Association of Nasopharyngeal Viruses and Pathogenic Bacteria in Children and Their Parents With and Without HIV

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Research

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Abstract

Background: Bacteria and respiratory viruses co-occur in the nasopharynx, and their interactions may impact pathogenesis of invasive disease. Associations of viruses and bacteria in the nasopharynx may be affected by HIV.

Methods: We conducted a nested case-control study in families with and without HIV in West Bengal India, to look at the association of viruses and bacteria in the nasopharynx of parents and children, when they are asymptomatic. Quantitative polymerase chain reaction, for 4 bacteria and 21 respiratory viruses, was run on 92 random nasopharyngeal swabs from children, and 77 swabs from their parents.

Results: In 67% of child samples bacteria was found; in 45% viruses were found and in 27% viruses were found with bacteria. *Staphylococcus aureus* (53%) was the most common bacteria, followed by *Streptococcus pneumoniae* (pneumococcus) (37%) in children and parents (53%, 20%). Human rhinovirus and adenovirus (14%, each) were most frequently detected in children living with HIV (CLH). Adenovirus was associated with pneumococcus in children (Odds ratio OR 6, 95% CI 1.12-31.9). Rhinovirus was associated with pneumococcus in CLH (OR 15.6, 1.66-146.4), and with increased pneumococcal density in children (Regression coeff 4.5, 1.14-7.9). CLH had 8.7 times increased pneumococcal and *S. aureus* colonization, in the presence of rhinovirus.

Conclusions: Viruses and bacteria were frequently co-detected and viruses specifically rhinovirus was associated with increased pneumococcal density in asymptomatic children regardless of HIV status. Positive associations were found between pneumococcus, *S. aureus,* and rhinovirus in CLH.

Introduction

The nasopharyngeal ecosystem composed of respiratory viruses and bacteria is dynamic [1, 2]. Its composition is affected by host, pathogen, and external factors [2, 3]. Underlying HIV infection may affect the density and balance of bacteria and viruses in the nasopharyngeal space.

HIV infected individuals have a high burden of invasive disease from respiratory viruses, and bacteria that may colonize the nasopharynx [4, 5]. HIV increases the risk of pneumococcal and *S. aureus* disease 40–300 times, and nasopharyngeal bacterial colonization usually precedes invasive disease [5, 6]. The association between pneumococcus and *S. aureus* is typically negative in healthy individuals, where if one is present the other usually is not, but in HIV infected individuals, particularly in children living with HIV (CLH), this negative interaction disappears, and CLH are more likely to have dual colonization [7–10].

The carriage density of potentially pathogenic bacteria correlates with pathogenesis, transmission, disease severity, and prognosis and is affected by underlying HIV infection [11–13]. In South Africa, higher carriage density of pneumococcus has been observed in archived nasopharyngeal swabs from CLH compared to HIV uninfected children (HUC) [14]. We also found increased pneumococcal density in

CLH compared to HUC [15]. Increased pneumococcal density appears to be an important prognostic marker of pneumococcal pneumonia, in HIV infected adults [13].

Respiratory viral infections increase the carriage density of bacteria, specifically pneumococcus, and may impact prognosis in CLH [16]. In hospitalized children with pneumonia in South Africa, 78% had viral infections and CLH specifically, had increased risk of death [17]. Influenza infection in particular was associated with pneumococcal pneumonia in this population.

Access to influenza and pneumococcal conjugate vaccines (PCV), need to reflect these differences in colonization and disease in individuals with HIV, especially in low- and middle-income countries. India has the largest population burden of pediatric HIV outside of sub-Saharan Africa [18] and acute respiratory illness (ARI), is the most common presentation for CLH in India [19]. Vaccine access to PCVs and influenza vaccines are limited for Indian CLH. Understanding nasopharyngeal ecology and the association between viruses with bacteria and bacterial density in high-risk families, could help inform evolving vaccine policy [20–22].

Studies have been focused on the epidemiology of ARI, but not necessarily on the interaction between commensal microbiota and viruses that have the potential to cause severe disease in HIV-infected individuals [19, 23]. This study investigates the bacteria and viruses within the nasopharynx, and the association of viruses with bacteria in high risk families in West Bengal where both children and adults are HIV infected, and access to influenza and pneumococcal vaccines are limited.

Materials And Methods

Study design

From February 2012 to October 2014, we conducted a prospective cohort study to look at the impact of *Haemophilus influenzae* type b conjugate vaccine and the 13-valent PCV (PCV13), in HIV infected and uninfected children and their unvaccinated parents, in West Bengal India [24, 25]. We banked over 1800 nasopharyngeal (NP) swabs collected from children and one of their parents at different visits. Nasopharyngeal calcium alginate swabs (Puritan) were collected in 1 mL of skim milk tryptone glucose glycerol (STGG) media, transported at -4°C, and banked within 1 hour at -80 °C, at the Indian Institute of Technology (IIT)-Kharagpur.

As part of this retrospective nested case-control study, a random selection of 169 archived NP swabs were analyzed: 92 swabs from 49 CLH and 43 HUC; and 77 from 44 parents of CLH (PCLH), and 43 parents of HUC (PHUC).

Multiplex quantitative real time PCR

Bacterial DNA and viral RNA/DNA was extracted from 400µL of NP swabs with the RTP pathogen kit (Cat No: 1040500200, Stratec®). The genomes were subjected to quantitative multiplex real-time reverse transcription-polymerase chain reaction (rRT-PCR), for detection of respiratory viruses including human

adenovirus, human bocavirus, human coronavirus (NL63, 229E, OC43 and HKU1229), enterovirus, influenza A virus, influenza A H1N1 virus, influenza B virus, human metapneumovirus A/B, human parainfluenza virus 1, 2, 3 and 4, human parechovirus, human rhinovirus, RSV A/B, and bacteria including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* type b, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae* as part of the FTD Respiratory pathogens 21 plus kit (Cat No: FTD-2+.1-64, FTD Diagnostics®). Samples with cycle threshold (Ct) value ≤38 were considered positive. Bacterial loads were calculated using standards provided with the kit, in genomic copies/mL and Log_{10} transformed.

Statistical Analysis

The data was entered into Epi Info 7 (CDC, Atlanta) and statistically analyzed by Stata version 13.1 (STATA Corporation). The bacterial and viral detection rates were defined as the percentage of samples that are positive for a particular bacterium or viral nucleic acid by PCR. Viral subtypes from one group were pooled since rates were low. Categorical variables were analyzed by χ^2 test, and Wilcoxon rank-sum test was used for continuous. The nutritional status of children was measured by weight-for-age-z scores (WAZ), and height-for-age-z scores (HAZ). Children with Z scores −2 to −3 were categorized as moderately malnourished and below −3 as severely malnourished [26]. CLH were categorized into immunologic categories based on CD4 counts [27]. Risk factor analysis for binary outcomes was done by logistic regression, and by linear regression for ordinal outcomes.

Results

Demographic characteristics

The demographics are described in Table 1. The median age of CLH was 4.8 years and 3.5 for HUC. CLH were malnourished and stunted as compared to HUC [WAZ for CLH -2.456 vs. -1.33 for HUC, p=<0.001]; [HAZ for CLH-1.89 vs. -.92 for HUC, p=<0.001]. 98% of mothers of CLH were HIV-infected. Mothers contributed 92% of all parental swabs.

Clinical characteristics

Nearly 36% (18/49) of CLH were on antiretroviral treatment (ART) and 51% were on Trimethoprim/Sulfomethoxazole prophylaxis (Table 2). 53% of parents of CLH were on ART. The majority of CLH had immune classification of severe [15/49, (30%)] or moderate [22/49, (45%)] disease. The median CD4 count was 650 cells/mm^3.

We noted the clinical history of children prior to NP specimen collection, and found 28% of CLH, and 21% of HUC, were sick in the past week. The major symptoms included fever, cough, rhinitis or diarrhea; however, during swab collection all children were afebrile (<100.4 °F). Over 23% of CLH had a history of ear infection within one month prior to study, compared to 4% in HUC (p=0.06).

Nasopharyngeal bacteria and viruses in children versus parents

A total of 169 NP swabs (92 from children; 77 from parents) were tested for viruses and 147 (83 from children, 64 from parents) for bacteria (Table 3). Bacteria were identified in 67% of the children, and 54% of parents. *Staphylococcus aureus* was the most common bacterium identified in children [53%, 44/83], followed by *Streptococcus pneumoniae* [37.3%, 31/83], and *Chlamydia pneumoniae* [2%, 2/83]. Similar trends were seen in their parents, [*S. aureus* (53%); pneumococcus (20%)]. Higher rates of pneumococcus were found in children compared to parents (p=0.02).

Pneumococcus with *S. aureus* were the most frequent bacteria co-detected (24%) in children. Viruses were detected in higher numbers (44%) in children than their parents (30%) (p=0.049), particularly rhinovirus (p=0.02). Viruses with bacteria were more frequently co-detected in children (26%), than virus alone (3%). For example, 13 out of 15 times rhinovirus was detected with bacteria, and adenovirus was found with bacteria 8 out of 9 times.

Nasopharyngeal microbes and viruses in children with and without HIV

Bacteria detection

Bacterial pathogens were tested in specimens from 43 CLH, 40 HUC, and viruses in 49 CLH, 43 HUC (Table 4). Samples for bacterial testing were lower due to the loss of reactions in bacterial standards.

Bacteria were identified in 70% of CLH and 65% of HUC. *S. aureus* was identified in an increasing fashion in CLH, as compared to HUC (63% vs. 42%; p=0.06). Similar pneumococcus and *C. pneumoniae* rates were found in CLH and HUC (37%; 2% each). Co-occurrence of pneumococcus with *S. aureus* was found more often in CLH (30%), than in HUC (17%), although not significant (p=0.17).

Respiratory virus detection

In the 92 samples tested, viruses were detected in 41% of CLH and 49% of HUC. Rhinovirus and adenovirus were most frequently detected in CLH (14% each), followed by bocavirus 4%, RSV 2%, coronavirus-229 2%, human metapneumovirus 2% and human parainfluenza-3 virus (2%). Of interest, viruses were identified mainly in children with stage 2 and 3 HIV disease (13/14), than stage 1 (1/14) (p= <0.001). 57% of CLH with viral positivity were not on ART. Rhinovirus was also most frequent in HUC (18%). Influenza B was identified only in HUC (9%). Being on an antibiotic in the past week, increased the risk of virus by 3.85 times, (95% CI 1.05-14, p=0.04).

Virus-bacteria co-detection

The co-occurrence of ≥1 bacteria with ≥1 viruses was found in 28% of CLH, and 25% of HUC. The co-occurrence was mainly found in children with stage 2 and stage 3 HIV disease (11/12), as compared to stage 1 (1/12) (p<0.001). Interestingly, wood used for cooking within households, increased the risk of

virus (OR 3.09, 1.0-9.21, p=0.042) and virus-bacteria detection (OR 4.75, 1.2-17, p=0.019) among children, suggesting indoor air pollution may increase the risk for viruses.

Nasopharyngeal bacteria and viruses identified in parents with and without HIV

Bacteria were tested in 33 PCLH and 31 PHUC specimens, and viruses in 44 PCLH and 33 PHUC. Bacteria were detected in 51% of PCLH and 58% of PHUC. *S. aureus* was most frequent in both PCLH (51%) and PHUC (58%). Similar rates of pneumococcus (20-21%) were detected in both parents, and their children. Viruses were detected in 34% of PCLH and 27% of PHUC. Adenovirus was most common in PCLH (11%), and RSV in PHUC (12%). Virus-bacteria co-detection was found in 16% of PHUC and 12% of PCLH.

Co-detection of nasopharyngeal bacteria with viruses

Virus-bacteria co-detections were found in 12 CLH and 10 HUC. Of the 12 CLH, 7 had co-occurrence of *S. pneumoniae* + *S. aureus* with viruses. The remaining 5 CLH had co-occurrence of viruses with pneumococcus (2), or *S. aureus* (3). Among the HUC, viruses were co-detected with pneumococcus (3), *S. aureus* (3), *S. pneumoniae* + *S. aureus* (3), or, *C. pneumoniae* (1). Among parents, viruses were co-detected only with *S. aureus* (8/64).

Nasopharyngeal carriage in children with and without history of symptoms

A history of respiratory symptoms in the past week was associated with more than 4 times increased risk of virus detection in children (OR 4.2; 1.5-11.7; p=0.005), and 3.65 times virus-bacteria co-detection (OR 3.65; 1.3-10; p=0.01). CLH with symptoms in the past week, had 6 times increased risk of virus detection (6.44; 1.62-25; p=0.008), as compared to 2 fold in HUC (2.6; .56-12; p=0.22). Bocavirus and RSV were identified only with symptom history, while rhinovirus and adenovirus were detected regardless of history.

Association of viruses with *S. pneumoniae* and its nasopharyngeal density in children

s. pneumoniae was associated with co-occurrence of viral species in children (OR 3.9) (Table 5). Particularly adenovirus, co-detection increased the likelihood of pneumococcal detection six fold (p=0.036).

In CLH, positive associations were found between pneumococcus with rhinovirus (OR 15), and between pneumococcus, *S. aureus,* and rhinovirus (OR 8.7). Increased pneumococcal density was seen with co-detection of viruses (Coeff 3.6), multiple viruses (Coeff 5), and rhinovirus specifically (Coeff 4.5).

Discussion

This is the first report from India about household level prevalence and association of respiratory viruses with bacteria in nasopharynx in parents and children in the context of HIV. A majority of the CLH had moderate to severe HIV disease and were not on ART, which increased their risk for bacteria and virus detection [28]. Familial bacterial carriage was evident, with parents having lower carriage of viruses and

bacteria, except *S. aureus*. Similar to our findings, others have reported more *S. aureus* carriage in CLH, as compared to HUC [29–31], while some did not [10]. The rates of pneumococcus in CLH were similar to studies from Brazil (28%) [32], and Indonesia (46%) [33]. While reasons are not clear, similar pneumococcal detection rates have been seen between CLH and HUC in India [25], Brazil [32], Mozambique [34], and South Africa [7]. Serotype specific carriage may provide more information.

The co-colonization of otherwise competing pneumococcus and *S. aureus* in the HIV-infected group could be immune-mediated due to decrease in pneumococcal-specific $CD4^{+}$T cells in HIV infected individuals [35]. The interference, or negative association, between pneumococcus and *S. aureus* seen in HIV uninfected children, has been observed following antiretroviral treatment in CLH [7].

Rhinovirus and pneumococcus are positively associated in healthy children under-2 [2, 36]. An Indian study reported rhinovirus with *S. aureus* in symptomatic HIV-affected individuals [37]. We found a positive association of rhinovirus, *S. aureus*, and pneumococcus in CLH.

Viral infections commonly cause hospitalizations in CLH, and rhinovirus and adenovirus in particular are responsible for LRTI and AOM in both HUC and CLH [3, 17, 38]. Higher rates of viruses were detected in children regardless of HIV, particularly rhinovirus. Rhinovirus has been frequently identified in asymptomatic children [2, 39]. The detection of virus in the nasopharynx may suggest asymptomatic carriage, subclinical infection, or prolonged viral shedding from recent symptomatic infection, or a past infection [40, 41]. CLH may shed viruses for a longer time, and this may increase their risk for secondary bacterial infections leading to pneumonia and AOM, and, also increase risk of infections within families where both adults and children are HIV infected [20, 42, 43].

Otitis media and LRTI cause significant morbidity and mortality in CLH. [44]. Bacterial and viral interactions specifically involving rhinovirus, adenovirus, pneumococcus, *S. aureus*, and nontypeable *H. influenzae* are particularly important in the pathogenesis of these conditions [3]. In studies in South Africa with HUC, CLH, and HIV exposed uninfected children, CLH had the same spectrum of pathogens for these conditions; however, they had increased risk of mortality [17].

Association of increased pneumococcal density with viruses, has been observed during asymptomatic [45], and symptomatic viral infections [16, 21, 46]. Viral detection with increasing pneumococcal density without producing symptoms, may suggest that viruses promote growth and transmission of bacterial colonizers in the nasopharynx, thus predisposing individuals to complications of upper respiratory tract infections [45]. Rhinovirus co-infections have been found with increasing pneumococcal density during episodes of ARI in young children in rural Peru [47]. A Finnish study showed rhinovirus circulation was associated with invasive pneumococcal disease (IPD) in children [42]. Mechanistic *in vitro* studies support that rhinovirus increases pneumococcal adherence, through the expression of platelet-activating factor receptor [48], and disrupts the epithelial barrier functions thus promoting the binding, translocation, and persistence of bacteria [49]. Similarly, adenovirus has been correlated with IPD in children [21, 50] and *in vitro* [51].

The major findings of this study are the detection of viruses with increasing pneumococcal density in asymptomatic children, and rhinovirus co-detections with pneumococcus in CLH compared to age-matched HUC. Association between pneumococcus, *S. aureus*, and rhinovirus in CLH, supports the increased risk of polymicrobial pneumonias in CLH. Pneumococcal conjugate vaccines impact the nasopharyngeal ecology and the risk of LRTI and AOM in adults and children with HIV [7, 52, 53].

The study is limited in its sample size, therefore confidence intervals were wide for OR, and we could not do multivariate analysis for association between microbes. The study is cross-sectional, thus, our observations are a snapshot of this dynamic process. The clinical relevance of viral activity with increasing pneumococcal density in asymptomatic children requires further study through well designed cohort studies.

Pneumococcal-viral interactions are complex. Larger longitudinal studies are needed to understand the viral-bacterial dynamics in high-risk families, in both asymptomatic, and symptomatic situations. Viral load, and host immune responses, may better differentiate asymptomatic versus active viral infections [54]. India has a huge burden of HIV and pneumonia. Pneumococcal vaccines are being rolled-out in the Indian UIP but seasonal influenza vaccines are still not part of government programs and neither are available in programs for HIV infected individuals. It is essential to think about access to vaccines for children and adults with HIV to mitigate respiratory infections [55]. This study is timely in looking at the association of pneumococcus with viruses in this high-risk group.

In conclusion this study demonstrates high detection of viruses and bacteria in asymptomatic children with and without HIV and shows that pneumococcal density increases in the presence of viruses.

Declarations

Ethics approval and consent to participate: The study was approved by the Institute Ethical Committee of Indian Institute of Technology-Kharagpur. Written informed consent forms were obtained from all study participants prior to start of the study

Consent for publication: Written informed consent forms obtained from all participants included consent for publication.

Availability of data and materials: The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: TK conceptualized the study, processed the samples, analyzed the data, interpreted the data and wrote the manuscript. RSD collected samples, processed samples and did data entry in EpiInfo. AC helped in running PCR. JC helped in the analysis of data and writing the manuscript. SDB conceptualized the study, conducted the field study, interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1
Demographic characteristics of children and their parents

	Children with HIV (CLH)	HIV uninfected children (HUC)	P
N	49	43	
Age years, median (IQR25, IQR75)	4.827 (3.8, 6.12)	3.53 (3.09, 4.04)	< **0.001***
Female child, n (%)	25 (51)	25 (58)	
WAZ median (IQR25, IQR75)	-2.45 (-3.63, -2.043)	-1.33 (-2.03, – .525)	< **0.001***
HAZ median (IQR25, IQR75)	-1.89 (-2.76, -1.186)	– .925 (-1.69, .015)	< **0.001***
Child school going, n (%)	27 (55)	28 (65)	0.223
Tuberculosis in house, n (%)	11 (22)	2(4)	**0.014**
Number of children in house, median (IQR25, IQR75)	2 (2, 2)	2(1, 2)	**0.001***
Number of rooms in house, median (IQR25, IQR75)	2 (2, 3)	2(2, 4)	0.45
Family income, median (IQR25, IQR75)	11362 (9478, 11362)	7594 (7594, 9478)	**0.0004**
Socioeconomic status[#]			
Lower, n (%)	0 (0)	2 (4.7)	-
Upper lower, n (%)	2 (4)	10 (23)	**0.006**
Middle, n (%)	20 (41)	29 (67)	**0.01**
Upper middle, n (%)	27 (55)	2 (4.7)	**< 0.00001**
Fuel used for cooking			
Gas, n (%)	6 (12)	21 (49)	**.00012**
Wood, n (%)	41 (84)	18 (42)	**.00003**
Coal, n (%)	2 (4)	0 (0)	-
Parents			
Mother alive, n (%)	47 (96)	43 (100)	-
Father alive, n (%)	27 (55)	43 (100)	-

*p < 0.05 significant; **Not applicable, [#]based on Kuppuswamy's Socioeconomic Status Scale 2012

	Children with HIV (CLH)	HIV uninfected children (HUC)	P
Mother HIV infected, n (%)	46 (98)	NA**	-
Father HIV infected, n (%)	22 (45)	NA	-
Mothers' age years, median (IQR25, IQR75)	30 (27, 33)	24 (22, 27)	**< 0.001***
Mothers' schooling years, median (IQR25, IQR75)	7 (3, 9)	9 (7, 10)	**0.0004***
Parent on ART during study, n (%)	26 (53)	NA	-

*p < 0.05 significant; **Not applicable, #based on Kuppuswamy's Socioeconomic Status Scale 2012

Table 2
Clinical history of children living with and without HIV

	Children with HIV (CLH)	HIV uninfected children (HUC)
N	49	43
Immune category, n (%)		
Normal	12 (24)	-
Moderate	22 (45)	-
Severe	15 (30)	-
Median CD4 count (IQR)	650 (392, 912)	-
On ART, n (%)	18 (36)	-
On TMP/SMX, n (%)	25 (51)	-
Median ART Duration (IQR), month	3 (2, 5)	-
HIV plasma load, median IQR	2.57E4 (3.54E3, 1.36E5)	-
Tuberculosis co-morbidity, n (%)	5 (10)	0
Ear infection history, n (%)	6/26 (23)	1/23(4)
Past week sickness history, n (%)	14 (28)	10 (23)
Fever, n (%)	7 (14)	5 (11)
Cough, n (%)	7(14)	6 (14)
Rhinorrhea, n (%)	2 (4)	5 (11)
Diarrhea, n (%)	1 (2)	0
Antibiotic intake during past week, n (%)	7 (14)	4 (9.3)

Table 3
Nasopharyngeal bacteria and viruses identified in children and their parents

	Children	Parents	P value
	No. (%)	No. (%)	
Bacterial detection			
N	83	64	
No. of individuals with no bacteria*	26 (31)	26 (40.6)	0.24
No. of individuals with bacteria**	56 (67)	35 (54.6)	0.11
No. of individuals with bacteria only***	34 (41)	27 (42)	0.88
Total *S. pneumoniae*[$]	31 (37)	13 (20)	**0.02**
Total *S. aureus*	44 (53)	34 (53)	0.98
Total *C. pneumoniae*	2 (2.4)	0	-
> 1 bacteria in one swab[#]	24 (29)	11 (17)	0.098
S. pneumoniae + S. aureus	20 (24)	11 (17)	0.30
S. pneumoniae + C. pneumoniae	1 (1)	0	-
Virus detection			
N	92	77	
Number of individuals with virus[##]	41 (44.5)	23 (30)	**0.049**
Single virus only[!]	3 (3)	9 (11.7)	0.03
Human rhinovirus	15 (16)	4 (5)	**0.02**
Human adenovirus	9 (10)	6 (8)	0.65
Human bocavirus	6 (6)	0	-
Influenza B virus	4 (4)	0	-
Respiratory Syncytial Virus	2 (2)	5 (6.5)	0.16
Human coronavirus	2 (2)	1 (1)	0.66

*Individuals not having any bacteria; **Number of individuals with bacteria detection singly or with other bacteria with or without virus; ***Number of individuals with bacteria detection singly or with other bacteria without virus; [$]Total bacteria detected individually or in combination with other bacteria or viruses in individuals; [#]Mixed bacteria detected with other bacteria; [##]Total virus detected individually or in combination with other viruses or bacteria; [!]One virus detected in an individual without bacteria; !Total viruses detected with bacteria

	Children	Parents	P value
Human metapneumovirus	2 (2)	0	-
Human parainfluenza virus	1 (1)	2 (2.6)	0.45
Human enterovirus	0	3 (4)	-
Viruses + bacteria co-detection			
N	83	64	
Total viruses + bacteria[!]	22 (26.5)	9 (4)	0.06
Single virus + bacteria	12 (14.4)	8 (12.5)	0.73
Mixed virus + bacteria	10 (12)	1 (1.5)	**0.01**

*Individuals not having any bacteria; **Number of individuals with bacteria detection singly or with other bacteria with or without virus; ***Number of individuals with bacteria detection singly or with other bacteria without virus; [$]Total bacteria detected individually or in combination with other bacteria or viruses in individuals; [#]Mixed bacteria detected with other bacteria; [##]Total virus detected individually or in combination with other viruses or bacteria; [!]One virus detected in an individual without bacteria; !Total viruses detected with bacteria

Table 4
Nasopharyngeal bacteria and viruses identified in HIV infected and HIV uninfected children

	HIV infected children	HIV uninfected children	P
	n, (%)	n, (%)	
Bacteria			
n	43	40	
No. of individuals with bacteria only*	18 (42)	16 (40)	0.86
No. of individuals with bacteria**	30 (70)	26 (65)	0.64
Total *S. pneumoniae*[***]	16 (37)	15 (37)	0.98
Total *S. aureus*[***]	27 (63)	17 (42)	0.06
Total *C. pneumoniae*[***]	1 (2)	1 (2)	0.96
> 1 bacteria in one swab	14 (32)	7 (17)	0.11
S. pneumoniae + S. aureus	13 (30)	7 (17)	0.17
S. pneumoniae + C. pneumoniae	1 (2)	0	-
Viruses			
N	49	43	
No. of individuals with virus[#]	20 (41)	21 (49)	0.43
Human rhinovirus	7 (14)	8 (18)	0.57
Human adenovirus	7(14)	2 (4)	0.12
Human bocavirus	2 (4)	4 (9)	0.31
Respiratory Syncytial Virus	1 (2)	1 (2)	0.92
Human coronavirus	1 (2)	1 (2)	0.92
Human metapneumovirus	1 (2)	1 (2)	0.92
Human parainfluenzavirus	1 (2)	-	-
Influenza B virus	-	4 (9)	-

*Number of individuals with bacteria detection singly or with other bacteria without virus; **Number of individuals with bacteria detection singly or with other bacteria with or without virus; [***]Total bacteria detected individually or in combination with other bacteria in individuals; [#]Number of individuals with viruses detected individually or in combination with other viruses or bacteria; [!]Total viruses detected with bacteria.

	HIV infected children	HIV uninfected children	P
Human enterovirus	-	-	-
Viruses + Bacteria			
N	43	40	
Total viruses + bacteria[!]	12 (28)	10 (25)	0.76

*Number of individuals with bacteria detection singly or with other bacteria without virus; **Number of individuals with bacteria detection singly or with other bacteria with or without virus; ***Total bacteria detected individually or in combination with other bacteria in individuals; [#]Number of individuals with viruses detected individually or in combination with other viruses or bacteria; [!]Total viruses detected with bacteria.

Table 5

Association between isolation of *Streptococcus pneumoniae* with respiratory viruses, and, between the density (Log copies/ml) of *Streptococcus pneumoniae* with viruses in the nasopharynx of children

	Pneumococcal colonization				Pneumococcal density			
	n[#]	OR*	95% CI**	P	n	Coeff[$]	95% CI	P
Overall children								
Virus presence	83	3.93	1.47–10.5	0.006	31	3.603	.44 – 6.7	0.027
1 virus	83	3.85	1.15–12.8	0.028	31	0.533	-3.2-4.3	0.77
> 1 virus	83	2.25	0.62–8.1	0.21	31	5.06	1.1-9	0.01
Rhinovirus	83	3.13	0.99–9.9	0.052	31	4.52	1.1–7.9	0.01
Adenovirus	83	6	1.12-32	0.036	31	1.62	-2.7-6	0.45
HIV infected children								
Rhinovirus	43	15.6	1.66–146	0.016	16	4.22	-0.2-8.6	0.06
Rhinovirus + *S. aureus*	43	8.7	1.42-54	0.019				
Adenovirus	43	4.16	0.66-26	0.12	16	-0.46	-6.1, 5.1	0.86
HIV uninfected children								
Rhinovirus	40	1	.20 – 4.9	1	15	2.7	-1.9-7.3	0.23
Adenovirus	40	NA***	NA	NA	15	2.77	-2.7, 8.3	0.3

[#]n, number of observations; *OR, odds ratio; **CI, confidence interval; [$]*Regression coefficient;* ***NA, not available.