Laboratory Diagnosis of Bacterial Infections of the Respiratory Tract

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Biological Safety Cabinet

- Process specimens in **biological safety cabinet**, as aerosols can result in laboratory-acquired respiratory infections

- Droplet contact, for example, inhalation of droplets (>5 micrometer in diameter) that cannot travel more than 3 feet / 1 meter
Normal Microbiota

- Normal microbiota / flora of the nasopharynx and oropharynx help prevent colonization of the upper respiratory tract
Pharyngitis

Table 54-1  Bacteria That Can Cause Acute Pharyngitis and/or Tonsillitis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Disease</th>
<th>Relative Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Pharyngitis/tonsillitis/rheumatic fever/scarlet fever</td>
<td>15% to 35%</td>
</tr>
<tr>
<td>Group C and G beta-hemolytic streptococci</td>
<td>Pharyngitis/tonsillitis</td>
<td>&lt; 3% to 11%</td>
</tr>
<tr>
<td><em>Arcanobacterium (Corynebacterium) haemolyticum</em></td>
<td>Pharyngitis/tonsillitis/rash</td>
<td>&lt; 1% to 10%</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Pharyngitis/disseminated disease</td>
<td>Rare*</td>
</tr>
<tr>
<td><em>Corynebacterium ulcerans</em></td>
<td>Pharyngitis</td>
<td>Rare</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>Pneumonia/bronchitis/pharyngitis</td>
<td>Rare</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Pharyngitis/enterocolitis</td>
<td>Rare</td>
</tr>
<tr>
<td>Human immunodeficiency virus-1</td>
<td>Pharyngitis/acute retroviral disease</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*Less than 1%.
Throat culture

- **Throat culture** remains the gold standard for the diagnosis of streptococcal pharyngitis

- Sensitivity: **90%** & Specificity: **99%**
<table>
<thead>
<tr>
<th><strong>Group A Streptococci (GAS)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid antigen</strong> detection test in 10-30 minutes. (1980s)</td>
</tr>
<tr>
<td><strong>Specificity</strong> $&gt; 95%$ &amp; sensitivity <strong>80-90%</strong></td>
</tr>
<tr>
<td><strong>Negative Result</strong> Should be cultured in <strong>Children &amp; Adolescence</strong></td>
</tr>
</tbody>
</table>
**Figure 3.11.8–2** Algorithm for laboratory diagnosis of streptococcal pharyngitis.
Nasal Culture

- Infection control of hospitalized patients to detect carriage of *Staphylococcus aureus* or *MRSA* as part of a *Staphyloccocal* outbreak
Sinus Puncture / Sinusitis

- *S. pneumoniae* 30 - 40%
- *H. influenzae* and *M. catarrhalis* each account for approximately 20% of cases

**Nosocomial sinusitis**
- S. aureus
- P.aeruginosa
- S.marcesens
- K.pneumoniae
- Enterobacter spp. & P.mirabilis
Tympanocentesis Fluid/ Otitis Media

- Lack of isolation of any pathogen has a **96% negative predictive value** for lack of a pathogen in the middle ear fluid

- *S. pneumoniae*
- *H. influenzae*
- *M. catarrhalis*
Major Causes of Acute Bronchitis

- Acute bronchitis is usually caused by viral agents.
- Bacterial:
  - *Bordetella pertussis* - deep nasopharyngeal swab
  - *Bordetella parapertussis*
  - *Mycoplasma pneumoniae*
  - *Chlamydophila pneumoniae*
Major Causes of Chronic Bronchitis

- *Haemophilus influenzae* / nonencapsulated
- *Streptococcus pneumoniae*
- *Moraxella catarrhalis*

- The role of bacteria in acute infections in these patients is questionable
Most Common Pathogens of Lower Respiratory Infections by Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td><em>Chlamydia trachomatis</em></td>
</tr>
<tr>
<td>Children</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>Infants</td>
<td>Influenza virus</td>
</tr>
<tr>
<td>5 to 18 months</td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>3 months to teens</td>
<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>Young adults (18 to 45 years)</td>
<td>Viruses</td>
</tr>
<tr>
<td>Older adults</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Institutionalized adults</td>
<td><em>Mycoplasma pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td><em>M. pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td><em>S. pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td><em>Legionella</em></td>
</tr>
<tr>
<td></td>
<td>Gram-negative rods</td>
</tr>
<tr>
<td></td>
<td><em>S. pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
</tbody>
</table>
It is estimated that 60% of children sporadically carry Streptococcus pneumoniae in their nasal passages by the age of 2 years.
PNEUMONIA

- Culture of lower respiratory secretions can be helpful, but in reality it is limited, with no agent isolated in 40% to 60% of cases.

- Low sensitivity (50%) of sputum culture for *S. pneumoniae*, especially if specimens are not immediately processed.
PNEUMONIA

- A delay in processing of more than 1 to 2 h may result in loss of recovery of fastidious pathogens, such as S. pneumoniae, and overgrowth of oronasal microbiota.

- Store specimen at 2 to 8 C until cultures.
Community Acquired Pneumonia - CAP

- For community-acquired pneumonia (CAP) in adults and the elderly, Streptococcus pneumoniae is the cause in 6 to 10% of all cases and 60% of the bacterial cases.
Ventilator-associated Pneumonia – VAP/ Hospital Acquired Pneumonia

- Specimen collected by bronchoscopy, which has an 82 to 91% sensitivity

- P aeruginosa,
- Enterobacter spp.
- Klebsiella spp.,
- Other Enterobacteriaceae
- MRSA
- Acinetobacter spp.
- S. pneumoniae
- Legionella
- H. influenzae
Sputum

- Sputum is among the least clinically relevant specimens received for culture in microbiology laboratories, even though it is one of the most numerous and time-consuming specimens.
SPECIMEN COLLECTION

Expectorated Sputum

- Food should not have been ingested for 1 to 2 hours before expectoration.
- Patient rinse mouth and gargle with water prior to sputum collection.
- For specialized cultures (e.g., mycobacteria and legionellae), supply sterile saline or water to gargle prior to collection.
Sputum Gram Stain

- Select the most purulent or most blood-tinged portion of the specimen
Sputum Gram Stain

- An acceptable specimen yields less than 10 squamous epithelial cells per low-power field (LPF) (100x)

- Many patients are severely neutropenic and specimens from these patients will not show white blood cells on Gram stain examination
Sputum Gram Stain

- Presence of 25 or more polymorphonuclear leukocytes per 100x field, together with few squamous epithelial cells – **Good Sputum**

- In *Legionella* pneumonia, sputum may be scant and watery / buffered charcoal yeast extract (BCYE)
Sputum Gram Stain

- Patients with atypical pneumonia (e.g., mycoplasma pneumonia) may produce no sputum or small amounts of sputum that is less purulent

- Gram-positive diplococci (57% sensitivity)

- Gram-negative rods suggestive of Haemophilus (82% sensitivity)
SPECIMEN COLLECTION

Induced Sputum

- Patients who are unable to produce sputum
- High diagnostic value in pneumocystis jiroveci, mycobacterium and fungi
Endotracheal Aspirate / Suction Tracheostomy

- Aspirate the specimen into a sterile leakproof cup

- **Endotracheal Aspirate Gram Stain**:  
  - Criteria used to reject ETAs from adult patients include greater than 10 squamous epithelial cells per low-power field or **no organisms** seen under oil immersion (1000X)
Bronchial washings

- Bronchial washings sample the major airways, which is the same area sampled by an endotracheal aspirate.

- Bronchial washings are acceptable for mycobacterial, Legionella, and fungal cultures.
Bronchoalveolar Lavage - BAL

- **BAL** sample is from the distal respiratory bronchioles and alveoli

- Large volume of sterile, nonbacteriostatic saline (greater than 140 ml /100-300)

- It is estimated that more than 1 million alveoli are sampled during this process

- Some 300 million alveoli are estimated to be present in the lungs
Quantitative culture methods
Serial dilution method is in italics
**Bronchoalveolar Lavage - BAL**

- Do not centrifuge specimens for **bacterial culture**
- \( > 10^4 \) CFU/ml in a BAL sample
- Count each morphotype individually

Taking the dilution factor of specimen effluents into consideration, this represents a **count of bacteria in the secretions of the lung of** \( 10^5 \) to \( 10^6 \) CFU/ml
**Bronchoalveolar Lavage - BAL**

- Use a centrifuge / cytocentrifuge to prepare BAL specimens for **Gram stain**.

- **Any organism** seen in a cytocentrifuged smear of BAL fluid is considered indicative of bacterial pneumonia.

- **Ciliated columnar bronchial epithelial** cells, goblet cells, or pulmonary macrophages.
Bronchial Brush Specimens / PSB

- This is the best specimen for viral culture and cytology studies

- Only PSB / Protected Specimen Brushings are acceptable for bacterial culture

- Place specimen in one ml of nonbacteriostatic saline
Lung Biopsy

- Submit in a sterile container(s) without formalin
- Prepare a touch prep for lung biopsy samples / Gram stain
Culture - Routine

- Blood Agar
- Chocolate Agar
- Mac Conkey
- MSA
Haemophilus influenzae

- Add a **10-IU bacitracin** disk to CHOC to inhibit upper respiratory microbiota and improve detection of H. influenzae
Streptococcus pneumoniae

- Add optochin disk to the second quadrant of BAP, to demonstrate inhibition by S. pneumoniae for direct detection on primary plates / Optional

- Some S. pneumoniae are bile resistant and others are resistant to optochin. No one test is 100% accurate
Incubation

- Incubate plates at 35 to 37 C in 5% CO₂ for a minimum of 48 h; 72 h is preferred

- For invasively collected lung cultures, extend incubation to 4 days
Culture examination/significant

- Colonies in the **first quadrant** of the plate, only if there is little or no other microbiota on the plate (e.g., 90% pure) and the **smear** suggests inflammation

- Large numbers in the **second** or greater quadrant of the plate
Urinary Antigen Test

- The sensitivity was 55% in a limited pediatric study (*S. pneumoniae*)

- Excellent specificity, the sensitivity (70 to 90%) is less than that of culture (*Legionella*)
Positive pneumococcal urinary antigen test result in adult patients hospitalized with community-acquired pneumonia (CAP) / Immunochromatographic test.

Specificity of the pneumococcal urinary antigen test was 96% and that its positive predictive value ranged from 88.8% to 96.5%.
Staphylococcus aureus

- S. aureus accounts for 1% to 9% of cases of CAP
- Mortality rate of up to 30%
- Necrotizing pneumonia with pulmonary hemorrhage has been associated with S. aureus isolates that produce PVL / Panton-Valentine leukocidin
**Staphylococcus aureus**

- Recent studies suggest that as many as 12% to 29% of adult and 35% to 50% of pediatric community-associated S. aureus infections are caused by MRSA, and the percentages continue to increase.

- More than 50% of nosocomial pneumonias caused by S. aureus are caused by MRSA strains.
Staphylococcus aureus

- **MSA media**

- In particular, thymidine-dependent strains of *S. aureus* may arise in patients treated with **long-term trimethoprim-sulfamethoxazole**

- These strains frequently present with colonies which are somewhat smaller, flatter, and grayer than the parent strain
Cystic Fibrosis

- Chronic lung infection is responsible for **75 to 85%** of deaths in patients with cystic fibrosis.

- The microbiota responsible for chronic lung disease in CF patients is very **stable**, with patients being infected with organisms such as *S. aureus* or *P. aeruginosa* for months to years.
Cystic Fibrosis / Specimen collection

- Deep pharyngeal swab in children, usually <10 years of age
- Sputum
- Endotracheal aspirates (on ventilated patients)
- BAL
Cystic Fibrosis

- **Do not use culture rejection criteria for sputum or endotracheal aspirates based on Gram stain quality**

- **Culture examination**: Any amount of selected pathogens in a patient with cystic fibrosis
Cystic Fibrosis

- *Pseudomonas aeruginosa* / AST at 24h
- Very Mucoid strain
- *Staphylococcus aureus*
- *Burkholderia cepacia complex* / BCSA
- *Stenotrophomonas maltophilia*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- Nontuberculous mycobacteria
Aspiration Pneumonia

- Anaerobes are implicated in disease, especially in the elderly

- Cultures are generally not cost-effective or helpful because they vary in sensitivity from 20 to 60%, possibly due to aeration during collection
Aspiration Pneumonia

- *Staphylococcus areus*
- *Enterobacteriaceae*
- *Pseudomonas*
- *Adnetobacter*
- *Moraxella catarrhalis*
Guidelines for reporting pathogens in lower respiratory cultures

- Report if present in significant amounts, even if not predominant:
  - *Moraxella catarrhalis*
Guidelines for reporting pathogens in lower respiratory cultures

- Report the following for inpatients only:
  - *Pseudomonas aeruginosa*; report AST
  - *Stenotrophomonas maltophilia*
  - *Acinetobacter*; report AST
  - *Burkholderia*; report AST
Guidelines for reporting pathogens in lower respiratory cultures

- Report if present in **significant amounts** and if it is the **predominant organism** in the culture, particularly if **smear** suggests infection with morphology consistent with isolate.

- **Staphylococcus aureus**; report AST

- Single morphotype of gram-negative rod (especially **Klebsiella pneumoniae**); report AST
Guidelines for reporting pathogens in lower respiratory cultures

- Examine for and always report:
  - *Streptococcus pyogenes*
  - *Nocardia*
  - *Francisella tularensis:*
    - G -coccobacilli that can grow on CHOC but do not grow on BAP, even with staphylococci for satelliting, weakly catalase positive and oxidase and urease negative
Guidelines for reporting pathogens in lower respiratory cultures

- Group B streptococcus in pediatric patients, if present, in any amount / report

- Other beta-hemolytic streptococci in significant amounts only if they are predominant

- Do not report Enterococcus & Coagulase-negative staphylococci unless the culture is 90% pure