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Acute Respiratory Illness in Rural Haiti

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ABSTRACT

Objectives: Acute Respiratory Infection (ARI) is the most common cause of childhood morbidity and mortality in developing countries, including Haiti. Our objective was to detect pathogens found in children with ARI in rural Haiti to help develop evidence-based guidelines for treatment and prevention.
Methods: Retrospective study of students with ARI at four schools in rural Haiti. Viral and/or bacterial pathogens were identified by qPCR in 177 nasal swabs collected from April 2013 through November 2015.
Results: Most common viruses detected were Rhinovirus (36%), Influenza A (16%) and Adenovirus (7%), and bacteria were *Streptococcus pneumoniae* (58%) and *Staphylococcus aureus* (28%). Compared to older children, children aged 3–5 years had more Influenza A (28% vs. 9%, p = 0.002) and Adenovirus detected (14% vs. 3%, p = 0.01). Similarly, *S. pneumoniae* was greatest in children 3–5 years old (71% 3–5yrs; 58% 6–15 years; 25% 16–20 years; p = 0.008). Children 3–10 years old presented with fever more than children 11–20 years old (22% vs 7%; p = 0.02) and were more often diagnosed with pneumonia (28% vs 4%, p < 0.001).
Conclusions: Younger children had increased fever, pneumonia, and detection of Influenza A and *S. pneumoniae*. These data support the need for influenza and pneumococcus vaccination in early childhood in Haiti.

Introduction

Hospital-based studies on children in Haiti have shown that acute respiratory infection (ARI) is the leading cause of child morbidity and mortality (Perry et al., 2005; Vinekar et al., 2015). Earlier studies in resource-poor settings have identified *Streptococcus pneumoniae*, *Haemophilus influenzae* type B and *Staphylococcus aureus* as important bacterial causes of ARI, and respiratory syncytial virus (RSV) and Influenza as important viral causes (Rudan et al., 2008). Many of these prior studies used technically difficult and labor intensive tests that included bacterial and viral culture (Rudan et al., 2008).

The use of diagnostic nucleic acid technology has increased detection of infectious agents associated with ARI. A multi-country study that included Haiti showed that agents detected in hospitalized children less than 5 years of age who had pneumonia included RSV, Influenza A and B, Parainfluenza viruses, Adenovirus and Human metapneumovirus (HMPV) (Bénet et al., 2017). In that same study, analysis of bacterial agents showed high detection of *Streptococcus pneumoniae* and *Mycoplasma*

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pneumoniae leading the authors to conclude that vaccination efforts for *Streptococcus pneumoniae* will be beneficial. Whereas hospital-based studies are informative, most patients do not require hospitalization and much less is known about ARI in school-aged children treated at outpatient clinics.

In 2012–2013 we assessed outpatient illnesses in children who attended four schools in rural Haiti (Gressier/Leogane) and showed that ARI was the most common complaint among 1,357 clinic visits (Beau De Rochars et al., 2015). The objective of this retrospective study is to analyze which infectious agents are detected in Haitian children presenting with ARI in the outpatient setting. Knowledge gained from this study will guide clinical care and public health vaccine campaigns.

Methods

Study participants/sample collection

Enrollment criteria were all students with complaints of fever and respiratory symptoms who attended one of four schools managed by the Christianville Foundation Network in Gressier, Haiti. The Christianville Foundation partners with University of Florida (UF) to address public health challenges in the Haitian community. These schools were previously described with one having pre-K through 12th grade with approximately 800 students, and three schools having pre-K through the 6th grade with 130–180 students each (Beau De Rochars et al., 2015). School clinic staff included one physician and two nurses who evaluated and managed patients as per standard clinic procedures, and obtained a nasal swab from each participant as part of routine clinical care using the BD universal viral transport kit (BD Company, Franklin Lakes, New Jersey). Given the lack of access to X-ray or pulse oximetry, criteria to obtain swab was based on clinical criteria of history of fever and respiratory symptoms. Diagnosis of upper vs lower respiratory infection was based on physical exam. Swabs were initially stored at −20 °C with testing anticipated soon after collection at the UF laboratory in Haiti; due to technical difficulties, de-identified coded swabs were transported in bulk on dry ice to UF and then stored at −80 °C until processed. When laboratory studies were completed, clinic staff retrospectively linked a limited clinical data set with results. The study protocol was reviewed and approved by the University of Florida IRB.

Nucleic acid isolation

Samples were thawed on ice and pulse vortexed 5 times. Total nucleic acid (including DNA and RNA) were then isolated using the RTP Pathogen kit per manufacturer's protocol (Stratec Biomedical, Birkenfeld, Germany).

Real time polymerase chain reaction (RT-PCR)

Total DNA and RNA were analyzed by multiplexed RT-PCR using the FTD Respiratory pathogen 21 plus kit (Fast-Track Diagnostics, Sliema, Malta) on an Applied Biosystems 7500 RT-PCR system. Targets were 18 viruses and 5 bacteria: Influenza A (FluA), Influenza B (FluB), Influenza A/H1N1 (FluA/H1N1), Rhinovirus (RV), Coronaviruses (NL63, 229E, OC43, HKU1), Parainfluenza (HPIV 1, 2, 3, 4), HMPV A/B (HMPV), Bocavirus (BV), *Mycoplasma*

Table 1
Percentage of school children with nasal swabs by school location, age, sex and month/year.

Total Patient Swab Samples (%)	177 (100)				
School	**Total**	**A**	**B**	**C**	**D**
Enrollment*	**1250 (100)**	**800 (64.0)**	**180 (14.4)**	**150 (12.0)**	**120 (9.6)**
Samples with enrollment data	167 (94.4)	125 (74.9)	28 (16.8)	10 (5.9)	4 (2.4)
Age		**3-5 years**	**6-10 years**	**11-15 years**	**16-20 years**
Samples with age data	177 (100)	65 (36.7)	57 (32.2)	27 (15.3)	28 (15.8)
Average age	8.6	3.8	7.8	12.6	17.5
Median age	7	4	8	13	17.5
Mode age	3	3	6	13	16
Sex		**Female**	**Male**		
Samples with sex data	177 (100)	96 (54.2)	81 (45.8)		
Year		**2013**	**2014**	**2015**	
Samples with year data	174 (100)	66 (37.9)	69 (39.7)	39 (22.4)	
No. of months		5	6	9	
Month-sample#		Apr-2 Sep-1 Oct-2 Nov-57 Dec-4	Jan-27 Feb-2 Mar-6 Apr-18 May-13 Jun-3	Feb-1 Mar-4 Apr-7 May-12 Jun-3 Aug-1 Sep-4 Oct-1 Nov-6	

*Estimated average enrollment during the collection time period.

pneumoniae, Respiratory syncytial viruses A/B (RSV), Adenovirus (HAdV), Enterovirus (EV), Parechovirus (HPeV), *Chlamydia pneumoniae* (CP), *S. pneumoniae*, *Haemophilus influenzae* type B (*H. influenzae* B), and *S. aureus*. Both FTD Respiratory Pathogen 21 plus kit controls and independent swab samples known to be positive for RSV, HAdV, Influenza and coronaviruses were used to validate the results.

Clinical exam

All patients were examined by the clinical staff. Fever was defined as greater than 37.9 degrees Celsius. Pneumonia was defined as history of fever with cough or congestion and clinical examination findings of crackles, grunting, or decreased breath sounds.

Statistical analysis

Two-sided Fisher's exact was used to compare the detection rates of agents by age groups, sex, seasonality and clinical parameters, with type I error controlled at 0.05. P-values were not adjusted for multiple comparisons. We also used the Fisher's exact test for the co-detection analysis. All tests were conducted using the R software version 3.3. Agents detected in 5% of patients were prioritized for analysis. Age groups for analytical comparisons were 3–5 years (Group A), 6–10 years (Group B), 11–15 years (Group C) and 16–21 years (Group D), with some comparisons using consecutive age groups combined. Eight children had samples obtained twice and their visits were separated by an average of 6 months (2–17 months). Given the passage of at least 2 months, each patient encounter (i.e., sample) was considered a unique entity for analysis.

Results

Demographics

Swabs were collected from 177 children attending four different schools during school attendance from April 2013 through November 2015: School A (75%), School B (17%), School C (6%) and School D (2%). The average age was 8.7 years (range = 3–20 years); female sex = 54%. A total of 76% of samples (133/174) with date information were collected during the September to June school year of 2013–14, two samples were collected in April 2013 (1%), and the remainder 22% (39) were collected from February-June (excluding December–January) and August–November of 2015 [Table 1].

Agents detected by age

Given that etiology of ARI changes by age, we first looked at agents detected by age. Younger children had FluA, HAdV and *S. pneumoniae* detected more often than older children, while *S. aureus* detection peaked in children with ages between the youngest and oldest age Groups. Analysis of viruses showed that more children in age Group A had FluA detected (28%) when compared to Groups B (10.5%, p = 0.02), C (4%, p = 0.01), and D (11%, p = 0.10). Significantly more children in age Group A had HAdV (14%) detected than children in Groups B–D combined (3%), p = 0.01. For bacterial agents, *S. pneumoniae* detection was greatest in children in the age Group A (71%) followed by Groups B (58%) and C (59%), with the least in D (25%). Significantly more Group A–C children had *S. pneumoniae* detected (64%) than those in Group D (25%), p < 0.001. Although *H. influenzae* B detection decreased with age, this difference was not statistically significant. For *S. aureus*,

Table 2
Viruses and Bacteria Detected in Children by Age.

	All Ages	Age (Group)				
	No. (%)	3-5 y (A)	6-10 y (B)	11-15 y (C)	16-20 y (D)	p-value
Total	177 (100)	65 (36.7)	57 (32.2)	27 (15.3)	28 (15.8)	
VIRUS						
RV	64 (36.2)	22 (33.8)	18 (31.6)	13 (48.1)	11 (39.3)	
FluA	28 (15.6)	18 (27.7)	6 (10.5)	1 (3.7)	3 (10.7)	p = 0.002[a]
FluA/H1N1	23 (13.0)	16 (24.6)	3 (5.3)	1 (3.7)	3 (10.7)	p < 0.001[a]
FluB	9 (5.1)	2 (3.1)	4 (7.0)	1 (3.7)	2 (7.1)	
HAdV	12 (6.8)	9 (13.8)	3 (5.3)	0	0	p < 0.01[a]
HPIV	12 (6.8)	4 (6.2)	4 (7.0)	1 (3.7)	3 (10.7)	
EV	11 (6.2)	3 (4.6)	4 (7.0)	3 (11.1)	1 (3.6)	
Cor	11 (6.2)	3 (4.6)	3 (5.3)	2 (7.4)	3 (10.7)	
HMPV	6 (3.4)	3 (4.6)	3 (5.3)	0	0	
HRSV	4 (2.3)	2 (3.1)	2 (3.5)	0	0	
HBoV	3 (1.7)	2 (3.1)	1 (1.8)	0	0	
HPeV	1 (0.6)	0	1 (1.8)	0	0	
BACTERIA	No. (%)					
C. pneumoniae	1 (0.6)	1 (1.5)	0	0	0	
M. pneumoniae	3 (1.7)	0	3 (5.3)	0	0	
H. influenzae b	6 (3.4)	4 (6.2)	2 (3.5)	0	0	
S. aureus	49 (27.7)	9 (13.8)	19 (33.3)	17 (63.0)	4 (14.3)	p < 0.01[b,c]
S. pneumoniae	102 (57.6)	46 (70.8)	33 (57.9)	16 (59.3)	7 (25.0)	p < 0.01[d]

Influenza A (FluA), Influenza B (FluB), Influenza A/H1N1 (H1N1), Rhinovirus (RV), Coronaviruses (229E, OC43, HKU1), Parainfluenza (HPIV 1, 2, 3, 4), HMPV A/B (HMPV), Bocavirus (BV), Respiratory syncytial viruses A/B (RSV), Adenovirus (HAdV), Enterovirus (EV), Parechovirus (HPeV), *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP), *S. pneumoniae* (SP), *Haemophilus influenzae* type B (HIB), and *S. aureus* (SA).
[a]Group A vs Group B–D.
[b]Group A vs Group B–C.
[c]Group B–C vs Group D.
[d]Group A–C vs Group D.
p Value obtained using two-sided Fisher's exact.

detection was highest in the Group B–C children (43%), with significantly less *S. aureus* detected in Groups A (14%) and D (14%), both $p < 0.01$ [Table 2].

The mean number of total agents detected per child in age Groups A, B and C was higher at 1.9–2.0 than in Group D at 1.2. Significantly more children in age Groups A (62%), B (58%) and C (74%) had 2 or more agents detected than children in Group D (32%), $p < 0.04$ for each comparison to Group D. Analysis of the number of viral agents detected showed no significant difference was seen between the proportion of children who had ≥1 viral agents detected between age Groups A (77%), B (67%), C (67%) or D (68%). However, significantly more bacterial agents were detected in age Groups A, B and C children than in Group D. The proportion of children with ≥1 bacterial agent(s) detected per age group were: Groups A (75%), B (82%), and C (81%) versus Group D (36%), $p < 0.001$ for each comparison to Group D.

Agents detected as a group

The most common viruses detected in children overall with fever and respiratory complaints (N = 177) were RV (36%), FluA (16%, including 13% due to FluA/H1N1) and HAdV (7%). More rarely detected were RSV, BV and HPeV. *S. pneumoniae* was the most common bacteria detected at 58%, followed by *S. aureus* at 28%, and *H. influenzae* B at 3%. [Supplemental Table S1]

Co-detection

Co-detection was common with 322 agents detected in 177 patient samples. At least one viral agent (range 1–3) was detected in 71% of children and at least one bacterial agent in 72% (range 1–3), with 18% having greater than 1 virus and 18% with greater than 1 bacterial spp. detected in the 177 patient samples. Only 10 children (5.6%) had no agent detected. The average number of any agent detected (viruses + bacteria) per child was 1.8 [Table 3]. Analysis by age groups showed no significant difference in viral detection. However, when looking at bacterial detection and co-detection of two or more agents, the oldest age group D had significantly less frequent detection ($p < 0.001$ for bacteria, $p = 0.031$ for co-detection of two or more agents).

More children had FluA as the sole pathogen (39%) than those who solely had HAdV (0%, $p = 0.017$), EV (0%, $p = 0.017$), or *S. pneumoniae* (19%, $p = 0.04$) detected. Co-viral detection was less common in children with FluA (21%) than in children with HAdV (83%, $p < 0.001$) and EV (100%, $p < 0.001$). Co-bacterial detection was also less common in children with FluA (57%) than in children with HAdV (100%, $p = 0.007$) and EV (91%, $p = 0.063$) [Table 4].

Evaluation for relationships between specific agents showed that EV was 100% correlated with concurrent RV detection (11/11, $p < 0.001$); both picornaviruses having possible correlates by PCR. In contrast, FluA and FluB was negatively correlated with RV detection ($p = 0.0022$ and $p = 0.027$, respectively). Evaluation between viral and bacterial correlates showed 100% of children with HAdV also had *S. pneumoniae* detected (12/12, $p = 0.0014$). Detection of *S. aureus* was negatively associated with that of FluA; despite 27.7% of all children having *S. aureus* detected, none of the 28 children with FluA detected were co-detected with *S. aureus*, $p < 0.001$. No other agents showed significant co-detection relationship.

Agents by sex

No significant differences were found between females and males regarding total viral plus bacterial agents, total viral or bacterial agents, or specific agents detected by PCR.

Table 3
Co-Detection in Children with ARI by Age.

		All Ages	Ages (Group)			
		No. (%)	3-5 y (A)	6-10 y (B)	11-15 y (C)	16-20 y (D)
Total		177 (100)	65 (36.7)	57 (32.2)	27 (15.3)	28 (15.8)
	Agents/Sample					
Virus	0	52 (29.4)	15 (23.1)	19 (33.3)	9 (33.3)	9 (32.1)
	1	94 (53.1)	34 (52.3)	30 (52.6)	15 (55.6)	15 (53.6)
	2	26 (14.7)	14 (21.5)	5 (8.8)	3 (11.1)	4 (14.3)
	3	5 (2.8)	2 (3.1)	3 (5.3)	0	0
Bacteria	0	49 (27.7)	16 (24.6)	10 (17.5)	5 (18.5)	18 (64.3)
	1	96 (54.2)	39 (60.0)	37 (64.9)	11 (40.7)	9 (32.1)
	2	31 (17.5)	9 (13.8)	10 (17.5)	11 (40.7)	1 (3.6)
	3	1 (0.6)	1 (1.5)	0	0	0
Virus or Bacteria	0	10 (5.6)	2 (3.1)	3 (5.3)	1 (3.7)	4 (14.3)
	1	65 (36.7)	23 (35.4)	21 (36.8)	6 (22.2)	15 (53.6)
	2	59 (33.3)	20 (30.8)	19 (33.3)	12 (44.4)	8 (28.6)
	3	34 (19.2)	15 (23.1)	10 (17.5)	8 (29.6)	1 (3.6)
	4	8 (4.5)	5 (7.7)	3 (5.3)	0	0
	5	1 (0.6)	0	1 (1.8)	0	0

Table 4
Co-Detection of Agents from 179 Children with Respiratory Complaints in an Out-patient Setting.

AGENT	FluA	RV	FluB	H1N1	229e	HKU1	OC43	HPIV3	HPIV2	HPIV4	HMPV	BV	RSV	HPeV	EV	HAdV	MP	SA	CP	HIB	SP
FluA	**28**	3		23			1									3				2	16
RV		**64**		3			2		1	1	2	3			11	5		17		2	41
FluB			**9**		1						1							4			4
H1N1				**23**			1									3				2	13
229e					**2**																
HKU						**2**												1			2
OC43							**7**									2	1	2		1	4
HPIV3								**3**										1			
HPIV2									**4**						1			1		1	2
HPIV4										**5**		1									2
HMPV											**6**					1		3			2
BV												**3**									2
RSV													**4**	1				1			4
HPeV														**1**							1
EV															**11**	1		2			8
HAdV																**12**		5		1	12
MP																	**3**				2
SA																		**49**		1	25
CP																			**1**		1
HIB																				**6**	5
SP																					**102**

	FluA	RV	FluB	H1N1	229E	HKU1	OC43	HPIV3	HPIV2	HPIV4	HMPV	BV	RSV	HPeV	EV	HAdV	MP	SA	CP	HIB	SP
Sole Agent	11	13	2	9	1	0	1	2	0	3	1	0	0	0	0	0	1	11	0	0	19
(%)	(39)	(20)	(22)	(39)	(50)	(0)	(14)	(67)	(0)	(60)	(17)	(0)	(0)	(0)	(0)	(0)	(33)	(22)	(0)	(0)	(19)
Co-Virus	6	23	2	6	1	0	4	0	1	1	3	3	1	1	11	10	1	29	0	6	72
(%)	(21)	(36)	(22)	(26)	(50)	(0)	(57)	(0)	(25)	(20)	(50)	(100)	(25)	(100)	(100)	(83)	(33)	(59)	(0)	(100)	(71)
Co-Bac	16	47	6	13	0	2	6	1	4	2	4	2	4	1	10	12	2	25	1	5	32
(%)	(57)	(73)	(67)	(57)	(0)	(100)	(86)	(33)	(100)	(40)	(67)	(67)	(100)	(100)	(91)	(100)	(67)	(51)	(100)	(83)	(31)

AGENT	RV	FluB	H1N1	BV	EV	SA	SP
FluA	0.0022					5.5e-5	
RV		0.027	0.018	0.046	8e-6		
FluB							
H1N1						6.6e-4	
HAdV							0.0014

p-Values are calculated for each pair of pathogens only if either one were detected in more than 5 samples. Only p-values <0.05 are shown.
Influenza A (FluA), Influenza B (FluB), Influenza A/H1N1 (H1N1), Rhinovirus (RV), Coronaviruses (229E, OC43, HKU1), Parainfluenza (HPIV 1, 2, 3, 4), HMPV A/B (HMPV), Bocavirus (BV), Respiratory syncytial viruses A/B (RSV), Adenovirus (HAdV), Enterovirus (EV), Parechovirus (HPeV), *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP), *S. pneumoniae* (SP), *Haemophilus influenzae* type B (HIB), and *S. aureus* (SA).

Seasonality

Of 174 samples that had date of collection data, 133 (76%) were collected from children during the school year of September 2013 through June 2014 for this one season evaluation. The number of children tested were not evenly distributed with 1–6 tested in Sep, Oct, Dec, Feb, Mar, Jun and 13–57 tested in Nov, Jan, Apr, May. Analysis of specific agents detected monthly showed seasonality was significantly associated with influenza detection. More children had FluA/H1N1 detected during the months of Oct–Dec 2013 (14/63, 22%) than children during the school year outside of those months (0/70, 0%, $p < 0.001$) [Figure 1]. Similarly, more children had any Flu (A/H1N1 + A/not H1N1 + B) detected in Oct–Dec 2013 (16/63, 25%) compared with those in the other 2013–14 school months combined (8/70, 11%, $p = 0.044$). Although no other agents with at least a 5% (7 of 134) detection rate were found to

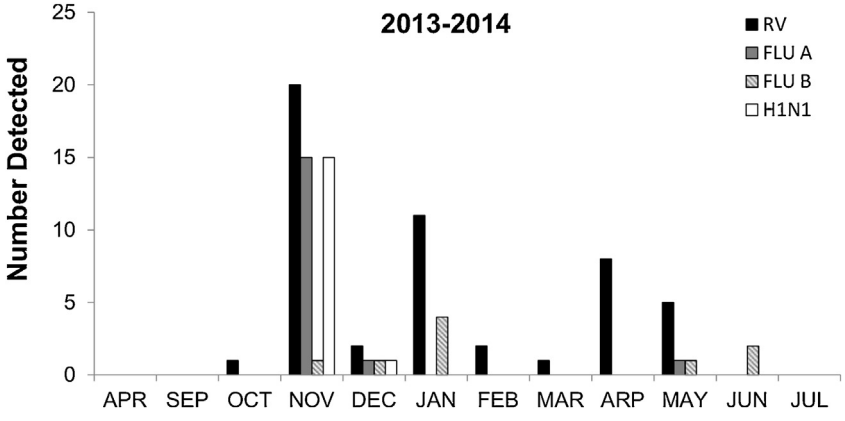

Figure 1. Distribution of agents detected by month of the year Apr 2013 to Jul 2014.

have seasonality, it was noted that all RSV detected occurred in Nov 2013 and RSV detection was more common in Nov 2013 (4/57, 7%) than in the other school year months combined (0/76, 0%, p = 0.032).

Clinical correlates

Of 158 children who had clinical data, primary diagnoses were Upper respiratory tract infection (URTI, excludes Group A *streptococcal* [GAS] pharyngitis) at 67%, Lower respiratory tract infection (LRTI) at 21% and 'Other' at 12%. Twenty-five (16%) children had more than 1 diagnosis. Fever was detected at the time of presentation in 28 (18%) children and was significantly more common in children with LRTI (24%) or with 'Other' diagnoses (53%) when compared to those with URTI (9%), $p \leq 0.04$ for both analyses [Table 5].

Fever and specific virus analysis showed a significantly higher percentage of children detected with FluA (50%), separately FluA/H1N1 (47%), and FluA or FluB (48%) had fever recorded compared to those with RV (12%). For bacteria detection, neither *S. pneumoniae* or *S. aureus* were associated with fever when compared to children without *S. pneumoniae* or *S. aureus*, respectively. Analysis of specific viral or bacterial agents detected showed more children with FluA or FluB had LRTI (33%) compared to URTI (15%) [Table 5]. None of the 6 children with primary diagnosis of Tonsillitis had any viruses detected, whereas 5/8 (63%) with primary diagnoses of Parasitosis or febrile syndrome had FluA/H1N1 or HAdV detected. [data not shown]

Fever and LRTI separately were associated with the younger age groups. Significantly more children aged 3–10 years had fever detected (22%) when compared to children aged 11–20 years (7%, p = 0.02). Similarly, LRTI was more common in 3–5 year old children (36%) compared to those 6–10 years (19%), 11–15 years (8%), and 16–20 years (0/21, 0%), and this was statistically significant when comparing Groups A–B (28%) to C–D (4%), $p < 0.001$.

Evaluation of medications given showed only 2 of 158 (1.3%) children did not receive any medications. Antibiotics were given to 44 (27.7%) children that included 31 (94%) diagnosed with LRTI with the rest attributed to primary- or co-diagnoses of tonsillitis, bacterial skin, urinary tract or sinus infections. Of the 31 with LRTI, 30 received Amoxicillin and 1 co-trimoxazole. Overall, the three most common medications given were acetaminophen (74%, 117), mucolytic +/− beta agonist +/− expectorant mix (52%, 83), and Vitamin C (47%, 74). For the 101 children with uncomplicated URI diagnosis (no concurrent known bacterial infections), 22% received one, 69% two, and 7% all three of these drugs. Compared to children diagnosed with uncomplicated URI, children with LRTIs or 'Other' diagnoses did not significantly differ in percent receiving these medications.

Table 5
Clinical Diagnoses, Fever, Agents and Age Correlations.

	Total	Diagnosis vs Fever		Diagnosis vs Age Group[a]		
	No. (%)	Fever	p-value	Group A-B	Group C-D	p-value
All	158 (100)	28 (17.7)		112 (70.9)	46 (29.1)	
Diagnoses						
URTI	106 (67.1)	10 (9.4)		68 (60.7)	38 (82.6)	p<0.01
LRTI	33 (20.9)	8 (24.2)	p=0.04[b]	31 (27.7)	2 (4.3)	p<0.001
'Other'	19 (12.0)	10 (52.6)	p<0.01[c]	13 (11.6)	6 (13.0)	
tonsillitis	6 (3.8)	3 (50.0)				
parasitosis	4 (2.5)	3 (75.0)				
febrile s.	4 (2.5)	4 (100)				
'Various'	5 (3.2)	0 (0.0)				

		Agent vs Fever		Agent vs Diagnosis		
	Total=158	Fever=28	p-value	LRTI=33	URTI=106	p-value
Virus						
FluA	22 (13.9)	11 (50.0)	p<0.001	8 (24.2)	10 (9.4)	
FluA/H1N1	17 (10.8)	8 (47.1)	p<0.003	6 (18.2)	7 (6.6)	
FluA or B	31 (19.6)	15 (48.4)	p<0.001	11 (33.3)	16 (15.1)	p<0.05
FluB	9 (5.7)	4 (44.4)	p=0.053	3 (9.1)	6 (5.7)	
HAdV	10 (6.3)	4 (40.0)		2 (6.1)	5 (4.7)	
RV	59 (37.3)	7 (11.7)		9 (27.3)	45 (42.5)	
EV	9 (5.7)	1 (11.1)		0 (0.0)	9 (8.5)	
Bacteria						
S. aureus	45 (28.5)	8 (17.8)		6 (18.2)	30 (28.3)	
S. pneumoniae	92 (58.2)	18 (19.6)		21 (63.6)	61 (56.6)	

Age Group[a]	**Total=158**	**Fever=28**		**LRTI=33**	
3-5 y (A)	59 (37.3)	10 (16.9)		21 (35.6)	
6-10 y (B)	53 (33.5)	15 (28.3)		10 (18.9)	
11-15 y (C)	26 (16.5)	1 (3.8)		2 (7.7)	
16-23 y (D)	20 (12.7)	2 (10.0)		0 (0.0)	
Group A-B	112 (70.9)	25 (22.3)	p=0.02[d]	31 (27.7)	p<0.001[d]
Group C-D	46 (29.1)	3 (6.5)		2 (4.3)	

[a]Age Group: 3–5 y (A); 6–10 y (B); 11–15 y (C); 16–20 y (D).
[b]Fever in URTI vs LRTI.
[c]Fever in URTI vs Non-RTI, with/without Febrile Syndrome that by definition has fever at presentation.
[d]Group A–B vs Group C–D.
p Value obtained using two-sided Fisher's exact.

Discussion

This is one of the largest retrospective studies of pediatric outpatients with ARI in rural Haiti. The overall findings of RV as the most common virus detected followed by Influenza in Haiti is comparable to that found in studies of children in other tropical, resource-poor countries (Hoffman et al., 2012; Schlaudecker et al., 2012; Taylor et al., 2017). However, HMPV in children with ARI was detected less often (3%) in this study compared to similar studies (11–14%) (Al-Sonboli et al., 2006; Banerjee et al., 2007; Taylor et al., 2017). These and other studies showed HMPV caused a high percentage of severe ARI in those <2–5 years of age (Ali et al., 2013; Panda et al., 2014), suggesting the lower detection rate seen in this study may be due to the older median age in this study, and differences between outpatient study compared to previous inpatient studies.

RSV detection was significantly lower at only 2.3% of tested patients compared to previously published reports of 30–40% in other developing countries where the bulk of the children were seen in hospitals or emergency rooms (McCracken et al., 2013; Dawood et al., 2015; Bouzas et al., 2016). This low level of detection may have been due to initial storage of nasal swabs at −20 °C which has been found to decrease RSV detection (Nunes and Moura, 2006) and our patients being 3–20 years old when the highest rate of the RSV positivity are typically among children ≤2 years of age. Low detection of RSV was also seen in a study of 4,242 pediatric patients that showed detection of RSV infection at 3.4 −7.2% in older children 3–14 years of age compared to 18.7–29.2% in 4 months to 2 years old (Liu et al., 2014). It is possible that by the time Haitian children are 3 years of age, the risk for significant infection has greatly declined in comparison to those in less resource poor countries where RSV and HMPV can continue to cause significant disease in those <5 years old. Future studies on RSV and HMPV that included children <3 years of age in both out- and in-patient setting could help better understand their clinical impact.

Consistent with other studies is the finding that detection of Influenza (A, including A/H1N1, and B) was significantly associated with increased clinical symptoms of fever at presentation and LRTI diagnosis especially in younger children (LaForce et al., 1994; Cox and Subbarao, 2000; Descalzo et al., 2016). Introduction of FluA and FluB vaccine in younger and school aged children could significantly decrease its clinical impact, including decrease spread to siblings and adults at home.

A study that ascertained peak influenza activity in 70 tropical or subtropical countries suggested that timing of influenza vaccine in Haiti should be in April based on data from nearby countries including Cuba and Dominican Republic (no Haiti data) (Hirve et al., 2016). Yet in this evaluation of children in Haiti for the school year of 2013–14, the peak detection of influenza appeared to be in Oct–Dec 2013. This finding suggests that immunization in Haiti should begin by August/September, similar to October for Mexico, Guatemala and Jamaica in the 70 nation study. Increased testing in Haiti for peak detection over several years is needed to determine best timing of influenza vaccinations.

The high detection rate of *S. pneumoniae* (58%) and *H. influenzae* B (3.4%) compared to affluent countries may reflect the lack of *S. pneumoniae* vaccine in the immunization program in Haiti and limited *H. influenzae* B vaccinations which only began in late 2012 (Mbelle et al., 1999; Abdullahi et al., 2008; Agrawal and Murphy, 2011; WHO, 2013a; Adegbola et al., 2014). Agent-specific vaccination has been shown to decrease *S. pneumoniae* and *H. influenzae* B colonization and subsequent infection in children (Dagan et al., 2002; Agrawal and Murphy, 2011; Alvarez et al., 2016). The decrease in *S. pneumoniae* and *H. influenzae* B detection with increased age is likely attributed to protective antibodies developing due to infections (Mbelle et al. 1999; Dagan et al., 2002; Peraza et al., 2004). Despite this decrease, the high colonization rates and associated risk for infection strongly supports introduction of *S. pneumoniae* and continuation of *H. influenzae* B vaccine in those <5 years of age (WHO, 2013b; WHO, 2012). Furthermore, future determination of the *S. pneumoniae* serotypes found in 58% of the overall cohort could help identify the usefulness of available vaccines.

Although multiple studies of LRTI in developing countries have implicated infection with *S. pneumoniae*, *H. influenza* type B and *S. aureus* as the major bacterial causes of severe pneumonia (Shann, 1995), this study detected high rates of these pathogens in non-severe outpatients. Possibly the problem of high rates of colonization could have masked some primary or secondary bacterial infections. Future studies may benefit from blood cultures along with PCR to assess disease burden and from more extensive patient follow-up. In addition, community case-control studies and studies where the incidence of pneumonia is measured pre- and post-vaccination may clarify disease burden and attributable fraction of colonization versus active disease from these pathogens (Rudan et al., 2008; Levine et al., 2012; Morpeth et al., 2017).

The 20% incidence of LRTI in this ARI study is consistent with previous findings that 10–20% of children with ARI may develop pneumonia (Panda et al., 2014). Virtually all patients who had LRTI diagnosed were treated with an antibiotic considered appropriate by the World Health Organization (WHO, 2014). However, >50% of patients received one or more “common cold” medications that are generally not advised by WHO, particularly in young children (WHO, 2001). The risks/benefits of such medications are an issue in both resource rich and poor countries, but the latter have an additional fiscal burden related to treatments that may have unproven efficacy and potential toxicities.

Limitations of the study are related to the developing world setting. As discussed above, due to the use of nasal swabs with PCR based detection, we can comment on the pathogens detected at the time of symptoms but cannot directly point to the pathogen as the etiology. The lack of corresponding tests for the diagnosis of pneumonia such as chest x-ray, pulse oximetry, or blood culture is due to lack of resources in the rural clinic setting. Another resource related issue is the skewing of the patient population to the younger age group since only one out of the four schools provides middle school or high school education. Also there are gaps in data during the vacations and holidays when schools are closed such as for winter vacation in December which may present a bias in the data. Lastly, since the school clinic was in location A, children who were ill in other locations enrolled on the study were less able to travel to the clinic which likely presented a bias based on resource distribution.

In conclusion, school-aged children in rural Haiti between 3 and 5 years old had Influenza A and *Streptococcus pneumoniae* detected and pneumonia diagnosed more often than older children. The children presenting with these agents detected in this outpatient study were less ill compared to previous inpatient studies. Future research should include community-based case-control studies to assess colonization versus active infection. This study also supports ongoing advocacy for influenza and pneumococcal vaccines in young Haitian children. Pre-post studies (e.g. a stepped wedge design) during these vaccination campaigns would define the impact of vaccination and further characterize the morbidity and mortality associated with the pathogens detected herein.

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Declarations of interest

None.

Ethical approval

We have read and complied with the policy of the journal on ethical consent as stated in the Guide to Authors. This study was reviewed and approved by the University of Florida IRB.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2019.02.003.

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