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ORIGINAL ARTICLE

Virus and *Mycoplasma pneumoniae* prevalence in a selected pediatric population with acute asthma exacerbation

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Abstract

Objective: To determine the prevalence of viral and atypical bacteria *Mycoplasma pneumoniae* infection in children experiencing asthma exacerbation and compare positive and negative subjects with regard to exacerbation severity, need for hospitalization, and treatment. **Methods:** One hundred sixty-nine asthmatic children aged 2–15 years old who were admitted to emergency rooms in Bogotá, Colombia for acute asthma exacerbation were interviewed. Nasopharyngeal aspirates were taken for DNA and RNA extraction. *M. pneumoniae* and virus were detected by PCR using specific primers. **Results:** The prevalence of *M. pneumoniae* and viral infection in the study population was 12.4% and 83.7%, respectively. All subjects positive for *M. pneumoniae* were also positive for viral infection. Rhinovirus was the most frequently detected viral agent. No significant differences in severity of asthma exacerbations or in need for hospitalization between the virus or *M. pneumoniae* positive and negative groups were observed. A significantly lower percentage of *M. pneumoniae* positive subjects had used inhaled steroids over the six months prior to asthma exacerbation compared to *M. pneumoniae* negative subjects (38.1% vs. 68.2%), suggesting that inhaled corticosteroids may have a protective effect against *M. pneumoniae* infections. **Conclusions:** The *M. pneumoniae* and virus prevalence found in this study were similar to those described in the literature. The 100% co-infection rate observed suggests that viral infection can predispose patients to *M. pneumoniae* infection, and that this interaction may trigger asthmatic exacerbation. Further studies should be done to confirm the protective effect of inhaled corticosteroids on *M. pneumoniae* infection in patients with asthma exacerbations.

Keywords

Asthma exacerbations, atypical bacteria, inhaled corticosteroids, PCR, viral infection

History

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Introduction

Asthma is the most common chronic disease in children [1]. According to the global initiative for asthma (GINA) and the World Health Organization [2,3], asthma has become a global public health problem, with prevalence increasing over the past two decades. In Colombia, the prevalence of asthma among the general population has increased from 10.4 to 12% in ten years, reaching 18.9% in children under 4 years old [4–7]. Asthma has huge negative impact on quality of life and health care costs particularly derived from asthmatic exacerbations being [2,3,8,9] one of the most common causes of hospitalization among children [10].

Most acute asthma exacerbations in children and adults are caused by respiratory viruses [11]. Atypical bacteria such as *M. pneumoniae* and *Chlamydia pneumoniae* are also known to trigger asthma [12]. However, the role of these bacteria in

asthma attacks is still controversial. A wide range of prevalence for these atypical bacteria has been reported, from 5 to 46% [13–21]. Specifically *M. pneumoniae*, has been detected in ~8% of adults and 22.5% of children experiencing acute asthma exacerbations [15,22]. However, Maffey et al. [23] observed a lower prevalence of *M. pneumoniae* (4.5%) in pre-school age children with asthma exacerbations. This variability may reflect differences in study populations, detection techniques, or, in the case of *M. pneumoniae*, which occurs endemically with epidemics every 3–4 years, by seasonal changes in distribution [24,25].

Conventional culture and serological methods used for the detection of *M. pneumoniae* and viruses have limitations. They are time-consuming and lack sensitivity or specificity, respectively. An alternative molecular approach involving *in vitro* amplification of *M. pneumoniae* or viral specific nucleic acid targets by PCR may be a more accurate diagnostic method.

The aim of this study was to determine the prevalence of virus and *M. pneumoniae* infection, using molecular techniques, in a selected population of children between the ages of 2 and 15 years with asthma exacerbations, and to

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investigate potential differences between virus and *M. pneumoniae* positive and negative subjects with regard to exacerbation severity, need for hospitalization, length of hospital stay and treatment. In addition, we sought to establish the seasonality of *M. pneumoniae* and viral agents during the sample collection period.

Methods

Study design

A cross-sectional study was undertaken to determine the prevalence of viral and *M. pneumoniae* infection in children with acute exacerbation between December 2010 and March 2012. The study was approved by the Research Ethics Committees of the participant institutions, which complied with the current rules of Resolution No. 8430 of 1993 and was classified as minimal risk.

The study sample was made up of 169 children aged 2–15 who had been diagnosed with asthma at least 6 months prior to being admitted to the Emergency Room at the following third-level hospitals: Fundación CardioInfantil Instituto de Cardiología (FCI) and Fundación Neumológica Colombiana (FNC) in Bogotá, Colombia. The legal representatives of the children included in the study signed an informed consent and the child consented as appropriate.

The presence of more than one of the following symptoms was established as evidence of a high probability of asthma: wheezing, coughing and breathing difficulty, particularly if the symptoms were frequent or recurrent (minimum 2 episodes for this study), and if they occurred independently of colds and in response to exercise [26]. We used GINA guidelines for classifying the asthma severity level. An asthma exacerbation was defined as any episode characterized by progressive increasing difficulty in breathing, a sensation of not being able to get enough air in the lungs, wheezing, coughing, thoracic pain or oppression, or a combination of these symptoms [4,9,27]. The degree of severity of the exacerbation was classified according to the pulmonary index [28–30]. Children who showed symptoms of bronchospasm with diagnosis different from asthma, including bronchiolitis, cystic fibrosis and first wheezing episode in 2-year-old children, were excluded. Community acquired pneumonia was defined as the presence of cough and dyspnea less than 15 days plus alveolar infiltrates in chest radiography acquired out of hospital context. All patients were examined by a pediatrician or pulmonologist who confirmed the asthma diagnosis and established the degree of severity of the crisis.

Sample collection and nucleic acid extraction

Nasopharyngeal aspirates were collected from each patient, stored at 4°C and then transported on ice to the laboratory where they were processed. All samples were brought to a volume of 1.2 mL using PBS pH 7.4 and were then centrifuged at 1900 rpm for 10 min at 4°C. Extraction of DNA and RNA was performed using the Wizard DNA Clean-Up System kit (Promega, Madison, WI) and QIAampViral RNA Mini Kit (QIAGEN, Hilden, Germany) respectively. DNA and RNA were stored at –20°C until further processing and remaining aspirate samples were stored at –80°C.

Detection of viral agents

Viral agents were detected using reverse transcription and polymerase chain reaction (RT-PCR) from viral RNA or conventional PCR from viral DNA, as appropriate. Protocols used for detection of Influenza A, B and Parainfluenza 1, 2 and 3 viruses were based on those described by Bellau-Pujol et al. [29] and modified by Rojas et al. as follow. The reaction mixture contained 2.5 µL of 10× PCR Rxn Buffer, 2 µL de 25 mM MgCl₂, 1 µL of 10 nM dNTPs, 0.5 µL of each primer 10 nM, 0.2 µL of Taq polymerase 1U and 2 µL of cDNA, in a final volume of 25 µL. For detection of Adenovirus and Picornaviruses specific standardized PCR or RT-PCR primers and protocols described by Kidd et al. 1996 and Kumar et al. 2005, respectively were used [30,31]. The RT-PCR reaction products of samples positive for Picornavirus were sequenced to distinguish between Rhinovirus and Enterovirus and determine the subtypes. RSV was detected using PrimerDesign™ genesig kit for the quantitation of respiratory syncytial virus (all species) (PrimerDesign™ Ltd., UK) following the manufacturer's instructions. All samples that showed the presence of a viral-specific PCR fragment were considered positive. Reactions were validated using viral nucleic acid purchased from ATCC and/or known sequenced samples as positive controls and molecular biology grade water as blank reaction. Samples that did not produce a PCR product but amplifies a specific human β-globin fragment of 110 bp were considered negative. Inconclusive reactions were repeated; products were sequenced if necessary. All negative samples were tested in order to rule out the presence of PCR inhibitor using specific primers for the human β-globin.

Detection of *M. pneumoniae*

Conventional PCR was used to detect the presence of *M. pneumoniae* in samples using primers specific for the *M. pneumoniae* P1 gene. Initial screening was done with primers P1-178 and P1-331, which generate an amplicon of 153 bp. The primers and basic protocol used for the PCR was taken from Qasem et al. (2002) [31]. An ATCC 15531 *M. pneumoniae* strain was used as a positive control for *M. pneumoniae* P1 PCR. Samples that tested negative for *M. pneumoniae* by this PCR analysis were subjected to PCR using primers specific for the human β-globin in order to rule out the presence of PCR inhibiting agents in those samples [32]. The samples that showed the presence of the expected 153 bp P1 fragment were considered to be positive for *M. pneumoniae*. Molecular typing of *M. pneumoniae* was carried out using semi-nested PCR amplification and sequencing of the RepMp2/3 repetitive region of the P1 gene, as described by Dumke et al.

Data management and statistical analysis

Statistical software (Chicago, IL) SPSS version 15 was used for data management. For continuous variables, the assumption of normality was evaluated using the Kolmogorov–Smirnov test. If normality was confirmed, standard averages and deviations were used; otherwise, medians and interquartile ranges were used. Proportions were calculated for qualitative variables.

The size of the sample was calculated using the prevalence of asthma in children in Bogotá (12%), the percentage of asthmatic patients who had acute asthma exacerbation (35%) and the expected prevalence of *M. pneumoniae*, which we took to be 20% [27,17]. With 6% precision, a 5% type I error and a two-tailed hypothesis, the sample size was calculated to be 171 subjects. Consecutive cases of children with asthma exacerbations from the two study institutions were considered.

To calculate prevalence, we determined the percentage of the total number of subjects that tested positive for virus or *M. pneumoniae* by PCR. Prevalence is reported as a ratio with 95% CI. The groups with and without virus, and with and without *M. pneumoniae* were compared. Chi-square statistic and Fisher's exact test were used in the case of qualitative variables when the expected frequencies were less than five. For continuous variables, the comparisons were done with Student's *t*-test for independent samples and the Mann-Whitney *U*-test otherwise. All values of *p* were two-tailed and values under 0.05 were considered statistically significant.

Results

Study population

One hundred and seventy-five male and female children between the ages of 2 and 15 years with acute asthma exacerbation were enrolled. Seventy-eight patients (46.1%) had moderate asthma and were receiving low and medium doses of inhaled corticosteroids (ICS). Thirty-one patients (18.9) had severe asthma and were receiving treatment with combined therapy (intermediate dose of ICS+LABA or leukotriene antagonist). Sixty patients (35%) were not receiving treatment and we can't reliably classify their severity. Samples from six of these subjects failed to give PCR results with *M. pneumoniae* P1 and β -globin primers used as a positive control; these six subjects were excluded. Thus, 169 children were included in the overall analysis. Over half of the subjects had been treated with steroids or inhaled short acting β 2 agonist bronchodilators.

M.pneumoniae and virus prevalence

Nasopharyngeal aspirates were collected from each study subject. DNA and RNA were extracted from the aspirates and subjected to PCR or RT-PCR with primers specific for *M. pneumoniae* and each of eight viruses: Adenovirus, RSV, Influenza A, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Picornavirus. Picornavirus-specific PCR products were sequenced to differentiate between Rhinovirus and Enterovirus and to determine subtype. As shown in Table 1, the *M. pneumoniae*-specific PCR product was detected in 21 of the 169 samples, corresponding to a prevalence of 12.4%. Of the 66 subjects aged 5 and under, 12 tested positive for *M. pneumoniae* by this assay, corresponding to a prevalence of 18.2% (IC 95% 8.7–27.6). Of the 103 subjects over the age of five, 9 tested positive, corresponding to a prevalence of 8.7% (IC 95% 3.2–14.3).

Viral-specific PCR products were detected in 141 of the 169 samples, corresponding to a prevalence of 83.4%. All the *M. pneumoniae* positive samples were also positive for at least one virus. Of the specific viruses tested, Rhinovirus was the

Table 1. Prevalence of virus and *M. pneumoniae* (total *n* = 169).

	<i>N</i>	%
<i>M. pneumoniae</i>	21	12.4
Virus	141	83.4
Adenovirus	3	1.8
Respiratory syncytial virus	13	7.7
Influenza A virus	47	27.8
Influenza B virus	0	0
Parainfluenzavirus 1	9	5.3
Parainfluenzavirus 2	0	0
Parainfluenzavirus 3	3	1.8
Picornavirus	128	75.7
Rhinovirus	125	73.9
– Rhinovirus type A	36	21.3
– Rhinovirus type B	6	3.6
– Rhinovirus type C	83	49.1
Enterovirus –68	3	1.8

most frequently detected, with 125, or 73.9%, samples positive for some form of Rhinovirus. Of those, 83 were subtype C, 36 were subtype A and 6 were subtype B. The next most frequently detected virus was Influenza A, found in 47 of the 169 samples (27.8%). RSV was detected in 13 samples (7.7%). Adenovirus, Parainfluenza 1, Parainfluenza 3 and Enterovirus (subtype 68) were found in 3 samples. No samples tested positive for Influenza B or Parainfluenza 2. 18 samples tested positive for more than one virus: 10 with RSV and Rhinovirus; 3 with Adenovirus and Rhinovirus; 3 with Influenza A and Parainfluenza1; 2 with Parainfluenza 1 and 3.

Among 169 patients, 10.6% (*n* = 18) had fever, defined as temperature higher than 38 °C. Two of them (1.2%) had positive PCR for *M. pneumoniae*. Two patients (1.2%) with negative PCR for *M. pneumoniae* presented acute otitis media as extrapulmonary manifestation.

Presence of virus or *M. pneumoniae* is not associated with characteristics of patients or of their asthma exacerbations

Comparison between two groups (No virus or MP group or negative patients and virus+Mycoplasma group or positive patients, there were no differences in terms of demographic and clinical characteristics. However, among the group that tested positive for *M. pneumoniae*, a significantly lower percentage had used inhaled steroids over the six months prior to the exacerbation compared to those without *M. pneumoniae* (*p* = 0.018; Table 2). There were no significant differences between virus and *M. pneumoniae* positive and negative groups when comparing the severity of acute exacerbations, need for and length of hospitalization, the frequency of community acquired pneumonia (determined by chest X-ray and clinical manifestations), the use of antibiotics or short cycles of steroids to manage the acute asthma exacerbation, and hospital readmission (Table 3).

Virus and *M. pneumoniae* seasonality and subtypes

The seasonal pattern of virus detection throughout the sampling period is shown in Figure 1. Rhinovirus, the most common virus found, occurred throughout the whole period but peaked during the months of February and March of 2011

Table 2. Demographic and clinical characteristics of study population.

	Total group <i>n</i> = 169	No virus or MP <i>n</i> = 25	Virus alone <i>n</i> = 123	MP + Virus <i>n</i> = 21	<i>p</i>
Age (years)	7.2 ± 3.4	6.7 ± 3.1	7.5 ± 3.4	6.3 ± 3.6	0.252
≤5 years	66 (39.1%)	10 (40%)	44 (35.8%)	12 (57.1%)	0.178
Female gender	77 (45.6%)	13 (52%)	55 (44.7%)	9 (42.9%)	0.773
Weight (kg)	27 ± 12.6	25.9 ± 14.3	27.8 ± 12.5	23.9 ± 11	0.386
Height (cm) (<i>n</i> = 150)	122.9 ± 20.1	120.9 ± 19.4	123.9 ± 19.9	119.4 ± 22.6	0.590
BMI (<i>n</i> = 150)	17.3 ± 3	17.5 ± 3.1	17.4 ± 3.0	16.4 ± 2.1	0.404
SABA	125 (74%)	20 (80)	93 (75.6%)	12 (57.1%)	0.155
Anticholinergic	5 (3%)	1 (4.0)	4 (3.3%)	0 (0%)	0.680
Inhaled corticosteroid	109 (64.5%)	19 (76.0)	82 (66.7%)	8 (38.1%)	0.018
Antileukotriene	32 (18.9%)	6 (24.0)	24 (19.5%)	2 (9.5%)	0.437
LABA	5 (3%)	0 (0.0)	5 (4.1%)	0 (0%)	0.382
Antihistaminic	40 (23.7%)	9 (36%)	25 (20.3%)	6 (28.6%)	0.208
Oral corticosteroid	3 (1.8%)	1 (4%)	2 (1.6%)	0 (0%)	0.576

MP: *Mycoplasma pneumoniae*; SABA short-acting beta agonists; LABA: long-acting beta agonists; *p*: comparison between No virus or MP group and MP + virus group.

Table 3. Characteristics of asthma exacerbations.

Variable	Total group <i>n</i> = 169	No virus or MP <i>n</i> = 25	Virus alone <i>n</i> = 123	MP + Viruses <i>n</i> = 21	<i>p</i>
Respiratory rate (per min)	30.1 ± 7.9	29.8 ± 7.2	30.1 ± 7.8	30.4 ± 9.0	0.964
SpO ₂	88.6 ± 4.2	88.5 ± 4.2	88.7 ± 4.4	87.8 ± 3.0	0.662
Heart rate (per minute)					
<100	17 (10.1%)	4 (16.0%)	13 (10.7%)	0 (0%)	0.434
100–120	51 (30.4%)	8 (32.0%)	37 (30.3%)	6 (28.6%)	
>120	100 (59.5%)	13 (52.0%)	72 (59.0%)	15 (71.4%)	
Severity of exacerbations					
Mild	66 (39.1%)	11 (44.0%)	47 (38.2%)	8 (38.1%)	0.705
Moderate	92 (54.4%)	12 (48.0%)	67 (54.5%)	13 (61.9%)	
Severe	11 (6.5%)	2 (8.0%)	9 (7.3%)	0 (0%)	
Need for hospitalization	41 (24.3%)	3 (12.0%)	34 (27.6%)	4 (19.0%)	0.210
Hospitalization length (days) (<i>n</i> = 41)	3.2 ± 1.8	2.7 ± 1.2	3.2 ± 1.9	3.8 ± 2.1	0.741
Community acquired pneumonia	21 (12.4%)	2 (8.0%)	16 (13.0%)	3 (14.3%)	0.758
Intensive Care Unit	3 (1.8%)	0 (0%)	3 (2.4%)	0 (0%)	0.565
Mechanical ventilation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
Antibiotic use	29 (17.2%)	3 (12.0%)	22 (17.9%)	4 (19.0%)	0.753
Course of steroids	139 (82.2%)	17 (68.0%)	104 (84.6%)	18 (85.7%)	0.129
Hospital readmissions	14 (8.3%)	1 (4.0%)	13 (10.6%)	0 (0%)	0.188

MP: *Mycoplasma pneumoniae*

Community acquired pneumonia: presence of cough and dyspnea less than 15 days plus alveolar infiltrates in chest radiography acquired out of hospital context.

and February 2012. Influenza peaked in June 2011 and February 2012 presenting a bimodal pattern. Similar to Rhinovirus, RSV cases occurred throughout the whole period but unlike Rhinovirus, RSV presentation frequency was low and cases were preferentially detected between March and May 2011. Seasonality analysis was not done for the other viral agents due to low numbers of positive samples.

Seasonality of *M. pneumoniae* infection in this population is shown in Figure 2. Totally 85.7% of the *M. pneumoniae* positive samples were detected between the months of December 2011 and February 2012. We also determined the subtype of *M. pneumoniae* in four *M. pneumoniae* positive samples from the current study and, for comparison, three *M. pneumoniae* positive samples collected in 2005 as part of a different study. *Mycoplasma pneumoniae* subtypes, determined by PCR amplification and sequencing of a repetitive region of the P1 gene, were defined according to Dumke et al. 2006 [33]. We found that the three samples from the earlier study carry type 1 *M. pneumoniae* and the four from this study carry type 2.

Discussion

This study describes the prevalence of viral and *M. pneumoniae* infection in 169 children between 2 and 15 years of age who visited an emergency room due to acute asthma exacerbation, as determined by PCR-based detection methods. The overall prevalence of viral infection in this group of patients was 83.4%. Previous studies have reported 32% to 85% of children with asthma exacerbation to have viral respiratory infection, as determined by PCR detection methods [33–35]. Rhinovirus was the most frequently found virus in the present study (71%) and especially Rhinovirus Type C (47%). This confirms previous reports of Rhinovirus type C prevalence of 50% to 60% in children with asthma exacerbation, and supports the current hypothesis that Rhinovirus type C plays a significant role in allergic diseases and asthma in particular [33,36–39].

The overall prevalence of *M. pneumoniae* infection in the current study was 12.4%, which is within the range reported in previous studies on children with acute asthma

Figure 1. Monthly distribution of respiratory viruses associated with asthma exacerbations in a selected population of children, Bogotá, Colombia (December 2010–February 2012).

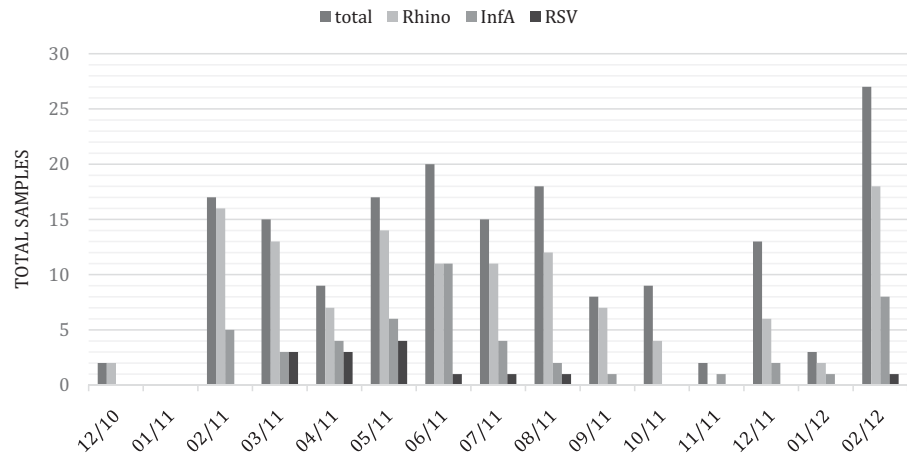
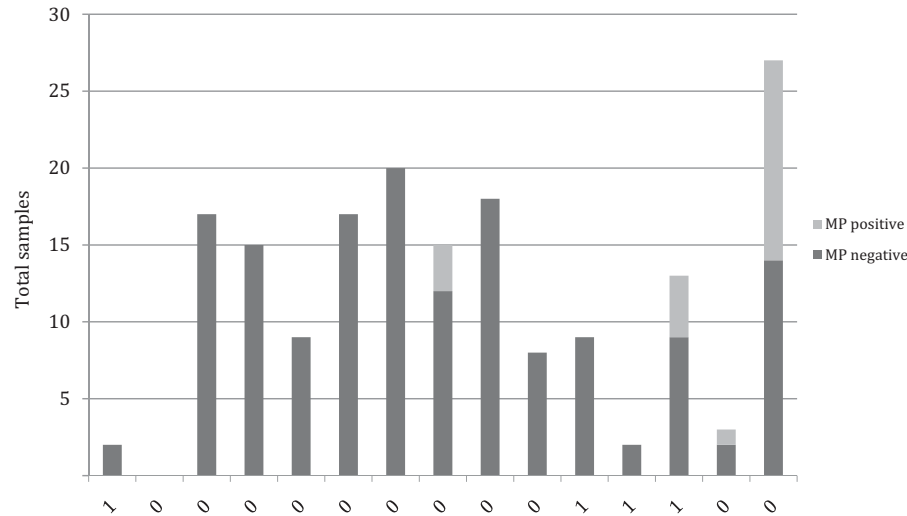


Figure 2. *Mycoplasma pneumoniae* distribution (December 2010–February 2012).



exacerbation [15,21]. We found a clear tendency to a greater prevalence of *M. pneumoniae* in the under-five group without statistically significant difference (18.2% [IC 95%: 8.7–27.6] vs. 8.7% [IC 95%: 3.2–14.3]), which may be explained by the low number of *M. pneumoniae* positive subjects in this study. Our study shows a higher *M. pneumoniae* prevalence in pre-school children with acute asthma exacerbation than the 4.5% and 10% prevalence previously reported by Maffey et al. [21] and Esposito et al. [15], respectively.

It is noteworthy that 100% of patients with *M. pneumoniae* in this study presented co-infection with some type of virus. While this finding is common in respiratory infection [40], no studies show similar results in chronic obstructive pulmonary disease and asthma exacerbation [41]. However, there is an evidence supporting the interaction between virus and asthma. Viral respiratory tract infections often precede bacterial infections in both healthy subjects and those with chronic lung disease [42]. Oliver et al. observed virus-induced damage to host antibacterial defense in alveolar macrophages, suggesting that viral infections could facilitate further bacterial infection [43]. As we did not perform serology tests for *M. pneumonie*, we could not reliably differentiate infections from colonization due to this microorganism. In the same sense, it is not possible for us to define the true role of

M. pneumoniae or virus as trigger or cause of asthma exacerbations. As a main descriptive study, we define the prevalence as the frequency of the presence of each micro-organism but we can't establish the causality role

The majority of the patients were recruited in the Emergency Department and proceeds from different institutions. So, a complete history of the status control of these children was not available. However, the mentioned proportions of patients without treatment and the exacerbation condition suggest a significant number of children were not well controlled asthma.

We found no significant difference in severity of asthma exacerbation or need for hospitalization in the presence of viral agents or *M. pneumoniae*/virus co-infection, similar to findings in previous studies [44,45]. Imamura et al. did report a relationship between Enterovirus-68 and increased severity of asthma exacerbation [46]. All three of the patients in the present study who were found to have Enterovirus-68 were hospitalized, but due to less number we could not draw definite conclusions about a relationship between this virus and the need for hospitalization.

The results of this study showed significantly lower frequency use of inhaled steroids in the previous 6 months in the *M. pneumoniae* positive group compared to the

negative group. This finding suggests a possible protective effect of inhaled corticosteroids on the presence of *M. pneumoniae*. Inhaled corticosteroids are effective for the control and management of asthma symptoms due to their anti-inflammatory properties [47]. Inhaled corticosteroids have been shown to reduce the amount of TLR2 mRNA and, therefore, the production of pro-inflammatory cytokines and cellular infiltration in the airways in mice [48]. It is clear that the use of corticosteroids could also be beneficial, in addition to antibiotics, for the treatment of *M. pneumoniae* infection. Administration of corticosteroids may help to relieve symptoms in severe cases of *M. pneumoniae* infection that do not respond to antibiotic treatment [49]. While the mechanism of this beneficial action of corticosteroids against *M. pneumoniae* infection manifestations is not clear, it is possible that the pathogenesis of *M. pneumoniae* infection is mediated by the immune system and thus the physiopathological effects of the bacteria, including airway inflammation, could be reduced by the use of immunomodulatory drugs like corticosteroids. In fact, it had been shown that *M. pneumoniae* infection can cause bronchial hyperactivity and airway inflammation in rats [33]. Sasaki et al. support the hypothesis that inhaled corticosteroid did not influence the infectious load of *Chlamydia* or *Mycoplasma pneumoniae* [48,50]. In contrary, present data indicate that the use of inhaled corticosteroid can be associated with lower prevalence of *M. pneumoniae* infection, suggesting that corticosteroids may also have a direct effect on bacterial load. The possibility that the use of inhaled corticosteroids could reduce adherence or colonization of the airways by *M. pneumoniae* needs further investigation.

There was no difference between *M. pneumoniae* positive and negative subjects with regard to the frequency with which their acute asthma exacerbation were treated with antibiotics and their outcomes. No clinical characteristics were found to support the use of antibiotics in *M. pneumoniae* positive population. We suggest that the use of antibiotics is not justifiable in all patients with an acute asthma exacerbation but they must be considered, as suggested by other studies, for children with persistent symptoms who do not improve with conventional treatments [23].

The typing of four samples from the present study and of three from an earlier study done by Escobar et al. [51], allowed us to detect the presence of the two types of genomes that have circulated among the Colombian population in the last decade. Although no conclusive studies are available on the biological differences between the various *M. pneumoniae* populations, a phenomenon of subtype change from one epidemic to the subsequent one has been observed [36,37]. This may explain why, in the current study, the subtypes were grouped together in different periods of time, with type 1 in 2005 and type 2 between 2011 and 2012 [52].

M. pneumoniae prevalence was low during the first 12 months and higher during the last 3 months. This may suggest a seasonal pattern, but further study over longer periods of time would be needed to confirm this pattern. The variations in *M. pneumoniae* prevalence that we observed over time may be related to the epidemic periods of the bacteria [15,21], which appear to fluctuate between 3 and 7 years. Thus, according to the period in which the

prevalence is analyzed, different rates of prevalence can be obtained [53,54]. The samples that tested positive for *M. pneumoniae* were collected in the months of July, December, January and February, which are known to have low levels of rainfall (except December when rainfall is moderate) in our region [55]. It has been observed that the majority of *M. pneumoniae* epidemics in Europe begin in summer, peak in autumn and decrease in winter [56]. In tropical countries, where there are no clear seasons, the presence of the bacteria seems to increase in periods of low rainfall [57,58]. Further studies with larger numbers of subjects over longer times will be necessary to prove the seasonality of *M. pneumoniae*.

Limitations of this study:

We did not perform serology for *M. pneumoniae* in this study. So, we can't reliably differentiate between infections from colonization due to this microorganism. In the same sense, it is not possible for us to define the true role of *M. pneumoniae* or virus as a trigger or cause of asthma exacerbations. As a main descriptive study, we define the prevalence as the frequency of the presence of each microorganism, but we cannot establish the causality role.

Conclusion

Using PCR-based detection methods, we found the prevalence of *M. pneumoniae* and viral infection in a selected population of patients between 2 and 15 years of age with acute asthma exacerbation was 12.4% and 83.4%, respectively. Rhinovirus type C was the most common viral agent detected. These numbers are within the ranges reported in previous studies, validating the use of PCR detection methods as an alternative to culture and serological detection methods. All patients with *M. pneumoniae* also had viral infection, suggesting that viral infection can predispose patients to *M. pneumoniae* infection, and that this interaction may trigger acute asthma exacerbation. A significantly lower percentage of the *M. pneumoniae* positive subjects had used inhaled steroids over the six months prior to acute asthma exacerbation compared to the *M. pneumoniae* negative subjects, indicating that inhaled corticosteroids may have a protective effect against *M. pneumoniae* infections in patients with asthma exacerbations. Evaluation of *M. pneumoniae* infection rates over the time frame of this study shows a possible association of high infection rates and low rainfall, as described by previous studies.

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Declaration of interest

The authors declare that there are no conflicts of interest. This study was supported by Departamento Administrativo de

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