Dynamics of Bacterial Colonization With *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* During Symptomatic and Asymptomatic Viral Upper Respiratory Tract Infection

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Background. Virus is detected in about 80% of upper respiratory tract infections (URTIs) in children and is also detectable in the nasopharynx of 30% of asymptomatic children. The effect of asymptomatic viral infection on the dynamics of bacterial density and colonization of the nasopharynx has not been reported. The current study was performed to assess the presence and density of *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* in the nasopharynx of 4–7-year-old children during URTI and when well.

Methods. Nasal samples were obtained during 4 surveillance periods when children were asymptomatic and whenever they had symptoms of URTI. Respiratory viruses and bacterial pathogens were identified and quantified using polymerase chain reaction.

Results. The proportion of children colonized with all 3 bacteria was higher during visits for acute URTI than during asymptomatic surveillance visits. Mean bacterial densities were significantly higher at all visits for all 3 pathogens when a virus was detected. The differences between the means were 1.0, 0.4, and 0.7 $\log_{10}$ colony-forming unit equivalents per milliliter for *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* respectively, compared with visits in which virus was not detected. The percentage of children colonized and density were also higher at asymptomatic visits in which virus was detected than at visits in which virus was not detected.

Conclusion. The density and frequency of colonization with *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in nasal wash samples increase during periods of both symptomatic and asymptomatic viral infection. Increases in bacterial colonization observed during asymptomatic viral infection were nearly the same magnitude as when children were symptomatic.

Keywords. Upper respiratory infections; Streptococcus pneumoniae; Haemophilus influenza; Moraxella; colonization.

Upper respiratory tract infection (URTI) is the most common infectious disease of childhood and one of the most frequent indications for medical care [1]. Although URTIs are caused by respiratory viruses, the common local bacterial complications of URTI, acute otitis media, acute bacterial sinusitis and pneumonia, result from pathogenic bacteria found in the nose and nasopharynx. The current understanding of these complications is that viral URTI precedes bacterial infection and that alterations in mucosal conditions facilitate proliferation of bacteria in the respiratory tract and the development of secondary bacterial infections. Previous studies, performed using semiquantitative culture techniques, have supported this theory [2–4]. A virus is detected in about 80% of URTIs in children,

but notably ≥30% of asymptomatic children will also have a detectable virus in the nasopharynx, depending on the population studied [5–9]. The effect of asymptomatic viral infection on the dynamics of bacterial colonization of the nasopharynx has not been reported. The objective of the current study was to prospectively investigate the frequency and density of bacterial colonization during periods of symptomatic and asymptomatic respiratory viral infection in an observational cohort of 4–7-year-old children.

PATIENTS AND METHODS

Enrollment and Inclusion Criteria

Healthy children aged 48–96 months were recruited throughout the study period from 2 pediatric practices in Madison, Wisconsin, and followed up for 1 year. Children were excluded if they had an underlying condition, reported by the parent or noted in the medical record, that was likely to alter the natural history of URTI, including asthma, congenital or acquired immunodeficiency, craniofacial abnormalities, cystic fibrosis, allergic rhinitis, or a history of chronic sinusitis. Written, informed consent was secured, and assent was obtained from

children ≥7 years of age. Subjects received a small stipend for participation. The study was approved by the University of Wisconsin Institutional Review Board.

Procedures

Nasal samples were obtained at entry and during 4 surveillance visits (February, April, September, and December) when children were asymptomatic as verified by study nurses. If respiratory symptoms were present, the surveillance visit was postponed for 2 weeks. Parents were instructed to call the study nurse at the first sign of a URTI, which was defined as ≥48 hours of respiratory symptoms, including nasal congestion, nasal discharge, or cough. Nasal samples were obtained on day 2–3 (range, day 1–9) of illness by the study nurse. and a recovery sample was obtained at about day 15. However, because of variability in scheduling, the recovery swab sample was obtained 10–42 days after onset of URTI. A clinical assessment at the time of the visit assured that symptoms reflected illness confined to the upper respiratory tract.

Collection of Samples

Samples of nasal mucus were obtained using an established nose-blowing technique [10–12]. Saline nasal spray was sprayed into each of the child's nostrils. The study nurse then held a transparent plastic bag to the nose and occluded one of the nostrils, taking care to not touch the inside of the bag. The child was then instructed to blow their nose forcefully into the plastic bag. This procedure was then repeated with the other nostril. If children were unable to blow their nose, a sufficient sample was obtained by allowing the sprayed saline to drip into the plastic bag. The sample was placed in M4RT transport medium (Remel) and transported on ice to the processing laboratory.

Virus Identification

Diagnostic virology was performed on nasal samples by multiplex polymerase chain reaction (PCR; Respiratory Multicode Assay [EraGen Biosciences] or Respiratory Viral Panel [Luminex]) to test for the following viruses: respiratory syncytial virus (RSV; groups A and B), rhinovirus (RV; approximately 160 known types), parainfluenza (1, 2, 3, 4a and 4b), influenza (A, B and C), adenovirus (B, C and E), coronavirus (229E, NL63, OC43, HK, and severe acute respiratory syndrome), enterovirus, human bocavirus, and human metapneumovirus (A and B). Nasal specimens were also analyzed by means of partial sequencing to determine which RV types were present and differentiate closely related enterovirus from RV [13].

Bacterial Polymerase Chain Reaction

Nasal samples were also analyzed for *Streptococcus pneumoniae, H. influenzae*, and *Moraxella catarrhalis* by using quantitative real-time PCR. DNA was extracted with the BiOstic Bacteremia DNA Isolation Kit (Mo Bio Laboratories). Quantitative LytA PCR for the detection of *S. pneumoniae* [14] was combined with hpd PCR for the detection of *H. influenzae* [15] and copB PCR for the detection of *M. catarrhalis* [16], as described elsewhere. All primers and probes were obtained from Applied Biosystems, and the real-time PCR assay was performed with a 7300 Applied Biosystems instrument. Standard curves consisted of bacterial DNA extracted from known quantities of clinical isolates of each bacterium obtained from the University of Wisconsin Hospital Clinical Microbiology Laboratory, and PCR results were expressed as colony-forming unit equivalents (CFUe) per milliliter. The same standards were used throughout the study and were internally consistent. Colonization was defined as any detectable bacteria by PCR (≥1 CFUe/mL) in nasal samples.

Statistical Methods

Demographic characteristics were summarized in terms of means and standard deviation or percentages. The proportion of subjects colonized was analyzed using the generalized estimating equation approach, with a logit link function to account for multiple visits. Proportions were summarized along with the corresponding 95% confidence intervals, stratified by visit type and virus detection status. Tukey's honestly significant difference method was used to control the type I error when conducting multiple comparisons between visit types. Bacterial density values (in CFUe per milliliter) were log (base 10) transformed and analyzed using a linear mixed-effects model with subject-specific random effects. A compound symmetry correlation structure was used to account for correlations between repeated visits within the same subject. Residual plots were examined to verify model assumptions. All reported P values are 2 sided, and $P < .05$ was used to define statistical significance. Statistical analyses were conducted using SAS software (version 9.4; SAS Institute).

RESULTS

Invitations to participate in this study were sent to families of 4516 patients from 2 pediatric clinics after screening 6394 possible participants; 1878 subjects were not eligible owing to an underlying medical condition (Figure 1). From February 2012 to September 2015, a total of 279 subjects were enrolled. One subject was excluded after enrollment because nasal samples could not be obtained, 205 were followed up for an entire year, and the remaining 73 subjects had ≥1 surveillance sample taken. The demographic characteristics of enrolled subjects are shown in Table 1.

There were 847 surveillance, 363 acute URTI, and 298 recovery visits (Figure 1) A virus was detected in 34% of nasal samples obtained during surveillance visits, 81% of those obtained during acute URTI visits, and 32% of those obtained during recovery visits. The rates of detection of individual viruses at each visit type are detailed in Table 2. As expected, RV was the most common virus detected for all 3 visit types.
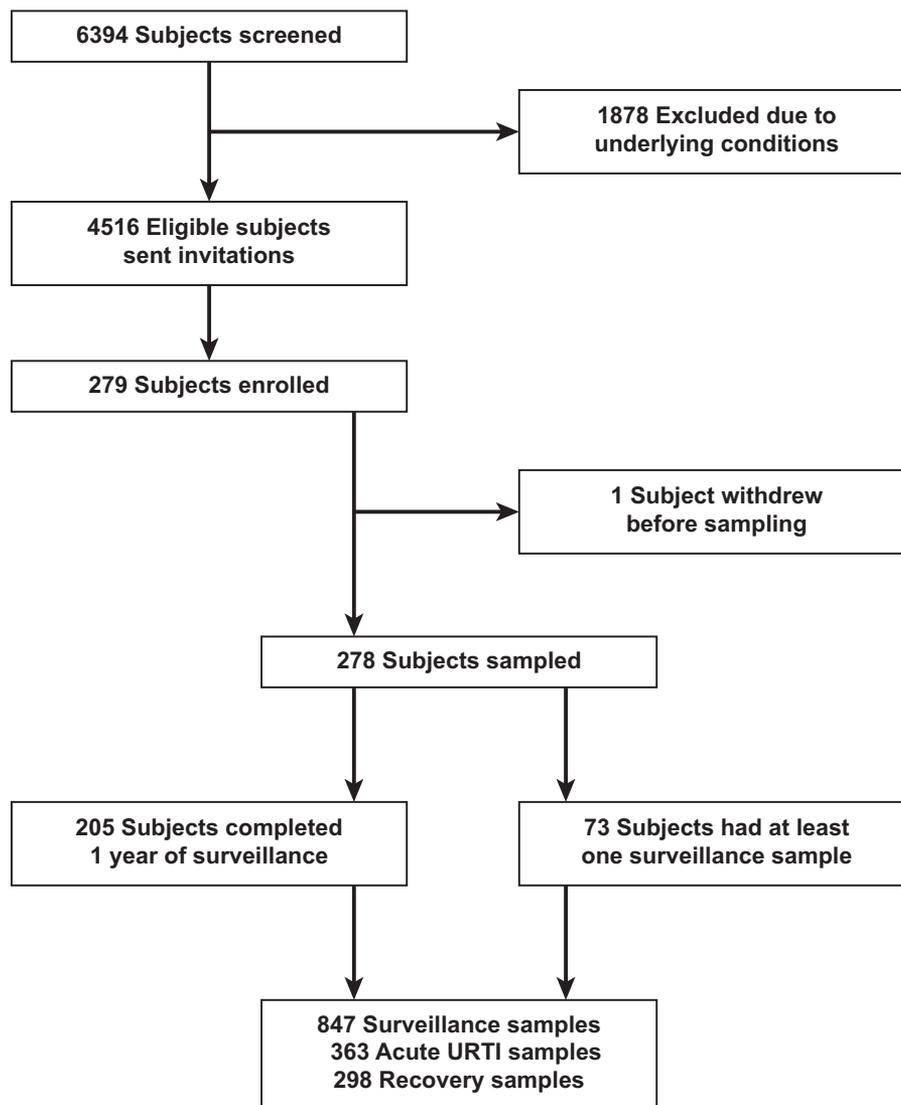
Figure 1. Enrollment of subjects. Abbreviation: URTI, upper respiratory tract infection.

The proportion of children colonized was determined at the initial surveillance visit for each bacterial pathogen, and these proportions are shown in Table 3. The proportion of children colonized was significantly higher for *S. pneumoniae* (54%) than for *Haemophilus influenzae* (26%) or *M. catarrhalis* (33%) (*P* < .001) and higher for *M. catarrhalis* than for *H. influenzae* (*P* = .04). Next we compared the proportion of children colonized at surveillance visits where virus was detected compared with visits where a virus was not detected. The proportion colonized was significantly higher when virus was detected than when not detected for all 3 respiratory pathogens (62% vs 47% for *S. pneumoniae* [*P* < .001], 28% vs 20% for *H. influenzae* [*P* = .007], and 43% vs 36% for *M. catarrhalis* [*P* = .03]; Table 4). Patients were often colonized with >1 bacterial pathogen. The proportions of children colonized with ≥1 pathogen at surveillance and acute visits are shown in Table 5. Colonization with

any 1 of the 3 bacteria was more common during acute visits than during surveillance visits (*P* < .001), as was the detection of any 2 bacteria (*P* < .001) or all 3 bacteria in the same sample (*P* = .05).

Bacterial densities in nasal samples obtained at study visits are shown in Figure 2. Densities were significantly higher at acute URTI visits for *S. pneumoniae, H. influenzae* and *M. catarrhalis,* demonstrating nearly a log increase in density compared with surveillance visits. In addition, bacterial densities for all 3 bacteria were significantly decreased at recovery visits compared with acute URTI visits. Bacterial densities at recovery visits dropped to near baseline surveillance levels for *S. pneumoniae* and *H. influenzae* but remained significantly above those for surveillance visits for *M. catarrhalis* (*P* < .05).

To isolate the effect of viral URTIs on bacterial density, the mean densities of bacteria were compared between visits at which a virus

Table 1. Subject Demographics (n = 278)

Demographic Variable	Subjects, %[a]
Age, mean (range), y	5.2 (4.0–7.7)
Female sex	46
Race	
American Indian or Alaska Native	1
Asian	5
African American	7
Other	6
Unknown or not reported	1
White	80
Hispanic ethnicity	7
Attends day care	83
Tobacco exposure	3
Maternal education level	
Graduate/professional	36
College degree	37
Some college	14
Vocational/technical training	5
High school or less	6
Not reported	1
Public insurance (Medicaid)	18

[a]Data represent No. (%) of subjects unless otherwise specified.

was detected and those at which a virus was not detected. Mean densities were significantly higher at all visits for all 3 bacterial pathogens (Figure 3A), when a virus was detected, with mean differences in density of 1.0, 0.4, and 0.7 $\log_{10}$ CFUe/mL for *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* respectively, compared with visits at which a virus was not detected ($P < .001$). The effect of viral infection on increasing bacterial density occurred regardless of whether symptoms were present. Figure 3B shows bacterial densities for asymptomatic visits at which a virus was detected compared with those at which a virus was not detected. At surveillance visits, the mean differences in density between these visits

Table 2. Virus Identification at Study Visits

Virus	Samples with Virus Detected, (%)		
	Surveillance Visits (n = 847)	Acute URTI Visits (n = 363)	Recovery Visits (n = 298)
AdV	2 (<1)	0 (0)	2 (1)
hBoV	12 (1)	2 (1)	3 (1)
CoV	24 (3)	24 (7)	11 (4)
EV	4 (<1)	7 (2)	1 (<1)
FluA	2 (<1)	5 (1)	0 (0)
FluB	2 (<1)	7 (2)	0 (0)
MPV	6 (2)	11 (3)	3 (1)
PIV	8 (1)	21 (6)	3 (1)
RSV	3 (<1)	15 (4)	2 (1)
RV	205 (25)	160 (44)	59 (20)
Mixed	20 (1)	43 (12)	10 (3)
Negative	559 (66)	68 (19)	204 (68)

Abbreviations: AdV, adenovirus; CoV, coronavirus; EV, enterovirus; FluA, influenza A virus; FluB, influenza B virus; hBoV, human bocavirus; MPV, metapneumovirus, PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus; URTI, upper respiratory tract infection.

Table 3. Colonization Frequency at Initial Surveillance Visits

Bacterial Pathogen	Children Colonized, No. (%; 95% CI) (n = 278)	Comparison	P Value[a]
Streptococcus pneumoniae	150 (54; 48–60)	*S. pneumoniae* vs *Haemophilus influenzae*	<.001
H. influenzae	72 (26; 21–31)	*S. pneumoniae* vs *Moraxella catarrhalis*	<.001
M. catarrhalis	93 (33; 28–39)	*H. influenzae* vs *M. catarrhalis*	.04

Abbreviation: CI, confidence interval.

[a]Based on generalized estimating equation analysis.

were 0.8, 0.4, and 0.4 $\log_{10}$ CFUe/mL for *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* respectively ($P < .01$).

Bacterial densities were analyzed for individual respiratory viruses (Table 6). Samples that had >1 virus detected were labeled as mixed. Samples in which RV was detected had significantly higher densities for all 3 bacterial pathogens than samples without detection of a virus. Samples with mixed virus detection showed significantly higher densities of *S. pneumoniae* and *M. catarrhalis* but not *H. influenzae.* Comparison between other individual respiratory viruses was not possible because of low numbers.

To further determine the role of viral infection in affecting bacterial colonization and density, we compared 4 groups: surveillance visits with no virus detected (SN), surveillance visits with virus detected (SV), acute URTI visits with no virus detected (AN), and acute URTI visits with virus detected (AV). Differences were adjusted for multiple comparisons. For *S. pneumoniae* (Figure 4A), the proportions of children colonized were similar for SV and AV visits, and both were significantly higher than for SN visits. The density of each bacterial species was grouped into 4 levels (1–100, 100–1000, 1000-10 000, or >10 000 CFUe/mL) for each of the visit types (SN, SV, AN, and AV). For *S. pneumoniae,* when a virus was detected, the greatest increase in the proportion of samples occurred in the group with >10 000 CFUe/mL. For *H. influenzae* (Figure 4B) colonization frequency was significantly higher for SV, AN, and

Table 4. Colonization Frequency at Surveillance Visits: Virus Versus No Virus

Bacterial Pathogen	Detectable Colonization, No. (%; 95% CI)		P Value[a]
	Virus Detected (n = 288)	No Virus Detected (n = 559)	
Streptococcus pneumoniae	178 (62; 55–68)	254 (47; 43–52)	<.001
Haemophilus influenzae	82 (28; 23–35)	111 (20; 16–24)	.007
Moraxella catarrhalis	125 (43; 38–49)	200 (36; 31–41)	.03

Abbreviation: CI, confidence interval.

[a]*P* values based on generalized estimating equation analysis

Table 5. Bacteria Detected

| Bacterial Pathogens Detected | Proportion Colonized, % (95% CI) | | P Value[a] |
	Surveillance Visits (n = 847)	Acute Visits (n = 363)	
None	29 (25–33)	16 (12–20)	<.001
Single pathogen			
Total	37 (33–41)	35 (29–41)	.49
S. pneumoniae	21 (18–24)	17 (12–21)	.13
Haemophilus influenzae	4 (2–5)	3 (1–5)	.73
Moraxella catarrhalis	13 (10–15)	15 (11–19)	.26
2 Pathogens			
Total	25 (22–29)	36 (31–41)	<.001
S. pneumoniae and *H. influenzae*	8 (6–10)	12 (8–15)	.04
S. pneumoniae and *Moraxella catarrhalis*	15 (12–17)	19 (14–24)	.1
H. influenzae and *M. catarrhalis*	2 (1–4)	5 (3–8)	.04
3 Pathogens: *S. pneumoniae, H influenzae,* and *M. catarrhalis*	9 (7–11)	13 (9–17)	.05

Abbreviation: CI, confidence interval.

[a]*P* values based on generalized estimating equation analysis.

AV visits than for SN visits. Again the greatest change in bacterial density occurred in the proportion of samples with >10 000 CFUe/mL. *For M. catarrhalis* (Figure 4C), the density of colonization was higher for both AN and AV visits than for SN visits, also with the greatest change in the >10 000 CFUe/mL group.

Bacterial density for all 3 pathogens was tracked over the time course of a URTI. The mean observed duration for

bacterial density to return to baseline levels was 13.6 days (range, 5–40 days). Overall 223 of 295 or 75% of URTIs (95% confidence interval, 70%–80%) showed a return to baseline of bacterial density by day 14 of illness for all 3 bacterial pathogens. The rate of return to baseline density did not differ between URTIs by virus type detected (data not shown).

DISCUSSION

In this study, dynamic changes in upper respiratory bacterial flora associated with viral infection were demonstrated. The density of all 3 of the common respiratory bacterial pathogens in nasal samples, *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* increased by nearly a factor of 10 between surveillance periods and episodes of symptomatic URTI. In addition, it has been shown for the first time that bacterial detection and density of all 3 sinus pathogens increase during asymptomatic viral infection. In general, bacterial density was lowest for asymptomatic visits at which virus was not detected and highest for acute URTI visits at which virus was detected. Bacterial densities decreased to near-baseline levels during recovery. The impact of virus detection on bacterial detection and density lasted about 14 days from the onset of symptoms.

The effect of virus on increasing bacterial density and colonization was greatest for *S. pneumoniae.* This large effect of virus detection on *S. pneumoniae* was also observed during asymptomatic viral infections. Interestingly, the proportion colonized for all 3 pathogens was similar between surveillance visits with virus detected and acute URTI visits with no virus detected. Furthermore, there were no differences in the frequency of
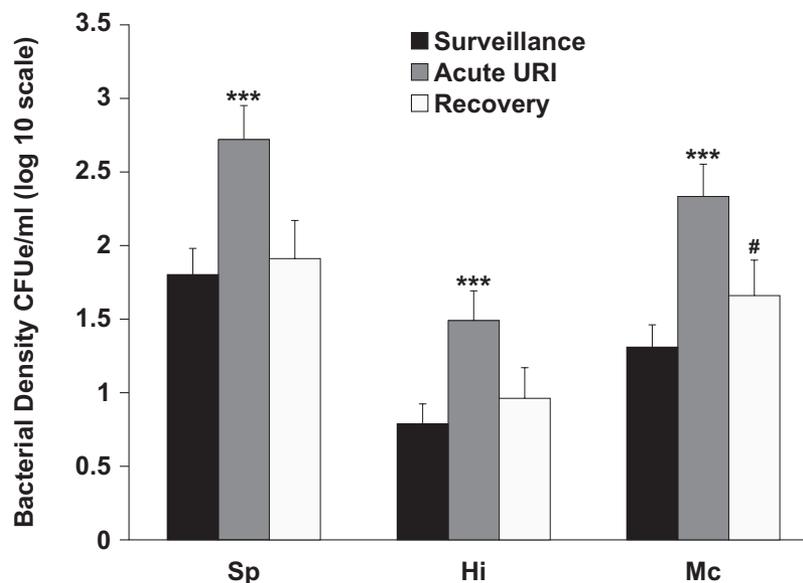
Figure 2. Bacterial density for *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* at surveillance visits (n = 847), acute upper respiratory tract infection (URTI) visits (n = 363), and recovery visits (n = 298). *P < .001 for surveillance versus acute URTI visits; †P < .01 for acute URTI versus recovery visits; ‡P = .03 for recovery versus surveillance visits. Error bars show 95% confidence intervals. Abbreviation: CFUe, colony-forming unit equivalents.
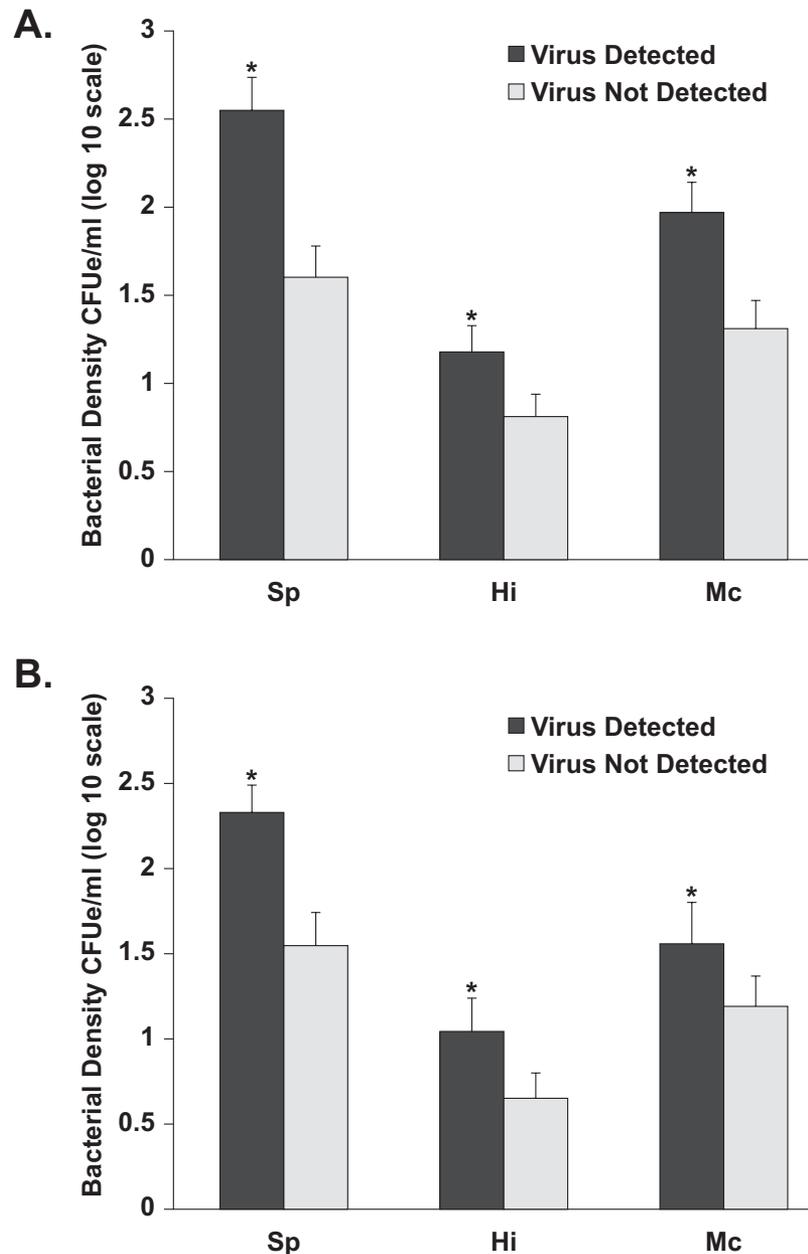
Figure 3. Bacterial density for *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* for all visits (n = 1508; *A*) and surveillance visits (n = 847; *B*) at which a virus was detected versus not detected. *$P < .01$ for virus detected versus not detected. Abbreviation: CFUe, colony-forming unit equivalents.

bacterial colonization between episodes of acute URTI with virus and those without virus. Accordingly, it is possible that the upper respiratory tract symptoms in acute episodes of URTI without virus are in fact caused by a virus not in our PCR panel or present in undetectable amounts.

For all 3 pathogens, bacterial density (but not colonization) was higher for symptomatic URTI visits at which a virus was detected than for surveillance visits at which a virus was detected. This suggests that increased density of pathogenic bacteria may itself be a cause of respiratory symptoms without viral coinfection or contribute to symptoms (not only complications) when

virus is present. A similar observation was made by Kloepfer et al [17] for *S. pneumoniae* and *M. catarrhalis* but not *H. influenzae* in a group of children with or without asthma who were followed up for 5 weeks in the autumn season. These differences (in bacterial species) may be explained by season or age, but the suggestion that bacterial species may cause respiratory symptoms is the same and may have therapeutic implications.

The proportion of children colonized in our study was higher than expected for *S. pneumoniae*, whereas *H. influenzae* and *M. catarrhalis* were detected at frequencies similar to those previously reported [4, 18–20]. A study done in infants

Table 6. Adjusted Mean Bacterial Density By Virus for All Visits[a]

| Virus | *Streptococcus pneumoniae* | | *Haemophilus influenzae* | | *Moraxella catarrhalis* | |
	No.	Bacterial Density, Mean (95% CI), Log$_{10}$ CFUe/mL	No.	Bacterial Density, Mean (95% CI), Log$_{10}$ CFUe/mL	No.	Bacterial Density, Mean (95% CI), Log$_{10}$ CFUe/mL
AdV	4	0.72 (−1.4 to 2.85)	3	2.12 (.36–3.89)	3	2.54 (.49–4.58)
hBoV	17	1.46 (.4–2.51)	7	1.32 (.45–2.2)	8	1.22 (.21–2.23)
CoV	59	1.79[b] (1.22–2.36)	8	0.67 (.19–1.14)	37	2.44[c] (1.9–2.98)
FluA	7	2.17 (.57–3.77)	5	2.6 (1.32–3.98)	4	3.1 (1.55–4.64)
FluB	9	2.18 (.80–3.57)	2	0.34 (−.82 to 1.5)	7	3.41 (2.06–4.76)
RV	424	2.57[c] (2.33–2.81)	125	1.24[c] (1.04–1.44)	189	1.82[c] (1.60–2.04)
MPV	20	2.58 (1.64–3.51)	6	1.14 (.36–1.92)	8	1.36 (.45–2.27)
RSV	20	2.84 (1.91–3.78)	7	1.3 (.52–2.08)	12	2.64 (1.73–3.55)
PIV	32	2.87 (2.13–3.62)	4	0.63 (0–1.25)	12	1.41 (.69–2.13)
Mixed	73	3.05[c] (2.54–3.56)	25	1.17 (.74–1.6)	46	2.68[c] (2.19–3.17)
EV	12	3.17 (1.96–4.38)	5	1.65 (.65–2.66)	3	1.38 (.21–2.56)
Negative	73	1.61 (1.43–1.79)	198	0.81 (.67–.96)	318	1.31 (1.15–1.47)

Abbreviations: AdV, adenovirus; CI, confidence interval; CoV, coronavirus; EV, enterovirus; FluA, influenza A virus; FluB, influenza B virus; hBoV, human bocavirus; MPV, metapneumovirus,; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.

[a]Based on a linear mixed-effects model.

[b]$P = .04$ for comparison with mixed results.

[c]$P < .02$ for comparison with negative results..

demonstrated a higher risk of URTI when subjects were colonized with *M. catarrhalis* and *S. pneumoniae* but not *H. influenzae* [20]. In contrast, our study did not demonstrate an increased risk of URTI with colonization with any of the bacteria tested. This difference may be due to the ages of populations studied and the methods used to detect colonization (culture vs PCR).

The results of our study affirm previous important insights into the pathogenesis of the bacterial complications of viral URTIs. Proliferation of *S. pneumoniae, H. influenzae,* and *M. catarrhalis* is thought to be a necessary step in the development of these conditions. Faden et al demonstrated an increase in the carriage rate of otopathogens in the nasopharynx of children with acute otitis media during URTI, compared with well children [21, 22]. Other more recent studies, using culture and quantitative PCR, have shown an increase in pneumococcal carriage [23] and density during acute URTI [24–26]. Xu et al [27] also recently reported that nasopharyngeal colonization rates of otopathogens during health were significantly lower than during URTI. Studies of otitis media and sinusitis have demonstrated that the presence of pathogenic bacteria in the nasopharynx markedly increases the risk of these complications [2–4, 28–31]. During URTI, there are complex interactions between bacteria and viruses [32, 33]. These observations suggest that the combination of virus and bacteria is necessary but not sufficient for the development of bacterial complications of URTI.

Detection of viruses during surveillance, when patients are asymptomatic, is not a new finding [6, 7, 34]. However, the implications of the finding that bacterial densities increase substantially during periods of asymptomatic viral infection are important and may have clinical relevance. These data allow us to understand the development of cases of acute otitis media, acute bacterial sinusitis, and their complications (mastoiditis and subperiosteal orbital cellulitis) in children without

respiratory symptoms or whose respiratory symptoms are extremely modest [35]. In nearly 50% of children presenting with complications of sinusitis, the complication is the first signal of the underlying sinus infection. The current findings demonstrate that, even in the absence of symptoms, viral infection enhances bacterial colonization and density. We can speculate that this enhancement of colonization, especially with *S. pneumoniae*, predisposes the patient to the complications of URTI.

The mechanism by which viral URTI increases bacterial colonization and density is not well understood. A cotton rat model of RSV infection demonstrated a higher frequency of colonization of nontypable *H. influenzae* than non-RSV infected rats during acute RSV infection. This was not associated with changes in bacterial adherence to epithelial cells or to changes in production of specific antibacterial antibody [36]. In vitro studies have shown that RV facilitates the binding, translocation, and persistence of bacteria by disrupting epithelial barrier function [37]. In addition, increased mucus production and host cell debris may enhance the nutritional milieu for bacterial growth during infection with influenza virus [38].

This study has several unique characteristics and possible limitations that should be considered. The longitudinal follow-up of subjects for 1 year allowed the avoidance of seasonal variation in viral infections. Sensitive PCR techniques permitted identification of viruses and quantitation of bacterial colonization. Obtaining nasal samples during surveillance and illness permitted new observations of increased bacterial density even when subjects had asymptomatic viral infection. Samples were obtained with a nasal blow technique rather than by nasopharyngeal swab sample for patient comfort and to improve adherence. This method may seem to be prone to inconsistency, given the potential for variations in dilution. However, this
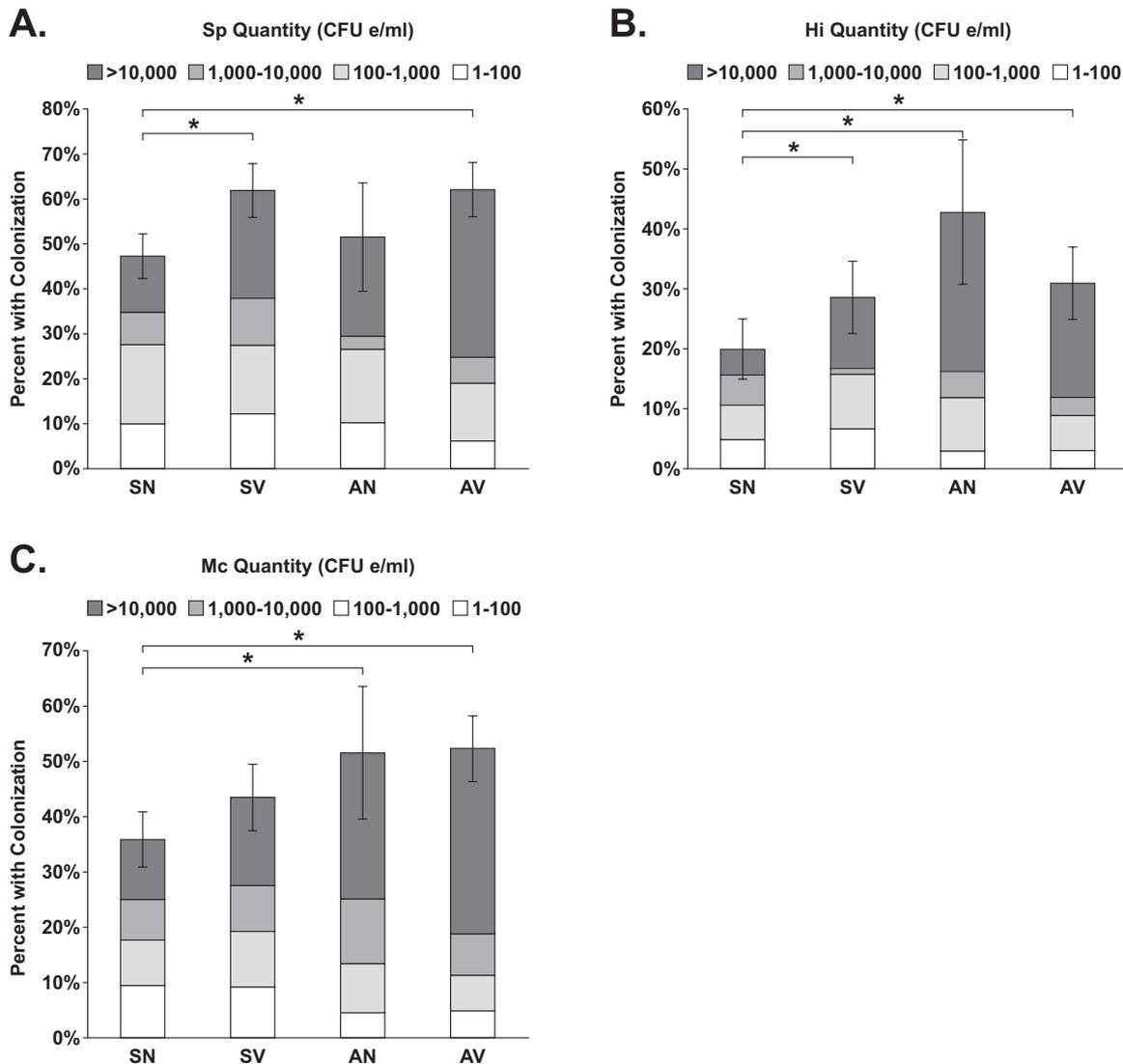
Figure 4. Bacterial colonization rates for *Streptococcus pneumoniae* (*A*), *Haemophilus influenzae* (*B*), and *Moraxella catarrhalis* (*C*) at surveillance visits with no virus detected (SN), surveillance visits with virus detected (SV), acute upper respiratory tract infection (URTI) visits with no virus detected (AN), and acute URTI visits with virus detected (AV). *$P < .05$ adjusted for multiple comparisons by Tukey's honestly significant difference method. Error bars represent 95% confidence intervals. CFUe, colony-forming unit equivalents. Abbreviations: Hi, haemophilus influenzae; Mc, moraxella catarrhalis; Sp, streptococcus pneumoniae.

technique is well established and has been used in many other studies of viral URTI [11, 12, 17, 39–43].

Although specimens obtained during URTI could theoretically have higher concentrations of mucus, and thus higher concentrations of bacteria, similar densities of bacteria were detected during asymptomatic viral infections. This latter observation suggests that an increased yield of mucous during URTI did not affect these results. In addition, our overall colonization proportions were higher than those previously reported, supporting the contention that the use of nasal wash samples did not underestimate the detection of bacterial pathogens. It is also notable that, despite the potential for dilution, the increases in bacterial density and colonization were very consistently found during viral URTI in this study and in others [17].

In conclusion, we have shown that the density and frequency of colonization with the bacterial pathogens *S. pneumoniae, H. influenzae,* and *M. catarrhalis* in nasal wash samples increase during periods of symptomatic and asymptomatic viral infection. In the current study, episodes of symptomatic respiratory infection with or without virus identification were similar with regard to bacterial colonization, suggesting that virus may be present even when undetected. Furthermore, the frequency of bacterial colonization observed during asymptomatic viral infection, especially for *S. pneumoniae,* was nearly of the same magnitude as that observed when children were symptomatic. These observations provide important insights into the pathogenesis of the bacterial complications of URTI, particularly in patients without respiratory symptoms. Further investigations that determine the precise mechanism of virus-bacterial interaction are needed.

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