: Aerobic bottle
  - **Purple**: Anaerobic bottle
  - **Pink**: Pediatric bottle

- Contain:
  - SPS
    - Anticoagulant
    - Antiphagocytic
  - Media
  - Resin
Blood Culture: Collection

• Proper collection site preparation is important for avoiding contamination by normal flora

• Collection sites:
  • Peripheral or IV catheter
  • If drawn from IV catheter, also draw from peripheral site

• Disinfect bottle tops with an alcohol wipe

• Disinfect puncture site using ChloroPrep
  • Scrub for 30 seconds
Blood Culture: Volume

- **Volume** is the most critical factor in pathogen recovery
- **Adults:**
  - Collect 2-3 blood culture sets per episode prior to antibiotic treatment
  - Order a minimum of 2 sets
  - Aerobic and anaerobic bottles should be drawn as a set
  - Optimum: 16-20 ml/set, 8-10 ml/bottle
  - Inoculate aerobic bottle first
  - Do not order more than 3 sets in a 24-hour period
Bloodstream infections

• Any organism present in blood is significant except **blood culture contaminants**
• Typically found in 1/2 blood culture bottles
  • Coagulase-negative staphylococci (CoNS)
  • Diphtheroids (Corynebacteria)
  • *Bacillus* spp.
  • *Propionibacterium* spp.
  • Viridans streptococcus
  • *Micrococcus* spp.
• True infection:
  • 2 sets of blood cultures must be positive
  • Patient shows signs and symptoms of a bloodstream infection
Automated blood culture

• Blood culture bottles are placed in automated blood culture instruments
• If bacteria are present, they multiply and produce CO2
• The machine detects CO2 and sounds an alarm when it reaches a certain threshold
Positive blood culture bottle work-up

Gram stain and culture

Gram stain: \textbf{Gram-positive} cocci in clusters
Blood “Traditional” Culture Workup (1)

1. **Pos Blood Culture**
   - **15 m**

2. **Gram Stain**
   - **16-20 h**

3. **Sheep’s Blood Agar Medium**
   - *Staphylococcus* spp.

4. **Coagulase Test**
   - **neg**
   - **pos**
Blood “Molecular” Culture Workup (2)

Pos Blood Culture

15 m

Gram Stain

1-2 h

GPCC+

Molecular Assay Results: MSSA or MRSA or CoNS

www.bd.com/geneohm
Final report

Gram Stain:  
**Gram-positive** cocci in clusters

Culture:  
*Staphylococcus aureus* (MRSA)  
- Clindamycin: **R**  
- Daptomycin: **S**  
- Linezolid: **S**  
- Oxacillin: **R**  
- Vancomycin: **S**

“MRSA isolated. Please check infection control policies.”
Case #2

- 25 year old female
- Presents with dysuria (painful urination)
- Frequency of urination
- Urinary tract infection (UTI)
- Challenge: pathogens from normal flora
Most common UTI pathogens

- **Community acquired**
  - Most common: *E. coli*
  - *Klebsiella*, other Enterobacteriaceae
  - *Staphylococcus saprophyticus*

- **Hospital acquired**
  - *E. coli*, *Klebsiella*, other Enterobacteriaceae
  - *Pseudomonas aeruginosa*
  - Enterococci
  - Staphylococci

Spot indole test

Positive

![Spot indole test](image1)

![Positive](image2)

*E. coli*
Urine collection and transport

• Must test within 2 hours of collection if stored at room temperature
• Must test with 24 hours if refrigerated
• Must test within 2 days if in boric acid preservative ("Grey top")
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Infection Prevention: Surveillance Cultures

- Different than diagnostic cultures
- Must order as “surveillance culture”
- Must send appropriate specimen
- Only tested for “targeted” pathogen
  - **MRSA**: nares swab
  - **Rectal swab**: carbapenem resistant *Enterobacteriaceae* (CRE)
Tests to Detect Antimicrobial Susceptibility
Antimicrobial Susceptibility Testing (AST)

Disk diffusion (Kirby Bauer)

Broth Microdilution MIC

**MIC = minimal inhibitory concentration (lowest concentration of drug that inhibits growth of the test bacteria)**

**Reported results:**
- **Susceptible (S)** – drug likely to work providing it can get to the infection site
- **Resistant (R)** – drug won’t work
- **Intermediate (I)** – drug may or may not work depending on site of infection and patient’s status
Disk Diffusion (Kirby Bauer)

- Pick colonies
- Prepare inoculum suspension
- Swab plate
- Add disks
- Incubate overnight
Measure zone sizes

Larger zone of clearing = more susceptible
### Zone Diameter “Breakpoints” (mm)

<table>
<thead>
<tr>
<th>Drug</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≥21</td>
<td>16-20</td>
<td>≤15</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥15</td>
<td>13-14</td>
<td>≤12</td>
</tr>
</tbody>
</table>
MIC Testing

MIC “Breakpoints” (µg/ml)

<table>
<thead>
<tr>
<th>Drug</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>2</td>
<td>≥4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
</tbody>
</table>
Commercial Antimicrobial Susceptibility Test Systems

- Etest
- Phoenix
- Vitek 2
- MicroScan
- Sensititre
Antimicrobial susceptibility testing (AST)

• Criteria on when to perform:
  • If 1 or 2 potential pathogens is isolated form culture
  • If it is likely that the bacteria are causing an infection
  • If bacteria have a susceptibility pattern that is unpredictable
Urine culture

• Report 1
  • > $10^5$ CFU/ml *E. coli*

Perform AST
  Significant quantity of potential pathogen
  *E. coli* is a common UTI pathogen
  No contaminants

• Report 2
  • > $10^5$ CFU/ml *Corynebacterium*
  • 40,000 CFU/ml *E. coli*
  • 10,000 CFU/ml yeast
  • 40,000 CFU/ml *Lactobacillus*

Do not perform AST
  Contamination likely: mixed
  Encourage re-collection if UTI is still suspected
Sputum culture

- Gram stain:
  - Many oral flora
  - Many Gram positive diplococci
  - Many WBCs

- Culture:
  - Many normal flora
  - Many *Streptococcus pneumoniae*

**Perform AST**

Good correlation of Gram stain with culture
Significant quantitate of a potential pathogen
Throat culture

• Culture
  • Many Group A Streptococcus

• Do not perform AST routinely
  • Group A Streptococcus is always susceptible to penicillin
  • Penicillin allergy

• Not necessary to perform AST on bacteria that are always (predictably) susceptible to the antimicrobial agents typically prescribed
AST on all organisms

- Why do we NOT perform AST on every potential pathogen isolated?
  - AST results on a report suggest that bacteria are causing an infection
  - Report results when NOT needed may lead to:
    - Unnecessary or inappropriate therapy
    - Selection of resistant bacteria
    - Patients on antibiotics are at higher risk for *Clostridium difficile* infections
    - Failure to look further to identify the true cause of the patient’s symptoms
**AST: Infection Prevention**

Review of **S, I, R** most important for IP

For MIC tests, must report S, I, R with or without MIC value.

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>Morganella morganii (iso)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC (MCG/ML)</strong></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>R</td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>&lt;=1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime/Avibactam</td>
<td></td>
</tr>
<tr>
<td>Ceftolozane/Tazobactam</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;=4</td>
</tr>
<tr>
<td>Colistin</td>
<td>R</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&lt;=0.5</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>I ²</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&lt;=1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>64</td>
</tr>
<tr>
<td>Oral Cephalosporins</td>
<td>I</td>
</tr>
<tr>
<td>Piperacillin + Tazobactam</td>
<td>&lt;=4</td>
</tr>
<tr>
<td>Tobramycin</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>&gt;=320</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
</tbody>
</table>
## Two *E. coli* urine isolates

<table>
<thead>
<tr>
<th>Agent</th>
<th>#1</th>
<th>#2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Ertapenem</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Pip-tazo</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

Note: Broad Spectrum drug results suppressed when “S” to narrow spectrum drugs

Isolate 1: “Typical” *E. coli* - NO “R”!

Isolate 2: Acquired “R” to all PO agents. Request *fosfomycin* – usually not tested routinely!
3 more *E. coli* isolates

Potential outbreak?

<table>
<thead>
<tr>
<th>Agent</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Ertapenem</td>
<td></td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>R</td>
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<tr>
<td>Nitrofurantoin</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Piper-tazo</td>
<td></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

CRE = carbapenem-resistant Enterobacteriaceae

CRE = R to doripenem, ertapenem, imipenem OR meropenem
The Cumulative Antibioticogram Report

• Analyzes data from routine antimicrobial susceptibility tests performed in the clinical laboratory
• Separate report prepared for each healthcare facility
• Primarily used to guide empiric therapy
• Sometimes used to monitor resistance
  • Changes in %S from year to year
Recommendations: Preparation of Cumulative Antibiogram

• Analyze/present data at least annually
• Include only species with $\geq 30$ isolates of each species
• Include diagnostic isolates
• Include the 1st isolate/patient; no duplicate patient isolates

Note: Often difficult to get 30 isolates in LTCFs
<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>No.</th>
<th>% Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumanii</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>62</td>
<td>100</td>
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<tr>
<td>Enterobacter aerogenes</td>
<td>63</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>131</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia coli (all)</td>
<td>1366</td>
<td>100</td>
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<tr>
<td>Klebsiella oxytoca</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>484</td>
<td>100</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>310</td>
<td>100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (all)</td>
<td>598</td>
<td>99</td>
</tr>
<tr>
<td>Urine isolates</td>
<td>993</td>
<td>100</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>484</td>
<td>99</td>
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<tr>
<td>Morganella morganii</td>
<td>73</td>
<td>98</td>
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<tr>
<td>Proteus mirabilis</td>
<td>310</td>
<td>99</td>
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<tr>
<td>Pseudomonas aeruginosa (all)</td>
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<td>99</td>
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<tr>
<td>Blood</td>
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<td>Respiratory</td>
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<td>97</td>
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<td>Body Fl/Wound/Tissue</td>
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<td>95</td>
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<td>Serratia marcescens</td>
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<td>Stenotrophomonas maltophilia</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>E.coli, ESBL (all)</td>
<td>354</td>
<td>100</td>
</tr>
<tr>
<td>Urine</td>
<td>264</td>
<td>100</td>
</tr>
<tr>
<td>K. pneumonia, ESBL (all)</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Urine</td>
<td>38</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th># total isolates tested</th>
<th>Aminoglycoside</th>
<th>βL</th>
<th>βL/βLi</th>
<th>Ampicillin/amoxicillin</th>
<th>Piperacillin/kazobactam</th>
<th>Cefazolin</th>
<th>Ceftazidime</th>
<th>Ceftiofur</th>
<th>Ceftazidime</th>
<th>Ceftazidime</th>
<th>Ciprofloxacin</th>
<th>Ertapenem</th>
<th>Meropenem</th>
<th>Trimmethoprim-Sulfamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>42</td>
<td>31</td>
<td>33</td>
<td>0</td>
<td>33</td>
<td>73</td>
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<td>-</td>
<td>37</td>
<td>29</td>
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<td>36</td>
<td>50</td>
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<tr>
<td>Gentamicin</td>
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Summary

• Assessment of patient’s clinical symptoms together with reliable clinical microbiology laboratory results are essential for accurate diagnosis of infections.

• Reliable clinical microbiology laboratory results are dependent on:
  • Appropriate collection and transport of specimens.
  • Accurate identification and antimicrobial susceptibility testing.
  • Good communication between healthcare providers and lab.

• Review of clinical microbiology laboratory results is key to identification of potential hospital transmission of microbes.

• Additional clinical microbiology laboratory tests may be needed for epidemiological investigations.

• A local cumulative antibiogram can help guide empiric therapy decisions and monitor “%S” for antimicrobial agents appropriate for common pathogens.
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