LytA Positive Streptococcus mitis/oralis Confound Interpretation of Pneumococcal Colonization Studies
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Introduction
Streptococcus pneumoniae is a significant cause of respiratory illness in older adults and colonization is considered a necessary precursor to invasive disease. Carriage studies may provide insights about the effects of vaccination in adults if cumulative incidence can be shown to be higher that previously demonstrated by earlier studies using traditional bacterial culture methods. The LytA PCR assay developed by the Center for Disease Control (CDC) has been used to screen samples and is felt to be sensitive and specific for S. pneumoniae. However, unless pneumococcal isolates are recovered from LytA + samples, there is a theoretical concern that results reflect Streptococcus mitis/oralis carrying homolog genes. As part of an ongoing longitudinal surveillance study in older adults assessing cumulative incidence of colonization, we assessed LytA + samples for the presence of S. pneumoniae versus LytA + Streptococcus mitis/oralis.

Methods
150 community-dwelling adults ≥55 years, were enrolled in Winter 2015 and Winter 2016. Medical, illness and vaccination with either 23-valent polysaccharide (PPSV23) or 13 valent conjugated vaccine (PCV) histories were collected at enrolment and surveillance visits. Subjects who were immunosuppressed or treated with antibiotics for > 4 weeks were excluded from the study. Nasopharyngeal (NP) and oropharyngeal (OP) samples were collected using flocked swabs in in skim milk-tryptone-glucose-glycerol (STGG) medium were obtained bimonthly. OP and NP samples were combined and processed with a sequential broth enhancement method and subjected to PCR using the LytA gene assay. A sample was considered positive if amplified at a cyclic threshold of ≤ 40. Broth samples found to be LytA + PCR positive were cultured on gentamicin blood agar plates as were their corresponding individual OP and NP samples and S. pneumoniae identified by morphology and susceptibility to optochin. Broth samples from which pneumococcus could not be recovered were evaluated for the presence of LytA + Streptococcus mitis/oralis by screening 2-3 optochin resistant alpha-hemolytic streptococci with distinct colony morphology for the LytA gene by PCR.

Step 1: Processing and PCR

\[ \text{LytA RT-PCR} \]

LytA (-): Discard Samples

LytA (+): Culture Samples

Step 2: Culture

\[ \text{LytA (+) Samples} \]

Culture broth, OP and NP samples on gentamicin agar plates

Optochin sensitive alpha hemolytic streptococci

Identify as S. pneumoniae

Optochin resistant alpha hemolytic streptococci

Identify as non-pneumococcal Streptococci

Step 3: Screen S. Mitis/Oralis

\[ \text{Non-pneumococcal Streptococci} \]

LytA RT-PCR

LytA (-): Discard Isolates

LytA (+): MALDI-TOF

Subject isolates to mass spectrometry for speciation

Results

From January 2015 – September 2016 285 LytA + samples were detected in 67 subjects for a cumulative incidence of pneumococcal carriage of 45% based on PCR. 25 subjects had multiple colonization events resulting in 98 discrete events during the study period lasting an average of 53 ±63 days. New events were defined as >3 negative routine visits prior to a new LytA PCR + visit. Pneumococcal isolates could be recovered from only 33% of LytA + samples which were cultured and 40% of colonization events.

Approximately 230 optochin resistant alpha-hemolytic streptococcal colonies were tested for the LytA gene. Four isolates initially tested positive though 3 could not be confirmed when individual colonies were re-tested. These colonies may have been mixed colonies of S. pneumoniae and S. mitis/oralis. A single non-pneumococcal alpha hemolytic streptococcus isolate was repeatedly positive for the LytA gene. This organism was confirmed as Streptococcus mitis/oralis by MALDI-TOF mass spectrometry.

Conclusions

We have confirmed the presence of LytA + Streptococcus mitis/oralis. Though our data suggests that LytA + Streptococcus mitis/oralis appears to be relatively uncommon (0.004% of clinical isolates) their presence may confound carriage studies based on PCR without culture confirmation. A second confirmatory PCR or DNA sequencing should be considered to improve specificity particularly in adult studies where lower titer carriage may impede confirmation by culture methods.

Table 1: All LytA (+) Subjects, Events and Samples

<table>
<thead>
<tr>
<th>Culture Pos., (%)</th>
<th>Colonized Subjects N = 67</th>
<th>Colonization Events N = 98</th>
<th>LytA (+) Samples N = 285</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Pos., (%)</td>
<td>35 (52)</td>
<td>37 (38)</td>
<td>58 (20)</td>
</tr>
<tr>
<td>Culture Neg., (%)</td>
<td>28 (42)</td>
<td>55 (56)</td>
<td>120 (42)</td>
</tr>
<tr>
<td>Not Cultured (%)</td>
<td>4 (6)</td>
<td>6 (6)</td>
<td>107 (38)</td>
</tr>
</tbody>
</table>

Table 2: Cultured LytA (+) Subjects, Events and Samples

<table>
<thead>
<tr>
<th>Culture Pos., (%)</th>
<th>Colonized Subjects N = 63</th>
<th>Colonization Events N = 92</th>
<th>LytA (+) Samples N = 178</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture Pos., (%)</td>
<td>35 (56)</td>
<td>37 (40)</td>
<td>58 (33)</td>
</tr>
<tr>
<td>Culture Neg., (%)</td>
<td>28 (44)</td>
<td>55 (60)</td>
<td>120 (67)</td>
</tr>
</tbody>
</table>

*Culture positive denotes samples from which S. pneumoniae was identified, culture by morphology and optochin susceptibility. Approximately 230 optochin resistant alpha-hemolytic streptococcal colonies were tested for the LytA gene. Four isolates initially tested positive though 3 could not be confirmed when individual colonies were re-tested. These colonies may have been mixed colonies of S. pneumoniae and S. mitis/oralis. A single non-pneumococcal alpha hemolytic streptococcus isolate was repeatedly positive for the LytA gene. This organism was confirmed as Streptococcus mitis/oralis by MALDI-TOF mass spectrometry.

*2-3 alpha hemolytic colonies from 83 cultures were screened for the presence of LytA + non-pneumococcal streptococci in clinical samples. Most colonization events (92/98) had samples from at least one visit screened for LytA + non-pneumococcal streptococci. In some cases colonies from multiple visits were screened per colonization event. We have confirmed the presence of LytA + Streptococcus mitis/oralis. Though our data suggests that LytA + Streptococcus mitis/oralis appears to be relatively uncommon (0.004% of clinical isolates) their presence may confound carriage studies based on PCR without culture confirmation. A second confirmatory PCR or DNA sequencing should be considered to improve specificity particularly in adult studies where lower titer carriage may impede confirmation by culture methods.

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