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Effect of prior vaccination on carriage rates of *Streptococcus pneumoniae* in older adults: A longitudinal surveillance study

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ABSTRACT

Introduction: Pneumococcus is a commensal of the upper respiratory tract and colonization is common in young children. Carriage studies have provided insights on vaccine effects in children and may also be useful for assessing vaccines in adults. However, culture based prevalence studies in older adults describe low colonization rates. Therefore, we assessed cumulative incidence of pneumococcal colonization in older adults using polymerase chain reaction (PCR) targeting the *lytA* gene and risk factors for carriage.
Methods: 100 community-dwelling adults ≥65 years were enrolled the winter of 2015 and followed biweekly for 12 months. Medical, vaccination and illness history as well as nasopharyngeal (NP) and oropharyngeal (OP) samples were collected. Combined OP and NP were incubated in enrichment broth and screened using real-time *lytA* PCR. Samples from new colonization events (*lyt*A PCR+) were cultured on gentamicin blood agar plates. Isolates identified by colony morphology as *S. pneumoniae* were serotyped using a multiplex combined immunoassay-PCR platform which classifies 96 serotypes. Cumulative incidence of pneumococcal carriage was calculated and risk factors for carriage assessed.
Results: The cumulative incidence of colonization was 41% by PCR and 14% by culture. Monthly prevalence ranged from 0 to 17% by PCR and 1 to 4% by culture with peaks in the spring and fall. Demographics were similar between colonized and never colonized subjects although colonized were younger (72.4 vs. 75.0 years, P = 0.06). Vaccination with any pneumococcal vaccine before or during study period was associated with decreased risk of becoming colonized ($p < 0.001$) as was vaccination with either the 13-valent conjugated pneumococcal vaccine (PCV13) or 23-valent polysaccharide vaccine (PPSV23) ($p < 0.001$).
Conclusion: Pneumococcal colonization in older adults as detected by *lytA* PCR is frequent and pneumococcal vaccination appears to be associated with decreased risk of carriage. Further study is needed to understand the biological significance of molecular detection of pneumococcus in adults.

1. Introduction

Streptococcus pneumoniae is a commensal of the human upper respiratory tract and an important pathogen in children and older adults. Colonization is considered a precursor to infection and the dynamics of pneumococcal carriage is an area of growing interest [1]. Children are colonized by pneumococci multiple times during the first years of life with rates exceeding 60% in some studies [2–5]. Adults living with preschool children also have high carriage rates, whereas for adults without contact with children, rates are substantially lower [2,5,6]. Although older adults are at increased risk for invasive pneumococcal disease, little data are available on carriage rates in this group. The few studies performed suggest colonization is infrequent in this population with rates ranging from 2 to 9% [5–10]. However, most studies used culture to assess colonization and may significantly underestimate rates because of the difficulty of recovering pneumococcus.

Detection of pneumococci in respiratory secretions by PCR has doubled colonization rates compared to conventional bacterial cultures [11–13]. A variety of gene targets have been used but have

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been associated with cross-reaction with non-pneumococcal oral streptococci, primarily the *Streptococcus mitis/oralis* group. Amplification of the autolysin gene (*lytA)* appears to be the most sensitive and specific PCR assay described and is recommended by the World Health Organization (WHO) for studies using non-culture detection [12,14,15]. However, colonization studies in adults using PCR are limited to point prevalence reports or studies of hospitalized persons or nursing home residents [10,11,16,17].

New pneumococcal vaccines are under development for older adults and designing efficacy studies with traditional microbiologic outcomes will be challenging. Because the incidence of bacteremia is low and microbiologic confirmation of pneumococcal pneumonia is notoriously difficult, other outcome measures are highly desirable. Since carriage studies in children have provided important insights into the effect of pneumococcal conjugate vaccines (PCV), it is possible that carriage could be a surrogate of vaccine effectiveness in adults [18–20]. Thus, comprehensive data on the prevalence, duration, and serotype distribution of pneumococcal carriage in older adults are needed. The objective of this surveillance study was to document cumulative incidence of pneumococcal colonization in older adults using PCR testing of NP and OP samples over 12 months and determine risk factors for colonization.

2. Methods

2.1. Study design

One hundred community-dwelling adults age ≥65 years, living in Rochester, NY were recruited January 2015 for one year of active surveillance for upper respiratory tract carriage of *S. pneumoniae*. Subjects were excluded if they were immunosuppressed or had an active infection requiring >4 weeks of antibiotics. At enrollment, demographic and medical data as well as NP and OP samples were collected. Vaccination status was first reported by the subject and confirmed by review of the electronic medical record (EMR) and/or contacting the subjects' primary care provider. Discrepancies between subject report and the EMR were reconciled to reflect the EMR date of vaccination. The University of Rochester and Rochester General Hospital institutional review boards approved the study and written informed consent was obtained from subjects.

Subjects underwent repeat NP and OP sampling twice a month, 14–21 days apart. An additional visit was conducted within 10 days of new pneumococcal detection to collect samples. Colonization was defined as at least one OP or NP sample positive by *lyt*A PCR. For subjects previously PCR+, a possible "new" event required the subject be PCR negative for >1 month (>3 negative samples 14 days apart).

2.2. Sample collection and processing

Regular and neonatal FLOQ swabs (Copan, Murrieta, CA) were used for sampling the OP and NP, respectively. Swabs were placed in separate glass vials containing 1 mL of skim milk-tryptone-glucose-glycerol medium and transported on ice to the laboratory. OP and NP samples were then combined (125μL each) and processed with sequential broth enhancement using LIM broth at a 1:5 dilution (Lim BBL, Becton, Dickinson and Co., Sparks, MD) and 12–18 h incubation at 37 °C with 5% CO_2 [21].

2.3. DNA extraction and PCR

DNA was extracted from 250μL of broth-enhanced specimens using RNA STAT-50 LS with 20 μg blue mussel glycogen and phenol-chloroform extraction. DNA pellets were reconstituted with 12μL of sterile water. Real-time PCR was performed using the assay developed by Carvalho et al. amplifying the conserved *lytA* gene [14]. The sensitivity of the assay was determined to be 100 cfu of bacteria per mL sample. The amplification mixture contained 10μL extracted DNA, 5 mM $MgCl_2$, 400 μM dNTP (dUTP replacing dTTP), 5U Taq polymerase, 1U uracyl DNA-glycosylase (UNG), and *lytA* primers and probe at 200 nM, final reaction volume 25μL. Amplification was performed on a BioRad iCycler for 42 cycles of 95 °C for 15 s and 60 °C for 1 min. A positive result was considered a cycle threshold value ≤40 in an assay with valid positive and negative controls.

2.4. Culture and serotyping

PCR positive broth samples and their corresponding individual NP and OP samples were cultured on gentamicin blood agar plates. *S. pneumoniae* was identified by colony morphology, susceptibility to optochin (BD BBL Taxo P Discs, zone of inhibition ≥14 mm) and bile solubility (lysis of 1.0 McFarland unit of bacteria in sodium deoxycholate solution) [22,23]. All isolates were suspended in 1 mL LIM broth with 20% glycerol and frozen at −80 °C. Serotyping was performed with a multiplexed immunoassay utilizing monoclonal antibodies to capsular polysaccharides combined with a multiplexed PCR assay targeting the capsular polysaccharide synthesis (*cps*) gene locus [24].

2.5. Statistical analysis

Sample size was calculated to detect a cumulative incidence of at least 20% with 80% power assuming a 5% point prevalence. Categorical variables were summarized by counts and proportions, and compared using Fisher's exact test. Continuous variables were expressed as mean ± standard deviation with comparisons made by Student's *t*-test.

Univariate and multivariate survival models, where event is defined as colonization, were used to assess the effects of prior pneumococcal vaccination and acute respiratory illness (ARI) on daily colonization rate, controlling for demographic and clinical variables. Log-rank test and Kaplan-Meier non-parametric estimation of colonization-free probability curves were used to compare days to colonization between groups. Intensity models were then applied to evaluate daily colonization rate by vaccination status or ARI adjusted for demographics of age, gender, chronic medical conditions (chronic obstructive pulmonary diseases [COPD], coronary artery disease, prior malignancy), household size, and contact with children. Multiple colonization events per subject were coded using a counting-process which defines intervals between colonization following termination of the first event as "survival" or pneumococcal colonization-free times. Vaccination variables were time-varying, such that the status of participants without pneumococcal vaccination at enrollment but vaccinated during the study would change immediately after they reported new vaccination. Since vaccination would not be expected to immediately protect against carriage, 2 subjects vaccinated within 2 weeks of a new event were counted as unvaccinated at the time of colonization. All other new vaccinations in colonized subjects occurred >2 months before or after colonization. A hazard ratio (HR) was calculated to determine daily colonization rates in vaccinated subjects relative to unvaccinated subjects, and differences considered statistically significant for $p < 0.05$.

3. Results

3.1. Cohort Demographics

One hundred subjects age ≥65 years were enrolled January-March 2015. Seventy-eight subjects completed 12 months of

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