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 *lyt*A 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 *lyt*A + 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 *lyt*A + samples for the presence of *S. pneumoniae* versus *lyt*A *+ 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 enrollment 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 *lyt*A gene assay. A sample was considered positive if amplified at a cyclic threshold of ≤ 40. Broth samples found to be *lyt*A + PCR positive were cultured on gentamicin blood agar plates as were their corresponding individual NP and OP 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 *lyt*A *+ Streptococcus mitis/oralis* by screening 2-3 optochin resistant alpha-hemolytic streptococci with distinct colony morphology for the *lyt*A gene by PCR.

Step 1: Processing and PCR

Samples Collected in STGG and subjected to sequential broth enhancement

LytA RT-PCR

LytA (-): Discard Samples

LytA (+): Culture Samples

Step 2: Culture

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*

non-pneumococcal Streptococci

2-3 optochin resistant alpha hemolytic streptococci selected from *S. Pneumoniae* negative plates

LytA RT-PCR

LytA (-): Discard Isolates

LytA (+): MALDI-TOF

Subject isolates to mass spectrometry for speciation

Results

•From January 2015 – September 2016 285 *lyt*A + 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 definted as >3 negative routine visits prior to a new *Lyt*A PCR + visit. Pneumococcal isolates could be recovered from only 33% of *lyt*A + samples which were cultured and 40% of colonization events.

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

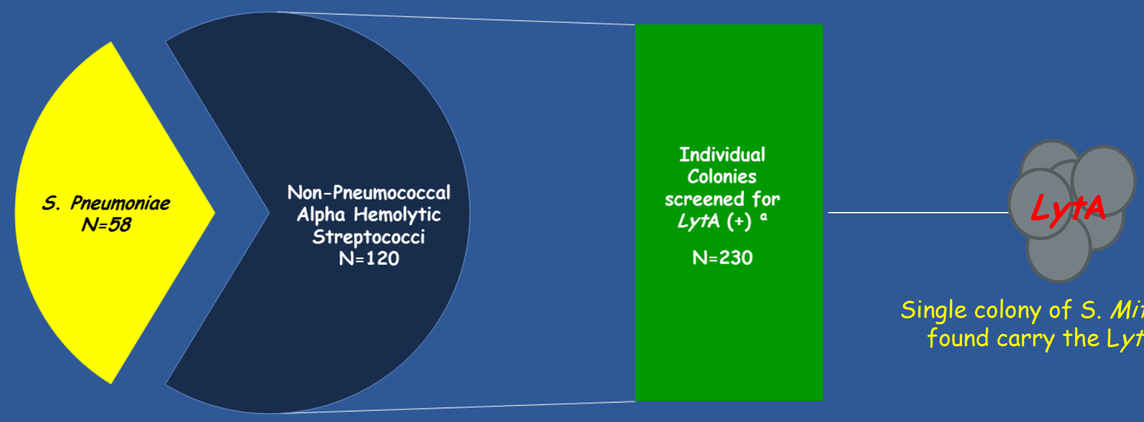
	Colonized Subjects N = 67	Colonization Events N = 98	LytA (+) Samples N = 285
Culture Pos., (%) [a]	35 (52)	37 (38)	58 (20)
Culture Neg., (%)	28 (42)	55 (56)	120 (42)
Not Cultured (%)	4 (6)	6(6)	107 (38)

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

	Colonized Subjects N = 63	Colonization Events N = 92	LytA (+) Samples N = 178
Culture Pos., (%)	35 (56)	37 (40)	58 (33)
Culture Neg., (%)	28 (44)	55 (60)	120 (67)

[a] Culture positive denotes samples from which S. *Pneumoniae* was identified on culture by morphology and optochin susceptibility.

Approximately 230 optochin resistant alpha-hemolytic streptococcal colonies were tested for the *lyt*A 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 *lyt*A gene. This organism was confirmed as *Streptococcus mitis/oralis* by MALDI-TOF mass spectrometry.



[a] 2-3 alpha hemolytic colonies from 83 cultures were screened for the presence of *Lyt*A + non-pneumococcal streptococci in clinical samples. Most colonization events (92/98) had samples from at least one visit screened for *Lyt*A+ non-pneumococcal streptococci. In some cases colonies from multiple visits were screened per colonization event.

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 low titer carriage may impede confirmation by culture methods.