Microbiological Diagnosis of pneumococcal infections

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**S. pneumoniae infections**

- *S. pneumoniae* is a major cause of pneumonia, meningitis, bacteremia, sinusitis, and otitis media, and it occasionally infects tissues at other sites
- IPD: pneumonia, meningitis, bacteremia and infections of other normally sterile sites
- Worldwide, WHO estimates IPD causes +/- 1.6 million deaths/year including 1 million children <5yrs
• Accurate and reliable detection of *S. pneumoniae* would thus be beneficial for both pneumococcal & nonpneumococcal disease
  - narrow-spectrum agents for *S. pneumoniae*
  - other antibacterials and antiviral agents

• Despite its importance, IPD (particularly pneumococcal pneumonia) can be surprisingly difficult to confirm microbiologically
Diagnostic testing for *S. pneumoniae*

2 fundamental questions to be asked:

- does the test identify *S. pneumoniae* specifically and
- does this detection adequately implicate *S. pneumoniae* as the causative pathogen of disease?

⇔ Distinction between colonisation and infection?
Laboratory Diagnosis of Pneumococcal Disease

- Microscopy and culture
- Antigen detection assays
  - Urinary antigen test
  - Ag test used on other body fluids
- Nucleic acid amplification tests
  - On normally sterile samples:
    - blood,
    - CSF
    - Pleural fluid
  - On respiratory samples
**Streptococcus pneumoniae**

- Gram positive cocci in pairs, diplococci
- Causing $\alpha$-hemolysis of blood agar
- Catalase negativity
- Optochin susceptibility:
  - But optochin-R pneumococci up to 10%
- Bile solubility
  - Discriminatory for optochin-R isolates
- No MALDI-TOF Identification
Guidelines for the management of adult lower respiratory tract infections. *Clinical Microbiology and Infection* 2011; 17: E1-E59

Woodhead M et al Eur Resp J 2005; 26:1138-1180
Rapid Sputum Examination by Gram staining has Diagnostic Value

- Sens: 57% increasing to 63% if ≤ 24 hrs antibiotics
  
  Roson B 2000; Butler JC 2003; Musher DM 2004

- 216 pts: 62% sputa with predominant morphotype in 65% Gram + diplococci; sens 68.2%, sp 93.8%
  

- **Old, simple, cheap rapid diagnostic test for etiology of CAP: can be useful in guiding AB treatment in +/- 25%**

- Sputum for routine gram stain and culture, if sputum is purulent and to be correlated with morphotype in gram stain (A3)

  **ERS Guidelines, 2005, Updates, 2011**
Culture based detection of *S. pneumoniae*

Advantages:
- low cost and high specificity: 85-95% but in adults
- both antibiotic susceptibility and serotype results possible

Difficulties:
- tendency of *S. pneumoniae* to autolyse
- antibiotic treatment prior to sampling
- low prevalence of detectable bacteremia
- colonisation vs infection in children?
- difficulty of obtaining good specimen
- description of *S. pseudopneumoniae*
Incidence of pneumococcal bacteremia

- rates of positive blood cultures in adults hospitalized with pneumonia are typically only 3%–8%

- in children even lower rates

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waskerwitz (1981)</td>
<td>5.8%</td>
</tr>
<tr>
<td>Dershewitz (1983)</td>
<td>4.3%</td>
</tr>
<tr>
<td>Carroll (1983)</td>
<td>10.4%</td>
</tr>
<tr>
<td>Bennish (1984)</td>
<td>4.3%</td>
</tr>
<tr>
<td>Jaffe (1987)</td>
<td>2.8%</td>
</tr>
<tr>
<td>Lee (1998)</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

→ Recommendations on blood cultures?

Value of Blood Culture in the Diagnosis of adult CAP

- Specificity: very high (100 %)
- Sensitivity low: positive in 4-29% of untreated cases; 34 % when initiated within 4 days after first symptoms

Butler JC et al J Infect Dis. 2003;187:1422

⇒ Most sensitive for *S. pneumoniae*
⇒ But… easy to sample and often the only source of information!

⇒ Blood cultures before initiation of AB therapy  (A3)

ERS Guidelines, 2005, Updates, 2011
The Management of Community-Acquired Pneumonia in Infants and Children Older Than 3 Months of Age: Clinical Practice Guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America

John S. Bradley,1,a Carrie L. Byington,2,a Samir S. Shah,3,a Brian Alverson,4 Edward R. Carter,5 Christopher Harrison,6 Sheldon L. Kaplan,7 Sharon E. Mace,8 George H. McCracken Jr,9 Matthew R. Moore,10 Shawn D. St Peter,11 Jana A. Stockwell,12 and Jack T. Swanson13

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Outpatient</th>
<th>Inpatient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NOT Recommended</strong></td>
<td><strong>Recommended</strong></td>
<td><strong>Recommended</strong></td>
</tr>
<tr>
<td>Comments</td>
<td>Non-toxic, fully immunized children treated as outpatients</td>
<td>Failure to demonstrate clinical improvement, progressive symptoms, or deterioration after initiation of antibiotic therapy</td>
</tr>
<tr>
<td>Strength</td>
<td>Strong</td>
<td>Strong</td>
</tr>
<tr>
<td>Evidence Quality</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Blood cultures: rationale

- **Outpatient**
  - Infrequently identifies pathogens (<2%)
  - False-positives more common than true positives at some hospitals
  - Rarely informs outpatient management

- **Inpatient**
  - Positive in ~3% of uncomplicated pneumonia
  - Positive in ~15% with empyema
  - Allows for culture-directed therapy when positive
  - Provides local epidemiologic data

Diagnostic Testing for pediatric CAP in 47 hospitals

- CXR (AP/lateral)
- Complete blood count
- Blood culture
- Serum electrolytes
- Viral studies
- C-reactive protein
- Arterial blood gas
- Erythrocyte sedimentation rate

Percent
A new **Immunochromatographic membrane test** (ICT) has been developed to detect capsular polysaccharide antigens PnC of *S.pneumoniae* in urine samples.

PnC is a common antigen for all pneumococcal serotypes. ICT has proven very useful in the rapid diagnosis of pneumococcal pneumonia in adults.
Early Diagnosis of Pneumococcal Pneumonia based on Urinary Ag

- Diagnostic yield increased up to 38.9% using ICT combined with conventional methods
- The test tends to be more sensitive for patients with versus those without bacteremia

Sequential approach:
Urinary antigen testing for high-risk patients for whom demonstrative results of a sputum Gram stain are unavailable.


Diagnositc efforts should be directed towards the most severely affected patients and the ones with greatest risk of death.

Ortega et al. Scand J Infect Dis 2005
Limited impact of *S. pneumoniae* U Ag test on adjustment of AB treatment

- Case Control study in 2 groups of pneumonia patients randomised:
  - PnAg performed: N= 139: 22/139 pos
  - PnAg not performed: N= 147

<table>
<thead>
<tr>
<th></th>
<th>Pn Ag group N= 139</th>
<th>Control N= 147</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute nr (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>51 (37%)</td>
<td>67 (46%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Narrowing</td>
<td>66 (47%)</td>
<td>73 (50%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Streamlining to peni or amoxi</td>
<td>17 (13%)</td>
<td>13 (9%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Stop macrolide</td>
<td>56 (40%)</td>
<td>54 (36%)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Implementation of Ag test: no result on change of AB prescription

Piso RJ et al Swiss Med Wkly 2012; 142: w13679
Empirical vs targeted AB in CAP based on results of Pn UAg

Narrowing treatment according to the UAg may be associated with a higher risk of clinical relapse; no outcome or economic benefits

Falguera M et al Thorax 2010; 65: 101-106
**S. pneumoniae** Urinary Ag test, According to Pneumococcal Colonization Status of Pediatric Patients, with or without Pneumonia

<table>
<thead>
<tr>
<th>Patients</th>
<th>With pneumococci in nasopharynx</th>
<th>Without pneumococci in nasopharynx</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with pneumonia</td>
<td>25/41 (61)</td>
<td>6/47 (13)</td>
<td>.001</td>
</tr>
<tr>
<td>Control children with dermatitis of diarrhea</td>
<td>43/80 (54)</td>
<td>25/118 (21)</td>
<td>.001</td>
</tr>
</tbody>
</table>

⇒ Antigen test does not distinguish children with pneumonia from controls without pneumonitis

Comparison of the manufacturer’s protocol and a protocol modified to increase specificity


<table>
<thead>
<tr>
<th></th>
<th>Children with pneumonia</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binax NOW method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manufacturer’s protocol</td>
<td>31/88 (35)</td>
<td>68/198 (34)</td>
<td>NS</td>
</tr>
<tr>
<td>Modified protocol</td>
<td>12/88 (14)</td>
<td>19/198 (10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

modified protocol: a positive reaction within 5 min, rather than within the full 15 min.

Specificity somewhat increased by reading within 5 min, but test does not differentiate pneumonia patients from controls.

Binax NOW S. pneumoniae Ag on other body fluids

- **on CSF samples** in pneumococcal **meningitis**:
  - Sens: 95%–100% and a spec of 100% : 30% more cases vs culture
    

- **pleural fluid** specimens from children and adults with
  - Sensitivity: 71% vs +/- 32% by culture

    Porcel JM et al Chest 2007; 131:1442–7

- **BAL samples**:
  - Sensitivity of 95% and a specificity of 87%
    

- The NOW test can also provide a rapid provisional identification of **S. pneumoniae in blood cultures** with positive results

# Real-Time in-house NAATs

<table>
<thead>
<tr>
<th>Ref, year</th>
<th>Assay</th>
<th>targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luo Y, 2012</td>
<td>PCR+ agarose GE</td>
<td><em>S. pneumoniae, H. influenzae</em> type b, <em>M. tuberculosis</em></td>
</tr>
<tr>
<td>Kim W, 2013</td>
<td>Mx PCR+ agarose GE</td>
<td><em>S. pneumoniae, S. mitis, S. oralis</em></td>
</tr>
<tr>
<td>Abdeldaim G, 2008</td>
<td>Quantitative RT-PCR</td>
<td><em>S. pneumoniae</em></td>
</tr>
</tbody>
</table>
Systematic Review and Meta-Analysis

- 29 studies published between 1993 - 2009 included
- Pneumococcal bacteremia for case definition and patients with bacteremia caused by other bacteria as controls:
  - Sens: 57.1%, Spec: 98.6%
- When the controls were patients suspected of having IPD without pneumococcal bacteremia:
  - Sens: 66.4%, Spec: 87.8%
- being a child was associated with low specificity

**Currently available PCR methods on blood for diagnosis of IPD lack sens and spec needed for clinical practice**

• 753 children 0–16 yrs with a diagnosis of CAP
• pneumococcal infection in 80/753 (10.6%) of patients by RT-PCR
• culture and RT-PCR simultaneously performed in 292 patients:
  - 45 (15.4%) pos by RT-PCR
  - 11 (3.8%) pos by culture  \[ P < .001 \]

• **RT-PCR: significantly more sensitive than culture in revealing bacteremic pneumonia**
RT-PCR and Mx PCR for diagnosis and Serotyping in Children with Culture - PID

All samples PCR positive for lytA gene Serotyping

- On normally sterile fluids
  - RT-PCR: 31/33 (93.9%)
  - MS-PCR: 24/33 (72.7%)
    - \( P = 0.047 \)
- On Npswabs
  - RT-PCR: 30/34 (88.2%)
  - MS-PCR: 19/34 (55.9%)
    - \( P = 0.007 \)

Both MS PCR and RT-PCR useful for pneumococcal serotyping but RT-PCR appears more sensitive

Azzari C et al PLoS ONE 2010; 5:e9282
### Added value of *S. pneumoniae* RT-PCR in IPD in blood

<table>
<thead>
<tr>
<th>cases</th>
<th>confirmed IPD</th>
<th>Probable pneumococcal infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>cases</td>
<td>1-3</td>
<td>4-5</td>
</tr>
<tr>
<td>age</td>
<td>4 – 7 years</td>
<td>10 -11 years</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td>pneumonia with pleurisy</td>
<td>pneumonia (with secondary pleurisy)</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>sterile</td>
<td><em>S. pneumoniae</em></td>
</tr>
<tr>
<td>Pleural fluid culture</td>
<td><em>S. pneumoniae</em></td>
<td>Not done</td>
</tr>
<tr>
<td>Blood real-time PCR</td>
<td>Pos</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Chantreuil J et al J Microbiol Exp 2015, 2: 00040
PCR for detection of IPD in children

- 76 children with IPD: PCR for Ply and LytA gene
  - Sensitivity PCR: 80%, specificity 98%
  - 5 additional cases identified vs standard techniques

pneumococcal meningitis: 122 cases
- 87/122 pos by culture: sensitivity 71%
- Among culture neg:
  - 35 pos by LytA PCR: sensitivity: 100%
- All 122 pos by ICT: sensitivity: 100%
• Children with confirmed meningitis (n = 82) or pneumonia (n = 13) prospectively recruited
• blood and CSF taken for pneumococcal DNA loads
• Median blood and CSF bacterial loads (log DNA copies/mL) were significantly higher in nonsurvivors than in survivors:
  - blood (3.80 vs. 2.97, \( P = 0.003 \)),
  - CSF (8.17 vs. 7.50, \( P = 0.03 \))

\[\text{High Pneumococcal DNA Loads are Associated With Mortality in Children With IPD}\]
Quantitative PCR for Diagnosis of *S. pneumoniae* Infection

- **First** prospective study on Q-PCR
- Based on ROC curve analysis
  - Ct with maximal sensitivity: 28.96
  - Corresponding to +/- 3.7x10^4 DNA c/ml
  - Sens: 90%; spec: 80%

- Significant increase in pathogens: with RQ-PCR (33.5%) vs culture (22.2%) (*p* < .05)
  - RQ-PCR corresp to >10^5 CFU/ml

⇒ **Quantitative PCR has favorable accuracy for diagnosis of pneumococcal pneumonia**


Quantitative DNA-based definition of pneumococcal pneumonia

- At detection limit of PCR
  - Sens: 98%
  - Spec: 84%
- Cut-off $10^4$ DNA copies/ml
  - Sens: 84%
  - Spec: 94%
- Mean Ct value significantly lower for samples with abundant growth

⇒ Quantitative PCR enables differentiation between pathogenicity and commensalism

Abdeldaim G et al Diagn Microbiol Infect Dis J 2008; 60: 143-50
Quantitative PCR for Diagnosis of S. pneumoniae Infection

70/184 (38%) patients with S. pneumoniae
- 15% by blood culture
- 20% by urinary Ag
- 15% culture positive sputa
- 27% by RQ-PCR
  - 82% of these also detected by other methods
  - 50% of these culture - , most of these treated with AB

⇒ RQ-PCR particularly valuable in patients treated with AB

Despite developments in laboratory diagnostics, a **microbiological diagnosis remains difficult in IPD**, particularly for pneumococcal pneumonia.

- **Culture-based** methods remain important.

- **Antigen based techniques** limited to adults but impact of positive tests is limited.

- The role of **nucleic acid amplification tests** has yet to be fully clarified especially of QR-PCR in respiratory samples.