PCR IN PNEUMOCOCCAL DISEASE DIAGNOSIS (& SURVEILLANCE)

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Current diagnostic approach

- **IPD case definition:**
  - Isolation of *Streptococcus pneumoniae* or detection of nucleic acid from a normally sterile site (blood, CSF, aspirated body fluid).

- **Advantage of culture:**
  - Isolate for serotyping & susceptibility; inexpensive

- **Disadvantage of culture:**
  - Insensitive and takes longer

- What about nonsterile sites (pneumonia etc.)?

- Is detection of *S. pneumoniae* enough?
Pneumococcal PCR

- Sensitive, specific, rapid
- Targets
  - *pyl* — pneumolysin (in *S. pseudopneumoniae*)
  - *lytA* — autolysin
  - *pspA* — pneumococcal surface protein
- Sensitivity: <1–10 cfu/mL; specificity *lytA* > *pspA* > *pyl*
- Multiplex:
  - other pathogens (e.g. meningitis)
  - antibiotic R/S genes (e.g. *pbp2b*, *ermB*, *mef*)
Evaluation

- Sterile sites (e.g. CSF, pleural fluid)
  - PCR is now “gold standard”
    - more sensitive (100%) than Gram stain (60%)  
    - faster and more sensitive than culture 
      - depends on previous antibiotics  
    - highly specific  
- Nonsterile sites (e.g. sputum, NPA/S)
  - How to distinguish colonisation from infection?  
  - ?Alternatives e.g. pneumococcal antigen tests?
### Bacteraemia/pneumonia diagnosis

- **Comparison of Binax pneumococcal urinary antigen with pyl/lytA PCR on EDTA blood**

<table>
<thead>
<tr>
<th>Condition (N)</th>
<th>Binax +ve (urine)</th>
<th>PCR +ve (blood)</th>
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<tbody>
<tr>
<td>Pneumococcal bacteraemia (58)</td>
<td>51 (sens 88%)</td>
<td>31 (sens 54%)</td>
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<tr>
<td>Non-pneumococcal bacteraemia (51)</td>
<td>2 (spec 96%)</td>
<td>2 (spec 96%)</td>
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<tr>
<td>Community-acquired pneumonia (77)</td>
<td>21 (27%)</td>
<td>6 (8%)</td>
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Nonsterile (respiratory) specimens

Wang et al & Gilbert Pediatr Pulmonol 2008;43:150

- 100 NPAs Chinese children <5 yrs; pneumonia
- Most already on antibiotics
- Investigation: culture; mPCR/RLB
  - 12 primer pairs: S. pneumoniae; H. influenzae (+ Hib);
    M. catarrhalis; S. pyogenes; S. aureus; B. pertussis;
    M. tuberculosis; M. pneumoniae; C. pneumoniae;
    L. pneumophila; P. aeruginosa; K. pneumoniae
- Amplicons - reverse line blot: membrane macroarray
Results - mPCR/RLB

Wang et al 2008

Reference strains ← Clinical specimens
Results
Wang et al 2008

- One or more pathogens identified: 65%
  - Culture – 23% (St pn 13, Hi 5)
  - mPCR/RLB +ve 63%
    - St pn 52 [alone 23]; Hi 28 [alone 11]
  - 8 other species (not L. pneumophila) identified
  - ≥2 species 13 specimens

- Advantages: mixed pathogens; sensitive
- Disadvantages: ?colonising vs infecting?
- Suitable for surveys > individual diagnosis
184 patients - community-acquired pneumonia

- Pneumococcal pneumonia 70 (38%)
  - Blood culture 15% (27/179)
  - NPS culture 27% (42/158)
  - Pneumococcal urinary antigen 20% (33/169)
  - Sputum culture ≥10^5 cfu/mL 15% (19/128)
  - Sputum RQ-PCR ≥10^5 cfu/mL 27% (34/127)

Of these 24/34 +ve by another method
Aim – causes (incl. pneumococcal serotypes)

13 Australian Children’s Hospitals study

Pleural fluid – culture & molecular testing

- lytA PCR + multiplex PCR for Hi, Mp, Cp
- lytA +ve – serotype identified

mPCR/RLB: 2 sets of primers for all 90 serotypes (+pyl)

- 23 for common serotypes; 34 for less common
- (32 crossreacting)

Amplicons detected by RLB

*supported by GlaxoSmithKline
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<tbody>
<tr>
<td>15F15AAP</td>
<td>16FAp</td>
<td>16AAP</td>
<td>17AAP</td>
<td>19B19CAP</td>
<td>21Sp</td>
</tr>
<tr>
<td>32F32AAP</td>
<td>33B33DAP</td>
<td>33CAP</td>
<td>34SP</td>
<td>35F47FAp</td>
<td>35A35C42Sp</td>
</tr>
<tr>
<td>47AAP</td>
<td>48AP</td>
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Pleural fluid specimens: $N = 128$

- Culture $+ve$ 40
  - 14 Stpn
  - 26 other spp.

- Culture $-ve$ 88

- $lytA$ PCR
  - $-ve$ $N=37$
  - $+ve$ $N=51 (58\%)$

- Multiplex PCR
  - $H. influenzae$ 3
  - $C. pneumoniae$ 1
  - $M. pneumoniae$ 1

Co-infections $n = 3$
- HI + Stpn = 2
- CP + Stpn = 1

ARNiE – preliminary results
Positive Stpn result
Culture = 14 (11%)
PCR = 65 (51%)

Serotype identification
n = 40+/65 (19A, 3, 7)
• 33 by mPCR/RLB
• + ≥ 7 by cpsAB/wzg sequence
Conclusions

- Pneumococcal PCR sensitive, specific, rapid
- Gold standard for sterile sites
  - Allows serotype identification
- Less sensitive/specific than pneumococcal UA for diagnosis of pneumococcal pneumonia in adults
- mPCR/RLB can identify multiple respiratory pathogens - studies of pneumonia aetiology
Acknowledgements

CIDM

- mPCR/RLB: Fanrong Kong
  - Serotype identification: Fei Zhou
  - Respiratory pathogens: Yajuan Wang (Beijing CH)
- Empyema PCRs – Julia Warning, Kiran Thapa
- Serotyping – Shahin Oftadeh

Collaborators

- ARNiE study – Adam Jaffe, Roxanne Strachan
Pneumococcal *cps* cluster
– serotype identification

- Relatively conserved region - regulation & export
- Relatively conserved region - sugar synthesis

- Cassette structure - flanked by *dexB*, *aliA*
- Relatively conserved genes at each end but variable in the middle
  - *cps A-D* all serotypes; *cps M-O* most serotypes
  - *cps H-L* serotype specific (exc serotype 3)