Laboratory Diagnosis of Respiratory Tract

Mohammad Rahbar
Associate Professor
Iranian Reference Health Laboratory
Anatomy of the upper respiratory tract

- Specimens
  - Oropharynx (throat)
  - Nasopharynx
  - Epiglottis
  - Nose

- Endogenous flora
  - Various aerobic and anaerobic organisms including some that are agents of respiratory tract infection
Respiratory Specimens

- Upper respiratory tract specimens
  - Throat
    - Detection of streptococcal pharyngitis
Respiratory Specimens

- Upper respiratory tract specimens
  - Nose
    - detection of MRSA carriers
  - Nasopharyngeal swabs
    - diagnosis of *Bordetella pertussis*
  - Nasopharyngeal swabs and washings
    - diagnosis of viral disease
Direct antigen testing

- Detects group A antigen in exudate
- Sensitivity varies with kit
- Tend to miss low numbers
- Culture follow up essential for pediatric patients
- Culture follow up at MD’s discretion for adult patients
Streptococcus pyogenes

- 15-35% of bacterial pharyngitis
  - 30% in children
  - 5-10% in adults
- Nonsuppurative sequelae
  - Acute rheumatic fever
  - Acute glomerulonephritis
- Suppurative sequelae
  - Peritonsillar abscess
## Culture methods

<table>
<thead>
<tr>
<th>Medium</th>
<th>Method</th>
</tr>
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<tbody>
<tr>
<td>Sheep blood agar</td>
<td>Aerobic</td>
</tr>
<tr>
<td>with stabs</td>
<td></td>
</tr>
<tr>
<td>Sheep blood agar</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Sheep blood agar</td>
<td>5-10% CO$_2$ or anaerobic</td>
</tr>
<tr>
<td>with TMP/SMX</td>
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</tbody>
</table>

Examine for β-hemolytic colonies at 24 and 48 hr.
Identification

- Large-colony Group A (\textit{S. pyogenes}) (>0.5 mm)
  - PYR positive (as is \textit{Enterococcus})
  - Bacitracin susceptibility (0.04U)
    - Sensitivity 99.6\%, Specificity 85\%, i.e., presumptive
    - Confirm with Group A antigen detection

- Large-colony Group C & G
  - MUG positive (4-methyl-umbelliferyl- \textit{β}-D-glucuronide)

- Minute colony \textit{β}-streptococci
  - \textit{Streptococcus anginosus} - not agents of pharyngitis
  - Group A – PYR negative
  - Groups C, G, F, nongroupable (MUG negative)
Arcanobacterium hemolyticum

- Formerly Corynebacterium hemolyticum
  - Catalase negative
- Pharyngitis in teens and young adults (10 – 20 y/o)
  - Rash (like scarlet fever), no RHD or AGN
  - Invasive disease occurs
- May respond poorly to penicillin

Culture
- Best: CO₂ 48 hr, ppt, β-hemolytic, black dot in center
  - Anaerobically: slower growth
- If β-hemolytic colonies not A,C or G, Gram stain
  - If gram-positive rods, do API CORYNE
Pertussis

- *Bordetella pertussis*
- Nasopharyngeal specimen
- PCR >> DFA > culture (direct inoculation)
  - Some PCR detect *B. pertussis/parapertussis*
  - DFA and culture no longer recommended for diagnosis
Epiglottitis

- Inflammation & edema of epiglottis
- Medical emergency
- Usually due to *Haemophilus influenzae* type b
  - Blood cultures
  - Swab epiglottis for culture only after artificial airway established
- Include chocolate agar
“The culture of lower respiratory specimens may result in more unnecessary microbiologic effort than any other type of specimen.”
Raymond C Bartlett
Lower Respiratory Tract Infections

Epidemiology

- Pneumonia is the sixth leading cause of death in US
- Increasing numbers of patients at risk
  - Aging population
  - Increase in patients with immunocompromising conditions
- Overtreatment has lead to resistance
  - Multidrug resistant *Streptococcus pneumoniae*
  - Resistance among hospital acquired pathogens such as *Acinetobacter, Pseudomonas aeruginosa* and others
Lower Respiratory Tract Infections

- Major sections
  - Clinical aspects of diseases of LRT
  - Specimen collection
  - Specimen processing
  - Interpretation of bacterial cultures
  - Most common pathogens
Categories of Lower Respiratory Tract Infections

- Acute bronchitis
- Community acquired pneumonia
- Hospital acquired pneumonia
- Pneumonia in the immunocompromised host
### Community Acquired Pneumonia

#### Etiologic Agents

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>66</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>1-12</td>
</tr>
<tr>
<td><em>M catarrhalis</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Legionella</em> species</td>
<td>2-15</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>2-14</td>
</tr>
<tr>
<td><em>Klebsiella</em> species</td>
<td>3-14</td>
</tr>
<tr>
<td>Enteric gram negative bacilli</td>
<td>6-9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3-14</td>
</tr>
<tr>
<td><em>Chlamydia</em> species</td>
<td>5-15</td>
</tr>
<tr>
<td>Influenza viruses</td>
<td>5-12</td>
</tr>
<tr>
<td>Other viruses</td>
<td>&lt;1-12</td>
</tr>
<tr>
<td>Unknown</td>
<td>23-49</td>
</tr>
</tbody>
</table>

Community Acquired Pneumonia Diagnosis

Available Test Methodologies

- Sputum Gram stain and culture
- Blood cultures
- Serologic studies
- Antigen detection tests
- Nucleic acid amplification tests
Sputum Gram Stain and Culture

Proponents
- Demonstration of predominant morphotype on Gram stain guides therapy
- Accuracy is good when strict criteria are used
- Cheap, so why not?

Antagonists
- Poor specimen collection
- Intralaboratory variability (Gram stain interpretation)
- Low sensitivity and specificity
Sputum Collection

- Proper patient instruction
  - Food should not have been ingested for 1-2 h prior to expectoration
  - The mouth should be rinsed with saline or water
  - Patient should breathe and cough deeply
  - Patient should expectorate into a sterile container

- Transport container immediately to lab

- Perform Gram stain and plant specimen as soon as possible
Sputum Gram Stain

- Screen for acceptability
  - Examine specimen under low power (x 10 objective)
  - Examine 10 representative fields
  - Specimens that show few squamous epithelial cells (< 10/lpf) and many PMNs (> 25/lpf) are acceptable
  - Notify physician of unacceptable samples
Sputum Gram Stain
Unacceptable
Contamination with oral flora
Sputum Gram Stain
Good Quality
Sputum Gram Stain
Good Quality
Sputum Gram Stain

Good quality specimens

- Quantify number and types of inflammatory cells
- Note presence of bronchial epithelial cells
- Concentrate on areas with WBCs when looking for organisms
- Determine if there is a predominant organism (>10 per oil immersion field)
  - Semiquantitate and report organism with descriptive
  - If no predominant organism is present, report “mixed gram positive and gram negative flora”
Utility of the Gram Stain in Diagnosis of Pneumonia


- Prospective study
- Non immunocompromised patients hospitalized with CAP
- 1,000 bed hospital in Spain
- ER physicians instructed on sputum collection for Gram stain and culture

- Sputum collected **under supervision** of nurse or resident
  - Samples were processed immediately
  - Screened for epithelial cells
  - Screened for predominant morphotype (> 75% of the organisms seen)
  - Sputum planted to blood agar, chocolate agar and MacConkey agar

- Strictly defined clinical and diagnostic parameters
Utility of the Gram Stain in Diagnosis of Pneumonia


Results

- 190/533 (35.6%) patients had no sputum sample submitted (these patients were included in the calculations)
- 133/533 (25%) patients had a poor quality specimen
- 210/533 (39.4%) patients had a good quality specimen
- Overall sensitivity and specificity for pneumococcal pneumonia: 57% and 97%
- Overall sensitivity and specificity for H. influenzae pneumonia: 82 % and 99%
- Gram stain gave presumptive diagnosis in 80% of patients who had a good specimen submitted
- > 95% of patients in whom a predominant morphotype was seen on Gram stain received monotherapy
Gram Stain Reports

- Be as descriptive as possible
  - Moderate neutrophils
  - Moderate Gram positive diplococci suggestive of *Streptococcus pneumoniae*
  - Few bacteria suggestive of oral flora

- Keep report short—avoid line listing of all morphotypes present
Sputum and Endotracheal Suction Culture Evaluation

- Identify and perform susceptibility testing on 2-3 potential pathogens seen as predominant on Gram stain
- Alpha strep—rule out *S. pneumoniae*
- Yeast—rule out *Cryptococcus neoformans* only
- *S. aureus*, Gram negative bacilli
  - < normal flora, quantify and limit ID; no susceptibility
  - Add comment that organism not predominant on stain
- ID mould, Mycobacteria or *Nocardia spp.*

Diagnostic Tests for CAP

- **Outpatients**
  - Empiric therapy with a macrolide, doxycycline, or a fluoroquinolone

- **Hospitalized patients with CAP**
  - Gram stain and culture of sputum
  - 2 pretreatment blood cultures
  - Studies for Mtb, Legionella in select patients

- **Rationale**
  - To improve patient care
  - Advance knowledge of epidemiologically important organisms
  - Prevent antibiotic abuse
  - Reduce antibiotic expense

ATS Guidelines
Diagnostic Tests for CAP

- Empiric therapy for outpatients
  - Macrolide or tetracycline

- Hospitalized patients with CAP
  - 2 sets of pre-treatment blood cultures
  - Pleural fluid Gram stain/culture when appropriate
  - Studies for Legionella, Mtb, fungi in select patients
  - Sputum Gram stain/culture only if resistant or unusual pathogen is suspected
  - Avoid extensive testing

Hospital Acquired Pneumonia

- Most frequent nosocomial infection (30-33% of cases) among combined medical surgical intensive care units
- 83% are ventilator associated
- Etiologic agents
  - **Gram positive cocci**
    - *S. aureus* 17
    - *S. pneumoniae* 2-20
  - **Aerobic gram-neg bacilli**
    - *Pseudomonas aeruginosa* 60
    - *Enterobacter sp.*
    - *Klebsiella pneumoniae*
    - *Acinetobacter*
    - *Legionella*
  - **Anaerobes**
    - 10-20
  - **Fungi**
    - 0-10

Hospital Acquired Pneumonia Diagnosis

- American College of Chest Physicians: Clinical findings are not sufficient for definitive diagnosis
- Qualitative culture or endotracheal sputum has poor predictive value
- Bronchoscopy is recommended by many pulmonologists
  - Bronchial brushings
  - Bronchial washes
  - Protected specimen brushing
  - Bronchoalveolar lavage specimens (BAL)
  - Transbronchial biopsy
Respiratory Specimens

- Protected Brush Specimen
  - To procure uncontaminated lower airway secretions
  - Brush within 2 catheters
Respiratory Specimens

- **Bronchoalveolar Lavage (BAL)**
  - Samples large area of the lung
  - Performed using a bronchoscope
  - 100 to 250 ml of saline injected
  - Injected saline along with secretions is collected by aspiration

- **Transthoracic Aspiration**
  - Involves percutaneous introduction of a needle directly into the infiltrate
Bronchoalveolar Lavage (BAL) Specimen Acceptability

- Microscopic examination of Gram-stained smear
  - Acceptable
    - <1% of cells present are squamous epithelial cells
  - Unacceptable
    - >1% of cells present are squamous epithelial cells

Processing Bronchoscopy Specimens

- Bronchoscopy brush protected
  - Aerobic bacterial culture and Gram stain
  - Anaerobic bacterial culture
  - Limited volume

- Bronchoscopy brush, unprotected
  - No anaerobic culture
  - Limited volume

- Bronchial washings
  - Useful only for pneumonia caused by strict pathogens
  - Reasonable requests: Mtb, Fungi, *Legionella*, *Pneumocystis*

- Bronchoalveolar lavage
  - No anaerobe culture
  - Amenable to extensive testing for all opportunistic pathogens
Interpretation of Quantitative PSB/BAL

- Dilution Method
  - Quantify each morphotype present and express as CFU/ml

- Calibrated Loop Method
  - Quantify each morphotype present and express as $\log_{10}$ colony count ranges

- Thresholds for significance
  - PSB > $10^3$ CFU/ml
  - BAL > $10^4$ CFU/ml

Bronchoscopy Samples
Quantitative Methods

vortex 30-60 s  Final dilutions

Plate 0.1 ml  

Chocolate, blood  1:10

Dilute
0.1 ml to 9.9 ml saline

Plate 0.1 ml  Chocolate
blood  1:1000

Dilute 0.1 ml to 9.9 ml saline

Plate 0.1 ml  Chocolate
blood  1:100,000
Bronchoscopy Samples
Quantitative Methods

Calibrated loop method


PSB

vortex 30-60 s

BAL

Plate 0.1 ml

Plate 0.01 ml

Plate 0.001 ml

Chocolate

Chocolate

Chocolate

Final Dilutions

1:10

1:100

1:1000
Immunocompromised Patients
Suggested BAL Protocol

- Aerobic Gram stain quantitative bacterial culture
- Fungal stain and culture
- Mycobacterial stain and culture
- Viral culture/Respiratory DFA
- Pneumocystis DFA
- Legionella culture