Pathogens Causing Respiratory Tract Infections in Children Less Than 5 Years of Age in Senegal

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ABSTRACT

INTRODUCTION: While acute respiratory tract infections are the main cause of paediatric mortality and morbidity worldwide, pathogen patterns shift due to factors such as hygiene, vaccinations, and antibiotic resistance. Knowledge about current cause of respiratory infections is lacking, particularly in low- and middle-income countries. The aim of this study was to identity the various respiratory pathogens causing acute respiratory tract infections in children below 5 years of age visiting a sub-urban primary care clinic in Senegal.

METHODS: A case-control study was performed in September and October 2018. Oropharyngeal swabs were collected from cases; infants with fever and respiratory symptoms, and controls; children involved in the vaccination programme. Viral identification was conducted by polymerase chain reaction for 21 different viruses; bacteria were identified by culture studies. Associations between microorganisms, acute respiratory infection and severity of disease were calculated by multivariate regression adjusting for confounders such as age, sex, and living area.

RESULTS: Overall, 102 cases and 96 controls were included. Microorganisms were detected in 90.1% of cases and 53.7% of controls ($P < .001$). Influenza virus A (including H1N1), influenza virus B, respiratory syncytial virus (RSV), and *Streptococcus pneumoniae* were independently associated with acute respiratory tract infections. Co-detection of two or more pathogens was present in 49.5% of cases; 31.7% of cases had a pneumonia and 90.2% was treated with antibiotics.

CONCLUSIONS: This case-control study in a primary care setting in sub-Saharan Africa found influenza virus A and B, RSV, and *S pneumoniae* to be the main causes of acute respiratory tract infections in children below 5 years of age. We recommend evaluation of antibiotics prescription behaviour in this setting.

KEYWORDS: Acute respiratory tract infection, child, cause, case-control study, low- and middle-income country

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Introduction

Worldwide, the leading cause of paediatric mortality and morbidity in the postneonatal period remains lower respiratory tract infections (LRTIs). The WHO (World Health Organization) estimated that more than 800 000 of the 5.6 million total deaths in children less than 5 years old were caused by LRTIs in 2016.[1] A meta-analysis found a hospital-based case-fatality ratio of 6.1% in children in low-income countries with severe LRTI, compared with 3.9% in high-income countries.[2]

Children with LRTI can present with a highly variable package of symptoms including cough, tachypnoea, dyspnoea, fever, rhinitis, chest pain, abdominal pain, vomiting, diarrhoea, headache and lethargy. The WHO guidelines states tachypnoea as the main diagnostic criterion for pneumonia requiring an antibiotic.[3] However, distinction from upper airway infections and other illnesses such as asthma/bronchiolitis, anaemia or malaria can be challenging when minimal diagnostic tools are available.[4] Furthermore, in many low- and middle-income countries, treatment policies are empiric or based on foreign guidelines, due to a lack of local causal studies.[5] These could be reasons why research has discovered an overdiagnosis and overprescription of antibiotics to treat pneumonia in low-income countries.[6]

One of the biggest public health problems of this century is antimicrobial resistance. It threatens the effective treatment of major infections and is induced by an overuse and inappropriate usage of antibiotics.[7] This phenomena has major consequences: higher health-related costs because of the necessity of more expensive antibiotics, rising mortality rates due to

untreatable infections and an increasing danger for the immunosuppressed patient.[7,8]

Moreover, viral causes of LRTIs have become seemingly more important.[9] Ancient studies in mostly Western countries frequently described *Haemophilus influenza B (Hib), Streptococcus pneumoniae*, and *Moraxella catarrhalis* as major causative pathogens of LRTIs.[10,11] Nonetheless, while in 2000, *S pneumoniae* was responsible for 600 000 deaths in children, in 2015, this number dropped to 294 000 children.[12] Recent developments, such as the implementation of the pneumococcal conjugate vaccine and the Hib vaccine, are responsible for a reduction in the burden of these respiratory bacteria but may also have induced a shift in the pathogens causing LRTIs.[9,13]

Recent research in Senegal indicated viruses as main pathogens of all acute respiratory tract infections (ARTIs), both upper and lower. The frequency of virus detection was even higher than those found in several other countries.[14,15] Viruses most commonly found in Senegal were respiratory syncytial virus (RSV), adenovirus, rhinovirus, influenza virus A, enterovirus, human bocavirus, and parainfluenza viruses.[16] Furthermore, there are indications that nowadays ARTIs are more often caused by the sequential or concurrent interaction of different pathogens and that the presence of multiple organisms is linked with the existence of a LRTI and with severity of disease.[17,18]

To reduce paediatric mortality caused by LRTI, while fighting antibiotic resistance, more knowledge about the cause of respiratory infections, especially in low- and middle-income countries, is essential. Case-control studies have been conducted in Africa, investigating the association between different pathogens and pneumonia. These studies found strongest associations for *S pneumoniae, Bordetella pertussis*, RSV, influenza virus A, and human metapneumovirus.[19-24] However, these studies were conducted in hospitalized settings while most children with LRTI are treated in primary care clinics.

To the best of our knowledge, no case-control studies investigating ARTIs in primary care have been conducted in sub-Saharan Africa. Therefore, the aim of this case-control study was to investigate the prevalence of the different viruses and bacteria colonizing the airways and their association with the occurrence of ARTIs and severity of disease in children less than 5 years of age, visiting a sub-urban primary care clinic in Dakar, Senegal.

Methods

Study design and data collection

An unmatched case-control study was performed at the Institute de Pédiatrie Sociale (IPS), a primary care clinic in Dakar, Senegal, during September and October 2018. Institute de Pédiatrie Sociale is a free government health care clinic for children and pregnant women based in Pikine, a sub-urban area. Children with respiratory infections were enrolled using convenience sampling at the paediatric outpatient ward. Controls were enrolled during the vaccination programme, which took place once a week. Exclusion criteria for controls were fever and respiratory symptoms. Standardized forms were used to prospectively gather information concerning patient demographics, medical history, clinical signs, and symptoms at enrolment and treatment. Oropharyngeal swabs and bloods samples were taken in cases. One week after enrolment, symptoms and signs were evaluated by phone call in all cases. Every case was called a minimum of 3 times when parents did not respond. When a child had to be admitted to a hospital, daily follow-up of signs and symptoms was conducted. Data were imported in the data management software, Excel. Databases were anonymized.

Study population

This study has been approved by the Ethics Committee for Research of the Cheikh Anta Diop University of Dakar. Data were gathered after an informed consent statement had been signed by parents or a legal guardian. Children aged less than 5 years old with an acute respiratory infection defined as (antecedents of) fever and at least one respiratory symptom were found suitable for inclusion. Cases were excluded if the symptoms began >10 days before enrolment and/or if antibiotics were taken within 48 hours before enrolment. Exclusion criteria for controls were signs of fever or respiratory symptoms. Fever was defined as a rectal or ear temperature of ⩾38.0°C or an axillary temperature of ⩾37.5°C. Antecedents of fever had to occur within 24 hours before enrolment. Respiratory symptoms included cough, thoracic pain, sore throat, dyspnoea, chest indrawings, tachypnoea, cyanosis, and rhinorrhoea. Tachypnoea was defined using the WHO definition of a respiratory rate >60 breaths/min in children <2 months of age, >50 breaths/min in children 2 to 12 months of age, and >40 breaths/minute in children ⩾1 year of age.[3] Tachycardia was defined as >160 beats per minute (BPM) for infants <2 years of age and >140 BPM for children 2 to 12 years of age.[25] Elevated C-reactive protein (CRP) suggestive for severity of disease was defined as ⩾50 mg/L.[26,27] Leucocytosis was present if leucocytes are increased to more than 11 000/mm^3. Pneumonia was defined using the WHO guidelines as tachypnoea with cough and/or dyspnoea.[3] Alarming signs were defined as tachypnoea, chest indrawing, or hypoxemia.

Sampling and microbiological analyses

In all cases and controls, oropharyngeal samples were collected. All samples were analysed by the biotechnology unit of the laboratory of bacteriology of University Hospital Aristide Le Dantec and by the virology unit of Pasteur Institute. The samples were stocked and transported following the protocols. For bacterial samples, bacteria were isolated from appropriate culture media. Morphological, cultural, and biochemical or antigenic characters were studied. Strains were determined and

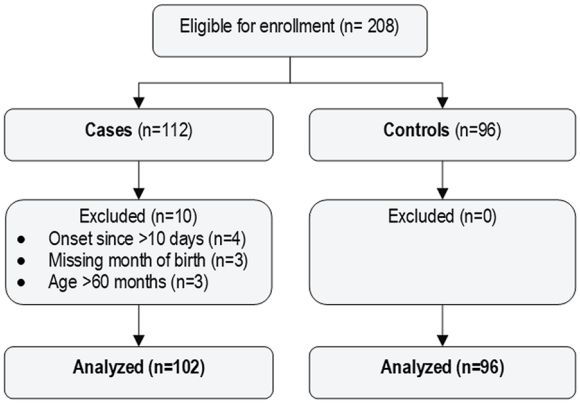

Figure 1. Inclusion and exclusion of cases and controls.

considered for infection if the bacterial load was at least 10^5 CFUs/mL. All samples were tested using polymerase chain reaction (PCR) with a Bio-Ras CFX-96 thermo cycler and the FTD Respiratory pathogens 33 (Fast-track diagnostics), capable of detecting 21 viruses and 12 bacteria (Supplement 1). In addition, blood samples were collected in cases and CRP and leucocytes were determined by the laboratory of IPS. C-reactive protein was determined using the CRP Latex Kit 850A (Lorne Laboratories, Berkshire, UK).

Statistical analysis

Based on G*Power 3.1.9.2 version, a sample size of 182 was yielded, when using logistic regression, an alpha of .05, and a small-to-medium effect size with an odds ratio (OR) of 1.60.[28] Therefore, a sample size of 198 is sufficient to detect microorganisms as a predictor of acute respiratory infection. Baseline characteristics were compared between cases and controls. Continuous variables were expressed as medians and ranges. They were compared using the Mann-Whitney *U* test. Categorical variables were expressed as frequencies and proportions and were compared using the chi-square test. To identify potential predictive factors, a univariable logistic regression was used. Thereafter, established predictors of the outcomes (occurrence of respiratory infection and severity of disease) ($P < .1$) were included in multivariable logistic regression. Data were presented as ORs and 95% confidence intervals (CI). $P < .05$ was considered significant. Epi Info was used for analysis of the data.

Results

Study population

Of 208 eligible children, 102 cases and 96 controls were analysed (Figure 1). Ten cases were excluded from analysis, due to an onset of disease of more than 10 days before enrolment, missing month of birth, or an age of more than 60 months. Approximately 5% of all assessed controls declined to participate in the study, mainly based on fear of discomfort for their child or possible disapproval of the other parent. None of the cases declined. Overall, 96 (48.5%) children were male, and the mean age was 18.0 (±12.4) months. Overall vaccination coverage was 97.7%. Table 1 shows a summary of baseline characteristics of the included cases and controls at enrolment. A significant difference of means was found in mean age among case and control group, respectively, 23.5 (±13.2) and 12.2 (±8.0) with a *P* value of <.001. Furthermore, cases more often lived in other areas in and outside of Dakar, whereas more controls than cases lived in the neighbourhood near the Pikine clinic, respectively, 36 (35.3%) and 63 (65.6%) with a *P* value of <.001.

Description of cases

Among all cases, 31.7% had a pneumonia based on WHO criteria. The main respiratory symptoms were rhinitis and cough, in 90 (88.2%) and 89 (87.3%) of cases, respectively. In all, 19.8% had a tachycardia and 32.7% a tachypnoea. None of the children had a hypoxemia during consultation. On physical examination, 52 (51.0%) cases had abnormal lung sounds of which rhonchi were the main type. An elevated CRP was seen in 72.8% of cases with a mean CRP of 47.2 (±41). In all, 46.2% had a leucocytosis with a mean number of leucocytes of 11 527.8 (±5654.5). Malaria rapid diagnostic tests were performed on only 32 cases, but all were negative. Data on treatment were available in 92 cases. Antibiotics were prescribed in 90.2% of those. Data on follow-up were only available in 51 cases. In 78.1% of those, symptoms had passed within 1 week. None of the children had to be hospitalized. Table 2 shows a summary of clinical signs and symptoms and management of cases.

Microbiological analysis

For detection of bacteria, only culture was used, because when using PCR, equal amounts of bacteria were found in cases and controls. The reason for this is that a better differentiation between bacterial colonization and infection can be made using cultures.[29] At least 1 pathogen was detected in 90.1% of cases and 53.7% of controls. Bacteria were detected in 62.8% of cases and 25% of controls, of which *S pneumoniae* (73.6%) and *H influenzae B* (24.6%) were the most prevalent. Viruses were detected in 75.3% of cases and 42.1% of controls and influenza virus A (28.9%), RSV (17.8%), and influenza virus B (10.4%) were most common. In 49.5% of cases, 2 or more pathogens were detected.

Univariate analysis shows that *S pneumoniae*, RSV, influenza virus A (including H1N1) and B were significantly associated with the occurrence of ARTI (Table 3). Multivariate analysis confirmed these correlations. The following pathogens were related to illness, in order of strength of association: Influenza virus B (adjusted odds ratio [aOR]: 7.2, CI: 1.2-42.9), influenza virus A (aOR: 5.8, CI: 1.9-17.5), RSV (aOR: 5.2, CI: 1.6-16.5) and *S pneumoniae* (aOR:

Table 1. Baseline characteristics.

CHARACTERISTIC	CASES (N = 102)	CONTROLS (N = 96)	*P* VALUE
Categorical variables, No. (%)			
Sex, male	50 (49.0)	46 (47.2)	.887
Comorbidities	8 (7.8)	4 (4.1)	.375
Difficulty at birth	10 (9.8)	5 (5.2)	.285
Vaccinations up-to-date (including PCV and Hib vaccine)	96 (94.1)	96 (99.0)	.119
Household size >10	41 (40.2)	32 (33.3)	.377
Travelling last 2 wk within Senegal	20 (19.6)	16 (16.5)	.586
Travelling last 2 wk outside Senegal	5 (5.2)	1 (1.0)	.111
Antibiotic use last 2 wk	12 (12.4)	14 (13.7)	.835
Breastfeeding until now or >1.5 y	80 (82.5)	91 (89,22)	.221
Living area Pikine	36 (35.3)	63 (65.6)	<.001
Continuous variables, mean (±SD)			
Age	23.5 (±13.2)	12.2 (±8.0)	<.001

Abbreviations: PCV, pneumococcal conjugate vaccine.

Table 2. Clinical signs and management.

VARIABLES	CASES (N = 102)
Categorical variables, No. (%)	
Pneumonia WHO	32 (31.7)
Tachycardia[a]	20 (19.8)
Tachypnoea[a]	33 (32.7)
Symptoms	
•Rhinitis	90 (88.2)
•Cough	89 (87.3)
•Thoracic pain	40 (39.2)
•Throat pain	15 (14.7)
•Chest indrawings	6 (5.9)
•Otitis	2 (2.0)
•Vomiting	31 (30.4)
•Diarrhoea	21 (20.6)
Abnormal lung sounds	52 (51.0)
•Rhonchi	41 (40.2)
•Crepitations	6 (5.9)
•Wheezing	8 (7.9)
•Difference right/left	5 (4.9)
Temperature ⩾39°C	31 (31.0)
Hypoxemia[b]	0 (0)
CRP >50 mg/L[a]	39 (38.6)
Leucocytosis	48 (46.2)
Antibiotics[c]	83 (90.2)
•Amoxicillin	23 (25.0)
•Augmentin	8 (8.7)
•Cephalosporin	35 (38.0)
•Macrolide	5 (5.4)
•Fluoroquinolone	1 (1.1)
Bronchodilators	28 (30.8)
Antihistamines	66 (72.5)
Antipyretics	59 (64.1)
Steroids	6 (6.6)
Symptom free after 1 week[d]	32 (78.1)
Continuous variables, mean (±SD)	
CRP	47.2 (±41)
Leucocytes	11 527.8 (±5654.5)

Abbreviations: CRP, C-reactive protein; WHO, World Health Organization.
[a]Missing data in 1 case.
[b]Missing data in 17 cases.
[c]Missing data in 10 cases.
[d]Missing data in 51 cases.

Table 3. Microbiological findings for respiratory samples of cases and controls with OR for illness.

MICROORGANISM	NO. (%)		OR (95% CI)	
	CASES (N = 102)	CONTROLS (N = 96)	UNADJUSTED	ADJUSTED[A]
⩾1 pathogen	91 (90.1)	51 (53.7)	7.9 (3.6-16.9)	8.5 (3.3-22.0)
Bacteria (⩾1)	64 (62.8)	24 (25.0)	5.1 (2.7-9.3)	5.3 (2.3-12.3)
Streptococcus pneumoniae	49 (48.0)	18 (18.8)	3.0 (2.1-7.6)	3.0 (1.2-7.4)
Acinetobacter spp.	4 (3.9)	0 (0)	—	
Haemophilus influenzae B	10 (9.8)	5 (5.2)	2.0 (0.7-6.0)	
Klebsiella pneumoniae	1 (1.0)	0 (0)	—	
Streptococcus beta-hemolytic	4 (3.9)	0 (0)	—	
Mycoplasma pneumoniae[b]	4 (4.0)	4 (4.2)	0.9 (0.2-3.9)	
Viruses (⩾1)[c]	74 (73.3)	37 (39.0)	4.3 (2.3-7.9)	4.3 (2.0-9.1)
Coronavirus NL63	1 (1.0)	3 (3.2)	0.3 (0.0-3.0)	
Adenovirus	8 (7.9)	5 (5.3)	1.5 (0.5-4.9)	
Enterovirus	6 (5.9)	8 (8.4)	0.7 (0.2-2.1)	
Parechovirus	0 (0)	3 (3.2)	—	
Rhinovirus	5 (5.0)	7 (7.4)	0.7 (0.2-2.1)	
RSV	17 (16.8)	7 (7.4)	2.5 (1.0-5.4)	5.2 (1.6-16.5)
PIV 2	1 (1.0)	0 (0)	—	
Influenza A virus	32 (31.7)	7 (7.4)	5.8 (2.4-14.0)	5.8 (1.9-17.5)
Influenza A virus (H1N1)	30 (29.7)	8 (8.4)	4.6 (2.0-10.6)	4.6 (1.6-)
Influenza B virus	12 (11.9)	2 (2.1)	6.3 (1.4-28.8)	7.2 (1.2-42.9)
Human bocavirus	1 (1.0)	2 (2.1)	0.5 (0.0-5.2)	
Co-detection	50 (49.5)	20 (21.1)	3.7 (2.0-6.9)	

Abbreviations: CI, confidence interval; PIV, parainfluenza virus; OR, odds ratio; RSV, respiratory syncytial virus.
[a]Adjusted for age, sex, living area, significant pathogens (*S pneumoniae*, influenza A virus, influenza B virus, RSV), and co-detection. Only significant adjusted ORs are displayed in the adjusted column.
[b]Bacteria detected by polymerase chain reaction.
[c]Missing data on virus detection in 2 cases.

3.0, CI: 1.2-7.4). Respiratory syncytial virus was only significantly related to illness in children less than 2 years of age. Likewise, the prevalence was higher in this age group. *Legionella pneumophila, B pertussis, Chlamydia pneumoniae*, Coronavirus 229, Coronavirus OC43, Coronavirus HKUI, PIV (parainfluenza virus) 1, PIV 3, PIV 4, human metapneumovirus, and influenza virus C were investigated, but not detected in cases or controls. The prevalence of pathogens in cases and controls is illustrated in Figure 2.

Table 4 shows the prevalence of different co-detections. *Streptococcus pneumoniae* was found in most of the co-detections (76.0%). It was most frequently found in combination with influenza virus A, in 17 cases. Associations between all possible specific co-detections and illness were analysed, but no additional relationship was found, other than a cumulative association of the present pathogens.

Analysis of cases. Treatment with antibiotics was not significantly related to alarm signs: tachypnoea (OR: 3.9, CI: 0.5-33.0), withdrawals (OR: 0.2. CI: 0.0-1.1), and hypoxemia (OR: undefined). None of the pathogens was significantly associated with those alarm signs or an elevated CRP or leucocytosis. No difference in elevated CRP or leucocytosis was found between bacteria and viruses.

Discussion

This case-control study investigates the prevalence of the different viruses and bacteria colonizing the airways and their

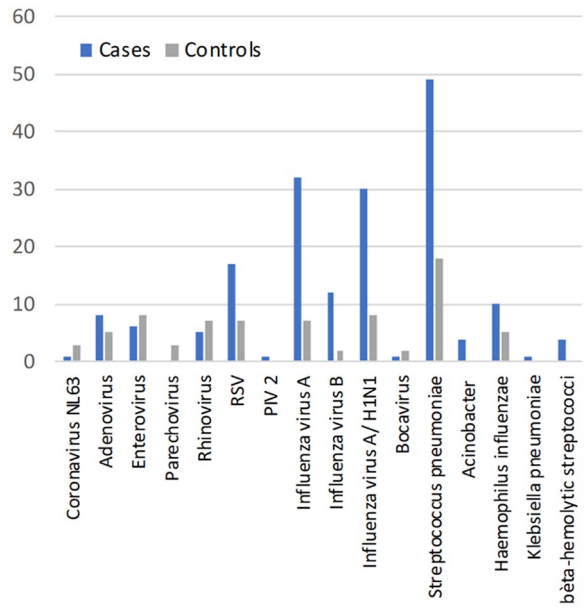

Figure 2. Detected pathogens in the oropharynx of cases and controls. PIV indicates parainfluenza virus; RSV, respiratory syncytial virus.

Table 4. Involvement in co-detections of pathogens in cases.

PATHOGENS	NO. OF TIMES INVOLVED IN CO-DETECTIONS
Streptococcus pneumoniae	38
Influenza A	20
RSV	11
Influenza B virus	9
Haemophilus influenzae	8
Enterovirus	6
Adenovirus	6
Mycoplasma pneumoniae	4
Streptococcus beta-hemolytic	3
Acinobacter spp.	3
Rhinovirus	2
Coronavirus NL63	1
Bocavirus	1
Klebsiella pneumoniae	1
PIV2	1

Abbreviations: PIV, parainfluenza virus; RSV, respiratory syncytial virus.

association with the occurrence of ARTIs in children visiting a sub-urban primary care clinic in Senegal.

Dieng et al studied pathogens in children with ARTI in Senegal and found a total of 78 bacteria in 162 children. They found a lower prevalence of *S pneumoniae* (18% of cases) in comparison with our study (48% of cases) and a prevalence of *M cattharalis* of 15% where we did not detect this pathogen. Dieng et al used different swab sampling techniques (bronchoalveolar lavage, sinus fluids, and throat swab) depending on the site of infection. *Moraxella cattharalis* causes otitis media and sinusitis rather than LRTI, possibly explaining the higher detection rate.[30] Furthermore, their study covered a full year, including all seasons. Although our study was conducted during 2 months, it covered the rainy season, which is known for its respiratory infections.[15]

Streptococcus pneumoniae was the most prevalent bacteria in our sample. Recent case-control studies in low-income countries found similar prevalence in cases and controls, even though those countries have lower vaccination coverages.[19,21,24,31] In Western countries with a similar vaccination coverage to Senegal, *S pneumoniae* rates in children with ARTI are evidently lower than we detected.[32] Possibly, disease burden of *S pneumoniae* is high in Senegal due to serotype replacement following the introduction of the pneumococcal vaccine, as has occurred in many other countries.[33] *Staphylococcus aureus* is a bacteria frequently detected in these studies.[19,21,24] They used nasal/nasopharyngeal swabs, but the literature does not mention major differences in detection of *S aureus* between nose or throat.[34] Another explanation is the use of PCR for detection of bacteria.

Numerous studies in sub-Saharan Africa have been conducted studying the prevalence of viruses in children with an ARTI. Equal total amounts of viruses and a similar prevalence of RSV and influenza viruses were detected when comparing with our results, but these studies, including some recent Senegalese studies, found significantly higher percentages of adenovirus, enterovirus, and rhinovirus.[14,15,35,36] Results from case-control studies conducted in low- and middle-income countries show a similar prevalence of all viruses in healthy children, except that the average prevalence of rhinovirus in those studies is approximately 30%, whereas we found rhinovirus in 7.4% of controls.[19,21-24] The most obvious possible reason for the differences in virus prevalence is our short study period, given that virus prevalence is highly seasonal.[37]

This study did not find an association between different pathogens and alarm signs (tachypnoea, tachycardia, hypoxemia, or an elevated CRP). Concordant to our findings, no association was found between alarming signs and specific pathogens in previous research.[38] Both viral and bacterial infections can cause either mild or severe infections in children. Other studies found an association between viral or bacterial aetiology and an elevated CRP too weak to rule out bacterial infection in clinical practice.[26,39,40] We did not find this association, presumably because of a small number of severely ill children.

Colonization by more than one pathogen is believed to influence the occurrence and severity of illness, by stimulating each other's adherence and decreasing clearance.[41] Co-detections were seen in 49.5% of cases and 17.7% of controls. Most of the co-detections in our study were caused by *S*

pneumoniae combined with influenza virus A. In a review by Brealey et al, the prevalence and clinical significance of co-detections in children with ARTI were studied. They found a similar prevalence to what our study shows. After adjusting for pathogens significantly related to illness, no relation was detected between the presence of a co-detection and illness in our study, which means that we did not detect a synergic effect in co-infection, possibly due to a small sample size.

In investigating the cause of ARTI in low-income countries, most studies are done in hospital setting and case-control methods are rarely used. Nevertheless, in these studies, similar pathogens were found with similar significant associations with illness compared with our study.[19-24,42,43] This could mean that the same pathogens are responsible for both upper and lower ARTIs. Furthermore, it confirms a generally shifting pattern towards viruses of pathogens causing ARTIs. Some pathogens were more prevalent in other settings, such as human metapneumovirus, PIV, and *Mycoplasma pneumoniae*.[19,22,24,42,43] Low prevalence of these pathogens was found in our study, possibly due to seasonality or their possible higher prevalence in severe infections.

Notable is the overprescription of antibiotics in our population. Approximately 90% of cases have been treated with antibiotics, whereas only 31.7% had a pneumonia and should have been treated as such following WHO criteria. Moreover, most of the antibiotic treatments concerned broad-spectrum antibiotics, whereas a narrow-spectrum penicillin is recommended in the primary care setting. Literature confirms that practices are similar in other low-income countries.[44-46]

The main strength of this study is the inclusion of all ARTIs within a primary care clinic. This gives insight into the pathogens causing the infections. Other strengths are the large number of pathogens tested for, with PCR and culture testing for bacteria, and the inclusion of treatment methods. An additional strength is that all data were gathered by one researcher, hereby decreasing the risk of variation in swabbing technique.

There are also limitations to be mentioned. We could not properly adjust for some factors such as social status due to few data on these subjects. Furthermore, selection bias could have occurred, because no matching was done. The average age of controls is lower than the average age of children with a respiratory infection, probably due to the young age of children enrolled in vaccination programmes. However, we did not discern significant heterogeneity regarding pathogens in different age groups (except for the mentioned differences in prevalence of RSV) or living areas. Another limitation is the use of only throat swabs. The nasopharyngeal swab is found to be superior in detecting influenza virus, *S pneumoniae*, and *Moraxella species*, but oropharyngeal swabs are superior for the detection of *M pneumoniae* and *H influenzae*.[47-50] It is preferable to use both techniques, but limited resources inhibited that. There was a lack of a gold standard such as chest X-ray for pneumonia. By taking a clinical definition of ARTI, there is possible overlap with other feverish diseases such as malaria and gastrointestinal illnesses. Other limitations are the short duration of the study and a lack of follow-up data of patients due to nonresponse to phone calls. We might have missed data on severity of illness and hospitalizations.

Conclusions

This case-control study conducted in Senegal investigates the cause of ARTIs in children less than 5 years of age in a primary care setting. In this population, illness was mainly caused by influenza virus A and B, RSV, and *S pneumoniae*, which confirms a generally shifting pattern towards viruses in pathogens causing ARTIs. We suggest continuation of this case-control study for a full year, and further research on serotypes and resistance patterns of bacteria present in children with ARTI. These findings could lead to improvement of vaccinations against respiratory pathogens. We recommend reduction of antibiotics prescription, especially broad-spectrum antibiotics, for children with an ARTI visiting a primary care clinic.

Author Contributions

RBK, ADG, AD and TDVI contributed to the design and implementation of the research. Samples were analyzed by AF, ADG, AmB, AbD and MN. RBK analyzed the data and wrote the manuscript with consultation from all authors. ADiallo supervised the project at the clinic. CSBB supervised the study.

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