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Polymicrobial Acute Respiratory Infections in a Hospital-Based Pediatric Population

Margaret L. Chorazy, PhD, MPH[a], **Mark G. Lebeck, MS**[b], **Troy A. McCarthy, BA**[c], **Sandra S. Richter, MD**[d], **James C. Torner, PhD, MS**[a], and **Gregory C. Gray, MD, MPH**[e]

[a]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA, USA

[b]Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA, USA

[c]Center for Biocatalysis and Bioprocessing, College of Medicine, University of Iowa, Iowa City, IA, USA

[d]Department of Clinical Pathology, Cleveland Clinic, Cleveland, OH, USA

[e]Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA

Abstract

Background—The clinical impact of polymicrobial respiratory infections remains uncertain. Previous reports are contradictory regarding an association with severe disease.

Methods—Three hundred and forty-six specimens from children with acute respiratory illness identified at the University of Iowa Hospitals and Clinics Clinical Microbiology Laboratory (CML) were evaluated by DFA, and/or viral culture by CML and later by molecular study for the presence of influenza, parainfluenza (HPIV), respiratory syncytial virus (HRSV), adenovirus (HAdV), human metapneumovirus (HMPV), rhinovirus (HRV), and human bocavirus (HBoV). Demographic and clinical data were abstracted from medical records.

Results—Multiple viruses were detected in 46 (21.7%) of 212 virus-positive specimens with the most frequent virus-virus combinations being HRV-HRSV (n=12), HRV-HBoV (n=6), and HRV-HPIV 3 (n=4). Risk factors for coinfection included: male gender (OR 1.70, 95% CI 0.83–3.46), 6 mos-1 yr age (OR 2.15, 95% CI 0.75–6.19), and history of immunosuppression (OR 2.05, 95% CI 0.99–4.23). Children with viral coinfections were less likely than children with single virus infections to be admitted to an intensive care unit (OR 0.32, 95% CI 1.12–9.17), however, this may be explained by undetected viral-bacterial coinfections.

Conclusions—HRV, HRSV, HBoV and polymicrobial infections were prevalent in this study. While the cross-sectional design could not easily examine polymicrobial infection and disease severity, prospective, population-based research regarding the clinical impact of such infections is warranted.

Keywords

acute respiratory infection; coinfection; epidemiology; polymicrobial infection

Corresponding author information: Margaret L. Chorazy, MPH, PhD, Department of Epidemiology, College of Public Health, The University of Iowa, 105 River Street, S449 CPHB, Iowa City, IA 52242, Telephone: 319-384-1559/Fax: 319-384-4155, margaret-chorazy@uiowa.edu.

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INTRODUCTION

Acute respiratory infections (ARIs) account for an estimated 75% of all acute morbidities and are the leading cause of hospitalization for infants and young children in developed countries.[1] Viral pathogens are the most common cause of ARIs, including influenza virus, human parainfluenza virus (HPIV), human respiratory syncytial virus (HRSV), human adenovirus (HAdV), and human rhinovirus (HRV). Additionally, the importance of newly recognized viruses such as human metapneumovirus (HMPV), human bocavirus (HBoV), human coronaviruses (HCoV), and human polyomaviruses (HPyV) is becoming increasingly evident.

However, the relative importance of mixed infections, sometimes termed polymicrobial infections, has yet to be determined and constitutes an area of active research.[2] The use of molecular detection techniques has more readily allowed for the simultaneous detection of pathogens in respiratory specimens though few studies have attempted to systematically address the clinical importance of these polymicrobial infections.[3–22] Furthermore, the interpretation of results from these studies is complicated by the numerous differences in study design including the methods of pathogen detection employed, the composition of the respiratory pathogen panel included in analysis, and the specific patient population being studied. However, results from recent studies suggest a role for polymicrobial infections as a cause of severe viral ARIs.[4, 5, 8, 9,12, 14, 18, 23–25]

Our primary objectives were to describe the epidemiology of polymicrobial ARI in children and to investigate the association of polymicrobial infection and severity of illness. The central hypothesis of the study was that among children with ARI, those who were infected with multiple viruses were more likely to have severe illness than those individuals with single virus infections.

MATERIAL AND METHODS

Specimen collection and clinical data

We conducted a retrospective, cross-sectional study of frozen, archived respiratory specimens from 421 children under the age of 10 years collected from March 28, 2008 through June 30, 2009 and stored by the University of Iowa Hospitals and Clinics (UIHC) Clinical Microbiology Laboratory (CML). This convenience sample included inpatients and outpatients.

Respiratory specimens were linked to the patient medical record to collect demographic and clinical data including - gender, age, race/ethnicity, zip code of primary residence, payor for services provided, clinic visited, specimen collection date, specimen source, clinical signs and symptoms, clinical diagnosis, patient disposition, admission/discharge date, other CML results, exposure to second-hand tobacco smoke, use of antimicrobials, history of chronic respiratory condition, history of immunosuppression (includes history of cancer, transplant, and other primary or secondary immunodeficiencies), requirement for supplemental oxygen and/or mechanical ventilation, and bronchodilator administration. The study was approved by the University of Iowa Institutional Review Board.

Specimen processing

Serial clinical viral diagnostic specimens were tested by the CML as ordered by physicians. These specimens included nasopharyngeal swab/wash/aspirate, tracheal aspirate, broncheoavleolar lavage, and bronchial wash. The CML used direct immunofluorescent assays and/or viral culture to detect influenza A and B, HPIV 1–3, HRSV, and HAdV. A 1–

2 ml aliquot of remaining processed specimen was then diluted with an equal amount of 20% MEM and preserved at −80°Cuntil retrieved for later molecular study in the Center for Emerging Infectious Diseases (CEID).

The MagMax-96 Total RNA Isolation Kit (Applied Biosystems/Ambion, Foster City, CA) and KingFisher Magnetic Particle Processor (Thermo Scientific, Vantaa, Finland) were used to extract viral nucleic acids from respiratory specimens. Previously described PCR and RT-PCR assays were used to detect HAdV, HBoV, HRV, andHMPV.[26–30] Eachof these viruses were tested for separately in each clinical specimen. PCR products were visualized on an ethidium bromide stained 1% agarose gel, and PCR-positive samples were submitted for DNA nucleotide sequencing. Gene sequencing was performed on an Applied Biosystems Model 3730xl (96-capillary) DNA sequencer. The forward and reverse sequences were combined using BioEdit software (Ibis Biosciences, Carlsbad, CA) and were compared with nucleotide sequences submitted to NCBI GenBank. Specimens that yielded identity scores of ≥ 90% were considered good genotypic matches.

In order to identify HRV A, B, or C, amplified nucleotide sequences from HRV-positive specimens were aligned and neighbor-joining phylogenetic trees were generated using a maximum composite likelihood method. Bootstrap analysis was completed using 1000 repetitions. Alignment and phylogenetic analyses were performed using Mega 4.0 software.[31]

Statistical analyses

Primary analyses were limited to either confirmed (ARI-related ICD-9 diagnosis code) or suspected (signs and symptoms consistent with ARI documented in the medical record) ARI and excluded duplicates. Coinfection was defined as a sample with a positive test result for 2 or more respiratory viruses from tests performed by CML and/or CEID. Though other viral, bacterial, and fungal clinical microbiology results may have been available in the medical record for an included episode of ARI, this information was not included in the primary analysis. Specimens collected from individuals for whom medical record abstraction was not possible were excluded.

Demographic and clinical covariates were studied to identify host risk factors associated with polymicrobial ARI. To be included in analysis, a specimen must have been positive for at least one virus. Bivariate analyses such as Pearson’s chi-square test, Fisher’s exact test, and bivariate logistic regression were used to examine potential risk factor associations with respiratory coinfection. Beginning with a saturated model, manual backwards elimination and multivariate logistic regression modeling were used to identify the model that best predicted the occurrence of polymicrobial infections in this population.

To investigate the association of polymicrobial infections with severe illness, we conducted additional case-control analyses of the cross-sectional data. The exposure of interest was presence of any respiratory viral coinfection, and subjects with an ARI caused by a single virus served as the comparison group. A case was defined as a child who was hospitalized and admitted to the intensive care unit (ICU) as a result of ARI. Children who were hospitalized but not admitted to the ICU during this time period served as controls. Bivariate analyses were performed to identify covariates of interest, potential confounders associated either with exposure oroutcome, and potential effect modifiers (i.e., history of chronic respiratory disease and immunosuppression). Identified confounders remained in the final model even if the confounder itself was not statistically significant. Bivariate logistic regression was used to determine crude unadjusted odds ratios and 95% confidence intervals for ICU admittance. Odds ratios and 95% confidence intervals were adjusted for the effect of potential confounders (identified in the literature or in bivariate analyses) using

multivariate logistic regression. Interaction terms representing potential effect modifiers were included in analyses and remained in the final model if significant. Beginning with a saturated model, manual backwards elimination and multivariate logistic regression modeling were used to decide which of the remaining covariates of interest identified in bivariate analyses were to be included in the model. This process was repeated for secondary analyses comparing virus-bacteria coinfection to single virus infection among children for whom a bacterial diagnostic test (typically culture) was ordered during the same episode of ARI.

RESULTS

Overview of specimen population

A total of 116 specimens were collected during the study period but were unavailable for the following reasons – 39 virus-negative specimens had no remaining volume to archive following routine microbiological testing, 75 virus-positive specimens were not archived at the discretion of the technician, and 2 virus-positive specimens were set aside for validation of in-house diagnostic assays. These 116 specimens represented 105 unique individuals and 100 children with accessible medical records.

Primary analysis was limited to the first specimen collected from the first ARI episode per child during the study period (n=421), specimens from children with confirmed or suspected ARI (n=349), and children with accessible medical records (n=346).

Summary of selected demographic and clinical characteristics of the study population with accessible medical records

Among the 346 children with confirmed or suspected ARI, 54.3% were male, 85.8% were under the age of 5 years (mean age 2.21 years), 73.3% were Caucasian, 47.2% used Medicaid as the primary payor for medical services, and 60.0% resided in an urban area. A large proportion of the children were hospitalized (76.3%); and of those that were hospitalized, 28.0% were in an ICU at the time of specimen collection. The median length of hospitalization was 4 days. Antimicrobials were frequently administered prior to the initial visit (30.1%), any time during the visit (58.7%), and as take-home prescriptions (28.6%).Specimens most often originated from nasopharyngeal washes (87.4%). Respiratory viruses were most commonly detected in the winter and spring months (75.1% detected from January through June). Additional information regarding clinical symptoms, diagnoses, and interventions such as oxygen supplementation can be found in Table, Supplemental Digital Content 1, http://links.lww.com/INF/B462.

Among the 100 children excluded from the study due to unavailable specimens, 60.0% were male, 90.0% were under the age of 5 years (mean age 1.64 years), 75.0% were white, and 78.0% resided in an urban area. Less than half of the children (44.0%) were hospitalized, and of those that were hospitalized, 27.3% were ever patients in an ICU. The median total length of stay was 2 days. Specimens most often originated from nasopharyngeal washes (84.0%). Respiratory viruses were most commonly detected in the winter and early spring months (January through March).Compared to children for whom specimens were included in the study, children for whom specimens were unavailable were younger ($p=0.035$), were more likely to reside in an urban area ($p=0.009$), and were less likely to be hospitalized ($p<0.001$).

Single virus infection and coinfection prevalence estimates

A virus was identified in 56.3% of the 421 respiratory specimens. When limiting the analysis to the 346 respiratory specimens from children with confirmed or suspected ARI

and available medical record data, a virus was identified in 61.3% of the specimens (Table 1). A coinfection was identified in 21.7% of the 212 virus-positive specimens. HRV (27.5%)was the most prevalent virus detected. Of the 95 HRV-positive specimens, HRV A was the most common group detected (46.3%) followed by HRV C (41.1%), and non-typeable HRVs (12.6%). Coinfections were detected more often for HBoV (53.5% of 28HBoV-positive specimens) and HAdV (53.3% of 15 HAdV-positive specimens). Among the 46 specimens with detected coinfections, the most frequent virus-virus combination was HRV-HRSV (n=12).Among the 95 HRV-positive specimens, 58.3% of the non-typeable HRV specimens, 36.4% of the HRV A specimens, and 30.8% of the HRV C specimens were involved in coinfections. Additional information regarding the frequency of specific viral coinfections can be found in Table, Supplemental Digital Content 2, http://links.lww.com/INF/B463.

Host factors associated with viral coinfection

While none of the covariates in the final model were statistically significant (p>0.05), the results were suggestive. Males were at increased odds of coinfection (OR 1.70, 95% CI 0.83–3.46). Children aged 6 months to 1 year had increased odds of coinfection as compared to children aged less than 6 months (OR 2.15, 95% CI 0.75–6.19) and the odds of coinfection decreased with increasing age after 1 year, though this trend was not statistically significant. Children with a history of immunosuppression had increased odds of coinfection (OR 2.05, 95% CI 0.99–4.23). Additional information regarding prevalence and odds ratios of viral coinfection by risk factor can be found in Table, Supplemental Digital Content 3, http://links.lww.com/INF/B464.

Modeling odds of ICU admission associated with viral coinfection

The unadjusted odds ratio for ICU admission associated with virus-virus coinfection was 0.30 (95% CI 0.09–1.04) (Table 2). Bivariate analyses suggest a significant trend for the association between urban/rural residence and ICU admission; as characterization of residence became more rural, the odds of ICU admission decreased. A significant trend was also observed for the association between tobacco smoke exposure and ICU admission. History of chronic respiratory condition and history of immunosuppression did not significantly modify the association between coinfection and ICU admission. After controlling for potential confounders, the adjusted odds ratio was 0.32 (95% CI 0.08–1.27). Male gender (OR 3.11, 95% CI 1.20–8.06), history of immunosuppression (OR 3.20, 95% CI 1.12–9.17), history of prematurity (OR 5.06, 95% CI 1.61–15.93), and leukocytosis (OR 4.44, 95% CI 1.68–11.74) were significantly associated with increased odds of ICU admission in the final model.

Secondary analysis: viral-bacterial coinfection

Of217 children hospitalized with confirmed or suspected ARI for whom abacterial diagnostic test was ordered, a single virus was detected in 67 (30.9%) children and coinfections involving viruses and/or bacteria were detected in 111 (51.2%) children (Table 3). Compared to children who did not have a bacterial test ordered, those who did were more likely to be older (age 1–5 years p=0.035, older than 5 years p=0.025), have a history of cancer (p=0.022), have an elevated white blood cell count (p=0.048), have a fever (p=0.005), and to be hospitalized (p<0.001). They were less likely to have nasal congestion/runny nose (p=0.002) and wheeze (p<0.001).

The unadjusted odds ratio for ICU admission associated with virus-bacteria coinfection was 6.00 (95% CI 2.51–14.33). After controlling for potential confounders, the adjusted odds ratio was 5.58 (95% CI 1.95–15.96) (see Table, Supplemental Digital Content 4, http://links.lww.com/INF/B465, prevalence and odds ratios of virus-bacteria coinfection among

hospitalized, virus-positive children). History of prematurity (OR 3.17, 95% CI 1.03–9.77) was also significantly associated with increased odds of ICU admission. When children with virus-bacteria coinfections were removed from the analysis of virus-virus coinfection versus single virus infection, the observed odds ratio for ICU admission was 0.53 (95% CI 0.11–2.49).

DISCUSSION

Viral detections and polymicrobial infections were common in our study of pediatric inpatients and outpatients with ARI. Many children were hospitalized, and of those that were hospitalized, approximately one-third were patients in an ICU at the time of specimen collection.

We hypothesized that certain host-specific risk factors were associated with the likelihood of viral coinfection. Male gender, age between 6 months to 1 year, and history of immunosuppression were associated with increased odds of viral coinfection ($p>0.05$). Few studies have identified host factors that may predispose a child to respiratory coinfections. In a study of patients less than 14 years old hospitalized with ARI in China, Peng et al. noted that coinfection was more common in children aged 3 to 6 years.[15] In a study of community-acquired pneumonia (CAP) in hospitalized and non-hospitalized children aged less than 3 years old, Cilla et al. noted that children aged less than 12 months were more likely to have a viral coinfection.[8]

We hypothesized that children with viral coinfections were more likely to have severe ARI. After controlling for potential confounders, the odds ratio for ICU admission associated with virus-virus coinfection was not significant (OR 0.32, 95%CI 0.08–1.27). With respect to virus-virus coinfections, our results are similar to studies that have found no association with severity of illness.[6,9,10,12,14] In our study, however, children with viral coinfections were less likely to be admitted to the ICU than children with single virus infections. Similar results were observed by Martin et al. -viral coinfections were associated with decreased risk of oxygen requirement, extended hospital stays, and admissions to inpatient or intensive care units.[32]

As this seemingly protective effect of polymicrobial infection was unexpected, we hypothesized that concurrent bacterial infections were influencing the viral coinfection results. We limited secondary analyses to virus-positive, hospitalized children with confirmed or suspected ARI for whom a bacterial test (most often culture) had been ordered during the same hospitalization that a viral test had been ordered. Children with virus-bacteria coinfection, as compared to children with single virus infection, were more likely to be admitted to an ICU (OR 5.58, 95% CI 1.95–15.96) even after controlling for potential confounders In our primary analyses, children with single virus/single bacterium coinfections would have been classified as having a single virus infection since bacterial data was not included. Furthermore, it is likely that some participants for whom cultures had not been ordered had a concurrent bacterial infection. Though these undetected bacterial cases would be distributed between single virus infections and virus-virus coinfections, we suspect that a higher proportion of single virus infections would also be positive for bacterial pathogens as evidenced by the data presented in Table 3(virus-bacteria coinfections represented 26.6% of virus-positive specimens whereas virus-virus-bacteria coinfections represented only 5.7% of virus-positive specimens). Given that a substantial proportion of children with virus-bacteria coinfections would have been classified as having single virus infections in our primary analyses and that our secondary analyses suggest these children are at increased risk of ICU admission, children with virus-virus coinfections would appear to be less likely to be admitted to the ICU as compared to children with single virus infections

if bacterial coinfections are not considered. When children with virus-bacteria coinfections were removed from the analysis of virus-virus coinfection versus single virus infection, the observed odds ratio moved closer to the null. This suggests that at least part of the observed protective effect of virus-virus coinfection ($p>0.05$) can be explained by virus-bacteria coinfection, and that perhaps undetected bacterial coinfections could account for the remaining effect.

The epidemiologic and clinical importance of mixed respiratory infections are areas of ongoing research. Coinfection rates vary widely among studies and are estimated to account for 8.4% to 36.1% of ARIs for which at least one virus was detected.[4, 5, 7, 8, 10, 15–17, 20, 21] Results from some studies suggest that children infected with two or more viruses do not have more severe clinical illness than children infected with only one virus.[7, 10, 11, 13, 15] However, results from other studies have suggested an association between respiratory coinfections and severe illness.[4, 5, 8, 12, 14, 18, 21, 25] In the afore mentioned study of CAP by Cilla et al., age and viral coinfection were shown to be independent risk factors for hospitalization.[8] In a study of patients with LRTIs by Bharaj et al., a high proportion of children with mixed infections had severe or very severe disease.[4]

Examples of coinfecting bacterial and viral pathogens are common in the literature, but reports tend to be virus-specific.[33] For example, most deaths associated with epidemics of influenza are associated with secondary bacterial infections including *Streptococcus pneumoniae* and *Haemophilus influenzae*. Associations between HAdV and *Bordetella pertussis* have been noted in severe respiratory disease in children. Evidence describing the importance of viral and bacterial cooperation in cases of pneumonia is growing.[34–37] Jennings et al. conducted a study among patients hospitalized with CAP and demonstrated that HRV-pneumococcal coinfection was independently associated with severe pneumonia.[12] Templeton et al. included both inpatients and outpatients in their study of CAP and demonstrated that HRV-bacterial or HCoV-bacterial coinfections were independently associated with severe pneumonia.[18] Another study of children with CAP suggested that mixed viral-bacterial co-detections were associated with treatment failure.[35] Additional evidence of the interaction between bacterial and viral pathogens comes from animal studies.[33] Several mechanisms have been postulated to explain this interaction.[33] Viruses may increase the ability of bacteria to infect or adhere to mucosal surfaces through changes induced in host cell membranes. It has also been hypothesized that exudates on mucosal surfaces resulting from viral infection may increase bacterial growth. The host immune defense against bacteria could also be affected by viral infection through the inhibition of nonspecific phagocytosis. Orperhaps viral infection exacerbates the effect of bacterial toxins.

We must acknowledge the limitations of this study. The use of archived specimens proves to be problematic with respect to biases associated with sampling and exposure misclassification. First, not all individuals with ARIs may be symptomatic; and furthermore, not all symptomatic individuals with ARI may seek medical care. Only individuals who sought medical care and for whom a viral diagnostic test was ordered were eligible for inclusion into this study. Therefore, it is likely that certain cases of ARI (e.g., symptomatic infections requiring medical attention or more severe infections eliciting increased effort to identify an etiologic agent) may be over-represented in our population.

Some of the eligible specimens collected during the study period were not available for study. If children whose specimens were excluded were more likely to be coinfected than those children whose specimens were included, then our observed measures of association between coinfection and ICU admission may have been biased.

Underestimation of respiratory coinfections likely occurred for several reasons. First, this study was limited to a specific set of viruses. Second, if a clinician ordered a virus-specific DFA in addition to a viral culture panel and the DFA was positive, culture would not be completed and the likelihood of co-detection by the CML is reduced. Among the 105 specimens not included in this study, which were therefore limited to CML's routine testing procedures alone, only 2 viral coinfections were detected. Third, the viral culture and DFA methods utilized for influenza A and B, HPIV1-3, HRSV, and HAdV are less sensitive than the molecular methods utilized for HRV, HBoV, HMPV, and HAdV. Finally, only a limited proportion of our sample had information available in the medical record regarding bacterial pathogens.

This was a cross-sectional study using archived respiratory specimens that were collected as a part of routine medical care. As such, we are unable to establish causality with regard to coinfections and severe ARI in children as we cannot firmly establish a temporal sequence of events. Unless multiple samples were taken over the duration of the illness, little can be done to address this problem or to identify the significance of concurrent versus consecutive infections. Over-estimation of respiratory coinfections due to co-detection of asymptomatic viral shedding post-infection and acute infection with a second virus may have also occurred. Among the 407 children for whom medical records were available, 3.5% of virus-positive children did not have a symptomatic ARI. Molecular methods may over-estimate the presence of viable virus through detection of viral particles or nonviable virus.

Finally, data quality and completeness were expected to vary among covariates selected for abstraction from electronic medical records. Any misclassification of clinical covariates is expected to be non-differential as the abstractor was blinded to coinfection and illness severity. Exclusion of incomplete information from analysis may lead to biased estimates of association as this assumes that the observations with complete data are representative of all observations. Furthermore, exclusion of observations may result in an insufficient sample size for analyses.

Despite these limitations, the methodology of the current study sets it apart from earlier studies. Unlike many of its predecessors, this study was designed with an *a priori* hypothesis in mind concerning a role for coinfections in severe ARI. Furthermore, few studies have attempted to control for potential confounders, and to our knowledge, none have explored the role of potential effect modifiers.

The University of Iowa Hospitals and Clinics is a comprehensive academic medical center and regional referral center. Our results may not be applicable to other hospital-based settings due to the composition of the patients seeking care at UIHC and the behavior of the physicians providing care; however, the underlying biological theory suggests that an association could still exist if virus-bacteria coinfections do in fact result in more severe illness, though the measure of association may be attenuated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Single virus infection and coinfection prevalence estimates

Virus	Single virus infection, n (% Total Positive[a])	Coinfection, n (% Total Positive[a])	Total Positive, n (% Total Specimens[b])	95% CI[c]
HAdV	7 (46.7)	8 (53.3)	15 (4.3)	2.4–7.1
HRV	61 (64.2)	34 (35.8)	95 (27.5)	22.8–32.5
HBoV	13 (46.4)	15 (53.5)	28 (8.1)	5.4–11.5
HMPV	19 (70.4)	8 (29.6)	27 (7.8)	5.2–11.2
CoxS	0 (0)	1 (100)	1 (0.03)	0–1.6
Flu A	5 (100)	0 (0)	5 (0.1)	0.5–3.3
Flu B	5 (100)	0 (0)	5 (0.1)	0.5–3.3
HRSV	43 (65.2)	23 (34.8)	66 (19.1)	15.1–23.6
HPIV 1	1 (100)	0 (0)	1 (0.03)	0–1.6
HPIV 2	1 (100)	0 (0)	1 (0.03)	0–1.6
HPIV 3	11 (61.1)	7 (38.9)	18 (5.2)	3.1–8.1
HPIV NOS	0 (0)	3 (100)	3 (0.1)	0.01–2.5
Total	166 (78.3)	46 (21.7)	212 (61.3)	55.9–66.4

Note: Flu A (influenza A virus), Flu B (influenza B virus), HPIV NOS (parainfluenza virus not otherwise specified).

[a]Denominator is virus-specific total number of positive specimens (row total).

[b]Denominator is total number of specimens from children with confirmed or suspected ARI and accessible medical records, n=346

[c]95% binomial confidence interval associated with prevalence percent estimate for total number of positive specimens.

Table 2

Prevalence and odds ratio of viral coinfection and ICU admission, by risk factor among hospitalized children[a]

Risk Factor	N	% Coinfected (95% CI)	Bivariate OR (95% CI)	p	% Admitted to ICU (95% CI)	Bivariate OR (95% CI)	p	Multivariate OR (95% CI)	p
Coinfection									
No	126				23.8 (16.7–32.2)	1.00		1.00	
Yes	34				8.8 (1.9–23.7)	0.30 (0.09–1.04)	0.057	0.32 (0.08–1.27)	0.104
Gender									
Female	70	15.7 (8.1–26.4)	1.00		15.7 (8.1–26.4)	1.00		1.00	
Male	90	25.6 (16.9–35.8)	2.24 (0.24–1.21)	0.134	25.6 (16.9–35.8)	1.85 (0.83–4.17)	0.134	3.11 (1.20–8.06)	0.020†
Age (years)[b]									
0 to < 1	79	19.0 (11.0–29.4)	1.00		26.6 (17.3–37.7)	1.00		1.00	
1 to < 5	63	22.2 (12.7–34.5)	1.22 (0.54–2.76)	0.635	17.5 (9.1–29.1)	0.58 (0.26–1.33)	0.199	0.52 (0.20–1.39)	0.192
> 5	18	27.8 (9.7–53.5)	1.64 (0.51–5.31)	0.408	11.1 (1.4–34.7)	0.35 (0.07–1.63)	0.179	0.27 (0.05–1.43)	0.124
Race									
Caucasian	114	21.1 (14.0–29.7)	1.00		19.3 (12.5–27.8)	1.00			
African-American	15	20.0 (4.3–48.1)	0.94 (0.25–3.59)	0.925	20.0 (4.3–48.1)	1.05 (0.27–4.03)	0.949		
Hispanic	14	14.3 (1.8–42.8)	0.63 (0.13–2.98)	0.556	14.3 (1.8–42.8)	0.70 (0.15–3.34)	0.652		
Other	5	40.0 (5.3–85.3)	2.50 (0.40–15.82)	0.330	40.0 (5.3–85.3)	1.03 (0.44–17.71)	0.277		
Medicaid									
No	82	22.0 (13.6–32.5)	1.00		18.3 (10.6–28.4)	1.00			
Yes	77	20.8 (12.4–31.5)	0.93 (0.44–1.99)	0.857	23.4 (14.5–34.4)	1.36 (0.63–2.94)	0.431		
Urban/Rural									
Urban	88	19.3 (11.7–29.1)	1.00		25.0 (16.4–35.4)	1.00			
Rural	72	23.6 (14.4–35.1)	1.29 (0.60–2.76)	0.510	16.7 (8.9–27.3)	1.62 (0.27–1.32)	0.203		
History of chronic respiratory condition[c]									
No	98	19.4 (12.1–28.6)	1.00		14.3 (8.0–22.8)	1.00			
Yes	62	24.2 (14.2–36.7)	1.33 (0.62–2.86)	0.470	32.3 (20.9–45.3)	2.86 (1.31–6.21)	0.008†		
History of any immunosuppressive condition[d]									
No	103	17.5 (10.7–26.2)	1.00		21.4 (13.9–30.5)	1.00		1.00	
Yes	57	28.1 (17.0–41.5)	1.84 (0.85–3.98)	0.120	21.1 (11.4–33.9)	0.98 (0.45–2.17)	0.964	3.20 (1.12–9.17)	0.030†
History of prematurity									
No	128	24.2 (17.1–32.6)	1.00		15.6 (9.8–23.1)	1.00		1.00	
Yes	32	9.4 (2.0–25.0)	0.32 (0.09–1.14)	0.078	43.8 (26.4–62.3)	4.20 (1.80–9.79)	0.001†	5.06 (1.61–15.93)	0.006†
Smoke exposure[e]									
None	46	21.7 (11.0–36.4)	1.00		10.9 (3.6–23.6)	1.00			
Direct	7	28.6 (3.7–71.0)	1.44 (0.24–8.57)	0.689	42.9 (9.9–81.6)	6.15 (1.06–35.80)	0.043†		
Indirect	22	27.3 (10.7–50.2)	1.35 (0.42–4.35)	0.615	27.3 (10.7–50.0)	3.08 (0.82–11.51)	0.095		
Missing	85								

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[a] Bivariate and multivariate analysis of selected risk factors and viral coinfection or ICU admission includes only virus-positive specimens from hospitalized children with confirmed (physician diagnosed) or suspected (physician-documented symptoms) ARI with accessible medical record information, without duplicates (n=160).

[b] p for trend 0.401(% coinfected bivariate analysis), 0.087 (% admitted to ICU bivariate analysis)

[c] Includes structural defects of the respiratory tract and asthma

[d] Includes history of cancer, transplant, and other primary or secondary immunodeficiencies

[e] p for trend 0.652 (% coinfected bivariate analysis), 0.030 (% admitted to ICU bivariate analysis)

[†] $p<0.05$

Table 3

Single virus infection and virus-bacteria coinfection prevalence estimates for specimens from hospitalized children with bacterial tests ordered

	Single infection	Coinfection			
Virus	**Virus, n (% Total Positive[b])**	**Virus + Virus n (% Total Positive[b])**	**Virus + Bacteria[a] n (% Total Positive[b])**	**2 Viruses + Bacteria[a] n (% Total Positive[b])**	**Total Positive n (% Total Specimens[c])**
HAdV	5 (50.0)	3 (30.0)	1 (10.0)	1 (10.0)	10 (4.6)
HRV	22 (42.3)	11 (21.2)	12 (23.1)	7 (13.5)	52 (24.0)
HBoV	9 (42.9)	9 (42.9)	2 (9.5)	1 (4.8)	21 (9.7)
HMPV	10 (47.6)	2 (9.5)	7 (33.3)	2 (9.5)	21 (9.7)
Flu A	1 (100)	0 (0)	0 (0)	0 (0)	1 (0.5)
Flu B	0 (0)	0 (0)	1 (100)	0 (0)	1 (0.5)
HRSV	14 (48.3)	6 (20.7)	8 (27.6)	1 (3.5)	29 (13.4)
HPIV All	6 (37.5)	6 (37.5)	2 (12.5)	2 (12.5)	16 (7.4)
Total	67 (54.0)	71 (13.7)	33 (26.6)	7 (5.7)	124 (57.1)

Note: Flu A (influenza A virus), Flu B (influenza B virus), HPIV All (parainfluenza virus 1–3 and not otherwise specified).

[a] Frequently detected bacteria included *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and other strep species, and *Klebsiella pneumoniae*.

[b] Denominator is virus-specific total number of positive specimens (row total).

[c] Denominator is total number of specimens, n=217.