Non-pneumococcal streptococci confounding PCR serotyping of Streptococcus pneumoniae in US colonized adults

Background & Objectives
- Carriage studies often used to evaluate impact of pneumococcal conjugate vaccine (PCV) on serotype-specific pneumococcal colonization rates
- Culture for pneumococci considered gold standard
- Molecular methods for detection and serotyping of pneumococci increasingly being used
  - Non-pneumococcal streptococci may carry homologs to pneumococcal-serotype genes.
- We evaluated use of PCR-serotyping for pneumococci in upper respiratory samples.

Methods
Study Setting and Population
- Outpatient clinics, senior residential communities, senior centers and health fairs in 4 US states (Georgia, Maryland, New York, and Tennessee)
- Healthy adults ≥ 65 years of age
- Adults were excluded if:
  - Reported severe immunocompromising condition (e.g. solid or hematologic malignancies, transplant recipient, end-stage renal disease)
  - Residents of nursing homes or skilled nursing facilities

Design
- Cross-sectional carriage survey from July 13, 2015 through March 31, 2016
- Nasopharyngeal (NP) and oropharyngeal (OP) swabs collected from each participant by trained staff
  - Participants without a paired NP and OP were excluded
  - Demographic and clinical characteristics collected via standardized questionnaire

Laboratory
- Isolation and detection of pneumococci
  - NP and OP stored and processed separately
  - STGG medium used for transport and storage of samples at -70°C
  - Broth enrichment followed by blood agar plate for culture isolation
  - Pneumococci identified by susceptibility to optochin and bile solubility
  - Real-time polymerase chain reaction (PCR) targeting lytA gene (pneumococci gene) on both OP and NP STGG samples in a subset of patients

Pneumococcal serotyping
- Quelling if a pneumococcal isolate was available
- Multiplex PCR on a subset of lytA-positive and lytA-negative specimens

Non-pneumococcal species
- Broth enrichment followed by blood agar plate for culture isolation of non-pneumococcal alpha-hemolytic streptococci species in lytA-negative samples
- Species approximation was determined by multi-locus sequence analysis of 7 housekeeping genes using eMLSA
- PCR for lytA and serotype deduction as well as immunochromatographic test (BinaxNow) performed on non-pneumococcal isolates

Table 1: PCR Serotype Deduction Results of lytA-positive and lytA-negative Specimens

<table>
<thead>
<tr>
<th>PCR Serotyping Results*</th>
<th>lytA positive Specimens</th>
<th>lytA negative Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=53)</td>
<td>(N=398)*</td>
<td></td>
</tr>
<tr>
<td>001, n (%)</td>
<td>9 (16.9)</td>
<td>41 (10.3)</td>
</tr>
<tr>
<td>004, n (%)</td>
<td>9 (16.9)</td>
<td>44 (11.1)</td>
</tr>
<tr>
<td>9V/9A, n (%)</td>
<td>6 (11.3)</td>
<td>42 (10.6)</td>
</tr>
<tr>
<td>005, n (%)</td>
<td>1 (1.9)</td>
<td>11 (2.8)</td>
</tr>
<tr>
<td>23A, n (%)</td>
<td>2 (3.8)</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td>19F, n (%)</td>
<td>1 (1.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>22F/12A/22B/44/46*n, n (%)</td>
<td>1 (1.9)</td>
<td>9 (2.3)</td>
</tr>
<tr>
<td>Others, n (%)</td>
<td>2 (3.8)</td>
<td>9 (2.3)</td>
</tr>
<tr>
<td>Negative for 37 serotypes tested by PCR, n (%)</td>
<td>34 (64.2)</td>
<td>282 (70.8)</td>
</tr>
</tbody>
</table>

*Not mutually exclusive / *PCR reaction groups these serotypes/*Only a subset had PCR serotyping done

Table 2: Non-Pneumococcal Streptococci Confounders on lytA-negative OP Specimens

<table>
<thead>
<tr>
<th>Isolate</th>
<th>PCR Typing</th>
<th>Immunochromatographic Test (BinaxNow)</th>
<th>Optochin</th>
<th>Bile Solubility</th>
<th>lytA Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus oralis (n=1)</td>
<td>st001</td>
<td>negative</td>
<td>resistance</td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>

Summary
- lytA PCR increased detection of pneumococci from 1.1% to 6.9% 
- Of the PCR positive specimens, 94% were from OP swabs 
- Serotype deduction by PCR of lytA-negative specimens also yield pneumococcal-specific serotypes 
- Non-pneumococcal streptococci carrying homologs of pneumococci-serotype genes isolated from lytA-negative OP specimens 
- Some of the Streptococcus mitis isolates were BinaxNow positive

Conclusion
- PCR serotyping for pneumococci can identify homologs to pneumococcal genes on other streptococci species leading to erroneous serotyping results and biased vaccine estimates 
- The almost 6-fold increase in pneumococcal positivity by PCR on OP specimens raises concerns about lytA specificity for pneumococci especially on those specimens