Assessment and Quantification of Nasopharyngeal *Streptococcus pneumoniae* Colonization Does Not Discriminate Between Children With Viral and Bacterial Respiratory Infection

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Introduction

Pneumonia causes a substantial amount of morbidity in children. Viruses, typical bacteria, and atypical pathogens all commonly cause disease - but bacterial pneumonia should be treated with antimicrobials. *Streptococcus pneumoniae* is generally considered to be the most important bacterial pathogen causing bacterial pneumonia in children.

Unfortunately, the microbiologic diagnosis of indeterminate paediatric respiratory infection is difficult. The identification of a viral/atypical pathogen in the nasopharynx does not preclude bacterial coinfection; WBC and serum CRP are only weakly predictive of bacterial aetiology; and there is substantial inter- and intra-observer variability in CXR assessment.

Some have recommended measuring pneumococcal nasopharyngeal load to identify bacterial infection; cutoffs of 3 log copies/mL, 3.9 log copies/mL, and 6.9 log copies/mL have all been proposed.

Methods

Prospective cohort study approved by the Hamilton Integrated Research Ethics Board.

- **Cohort 1:** all children aged >2mos admitted to the PICU of McMaster Children's Hospital (MCH) because of respiratory illness between Sep 2015-Oct 2016
- **Cohort 2:** previously healthy children aged >2mos admitted to MCH paediatric wards because of respiratory illness from Sep 2015-Jan 2020
- **Cohort 3:** previously healthy children aged 6mos – 10y diagnosed with non-severe community-acquired pneumonia in the MCH emergency department (ED) from Dec 2012-Mar 2014 and Aug 2016-Dec 2019

Via chart review, children in cohorts 1 and 2 were placed into the following categories:

a) definite viral infection syndrome (such as bronchiolitis)
b) pneumonia complicated by effusion (ie. definite bacterial infection)
c) 'indeterminate' pneumonia (ie. could plausibly be bacterial or viral in aetiology)

Participants in cohort 3 were all categorized as non-severe 'indeterminate' pneumonia.

Methods

Main outcome: NPS pneumococcal load
Covariates of interest: age, cohort of recruitment, disease category

Chi-square/Fisher exact testing was used to compare categorical variables. Forward stepwise logistic regression was done; those associated with a significant ($p<0.05$) change in the -2logL of the model were retained.

Participants' nasopharyngeal swabs (NPS) were collected in UTM and processed as follows:

- 200uL extracted (EasyMag), eluted in 55 uL
- 5 uL amplified for *S. pneumoniae* using previously published primers/probes (*lytA*)
- qPCR assay run on Rotor-Gene Q using QuantiTect Probe PCR kit
- Ct values <40 cycles considered positive
- 20 positive controls + 25 negative controls tested – no cross-reactivity with VGS
- positive samples quantified using a standard curve (serial dilutions of *lytA* cloned control)

Results

In total, there were:

- 206 participants recruited to Cohort 1
- 122 participants recruited to Cohort 2
- 179 participants recruited to Cohort 3

Table 1. Participant characteristics

Variable	Count (%)
Age category	
<24 months	220 (43%)
2 to <5 years	162 (32%)
5 to <10 years	92 (18%)
10 years and older	32 (6.3%)
Infection syndrome category	
Viral syndrome	160 (32%)
Indeterminate pneumonia, non-severe	179 (35%)
Indeterminate pneumonia, hospitalized	134 (26%)
Complicated pneumonia	34 (6.7%)
Pneumococcal NPS genomic load	
< 3 log copies/mL	286 (56%)
3-6.9 log copies/mL	153 (30%)
>6.9 log copies/mL	68 (13%)

Results (continued)

- the distribution of pneumococcal genomic load was roughly normal in those with detectable carriage

Table 2. Comparison of subjects in different cohorts

	Cohort 1	Cohort 2	Cohort 3
Age category			
< 2 y	**77 (37%)**	**67 (55%)**	**76 (43%)**
2 to <5 y	**61 (30%)**	**36 (30%)**	**65 (37%)**
5 to <10 y	**41 (20%)**	**15 (12%)**	**36 (20%)**
10 y and over	**27 (13%)**	**4 (3%)**	**1 (0.6%)**
Proportion with no detectable pneumococcal genomic load	**139 (67%)**	**65 (53%)**	**76 (42%)**
Median genomic load (25-75%ile) in those with detectable pneumococcal colonization, log copies/mL	6.23 (4.85-7.21)	5.77 (5.03-6.82)	6.30 (5.06-7.17)
Pneumococcal genomic load			
<3 log copies/mL	**140 (68%)**	**66 (54%)**	**80 (45%)**
3-6.9 log copies/mL	**46 (22%)**	**46 (38%)**	**61 (34%)**
>6.9 log copies/mL	**20 (9.7%)**	**10 (8.2%)**	**38 (21%)**

Bold indicates differences between categories, $p<0.001$.

Table 3. Comparison of subjects in different diagnostic disease categories.

	Viral	Pneumonia, indeterminate, nonsevere	Pneumonia, indeterminate	Pneumonia, complicated
Age category				
< 2 y	**93 (58%)**	**76 (43%)**	**49 (37%)**	**2 (5.9%)**
2 to <5 y	**36 (22%)**	**65 (37%)**	**43 (32%)**	**18 (53%)**
5 to <10 y	**22 (14%)**	**36 (20%)**	**26 (19%)**	**8 (24%)**
10 y and over	**9 (5.6%)**	**1 (0.56%)**	**16 (12%)**	**6 (18%)**
Proportion with no detectable pneumococcal genomic load	97 (61%)	**76 (42%)**	86 (64%)	21 (62%)
Median genomic load (25-75%ile) in those with detectable pneumococcal colonization, log copies/mL	6.12 (4.63-6.89)	6.30 (5.06-7.17)	5.80 (5.30-6.94)	6.26 (5.05-7.63)
Pneumococcal genomic load, log copies/mL				
<3 log	98 (61%)	**80 (45%)**	87 (65%)	21 (62%)
3-6.9 log	48 (30%)	**61 (34%)**	35 (26%)	9 (26%)
>6.9 log	14 (8.8%)	**38 (21%)**	12 (9.0%)	4 (12%)

Bold indicates differences between categories, $p<0.001$.

- pneumococcal carriage appeared to be associated with age category, cohort of recruitment, and disease diagnostic category

Results (continued)

Table 4. Associations with pneumococcal colonization >3 log copies/mL.

Covariate	Bivariate analyses		Multivariate analyses	
	OR (95%CI)	Wald p	OR (95%CI)	Wald p
Age category				
< 2 y	ref		ref	
2 to <5 y	1.27 (0.85-1.90)	0.25	1.28 (0.83-1.97)	0.27
5 to <10 y	0.39 (0.23-0.66)	0.001	0.39 (0.22-0.68)	0.001
10 y and over	0.16 (0.053-0.46)	0.001	0.22 (0.072-0.68)	0.008
Cohort				
1	ref		ref	
2	1.80 (1.14-2.85)	0.012	1.54 (0.94-2.53)	0.09
3	2.62 (1.73-3.98)	<0.001	2.20 (1.36-3.55)	0.001
Disease category				
Viral infection	ref		NS	
Indeterminate pneumonia, nonsevere	1.96 (1.27-3.02)	0.002		
Indeterminate pneumonia	0.85 (0.53-1.38)	0.52		
Complicated pneumonia	0.98 (0.46-2.10)	0.96		

NS, not significant.

Discussion

In this study, we did not find any association between respiratory disease category and *S. pneumoniae* nasopharyngeal carriage >3 log copies/mL, OR quantitative genomic load, when the effects of age and cohort/location of recruitment were taken into account.

In Canada, the assessment/quantification of NPS pneumococcal carriage does not appear useful to diagnose bacterial pneumonia in individuals or as a supplemental diagnostic aid in epidemiologic studies of paediatric respiratory infection.

Study limitations include:

- Cohorts 2 and 3 were convenience samples, so we cannot exclude selection bias
- Other potential confounders (sociodemographic status, daycare exposure, household crowding, genetic factors, vaccination status) were not adjusted for