RESPIRATORY SPECIMENS:
A REVIEW OF BEST PRACTICES
Objectives

1. Describe common and uncommon respiratory pathogens from the respiratory tract

2. Describe the utility of the Gram stain for the evaluation of respiratory specimens and as a diagnostic tool

3. Describe effective strategies for the work up and reporting of results from culture of respiratory tract specimens
The Respiratory Tract

- Oropharynx harbors large numbers of aerobic and anaerobic bacteria
- Sub-laryngeal bacterial colonization is minimal in the healthy host
- Different flora may be seen in those with underlying conditions
  - Immunosuppression
  - Diabetes mellitus
  - Alcoholism
  - Chronic lung disease
  - Broad-spectrum antimicrobial agents
Infection in the Lung

- Aspiration of bacterial into the alveoli is the most common mechanism initiating a pneumonic infection
- Asymptomatic aspiration commonly occurs, but organisms are usually cleared by the mucociliary apparatus
- Aerosol inhalation is a second, less frequent, mechanism for organisms to gain access to the LRT
- Hematogenous seeding of the lung from a distant focus of infection
Bacteria in the Respiratory Tract

Normal Respiratory Flora
- Corynebacterium spp.
- Coagulase negative staphylococci
- Staphylococcus aureus
- Neisseria spp.
- Haemophilus influenzae
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Gram negative bacilli

Oral flora – $10^{10}$-$10^{12}$ CFU/mL

Potential Pathogens
- Staphylococcus aureus
- Haemophilus influenzae
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Gram negative bacilli
# Bacterial Agents of Acute Pneumonia

<table>
<thead>
<tr>
<th>Common</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td><strong>Acinetobacter baumannii</strong></td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td><strong>Actinomyces species</strong></td>
</tr>
<tr>
<td><strong>Haemophilus influenzae</strong></td>
<td><strong>Bacillus species</strong></td>
</tr>
<tr>
<td>Mixed anaerobic bacteria from aspiration <em>(Bacteroides spp., Fusobacterium spp., anaerobic cocci, Prevotella/Porphyromonas spp.)</em></td>
<td><strong>Moraxella catarrhalis</strong></td>
</tr>
<tr>
<td>*<em>Enterobacteriaceae (Escherichia coli, Klebsiella pneumoniae, Enterobacter spp., Serratia spp.)</em></td>
<td><strong>Francisella tularensis</strong></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td><strong>Nocardia species</strong></td>
</tr>
<tr>
<td><strong>Legionella species (including L. pneumophila and L. mcdadei)</strong></td>
<td><strong>Pasteurella multocida</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Neisseria meningitidis</strong></td>
</tr>
</tbody>
</table>
Common Viral Agents of Acute Pneumonia

<table>
<thead>
<tr>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>Influenza A</td>
</tr>
<tr>
<td>Parainfluenza 1-3</td>
<td>Influenza B</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Respiratory Syncytial Virus</td>
</tr>
<tr>
<td></td>
<td>Human Metapneumovirus</td>
</tr>
<tr>
<td></td>
<td>Adenovirus</td>
</tr>
</tbody>
</table>
Pneumonia

Community Acquired
• Diagnosis is based on the presence of specific symptoms and suggestive radiographic features, such as pulmonary infiltrates and/or pleural effusion.
• Carefully obtained microbiological data can support the diagnosis but often fails to provide an etiologic agent.

Common Pathogens
• Mycoplasma pneumoniae
• Respiratory viruses
• Streptococcal pneumoniae
• Chlamydophila pneumoniae
• Haemophilus influenzae
• Staphylococcus aureus
Pneumonia

Health Care/Ventilator Associated, Hospital Acquired

- Viruses and fungi are rare causes of HCAP, HA, and VAP in the immunocompetent patient.
- The clinical diagnosis is based on imaging plus the presence of clinical features (fever, leukocytosis or leucopenia, purulent secretions)
- Determining the cause of the pneumonia relies on diagnostic testing.
- A smear lacking inflammatory cells and a culture absent of potential pathogens have a very high negative predictive value.

Common Pathogens in Immunocompromised Patients

- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Enterobacter spp*
- *Serratia marcescens*
- *Acinetobacter spp*
- *Stenotrophomonas maltophilia*
- *Staphylococcus aureus* and MRSA
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Legionella*
- *Aspergillus*
- Influenzae A,B
- HPIV
- Adenovirus
- Rhinovirus
- RSV
- MDROs
Acute Bronchitis

- Inflammation of the epithelial lining of the bronchi
  - Obstructs airflow
  - Shortness of breath and coughing
  - Production of thick mucus
- >85% caused by viruses—rhinovirus, adenovirus, influenza A and B, and parainfluenza virus
- May occur as a secondary bacterial infection following a viral upper respiratory tract infection
- May result from primary infection with one of several specific agents
  - *Bordetella pertussis*
  - *Mycoplasma pneumoniae*
  - *Chlamydophila pneumoniae*
- May become chronic
Viral Bronchiolitis

• Lower respiratory tract infection seen in children less than 2 years old with peak occurrence seen in 2-8 month old children

• Symptoms
  – Starts with symptoms of the common cold and progresses to involve bronchi and the bronchioles
  – Expiratory wheezing, tachypnea, retractions, irritability, and dehydration
  – Complications include conjunctivitis, otitis media, pneumonia

• Viral agents
  – Major agents: RSV (up to 80% of cases), parainfluenza virus type 3
  – Minor agents: adenoviruses, influenza, and rhinoviruses
Specimens for Diagnosis of Lower Respiratory Tract Infections

• Non-invasive specimens
  – Sputum
  – Tracheal aspirates
  – Blood cultures
  – Urine
  – Serum

• Bronchoscopic specimens
  – Bronchial wash/brush
  – Protected specimen brushings
  – Bronchoalveolar lavage
  – Transbronchial biopsy
  – Transbronchial needle aspirates

• Other invasive specimen types
  – Pleural fluid
  – Transthoracic-needle biopsy
  – Open lung biopsy
Bronchoalveolar Lavage (BAL)

- Performed following general inspection of the tracheobronchial tree and before biopsy or brushing
- Performed during flexible bronchoscopy
- Obtain specimens to rule out opportunistic infections in immunocompromised hosts
- Good general rule is to perform the lavage where the disease is most prominent radiographically
- In localized disease, lavage of the involved segment is most likely to yield the best results. In diffuse disease, the right middle lobe or lingula is often chosen to optimize fluid recovery.
- Typically involves the delivery of a total of 100 to 240 mL of fluid in 20 to 60 mL aliquots
- A lavage volume of 100 mL samples approximately one million alveoli (1.5 to 3 percent of the lung).
- Lavage fluid should be pooled into a single container
Respiratory Testing in the Micro Lab

1. What should we be doing with these specimens in the lab?
2. Are there new pathogens we should be looking for?
Utility of Gram Stain

• Rapid, inexpensive, informational
• Evaluation of specimen quality
  – Identify superficially contaminated specimens
  – Enhance discrimination between samples with potential pathogens vs. colonizing flora
• Presumptive organism ID
• Guide rational selection of preliminary antibiotic therapy
• Guides interpretation of culture results
Utility of Gram Stain

• Majority of the literature supports the clinical usefulness of gram stained sputum smears
• Wide range in reported sensitivity (35-96% and specificity (12-85%)
• Reference standard – sputum culture

• Multiple criteria for assessing Gram stain smears
Utility of Gram Stain

- Gram stain DOES NOT diagnose the presence of pneumonia
- Once pneumonia diagnosed Gram stain is useful in determining probable etiologic agent

A. sputum from a patient with pneumonia—Gram-positive, elongated cocci in pairs and short chains (Streptococcus pneumoniae)
B. a bronchoalveolar lavage specimen—Gram-negative intracellular rods (Klebsiella pneumoniae)
Utility of Gram Stain

• 50% of the information gleaned from sputum cultures is clinically misleading in the absence of correlation with direct gram stain results

• Selection of appropriate monotherapy 94% of the time when guided by bacterial morphotypes from the gram stain
# Sputum Culture in the Management of CAP

**No**
- Yield is variable and strongly influenced by the quality of the entire process
- Infrequent positive impact on clinical care
- Argue against the routine use of common tests, such as blood and sputum cultures.
- Optional in outpatients

**Yes**
- Cultures may have a major impact on the care of an individual patient and are important for epidemiologic reasons, including the antibiotic susceptibility patterns used to develop treatment guidelines
- Hospitalized patients with listed clinical indications

Mandell et al. 2007 CID44 (Suppl 2):S27-S72
## Non-Invasive Specimens

<table>
<thead>
<tr>
<th>Indication</th>
<th>Blood culture</th>
<th>Sputum culture</th>
<th>Legionella urine antigen test</th>
<th>Pneumococcal urine antigen test</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission to intensive care unit</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Endotracheal aspirate if intubated</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Asplenia</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cavitary infiltrates</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>Fungal and tuberculosis cultures</td>
</tr>
<tr>
<td>Chronic severe liver disease</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outpatient therapy ineffective</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Thoracentesis and pleural fluid cultures</td>
</tr>
<tr>
<td>Positive Legionella urine antigen test result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive pneumococcal urine antigen test result</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Recent travel (within past two weeks)</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Severe obstructive lung disease</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The benefit of a good quality sputum Gram stain

- Impact to therapy
  - Broadens initial empirical coverage for less common etiologies (*S. aureus* or gram-negative bacilli)
  - Early discontinuation of empirical treatment if results are negative

- Validates subsequent sputum culture results

Mandell et al. 2007 CID44 (Suppl 2):S27-S72
Work up of Respiratory Cultures

Specimen Quality

Premise:

• PMNs are an indication of infection or inflammation
• SEC indicate superficial contamination
• If a specimen contains a large amount of SEC, superficial contamination is likely the specimen should be recollected
• Extensive testing on heavily mixed cultures should not routinely be performed
### Screening Sputum Specimens for Acceptability

<table>
<thead>
<tr>
<th>Methods</th>
<th>Minimum Criteria for Specimen Acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of neutrophils/LPF (10-15 = +1; &gt;25 = +2); Mucus (+1); SEC/LPF (10-25=-1; &gt;25 =-2)</td>
<td>Score of &gt;0</td>
</tr>
<tr>
<td>Enumerate SEC/LPF</td>
<td>&lt;10 SEC/LPF</td>
</tr>
<tr>
<td>Enumerate Neutrophils/LPF</td>
<td>&gt;25 neutrophils/LPF</td>
</tr>
<tr>
<td>Enumerate SEC/LPF</td>
<td>&lt;25 SEC/LPF</td>
</tr>
<tr>
<td>Sum of neutrophils/LPF (1-75=+1; 76-150=+2; &gt;150=+3) and SEC/LPF (5-15=-1; 16-25=-2; &gt;25=-3)</td>
<td>Positive summation score</td>
</tr>
<tr>
<td>Ratio, neutrophils to SEC</td>
<td>&gt;10 neutrophils/SEC</td>
</tr>
<tr>
<td>Ratio, neutrophils to SEC</td>
<td>&gt;5 neutrophils/SEC</td>
</tr>
<tr>
<td>Enumerate SEC/LPF and presence/absence of organisms/OIF</td>
<td>&lt;10SEC/LPF and organisms present</td>
</tr>
<tr>
<td>Presence/absence of organisms/OIF</td>
<td>Organisms present</td>
</tr>
</tbody>
</table>

Screening Sputum Specimens for Acceptability

> 25 epithelial cells/lpf
[lpf, x10])

Interpretation: **Unsuitable for culture**

4+ (>25/lpf) neutrophils, no epithelial cells seen, 3+ (11-50/oif) Gram negative diplococci, 2+ (1-10/oif) yeast cells

**Interpretation: Suitable for culture**

Yeast cells = *Cryptococcus neoformans*; Gram-negative diplococci=*Moraxella catarrhalis*
Screening Sputum Specimens for Acceptability

>25 epithelial cells/lpf
Multiple bacterial morphologies suggesting oral contamination
**Interpretation:** **Unsuitable for culture**
High power view.

3+ neutrophils, 3+ Gram-positive diplococci
**Interpretation:** **Suitable for culture**;
Gram-positive diplococci = *Streptococcus pneumoniae*
**Mixed Flora**

- Used only with respiratory specimens
- Use of objective criteria (# of organisms present per OIF) to distinguish resident flora or colonizers from potential pathogens:

<table>
<thead>
<tr>
<th>Morphology</th>
<th>OK to Report if:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative bacilli</td>
<td>≥ 10 organisms/OIF</td>
</tr>
<tr>
<td>Moraxella</td>
<td>≥ 25 organisms/OIF</td>
</tr>
<tr>
<td>Staph</td>
<td>≥ 50 organisms/OIF</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>≥25 pairs/OIF</td>
</tr>
<tr>
<td>Aspiration event</td>
<td>&gt;50 organisms/OIF*</td>
</tr>
</tbody>
</table>

* intracellular gram-positive and gram-negative organisms in at least one field

Bartlett 1982 JAMA
Wright et al. 1990 Am J Med
Normandin et al. 1997 ASM C-91
Gram Stain Screening

- Use interpretive comments

DIRECT SMEAR SUGGESTS:
No neutrophils
Many squamous epithelial cells

Not representative of lower respiratory tract secretions. Culture not performed. Please consult Microbiology if clinical considerations warrant complete processing of this specimen. (Specimen will be held 5 days).
Work up of Respiratory Cultures

- No definitive guidelines for working up bacterial cultures
  - Standardized methods
  - Uniformity in work up and reporting of bacterial isolates
  - When to perform AST
Culture Set Up

• 5% sheep blood agar, MacConkey agar, and chocolate agar

• Add on Hemophilus plate?
  – Quad plate
    • Factor X (hemin) or V factor (Nicotinamide adenine dinucleotide (NAD)) along with the hemolytic reaction on horse blood
  – Remel Haemophilus Isolation Agar
    • Bacitracin and horse blood

• BCYE for Legionella
Work up of Respiratory Cultures

• Standardized pathogen list
• Basic correlation with Gram stain
  – Gram stain results used to guide the selection of potential pathogens in the culture that merit further identification and susceptibility testing
• Q-Score System

• Q234 System

• PMN-association System

Work up of Respiratory Cultures

Q-Score System

• Up to 3 organisms can be considered potential pathogens (PP) and be worked up (ID/AST) if from a good quality specimen (Q3)

• The lower quality of the specimen (e.g., the more SEC present) the fewer the organisms worked up (Q2, Q1)
Work up of Respiratory Cultures

Q-Score System

Q-SCORE = # of potential pathogens (PP) to work up

<table>
<thead>
<tr>
<th>Squamous cells (-)</th>
<th>0</th>
<th>-1</th>
<th>-2</th>
<th>-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Key:
0 = no cells
1 = 1-9/lpf
2 = 10-24/lpf
3 = ≥25/lpf

Q0 = no cult
Q1 = 1PP
Q2 = 2PP
Q3 = 3PP
Work up of Respiratory Cultures

Q-Score System

# PP in culture $\leq$ Q-score: work up PP with ID/AST

- (2PP) (Q3)

# PP in culture $>$ Q-score: Look to Gram stain

- (3PP) (Q2)

- Work up PP that were seen in Gram stain with ID/AST
- If all PP in the culture are seen in Gram stain = do not work up; perform morphological identification
Work up of Respiratory Cultures

Q234 System

- **Gram stain Quality Check:** PMN & SEC
  - Reject any sputum for culture according to normal protocol
  - Culture work up is based on number of PP present:

  2PP = Work up (< 2 PP)
  4 PP = morphological ID
  3 PP = Look to Gram stain

**NOTE:** If mixed flora > PPs = morph ID PP

- Work up to 2 PP if they are seen in the GS
- If all 3 PP are seen in the GS, morph ID all 3

Work up of Respiratory Cultures

PMN-Association System

• Success in microscopic evaluation relies on strict cytological criteria
  – >25 PMN, < 10 SEC
  – >25 PMN, <25 SEC
  – >10 PMN per SEC (10:1 ratio)

• Evaluate the presence of predominant morphotypes associated with WBC
• Work up organisms seen in association with PMN

Mixed flora
• 1 morphotype <10 organisms/OIF Helpful in predicting
• 1 morphotype >10 organisms/OIF primary pathogen
Work up of Respiratory Cultures

PMN-Association System

• Quantitation of organisms in smears inconsistent and inaccurate
  – Technologist variability
  – Do not report

• Do not rely on quantitation to determine relatedness to infection
  – PMN more predictive
Example 1: Sputum

GS: many PMN (+3), few SEC (-1), many enteric-like gram negative bacilli, moderate gram positive cocci suggestive of Staph, few Mixed flora (yeast)

CULT: moderate P. aeruginosa, moderate E.coli, moderate Staph aureus, few yeast

WORK UP:
Q Score (Q2=2PP):

Q234 (3PP):
Example 1: Sputum

GS: many PMN (+3), few SEC (-1), many enteric-like gram negative bacilli, moderate gram positive cocci suggestive of Staph, few Mixed flora (yeast)

CULT: moderate P. aeruginosa, moderate E.coli, moderate Staph aureus, few yeast

WORK UP:

Q Score (Q2=2PP): Work up E. coli and S. aureus

MID P. aeruginosa; Report Mixed flora

Q234 (3PP):
Example 1: Sputum

GS: many PMN (+3), few SEC (-1), many enteric-like gram negative bacilli, moderate gram positive cocci suggestive of Staph, few Mixed flora (yeast)

CULT: moderate P. aeruginosa, moderate E.coli, moderate Staph aureus, few yeast

WORK UP:
Q Score (Q2=2PP): Work up E. coli and S. aureus
MID P. aeruginosa; Report Mixed flora

Q234 (3PP): Work up E. coli and S. aureus
MID P. aeruginosa; Report Mixed flora
Example 2: Sputum

GS: many PMN (+3), moderate SEC (-2), many nonenteric-like gram negative bacilli, moderate Mixed flora

CULT: many P. aeruginosa, moderate Staph aureus, few viridans Strep

WORK UP:
Q Score (Q1=1PP):

Q234 (2PP):
Example 2: Sputum

GS: many PMN (+3), moderate SEC (-2), many nonenteric-like gram negative bacilli, moderate Mixed flora

CULT: many P. aeruginosa, moderate Staph aureus, few viridans Strep

WORK UP:
Q Score (Q1=1PP): Work up P. aeruginosa
MID S. aureus; Report Mixed flora

Q234 (2PP):
Example 2: Sputum

GS:  many PMN (+3), moderate SEC (-2), many nonenteric-like gram negative bacilli, moderate Mixed flora

CULT:  many P. aeruginosa, moderate Staph aureus, few viridans Strep

WORK UP:

Q Score (Q1=1PP): Work up P. aeruginosa
MID S. aureus; Report Mixed flora

Q234 (2PP): Work up P. aeruginosa and S. aureus
Report Mixed flora
Example 3: Tracheal Aspirate

GS: many PMN (+3), few SEC (-1), many Mixed flora (few enteric-like GNB; moderate gram positive cocci suggestive of Staph)

CULT: moderate diphtheroids, moderate coag negative Staph, few E.coli, rare Staph aureus

WORK UP:

Q Score (Q2=2PP):

Q234 (2PP):
Example 3: Tracheal Aspirate

GS: many PMN (+3), few SEC (-1), many Mixed flora (few enteric-like GNB; moderate gram positive cocci suggestive of Staph)

CULT: moderate diphtheroids, moderate coag negative Staph, few E.coli, rare Staph aureus

WORK UP:
Q score (Q2=2PP): Work up E. coli and S. aureus
Report Mixed flora

Q234 (2PP):
Example 3: Tracheal Aspirate

GS: many PMN (+3), few SEC (-1), many Mixed flora (few enteric-like GNB; moderate gram positive cocci suggestive of Staph)

CULT: moderate diphtheroids, moderate coag negative Staph, few E.coli, rare Staph aureus

WORK UP:
Q-Score (Q2=2PP): Work up E. coli and S. aureus
Report Mixed flora

Q234 (2PP): Report Mixed flora
MID E. coli and S. aureus **

** If mixed flora > PPs = MID PP
Premise for “Q” Systems

- Based on published prevalence of potential pathogen colonization of the oropharynx
- The more superficially contaminated the specimen, the higher the # of colonizing organisms present
- Quality of specimen is important in determining acceptability of specimen and extent of culture work up
- If organisms seen in smear, greater chance they are associated with an infective process
“Q” Systems Advantages

• Offers a consistent approach for interpreting cultures
  – Based on specimen quality
  – Based on organisms seen in Gram stain (organism seen on smear should be in a significant number in the specimen, >105/mL)
  – Limits number of organisms worked up from mixed cultures reporting of misleading information minimized

• All potential pathogens reported (may not perform full ID/AST)
Q System Caveats

• Gram stain sensitivity
  – Requires $10^4$-10$^5$ organisms per ml of fluid

• Standardization of Gram Stain
  – Specimen quality, type
  – Smear preparation and staining
  – Smear interpretation

• Culture and Gram Stain Correlation

• When not to apply the criteria
  – Legionella culture

QUANTITATIVE CULTURE
Quantitative Culture

• Evaluation of lower respiratory tract secretions obtained either bronchoscopically or via endotracheal aspiration without a bronchoscope
  – Quantities of bacterial growth above a threshold are diagnostic of pneumonia and
  – Quantities below that threshold are more consistent with colonization.

• The generally accepted thresholds are as follows:
  – Endotracheal aspirates, $10^6$ CFU/mL
  – BAL, $10^4$ CFU/mL
  – Protected specimen brush samples (PSB), $10^3$ CFU/mL

• These values have significance only when the samples have been obtained >72 hours before the initiation or a change of antibiotic therapy.
Methods for Quantitative Culture

• Two approaches for quantitative culture:
  • Serial-dilution method
    – Two 100-fold dilutions are made, and colony counts are obtained from 0.1-ml amounts of the diluted specimen inoculated onto media.
    – Counts are made from the plate containing between 30 and 300 colonies. The results are expressed as CFU per milliliter.
  • Calibrated-loop method,
    – 0.1 ml of PSB and 0.001 and 0.01 ml of BAL are inoculated onto agar media.
    – The results are expressed as log10 ranges of bacteria.
• All morphotypes should be quantitated and reported.
• Those organisms whose numbers approach or exceed the threshold for significance should be identified and have susceptibility testing performed.
• Those bacteria present in smaller quantities should not be completely characterized.
Cultures of respiratory secretions should be obtained from virtually all patients with suspected VAP

Noninvasive sampling with semi-quantitative cultures to diagnose VAP, rather than invasive sampling with quantitative cultures and rather than noninvasive sampling with quantitative cultures

Remain in favor of blood cultures for all patients with suspected VAP/HAP, although they don’t always correlate (25% positive from non-pulmonary source)

When the 5 trials were pooled via meta-analysis, sampling technique did not affect any clinical outcome, including mean duration of mechanical ventilation, ICU length of stay, or mortality

Strongly encourage diagnostic testing whenever the result is likely to change individual antibiotic management.
The guideline panel acknowledged that there is a potential that invasive sampling with quantitative cultures could lead to less antibiotic exposure if growth below defined thresholds is used as a trigger to stop antibiotics.

Remarks: Clinical factors should also be considered because they may alter the decision of whether to withhold or continue antibiotics.
EMERGING PATHOGENS
Corynebacterium sp.

- *C. pseudodiphtheriticum, C. striatum*
- Unlike *C. diphtheriae* and *C. ulcerans*, non-diphtheria corynebacteria do not produce toxins.
- Widely distributed in the environment
- Colonizers of the skin and mucosal membranes
- Patient-to-patient transmission in ICUs has been demonstrated for *C. striatum*
- Susceptible to vancomycin, linezolid

Nhan, TX et al. Microbiological investigation and clinical significance of Corynebacterium spp. In respiratory specimens. Diagnostic Microbiology and Infectious Disease 74 (2012) 236–241
Utility of Fungal Culture

• Histopathology alone is not sensitive enough to diagnose fungal infections
• Should be accompanied by immunostain, culture, and, when available, NAAT
• Has the ability to detect unsuspected fungi
Endemic Regions of the Systemic Mycoses
Histoplasmosis

Histoplasmosis in a State Where It Is Not Known to Be Endemic — Montana, 2012–2013

*MMWR Weekly* October 25, 2013 / 62(42);834-837

- Diagnosed in four Montana residents by four different physicians
- Three patients reported no recent travel outside of Montana and likely were exposed in Montana, which is west of areas where *H. capsulatum* is recognized as endemic
- 4th patient likely acquired her infection in Montana before traveling out of state (could have been acquired during travel to California)
- Three patients experienced diagnostic delays, likely in part because none reported recent travel to areas where *H. capsulatum* is endemic
Direct Examination of Clinical Specimens

BAL: macrophages with yeasts, Wright-Giemsa
Phenotypic Morphology in Culture

• Colonies on BA and BHI are glabrous or wrinkled and cream-to-brown in color
• Subcultures grown on Sabouraud’s dextrose agar are white, tan, or light brown with abundant aerial hyphae
• Buff-to-brown colonies produce sparse aerial hyphae and abundant macroconidia at first, then turn white with dense aerial hyphae on subculture
Microscopic Morphology

- Produces two types of conidia at 30°C
- Both produced singly at the tips of short, narrow conidiophores.
  - Large (8-15 micron), thick-walled, spherical/pear-shaped macroconidia with fingerlike projections (tuberculate macroconidia)
    - Tubercules are extension of the outer wall, are not cellular, and do not bud
  - Small (2-4 microns), oval microconidia with smooth to finely roughened walls
Sepedonium

- Confirmation of ID is recommended
Coccidioidomycosis

Notes from the Field: Coccidioides immitis Identified in Soil Outside of Its Known Range — Washington, 2013

MMWR Weekly May 23, 2014 / 63(20);450-450

• Three acute cases among residents of south central Washington reported during 2010–2011 were suspicious for local acquisition;
• None of the three patients had traveled within 22 months of illness onset to an area where coccidioidomycosis is known to be endemic
• Novel PCR used to detect Coccidioides DNA in six of 22 soil samples
• Viable mold from 4 out of 6 samples
Coccidioides spp.

- C. immitis consists of two taxa
  - Non-CA → Arizona, Texas, Mexico, and Argentina Group I
  - CA → California Group II
- Two species within the genus Coccidioides
  - “Coccidioides immitis” is restricted to isolates from California
  - “Coccidioides posadasii” for all other isolates belonging to this genus
Phenotypic Morphology in Culture

- Growth within 3-5 days
- Cottony, velvety, powdery, granular, smooth, or wrinkled colonies
- Usually white, but may be gray, buff, lavender, cinnamon, yellow or brown
- Can be leathery on blood based agar
Arthroconidia of Coccidioides sp.

• Arthroconidia formed within 5-10 days
  – Barrel- or cask-shaped and measure 2.5-4 μ by 3-6 μ
  – Liberated arthroconidia carry a portion of the walls of the intervening sterile segments (disjunctor cells)
  – Liberated arthroconidia survive desiccation and extreme temperatures, and germinate to produce hyphae
Malbranchea

• Confirmation of ID is recommended
RESPIRATORY VIRUS TESTING
NAAT Based Respiratory Virus Testing

**Yes**
- Infectious diseases present as a constellation of symptoms
- Infectious causes are broad and diverse
- Empirical response is to treat for everything
- Knowledge of the etiologic agent allows informed decisions
- On-demand, rapid
- Ability to exclude known viruses
- Labs can serve as sentinels
- Cost

**No**
- Panels may be too broad
- Should be risk based not specimen based
- Test for uncommon pathogens
- Multiplex may impact sensitivity for some targets
- Cost

Respiratory Pathogens in Hospitalized Patients

• The Centers for Disease Control and Prevention (CDC) Etiology of Pneumonia in the Community (EPIC)
• Incidence and microbiologic causes of community-acquired pneumonia requiring hospitalization among U.S. adults.
• Blood samples, acute-phase serum specimens, urine samples, and nasopharyngeal and oropharyngeal swabs were obtained from the patients as soon as possible after presentation.
• In the case of patients with a productive cough, sputum was obtained. Pleural fluid, endotracheal aspirates, and bronchoalveolar-lavage samples that had been obtained for clinical care were analyzed for the study.
• Only within collection of 72 hours
• Imaging studies were performed upon admission

Respiratory Pathogens in Hospitalized Patients

• A PCR assay was performed on nasopharyngeal and oropharyngeal swabs with the use of CDC-developed methods for the detection of adenovirus; *Chlamydophila pneumoniae*; coronaviruses 229E, HKU1, NL63, and OC43; human metapneumovirus (HMPV); human rhinovirus; influenza A and B viruses; *Mycoplasma pneumoniae*; parainfluenza virus types 1, 2, and 3; and respiratory syncytial virus (RSV).

• A real-time polymerase-chain-reaction (PCR) assay for legionella was performed on sputum regardless of the quality of the sample.

• PCR for bacterial targets


viruses detected in 27% and bacteria in 14%
EPIC Study Results

• Pathogens were detected in only 38% of adults
• Pediatric EPIC study
  – Pathogens were detected in 81% of children who had been hospitalized with community-acquired pneumonia
• HMPV, RSV, parainfluenza viruses, coronaviruses, and adenovirus were detected in 13% of the patients
• Among adults 80 years of age or older, the incidence of RSV, parainfluenza virus, and coronavirus each was similar to that of *S. pneumoniae*
• Contribution of viruses to hospitalizations of adults
• Prevalence of pneumococcal disease = 5%

Three pathogens were detected more commonly in patients in the ICU than in patients not in the ICU:

- *S. pneumoniae* (8% vs. 4%)
- *S. aureus* (5% vs. 1%)
- Enterobacteriaceae (3% vs. 1%)

Pathogens were detected less frequently in nasopharyngeal and oropharyngeal swabs obtained from asymptomatic controls than in swabs obtained from patients with pneumonia.

The incidences of influenza and of *S. pneumoniae* were almost 5 times as high among adults 65 years of age or older than among younger adults.

The incidence of human rhinovirus was almost 10 times as high among adults 65 years of age or older than among younger adults.

Rhinovirus Seasonality and Transmission

- In temperate climates reported a peak in incidence in the early fall, with a smaller peak in the spring
- Most common cause of respiratory viral illness during the spring, summer, and fall months, while
  - influenza virus and RSV predominate in the winter
- Initiated by intranasal and conjunctival inoculation, but not oral
- Survive in an indoor environment for hours to days at an ambient temperature and on undisturbed skin for 2 h
- In one study, HRV was transmitted via an aerosolized route to 56% of volunteers who played cards for 12 h with experimentally infected subjects
Asymptomatic Rhinovirus

- Asymptomatic infection is relatively common
- In children <4 years old, rates of asymptomatic infection range from 12 to 32% and tend to be higher in the youngest age groups
- HRV in 0% and 2% of asymptomatic adults
Rhinovirus

• Detected in 49% of children admitted to ICUs with lower respiratory tract infection
  – in approximately one-half of cases, no other respiratory pathogen was identified
• Can lead to an influenza-like illness, lower respiratory tract infections, chronic infections, and secondary bacterial infections, especially in immunocompromised patients, children with asthma, and adults with COPD
• Increases susceptibility to bacterial infection
  – Disrupts cell barrier function at tight junctions
  – Stimulating the immune system

Middle East Respiratory Syndrome
MERS-CoV

- A coronavirus
- Not detected by RVP panels
- Reported in Saudi Arabia in 2012
- Severe acute respiratory illness, including fever, cough, and shortness of breath
- 2 patients in US in May 2014 (IN and FL), both had traveled to Saudi Arabia
- Carried by camels
# Case Definition

## Patient Under Investigation (PUI)

A person who has both clinical features and an epidemiologic risk should be considered a patient under investigation (PUI) based on one of the following scenarios:

<table>
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<tr>
<th>Clinical Features</th>
<th>Epidemiologic Risk</th>
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| **Severe illness**  
Fever and pneumonia or acute respiratory distress syndrome (based on clinical or radiological evidence) | and  
A history of travel from countries in or near the Arabian Peninsula within 14 days before symptom onset, or close contact with a symptomatic traveler who developed fever and acute respiratory illness (not necessarily pneumonia) within 14 days after traveling from countries in or near the Arabian Peninsula.  
—or—  
A member of a cluster of patients with severe acute respiratory illness (e.g., fever and pneumonia requiring hospitalization) of unknown etiology in which MERS-CoV is being evaluated, in consultation with state and local health departments in the US. |

| Milder illness  
Fever and symptoms of respiratory illness (not necessarily pneumonia; e.g., cough, shortness of breath) | and  
A history of being in a healthcare facility (as a patient, worker, or visitor) within 14 days before symptom onset in a country or territory in or near the Arabian Peninsula in which recent healthcare-associated cases of MERS have been identified. |

| Fever or symptoms of respiratory illness (not necessarily pneumonia; e.g., cough, shortness of breath) | and  
Close contact with a confirmed MERS case while the case was ill. |

The above criteria serve as guidance for testing, however, patients should be evaluated and discussed with public health departments on a case-by-case basis if their clinical presentation or exposure history is equivocal (e.g., uncertain history of health care exposure).
Specimens for MERS-CoV Testing

Collection of all three specimen types (not just one or two of the three), lower respiratory, upper respiratory and serum specimens for testing using the CDC MERS rRT-PCR assay is recommended.

Lower respiratory specimens are preferred, but collecting nasopharyngeal and oropharyngeal (NP/OP) specimens, and serum, are strongly recommended depending upon the length of time between symptom onset and specimen collection.

Respiratory specimens should be collected as soon as possible after symptoms begin – ideally within 7 days. However, if more than a week has passed since symptom onset and the patient is still symptomatic, respiratory samples should still be collected, especially lower respiratory specimens since respiratory viruses can still be detected by rRT-PCR.

What’s Next?

• Laboratory diagnosis of infectious diseases increasingly relies on pathogen-specific tests = *a priori* knowledge of likely etiologic agents
• Many different pathogens can cause clinically indistinguishable symptoms
• PCR amplification of marker genes strategy introduces bias and ignores effects of the relevant viral and phage flora for which no marker gene exists
• Next-generation sequencing (NGS) allows for unbiased, hypothesis-free detection
• High-throughput DNA and RNA-seq possible, computational analysis lacking

Metagenomics-Based Pathogen Detection Tools

Summary

- A good Gram stain is useful for interpreting culture results
- Standardization may be a useful strategy
- Quantitative culture may not be required
- *S. pneumoniae* and Rhinovirus in hospitalized patients
- NAAT testing is required
- New pathogens coming to a location near you