The role of influenza, RSV and other common respiratory viruses in severe acute respiratory infections and influenza-like illness in a population with a high HIV sero-prevalence, South Africa 2012–2015

Marthi A Pretorius[1,2,3*], Stefano Tempia[4,5], Florette Treurnicht[3], Orienka Hellferscee[3], Mpho Seleka[3], Sibongile Walaza[3], Adam L. Cohen[4,5], Jocelyn Moyes[3,6], Ebrahim Variava[7,8],Halima Dawood[9,10],Cheryl Cohen[3,6], Marietjie Venter[2,11]

Affiliations:

[1]National Health Laboratory Service, Tshwane Academic Division, South Africa, [2]Department of Medical Virology, University of Pretoria, [3]Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa; [4]Influenza Division, Centers for Disease Control and Prevention, Atlanta, United States of America; [5]Influenza Program, Centers for Disease Control and Prevention, Pretoria, South Africa; [6]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [7]Department of Medicine, Klerksdorp Tshepong Hospital, Klerksdorp, South Africa; [8]Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [9]Department of Medicine, Pietermaritzburg Metropolitan Hospital, Pietermaritzburg, South Africa; [10]Department of Medicine, University of KwaZulu Natal, South Africa; [11]Global Disease Detection, Centers for Disease Control and Prevention, Pretoria, South Africa.

Corresponding Author's current address: Marthi Pretorius, National Health Laboratory Service, Tshwane Academic Division, South Africa, Department of Medical Virology, University of Pretoria, Pretoria, South Africa. Tel: +27123192287, Cell: +27823328211, email: marthi.pretorius@up.ac.za

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Abstract

Background: Viruses detected in patients with acute respiratory infections may be the cause of illness or asymptomatic shedding.

Methods: We compared the prevalence of 10 common respiratory viruses (influenza A and B viruses, parainfluenza virus 1, 2, and 3; respiratory syncytial virus (RSV); adenovirus, rhinovirus, human metapneumovirus (hMPV) and enterovirus) in patients hospitalized with severe acute respiratory illness (SARI), outpatients with influenza-like illness (ILI), and control subjects who did not report any febrile, respiratory or gastrointestinal illness during 2012-2015 in South Africa. We estimated the attributable fraction (AF) and the detection rate attributable to illness for each of the different respiratory viruses.

Results: We enrolled 1959 SARI, 3784 ILI and 1793 controls. Influenza virus (AF: 86.3%; 95%CI: 77.7%-91.6%), hMPV (AF: 85.6%%; 95%CI: 72.0%-92.6%), and RSV (AF: 83.7%; 95%CI: 77.5%-88.2%) infections were highly associated with severe disease, while rhinovirus (AF: 46.9%; 95%CI: 37.6%-56.5%) and adenovirus (AF: 36.4%; 95%CI: 20.6%-49.0%) were only moderately associated. The estimated detection rate associated with severe disease was: 20.2% for rhinovirus, 16.7% for RSV, 7.0% for adenovirus, 4.9% for influenza virus and 3.8% for hMPV. Similar patterns were observed for patients with ILI.

Conclusions: Influenza, RSV and hMPV can be considered likely pathogens if detected in patients with ILI and SARI while rhinovirus and adenovirus were commonly identified also among controls suggesting that they may cause only a proportion of clinical disease observed in positive patients. Nonetheless, given their high estimated detection rate attributable to illness, they may be important contributors to disease.

Keywords: Disease association, Respiratory Virus Infection, Severe Acute Respiratory Illness, Influenza Like Illness, Controls, Pneumonia, HIV, South Africa.

Introduction

Pneumonia is a leading cause of childhood mortality globally, with about 1.6 million new cases per year, of which 1.2 million occur in the developing world [1, 2]. Of these cases approximately 10% are severe enough to require hospitalization [2]. Before the worldwide availability of vaccines, *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae* type b were identified as the main bacterial causes of

pneumonia [2, 3]. Now, viruses are proportionally a much more common cause of pneumonia [2].

Respiratory viruses infections have been detected through the use of polymerase chain reaction (PCR) among patients hospitalized with lower respiratory tract infection (LTRI) in several studies [4-10]. While the use of sensitive PCR methods has significantly expanded the ability of laboratories to detect and identify pathogens, the clinical association between pathogen detection and disease remains difficult to interpret when considering viral shedding, replication and persistence of nucleic acids present during the pre- or post-syndromic phase of infection in the absence of comparison groups [5, 10, 11]. Without comparing to control groups, the clinical relevance of identifying some respiratory pathogens by PCR testing remains difficult to determine [10-14].

Understanding the contribution of respiratory viruses to illness would allow the prioritization of respiratory pathogens for inclusion in diagnostic tests, disease surveillance, vaccine development and treatment. Using control subjects, we estimated the attributable fraction of 10 common respiratory viruses among patients hospitalized with severe acute respiratory illness (SARI) and outpatients with influenza-like illness (ILI).

Materials and Methods

Study design and population

SARI Surveillance: Study samples were obtained from participants enrolled in a prospective hospital-based surveillance program for SARI initiated in February 2009. The methodology of this study has been previously described [15, 16]. For this study

participants were enrolled at 3 public hospitals in 2 provinces of South Africa (Edendale Hospital in a peri-urban area of KwaZulu-Natal Province; and Klerksdorp and Tshepong Hospitals (the Klerksdorp-Tshepong Hospital Complex, KTHC) in a peri-urban area of North West Province) from May 2012 through April 2015. Patients were enrolled if they presented with symptom duration ≤7 days, provided written informed consent and met any of the following age-specific SARI case definitions: (i) children aged 2 days to <3 months with a diagnosis of suspected sepsis or physician-diagnosed lower respiratory tract infection irrespective of signs and symptoms; (ii) children aged 3 months to <5 years hospitalized with physician-diagnosed LRTI including bronchitis, bronchiolitis, pneumonia and pleural effusion; or (iii) individuals aged ≥5 years with sudden onset of fever (>38°C or history of fever) and cough or sore throat and shortness of breath or difficult breathing with or without clinical or radiographic findings of pneumonia.

ILI and Control Surveillance: Study samples were obtained from participants enrolled in an active surveillance program for ILI and controls initiated in May 2012 and running through April 2015. Patients presenting with ILI and controls were enrolled at two outpatient clinics in the same catchment area to the above mentioned hospitals: Edendale Gateway Clinic, KwaZulu-Natal Province, and Jouberton Clinic, North West Province. An ILI case was defined as an outpatient of any age presenting with either temperature >38°C or history of fever, and cough of duration of ≤7 days. ILI cases that were referred for hospitalization subsequent to the visit were not eligible for enrolment.

A control was defined as an individual presenting at the same outpatient clinic with no history of fever, respiratory or gastrointestinal symptoms during the 14 days preceding the visit. The patients commonly presented to the clinic for visits such as dental

procedures, family planning, well baby visits, voluntary HIV counseling and testing or acute care for non-febrile illnesses. We aimed to enroll one HIV-infected and one HIV-uninfected control every week in each clinic within each of the following age categories: 0-1, 2-4, 5-14, 15-54 and ≥55 years.

A standardized questionnaire was used to collect demographic and clinical information from enrolled SARI and ILI cases and controls. In addition, for SARI cases hospital records were reviewed to assess disease progression and outcome (i.e., discharge, transfer or in-hospital death). Medical and symptom history was systematically verified by a trained nurse using a structured checklist. This information was obtained through medical chart review and interview with the patient or legal guardian for children less than 15 years of age.

Respiratory Virus detection

Respiratory specimens (i.e., nasopharyngeal aspirates for children <5 years of age and nasopharyngeal and oropharyngeal swabs from individuals ≥5 years of age) were collected from all enrolled patients (SARI, ILI and Controls), placed in viral transport medium, stored at 4-8°C and transported to the National Institute for Communicable Diseases within 72 hours of collection for testing. All specimens collected over the study periods were tested for the presence of 10 respiratory viruses (influenza A and B viruses, parainfluenza virus (PIV) types 1, 2 and 3, respiratory syncytial virus (RSV), adenovirus; rhinovirus; human metapneumovirus (hMPV), and enterovirus) using a real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay as described by Pretorius *et al.* [7]. Among consenting study patients, HIV status was established by

enzyme-linked immunosorbent assay (ELISA) or PCR assay depending on the patients' age [16].

Statistical Analysis

We implemented a multivariable multinomial regression model to determine the association between specific respiratory viruses among patients with SARI or ILI compared to controls enrolled from May 2012 through April 2015. Multinomial regression allows modeling of outcome variables with more than 2 categories and relates the probability of being in category *j* to the probability of being in a baseline or reference category. A complete set of coefficients are estimated for each of the *j* levels (patients with ILI or SARI in this analysis) that are compared with the baseline category (controls for this analysis) and the effect of each predictor in the model is measured as relative risk ratio (RRR). The association of the 10 viruses with mild (ILI) or severe (SARI) illness was assessed simultaneously using a multivariable model to adjust for the potential effect of co-infections. In addition, all estimates were adjusted for age (<1, 1-4, 5-24, 25-44, 45-64 and ≥65 years of age), HIV serostatus and underlying medical conditions other than HIV.

In addition, we implemented an age-stratified analysis among individuals aged <5 and ≥5 years of age to evaluate potential differences in disease association in young children and older individuals. For both analyses we also adjusted the effect of the viral covariates by age within each age strata (<1 and 1-4 years of age for children aged <5 years and 5-24, 25-44, 45-64 and ≥65 years of age for persons aged ≥5 years), HIV serostatus and underlying medical conditions other than HIV.

Subsequently we estimated the attributable fraction (AF) from the relative risk (RR) obtained from the multinomial model for each virus using the following formula: *AF=(RR-1)/RR*100*. Lastly, we adjusted the observed detection rate ($Prev_{Obs}=n/N$) for each virus among ILI or SARI cases by the corresponding AF to obtain the prevalence of each virus attributable to mild (ILI) or severe (SARI) illness (adjusted prevalence, $Prev_{Illness}$) using the following formula: $Prev_{Illness}=Prev_{Obs}*AF/100$. The analysis was performed using STATA 13.1 (StataCorp®, Texas, USA).

Ethical considerations

The SARI protocol was reviewed and approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) and the University of KwaZulu-Natal Human Biomedical Research Ethics Committee (BREC) protocol numbers M081042 and BF157/08, respectively. The ILI and controls protocol was reviewed and approved by BREC protocol number (BREC BF 080/12). This surveillance was deemed non-research by the U.S. Centers for Disease Control and Prevention.

Results

Characteristics of the Study Population and Detection of Respiratory Viruses

Over the study period, we enrolled 1959 SARI cases, 3784 ILI cases and 1793 controls. Children <5 years of age accounted for 73% (1431/1953); 28% (1075/3783) and 37% (658/1135) of SARI cases, ILI cases and controls, respectively. The HIV serostatus was known for 79% (1550/1959) of SARI cases, 87% (3280/3784) of ILI cases, and 92% (1643/1793) of controls. Among

individuals with known HIV serostatus, the HIV prevalence was 26% (410/1550) among SARI cases, 30% (974/3280) among ILI cases, and 43% (702/1643; reflecting the enrolment criteria) among controls. Among SARI and ILI cases the HIV prevalence was lowest among infants <1 year of age [SARI: 10% (75/740), ILI: 2% (3/304)] and highest among individuals 25-44 years of age [SARI: 89% (174/196), ILI: 59% (608/1035)].

A virus was identified in 70% (1381/1959) of SARI cases, 59% (2230/3784) of ILI cases and 36% (645/1793) of controls. Among SARI cases the most commonly detected viruses were rhinovirus (34%; 667/1959), RSV (20%; 391/1959) and adenovirus (29%; 379/1959). Rhinovirus (28%; 1064/3784), influenza virus (15%; 577/3784) and adenovirus (12%; 434/3784) predominated among the ILI cases. Rhinovirus (21%; 374/1793) and adenovirus (12%; 207/1793) were the most prevalent among controls (Table 1).

Attributable fraction of respiratory virus infection to mild or severe illness

In the main unstratified analysis using multivariable multinomial regression, all viruses except adenovirus where significantly associated with mild illness (ILI) and all viruses except PIV2 were associated with severe illness (SARI) (Table 1 and 4). Nonetheless, the level of association (*i.e.,* magnitude of the AF) varied across pathogens. Among ILI cases the AF was highest for influenza (adjusted AF [aAF]: 93.3%; 95% confidence intervals [95%CI]: 89.6%-95.7%), PIV2 (aAF: 90.8%; 95%CI: 60.5%-97.9%) and hMPV (aAF: 86.6%; 95%CI: 74.9%-92.9%) (Table 4). Among SARI cases the AF was highest for influenza (aAF: 86.3%; 95%CI: 77.7%-

Table 1: Association of respiratory viruses among patients (all ages) with SARI and ILI compared to controls in South Africa, 2012 – 2015 (Controls cases were used as the reference groups for the multinomial regression model, aRRR highlighted in bold indicate factors significant at $p<0.05$).

Factor	Control[a] n (%) N=1793	ILI n (%) N=3784	SARI n (%) N=1959	Multivariable Analysis ILI aRRR[b] (95% CI)	Multivariable Analysis SARI aRRR[b] (95% CI)
Influenza	25 (1.4)	577 (15.2)	111 (5.7)	**14.9 (9.6-23.2)**	**7.3 (4.5-11.9)**
Rhinovirus	374 (20.9)	1064 (28.1)	667 (34.1)	**2.1 (1.8-2.4)**	**1.9 (1.6-2.3)**
Adenovirus	207 (11.5)	434 (11.5)	379 (19.3)	1.1 (0.9-1.3)	**1.6 (1.3-1.9)**
Enterovirus	53 (3.0)	120 (3.2)	118 (6.0)	**1.7 (1.2-2.5)**	**1.9 (1.3-2.9)**
RSV	55 (3.1)	225 (5.9)	391 (19.9)	**2.7 (1.9-3.8)**	**6.1 (4.4-8.5)**
PIV 1	11 (0.6)	56 (1.5)	45 (2.3)	**4.0 (1.9-8.4)**	**4.3 (2.0-9.3)**
PIV 2	2 (0.1)	28 (0.7)	11 (0.6)	**10.9 (2.5-46.9)**	4.1 (0.8-21.6)
PIV 3	23 (1.3)	81 (2.1)	72 (3.7)	**2.9 (1.7-5.0)**	**2.6 (1.5-4.6)**
hMPV	13 (0.7)	141 (3.7)	86 (4.4)	**7.5 (4.0-14.1)**	**6.9 (3.6-13.4)**

[a] Reference group for the multinomial regression model. [b]Relative risk ratio (aRRR) adjusted by age, HIV serostatus and underlying medical conditions at multivariable analysis. Parainfluenza virus (PIV) 1, 2, 3; Respiratory Syncytial Virus (RSV); human metapneumovirus (hMPV).

Table 2: Association of respiratory viruses among children< 5 years of age with SARI and ILI compared to controls in South Africa, 2012 – 2015 (Controls cases were used as the reference groups for the multinomial regression model, aRRR highlighted in bold indicate factors significant at p<0.05)

Variable	Control [a]	ILI	SARI	Multivariable Analysis[c]	
				ILI aRRR[b]	SARI aRRR[b]
	n (%) N=658	n (%) N=1075	n (%) N=1431	(95% CI)	(95% CI)
Influenza	7 (1.1)	149 (13.9)	73 (5.1)	**24.0 (9.5-60.7)**	**12.7 (4.9-32.5)**
Rhinovirus	214 (32.5)	401 (37.3)	567 (39.6)	**1.6 (1.3-2.1)**	**1.8 (1.4-2.3)**
Adenovirus	122 (18.5)	212 (19.7)	323 (22.6)	1.1 (0.8-1.5)	**1.8 (1.4-2.4)**
Enterovirus	46 (6.7)	82 (7.6)	105 (7.3)	1.4 (0.9-2.2)	**1.6 (1.1-2.5)**
RSV	25 (3.8)	129 (12.0)	357 (24.9)	**4.1 (2.5-6.7)**	**9.9 (6.2-15.8)**
PIV 1	7 (1.1)	33 (3.1)	43 (3.0)	**4.1 (1.7-10.0)**	**4.8 (2.0-11.8)**
PIV 2	1 (0.1)	13 (1.2)	10 (0.7)	**12.1 (1.4-101.5)**	5.6 (0.6-49.4)
PIV 3	15 (2.3)	40 (3.7)	65 (4.5)	**3.2 (1.6-6.5)**	**2.8 (1.5-5.5)**
hMPV	4 (0.6)	53 (4.9)	79 (5.5)	**13.5 (4.1-44.9)**	**16.2 (4.9-53.4)**

[a]Reference group for the multinomial regression model. [b]Age and HIV adjusted relative risk ratio (aRRR) at multivariable analysis. [c]Only covariates significant at the multivariable analysis are reported. Parainfluenza virus (PIV) 1, 2, 3; Respiratory Syncytial Virus (RSV); human metapneumovirus (hMPV).

91.6%), hMPV (aAF: 85.6%; 95%CI: 72.0%-92.6%), and RSV (aAF: 83.7%; 95%CI: 77.5%-88.2%) (Table 4).

In the age stratified analysis among children <5 years of age all viruses except adenovirus and enterovirus where significantly associated with mild illness (ILI) and all viruses except PIV2 were associated with severe illness (SARI) (Table 2 and 4). Among SARI cases <5 years of age, the highest significant AF (≥90%) were observed for influenza, hMPV and RSV, while the lowest significant AF was observed for enterovirus (38.2%) (Table 4). In this group among viruses with significant AF the estimated detection rate attributable to illness (adjusted prevalence) was 22.4% for RSV, 18.1% for rhinovirus, 10.1% for adenovirus, 5.2% for hMPV, 4.7% for influenza, 2.9% for PIV3, 2.8% for enterovirus and 2.4% for PIV1 (Table 4).

Among individuals ≥5 years of age adenovirus, RSV and PIV1 were not significantly associated with mild illness (ILI) and adenovirus, PIV1-3 and hMPV were not significantly associated with severe illness (SARI) (Table 3 and 4). Among SARI cases ≥5 years of age the highest significant AF (>80%) were observed for enterovirus and influenza, while the lowest significant AF was observed for rhinovirus (42.7%). In this group among viruses with significant AF the estimated detection rate attributable to illness was 8.1% for rhinovirus, 5.9% for influenza, 3.4% for RSV, and 2.2% for enterovirus (Table 4).

Table 3: Association of respiratory viruses among patients ≥ 5 years of age with SARI and ILI compared to controls in South Africa, 2012 – 2015 (Controls cases were used as the reference groups for the multinomial regression model, aRRR highlighted in bold indicate factors significant at $p<0.05$)

Variable	Control [a]	ILI	SARI	Multivariable Analysis[c]	
				ILI aRRR[b]	SARI aRRR[b]
	n (%)N=1135	n (%)N=2708	n (%) N=522	(95% CI)	(95% CI)
Influenza	18 (1.6)	428 (15.8)	38 (7.2)	**12.3 (7.5-20.3)**	**5.3 (2.9-9.9)**
Rhinovirus	160 (14.1)	663 (24.5)	100 (19.0)	**2.5 (2.0-3.0)**	**1.7 (1.3-2.4)**
Adenovirus	85 (7.5)	222 (8.2)	56 (10.6)	1.1 (0.8-1.4)	1.2 (0.8-1.8)
Enterovirus	7 (0.6)	38 (1.4)	13 (2.5)	**3.7 (1.4-9.7)**	**7.9 (2.7-23.5)**
RSV	30 (2.6)	96 (3.5)	34 (6.4)	1.4 (0.9-2.2)	**2.2 (1.2-3.9)**
PIV 1	4 (0.3)	23 (0.8)	2 (0.4)	2.4 (0.7-8.7)	1.7 (0.3-10.3)
PIV 2	1 (0.1)	15 (0.6)	1 (0.1)	**10.6 (1.3-82.9)**	Not estimated
PIV 3	8 (0.7)	41 (1.5)	7 (1.3)	**2.7 (1.1-6.3)**	2.5 (0.8-7.5)
hMPV	9 (0.8)	88 (3.25)	7 (1.3)	**4.8 (2.3-10.1)**	1.7 (0.6-5.3)

[a]Reference group for the multinomial regression model. [b]Age and HIV adjusted relative risk ratio (aRRR) at multivariable analysis. [c]Only covariates significant at the multivariable analysis are reported. Parainfluenza virus (PIV) 1, 2, 3; Respiratory Syncytial Virus (RSV); human metapneumovirus (hMPV).

Table 4: Attributable fraction, observed prevalence and adjusted prevalence of respiratory viruses among patients with SARI and ILI in South Africa, 2012 – 2015.

Viruses	Influenza-Like-Illness			Severe Acute Respiratory Illness		
	Attributable Fraction (%) (95% CI)	**Observed Prevalence (%)**	**Adjusted Prevalence (%)[a]**	**Attributable Fraction (%) (95% CI)**	**Observed Prevalence (%)**	**Adjusted Prevalence (%)[a]**
Individuals of any age						
Influenza	**93.3 (89.6-95.7)**	15.2	14.2	**86.3 (77.7-91.6)**	5.7	4.9
Rhinovirus	**52.0 (44.0-58.9)**	28.1	14.6	**46.9 (37.6-56.5)**	34.1	20.2
Adenovirus	5.9 (-15.1-23.1)	11.5	0.7	**36.4 (20.6-49.0)**	19.3	7.0
Enterovirus	**41.8 (15.5-59.9)**	3.2	1.3	**49.0 (24.9-65.4)**	6.0	2.9
RSV	**63.1 (48.6-73.5)**	5.9	3.7	**83.7 (77.5-88.2)**	19.9	16.7
PIV 1	**75.3 (48.8-88.1)**	1.5	1.1	**76.9 (50.6-89.2)**	2.3	1.8
PIV 2	**90.8 (60.5-97.9)**	0.7	0.6	75.9 (-25.9-95.4)	0.6	0.5
PIV 3	**66.1 942.5-80.0)**	2.1	1.4	**62.0 (34.1-78.1)**	3.7	2.3
hMPV	**86.6 (74.9-92.9)**	3.7	3.2	**85.6 (72.0-92.6)**	4.4	3.8
Children <5 years of age						
Influenza	**95.8 (89.5-98.3)**	13.9	13.3	**92.1 (79.7-96.9)**	5.1	4.7
Rhinovirus	**38.2 (21.1-51.6)**	37.3	12.7	**45.7 (31.0-57.2)**	39.6	18.1
Adenovirus	9.3 (-21.6-32.3)	19.7	1.8	**44.9 (27.0-58.4)**	22.6	10.1
Enterovirus	29.4 (-9.7-54.6)	7.6	2.2	**38.3 (5.0-59.9)**	7.3	2.8
RSV	**75.7 (60.3-85.1)**	12.0	9.1	**90.0 (84.0-93.7)**	24.9	22.4
PIV 1	**75.5 (39.8-90.0)**	3.1	2.3	**79.3 (49.3-91.5)**	3.0	2.4
PIV 2	**91.7 (30.6-99.0)**	1.2	1.1	82.1 (-57.7-97.9)	0.7	0.6
PIV 3	**69.2 (38.6-84.6)**	3.7	2.6	**64.8 (31.8-81.8)**	4.5	2.9
hMPV	**92.6 (75.4-97.8)**	4.9	4.6	**93.8 (79.6-98.1)**	5.5	5.2
Individuals ≥5 years of age						
Influenza	**91.9 (86.6-95.1)**	15.8	14.5	**81.3 (65.3-89.9)**	7.2	5.9
Rhinovirus	**59.6 (50.2-67.3)**	24.5	14.3	**42.7 (21.6-58.1)**	19.0	8.1
Adenovirus	8.1 (-23.2-31.4)	8.2	0.7	16.8 (-27.1-45.5)	10.6	1.8
Enterovirus	**72.8 (28.3-89.7)**	1.4	1.0	**87.4 (62.9-95.7)**	2.5	2.2
RSV	28.2 (-13.2-54.5)	3.5	1.0	**54.8 (19.9-74.5)**	6.4	3.4
PIV 1	58.7 (-48.4-88.5)	0.8	0.5	40.0 (-270.1-90.3)	0.4	0.1
PIV 2	**90.6 (26.2-98.1)**	0.6	0.5	Not estimated	0.1	Not estimated
PIV 3	**62.9 (13.5-84.1)**	1.5	0.9	59.4 (-24.3-86.7)	1.3	0.8
hMPV	**79.2 (56.1-90.1)**	3.25	2.6	41.7 (-81.5-81.3)	1.3	0.6

[a]Observed prevalence adjusted by the AF to obtain the prevalence attributable to illness. Parainfluenza virus (PIV) 1, 2, 3; Respiratory Syncytial Virus (RSV); human metapneumovirus (hMPV).

Among ILI cases influenza had the highest AF and estimated prevalence associated with illness among children <5 years (AF: 95.8%; $Prev_{Illness}$: 13.9%) as well as individuals ≥5 years (AF: 91.9%; $Prev_{Illness}$: 14.5%) (Table 4).

Discussion

We assessed the association between virus detection and mild or severe illness relative to controls. The estimated detection rate attributable to illness reported in this study reflects a more accurate description of the prevalence of viruses causing respiratory disease in both children and adults in South Africa than reporting viral detection rates alone. Most of the viral pathogens evaluated in this study were found to be associated with mild or severe disease. Nonetheless, the magnitude of this association varied between pathogens. The results of our study suggest that influenza, RSV and hMPV infections are highly associated with severe respiratory illness in South Africa relative to controls, especially in children <5 years of age. While rhinovirus and adenovirus had the lowest estimated AF the estimated detection rate attributable to illness remained high indicating that, while these viruses could act both as pathogen and bystander, they could also be responsible for a substantial proportion of severe disease. RSV, rhinovirus, adenovirus, hMPV and influenza were the most common pathogens causing disease among SARI cases, especially in children <5 years of age.

Our findings differ from those of other studies which have used multiplex PCR to detect a viral aetiology in non-invasive respiratory specimens and used a control

group to interpret the findings. These studies found that fewer viruses were associated with disease. A study conducted among children ≤12 years of age hospitalized with pneumonia in the Kilifi District hospital in Kenya [5] reported that RSV was the most common virus identified (34% of cases), and the only virus associated with disease. A case-control study conducted among children ≤59 months in rural Kenya, also reported that only RSV was found to be significantly associated with severe pneumonia [10], while another study conducted in children reported that RSV and influenza were more commonly found among cases than controls [17]. A study conducted among adults also found a disease association with RSV, influenza, and hMPV, with influenza being the most common pathogen causing disease [18]. It should be noted that these studies had a limited number of controls potentially resulting in a lack of power to detect significant disease association for pathogens with low detection rates.

In our study RSV was found to be significantly associated with severe respiratory disease relative to controls across age groups. While RSV has been well documented as the leading cause of viral pneumonia in children <5 years of age not only in resource-limited setting but also in the developed world [2, 19], RSV is an increasingly recognized cause of severe disease in adults [20-24]. Several studies have shown that RSV infection is an important cause of illness in the elderly (≥65 years) and high-risk adults, with a disease burden similar to that of non-pandemic seasonal influenza [23, 25]. Although we found that RSV was associated with disease among both children and adults, other modeling studies conducted in South Africa have not found excess mortality or hospitalizations

associated with RSV among persons aged ≥45 years of age [26, 27]. This apparent contradiction may related to the fact that the burden of RSV among older adults in South Africa may be too low to be detected in modeling studies.

While rhinovirus has been shown to be less associated with illness (low attributable fraction) in both children and adults, the estimated detection rate attributable to illness remains elevated when compared to other pathogens, suggesting that rhinovirus may still causes a substantial proportion of clinical disease that manifests either as ILI or SARI. The high prevalence of rhinovirus among controls indicates that rhinovirus may potentially have an extended shedding period and can be detected in patients without symptoms. Several studies have reported the high positivity rate of rhinovirus in asymptomatic individuals and none so far have been able to give a clear cut indication of the role that rhinovirus plays in severe respiratory infection, although several have suggested that rhinovirus can act as both a bystander and a pathogen [28-30]. Similar results were obtained for adenovirus in this study.

In our study, influenza and hMPV were found to be significantly associated with severe disease relative to controls among children less than 5 years of age. Influenza has been described as one of the leading causes of pneumonia in children, the elderly, and adults with HIV infection [2, 16, 31, 32]. Since its initial description in 2001, hMPV has been reported worldwide. However, so far studies of hMPV have been limited; although it has been suggested that hMPV mirrors the epidemiology of RSV and influenza with more severe infections occurring in the very young, elderly and immune-compromised individuals [33, 34].

Our study has limitations that warrant discussion. First, several viruses were detected at low prevalence in the control group which would account not only for the high adjusted relative risk ratios but also for the wide confidence intervals. Second, comparing detection rate of pathogens among symptomatic patients with controls doesn't prove or disprove disease association in individual patients. Other approaches such as viral load and host interactions are needed to determine what role some of these viruses play in severe respiratory disease, while taking into account factors such as replication or persistence of nucleic acids present during the pre- or post syndromic phase of infection. Last, we did not adjust for the potential role of bacterial infections as this information was not available. The role of bacterial super-infection on severe illness following a viral infection cannot be excluded.

In conclusion, influenza, RSV and hMPV can be considered likely pathogens if detected in South African patients with ILI or SARI; whereas rhinovirus and adenovirus were commonly identified also among controls suggesting that they may cause only a proportion of clinical disease observed in positive patients. Nonetheless, given their high estimated detection rate attributable to illness, they may be important contributors to disease. The pathogens listed above had the highest AF or estimated detection rates attributable to illness and they may be considered for routine surveillance. This data together with other matched case-control studies like PERCH (Pneumonia Etiology Research for Child Health) [13] will provide useful information on how each pathogen impacts disease severity and may assist to better interpret surveillance data, to prioritize pathogens to be included in surveillance programs and to guide prevention interventions.

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