

# Detection of *Mycoplasma pneumoniae* in Mexican children with community-acquired pneumonia: experience in a tertiary care hospital

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**Purpose:** *Mycoplasma pneumoniae* is an important cause of community-acquired pneumonia (CAP). Information on the prevalence of *M. pneumoniae* in pediatric patients with CAP in Mexico is limited. The aim of this study was to detect *M. pneumoniae* in hospitalized pediatric patients with CAP.

**Patients and methods:** We performed a descriptive study in a tertiary-level pediatric reference center, obtaining 154 respiratory samples from patients under 18 years of age and diagnosed with CAP. *M. pneumoniae* was detected by real-time polymerase chain reaction (PCR) targeting the *p1* and *CARDS* genes. Complete blood cell count, measurement of C-reactive protein and detection of IgM and IgG anti-P1 were performed. Clinical, epidemiological and radiological data of the patients were analyzed.

**Results:** *M. pneumoniae* was detected by real-time PCR in 26.6% of the samples. 39% of the cases occurred during the spring season. A total of 83% of the patients with *M. pneumoniae* had some underlying disease; renal disease, autoimmune disease and primary immunodeficiencies had a significant association with *M. pneumoniae* CAP. Children under 6 years of age represented 53.7% of the cases. Fever and cough were the most frequent symptoms. IgM and IgG were positive in 1.9% and 14% of the patients, respectively. In the chest X-ray, 17.1% of the patients showed multifocal alveolar infiltrates pattern. The complications in this series were 26.8%. The mortality in this study was 4.9%.

**Conclusion:** This is the first report in Mexico about *M. pneumoniae* as a causal agent of CAP in a tertiary care pediatric hospital using real-time PCR and serology. *M. pneumoniae* was responsible for 26.6% of the cases and was frequent in children under 6 years of age. In addition, we described the clinical presentation in patients with underlying diseases.

**Keywords:** *Mycoplasma pneumoniae*, community-acquired pneumonia, pediatrics, Mexico

## Introduction

The most important clinical manifestation of *Mycoplasma pneumoniae* in the respiratory tract is community-acquired pneumonia (CAP). School-age children and adolescents are most often affected, but the disease occurs in people of all ages.<sup>1</sup> *M. pneumoniae* is found worldwide and throughout the year. However, in the US, it has been reported more frequently in the summer or early fall.<sup>2</sup> The course of CAP caused by *M. pneumoniae* is generally mild and self-limiting. It is estimated that 3–10% of the children with respiratory infection due to *M. pneumoniae* will develop CAP, of whom, up to 5% will require

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hospitalization,<sup>3</sup> and up to 10% of the hospitalized children will be admitted to the intensive care unit (ICU).<sup>4</sup>

Culture is still considered the gold standard for the diagnosis of *M. pneumoniae* infection, but slow growth (up to 6 weeks) and low sensitivity (0.04–61%) limit its use in the clinical microbiology laboratory.<sup>5–7</sup> The disadvantages of serology as a diagnostic method include variable sensitivity (62.2–80%),<sup>8,9</sup> the need for paired samples, complicated detection in immunosuppressed patients and ineffective diagnosis in the early phase of infection.<sup>10</sup>

Molecular techniques have emerged as an efficient diagnostic tool due to their high sensitivity (85–100%) and specificity (98–100%),<sup>11–13</sup> shorter turnaround time, and the fact that they require only a single sample without the need for viable *M. pneumoniae* organisms.<sup>1</sup> Given these advantages, real-time polymerase chain reaction (PCR) has become the main method for *M. pneumoniae* detection.<sup>14–16</sup>

The reported frequency of CAP due to *M. pneumoniae* in pediatric patients worldwide varies from 1.5% to 17.6%.<sup>17–19</sup> Studies conducted in Latin America report frequencies ranging from 0.74% to 43.8%.<sup>6,20–25</sup> Most of these reports are from a previously healthy pediatric population.

Due to the lack of a reference laboratory and personnel trained to perform the culture and the low availability of molecular methods, information on the prevalence of *M. pneumoniae* in pediatric patients with CAP in Mexico is limited.<sup>26–28</sup>

The aim of this study was to use real-time PCR and serology to detect *M. pneumoniae* in pediatric patients with CAP who were admitted in a tertiary hospital and to describe the clinical presentation, laboratory data and radiological findings of patients.

## Material and methods

### Location and study population

During a period of 16 months (November 2015–March 2017), we enrolled 154 pediatric patients (0–18 years old) with CAP who were admitted to the Instituto Nacional de Pediatría (INP), which is a tertiary-level pediatric reference center with 339 beds and a total of 7,830 discharges per year. The INP has all the pediatric subspecialties; however, the main population consist of immunocompromised patients (cancer, transplant, immunological disorders and primary immunodeficiencies).

## Clinical data

Demographic and clinical data as well as biological samples were obtained at the time of admission. The diagnosis of CAP was established based on the presence of the following criteria: Any respiratory signs and symptoms (cough, tachypnea, wheezing) and any sign of respiratory distress with or without the presence of fever presented in outpatient setting and that required hospitalization. None of these patients were admitted in the previous two weeks. Detailed chest examination was performed. Oxygen saturation was measured by pulse oximetry. All the patients have a chest X-ray and any clinical finding were correlated, those radiographs were interpreted by two independent physicians.

## Standardization of real-time PCR for *M. pneumoniae* detection

For the standardization of the real-time PCR, primers were designed using the Primer 3 program (v4.0.0), the target sequence for amplification was a segment of the genes *p1* adhesin with 338 bp (F: GTTAACGGCCTCCAGTCAAG, R: GTTTAGCCGCACTGGTTTGT) and Community-Acquired Respiratory Distress Syndrome (*CARDS*) toxin with 452 bp (F: CCGTGTGATTGAGAAGCCC, R: CATGACCAGGTTCAACGGGA) from the *M. pneumoniae* ATCC 155310D. The fragments obtained were cloned into the pDrive cloning vector (QIAGEN, Hilden, Germany) according to the instructions of manufacturer. Serial dilutions of the recombinant plasmids ( $10^7$ – $10^0$  DNA molecules/ $\mu$ L) were performed to determine the limit of detection of the method. The detection limit was  $C_T \leq 38.8$  for *p1* and at  $C_T \leq 38.7$  for *CARDS* (one copy of genomic DNA/ $\mu$ L).

## Biological samples

A nasopharyngeal swab was taken from each patient with a nylon swab (FLOQ Swabs, COPAN Murrieta, CA, USA). DNA extraction was performed using the QIAamp® DNA Mini kit (QIAGEN, Hilden, Germany) according to the instructions of manufacturer. The DNA was eluted in 200  $\mu$ L of nuclease-free water and stored at  $-20^\circ\text{C}$  until use. Two blood samples were taken: one for the complete blood cell count and another for the measurement of C-reactive protein (CRP) and the detection of IgM and IgG against P1 of *M. pneumoniae*. The interpretation of the hematological results was performed according to the age of the patients.<sup>29</sup> The blood samples were taken in the first week of admission.

## Detection of *M. pneumoniae* in respiratory samples

The detection of *M. pneumoniae* in respiratory samples was carried out by real-time PCR amplifying two genomic targets (*p1* and *CARDS*) with primers and probes described previously.<sup>11,30</sup> The human *RNaseP* gene was used as an internal amplification control.<sup>31</sup> The final volume of the reaction mixture was 25  $\mu$ L and consisted of 12.5  $\mu$ L of TaqMan Universal Master Mix II with UNG (Applied Biosystems, Foster City, CA, USA), 0.1  $\mu$ M each primer for *p1* and *CARDS*, 0.3  $\mu$ M primers for *RNaseP*, TaqMan probes for *CARDS* (0.2  $\mu$ M), *p1* (0.25  $\mu$ M) and *RNaseP* (0.1  $\mu$ M) and 4  $\mu$ L DNA. The reactions were performed in triplicate on the ABI-7500 Fast platform (Applied Biosystems). In order to confirm the amplification, in samples with  $35 > Ct \leq 38.8$  for the *p1* gene, the products were purified and sequenced using a 3500 XL system (Applied Biosystems). The sequences were analyzed with the blastn algorithm<sup>32,33</sup> to confirm the identity of the fragment.

## Enzyme immunoassay

The detection of IgM and IgG antibodies anti-P1 was performed using the Anti-*Mycoplasma pneumoniae* IgM-IgG human ELISA kits (Abcam, Cambridge, UK), following the instructions of manufacturer. The results were expressed in standard units and were interpreted as follows: positive test  $>11$  standard units, indeterminate 9–11 standard units and negative test  $<9$  standard units for both immunoglobulins.

## Statistical analysis

We compared the overall group of children with CAP with those with *M. pneumoniae* CAP. The JPM 11 software (SAS Institute Inc., Cary, NC, USA) was used. The variables were described as frequencies and percentages. Categorical variables were compared using Pearson's  $\chi^2$  test. Quantitative variables were analyzed using the Kruskal–Wallis test. A value of  $P < 0.05$  was considered statistically significant.

## Results

### Detection of *M. pneumoniae* by real-time PCR

Of the respiratory samples of 154 patients in the study, *M. pneumoniae* was detected in 41 (26.6%). The

amplification of the 2 genomic targets was positive in 14 respiratory samples (9.1%); the *p1* gene was detected in 26 samples (16.9%), and the *CARDS* gene was only positive in 1 sample (0.9%). The *RNaseP* gene showed values of  $Ct \leq 35$  in all the samples. The analysis of the *p1* gene sequences showed 99% identity according to the blastn algorithm.

### Characteristics of patients with *M. pneumoniae*

Among the patients with *M. pneumoniae*, 51.2% were girls, and 53.7% of cases occurred in patients under 6 years. The proportion of CAP cases occurring in patients over 6 years was higher in *M. pneumoniae* cases ( $n=19/42$ ) than in the overall study population, and this difference was statistically significant ( $P=0.001$ ). The age groups in which *M. pneumoniae* CAP were more frequent, were those patients under 2 years (31.7%) and those over 12 years (19.5%). The only group in which *M. pneumoniae* CAP was statistically significant were those patients between 10 and 12 years of age ( $P=0.043$ ). Regarding the seasonality, 39% of the cases occurred during spring ( $P < 0.001$ ) (Table 1).

A total of 83% of the patients with *M. pneumoniae* had some underlying diseases, with congenital malformations being the most frequent (29.3%). In comparison to all CAP cases, we found that *M. pneumoniae* CAP was more commonly associated with renal disease, autoimmune disease and primary immunodeficiencies. Only one patient with asthma had *M. pneumoniae* (Table 2).

### Clinical presentation

In 68.3% of the patients, the course of the disease was acute (less than a week). Fever and cough were the most frequent symptoms. The median duration of fever was three days (range 1–10 days), and the median duration of cough was seven days (range 1–90 days). Rhinorrhea was observed in 43.9% of the patients and vomiting in 22%. The presence of arthritis/arthralgia differed significantly in the *M. pneumoniae* group ( $P=0.004$ ) (Table 3).

In 95.1% of the patients, some level of oxygen desaturation was observed ( $SpO_2 < 92\%$ ), and 82.9% developed respiratory distress. The most frequent signs were crackles (65.9%) and wheezing (43.9%). These signs were not significantly different between both groups. (Table 4)

**Table 1** Demographic characteristics in patients with CAP

Characteristic	Total, n=154 (%)	<i>M. pneumoniae</i> , n=41 (%)	P-value
Female gender	68 (44.2)	21 (51.2)	0.288
≥ 6 years of age*	42 (27.3)	19 (46.3)	0.001
<6 years of age	112 (72.7)	22 (53.7)	
Median age in years (range)	3.08 (0.04–18.3)	5.41 (0.10–17.4)	
Age group			
<2	68 (44.2)	13 (31.7)	0.061
2, ≤4	20 (13)	6 (14.6)	0.714
>4, ≤6	24 (15.6)	3 (7.3)	0.088
>6, ≤8	8 (5.2)	3 (7.3)	0.475
>8, ≤10	6 (3.9)	3 (7.3)	0.186
>10, ≤12*	9 (8.5)	5 (12.2)	0.043
>12	19 (12.3)	8 (19.5)	0.103

Note: \*P<0.05 statistically significant.

Abbreviation: CAP, community-acquired pneumonia.

**Table 2** Underlying diseases in patients with CAP

Diseases	Total, n=142 (%)	<i>M. pneumoniae</i> , n=41 (%)	P-value
Underlying diseases*	102 (66.2)	34 (83)	0.024
Renal disease*	5 (3.2)	5 (12.2)	<0.001
Neurological disease	11 (7.1)	4 (9.8)	0.448
Neurodevelopmental disorder	9 (5.8)	2 (4.9)	0.758
Congenital malformation	40 (26)	12 (29.3)	0.574
Cardiovascular disease	12 (7.8)	4 (9.8)	0.584
Lung disease other than asthma	11 (7.1)	2 (4.9)	0.511
Asthma	16 (10.4)	1 (2.4)	0.051
Solid tumors	4 (2.6)	1 (2.4)	0.941
Leukemias and lymphomas	14 (9.1)	3 (7.3)	0.645
NNHD	9 (5.8)	3 (7.3)	0.639
Primary immunodeficiency*	9 (5.8)	7 (17.1)	<0.001
Autoimmune disease*	9 (5.8)	5 (12.2)	0.043
GERD	5 (3.2)	3 (7.3)	0.086

Note: \*P<0.05 statistically significant.

Abbreviations: NNHD, non-neoplastic hematological disease; GERD, gastroesophageal reflux disease; CAP, community-acquired pneumonia.

## Laboratory tests

A complete blood cell count was performed in 142 of 154 pediatric patients with CAP. Of the patients with *M. pneumoniae* (n=37), 18.9% had leukocytosis. The most frequent hematological findings were lymphopenia (56.8%) and monocytosis (59.5%). None of the patients had neutropenia or lymphocytosis. There was thrombocytosis in 8.1% of patients (P<0.001) and thrombocytopenia in 21.6%. In three patients, thrombocytopenia was due to their underlying disease (Table 5).

Measurement of CRP was performed in 112 of 154 pediatric patients with CAP. Among the patients

with *M. pneumoniae*, 32 samples were obtained. The measured values were in the range of 0.31–33.9 mg/L. Elevated values were observed (>0.8 mg/L) in 26 patients (81.3%).

The detection of anti-P1 antibodies was performed in 107 of 154 pediatric patients with CAP. IgM was detected in 2.8% (n=3) and IgG in 19.6% (n=21) of samples.

Of the patients with *M. pneumoniae* detected by real-time PCR (n=34), IgM was positive in two samples (1.9%; range 15.4–19.5 standard units; P=0.188) and IgG in 15 (14%; range 11.2–28.3 standard units; P<0.001). Of the patients negative for *M. pneumoniae* by real-time PCR, IgM was positive in one sample and IgG was positive in six.

**Table 3** Symptoms of patients with CAP

Symptoms	Total, n=154 (%)	<i>M. pneumoniae</i> , n=41 (%)	P-value
Acute course (<1 week)	120 (77.9)	28 (68.3)	0.083
Sub-acute course (>1 week)	34 (22.1)	13 (31.7)	0.083
Median duration of symptoms in days (range)	13 (3–90)	13 (3–90)	0.595
Fever	135 (87.7)	33 (80.5)	0.103
Median days of fever in days (range)	3 (1–19)	3 (1–10)	0.495
Median temperature in °C (range)	38.5 (37.3–40)	38.5 (37.9–40)	0.834
Cough	145 (94.2)	39 (95.1)	0.758
Median days of cough (range)	6 (1–90)	7 (1–90)	0.283
Rhinorrhea	78 (50.6)	18 (43.9)	0.313
Odynophagia	26 (16.9)	7 (17.1)	0.970
Apnea	2 (1.3)	0	0.391
Headache	7 (4.5)	2 (4.9)	0.905
Exanthema	1 (0.6)	0	0.546
Arthritis/arthralgia*	3 (1.9)	3 (7.3)	0.004
Seizures	3 (1.9)	0	0.292
Diarrhea	15 (9.7)	2 (4.9)	0.235
Abdominal pain	12 (7.8)	4 (9.8)	0.584
Vomiting	36 (23.4)	9 (22)	0.801

Note: \* $P < 0.05$  statistically significant.

Abbreviation: CAP, community-acquired pneumonia.

### Radiological findings

With respect to chest X-ray, an interstitial pattern was the most frequent finding (78%), followed by a bronchopneumonia pattern (29.3%). Multifocal alveolar pattern was statistically significant in the *M. pneumoniae* group (17.1%;  $P = 0.004$ ) (Table 6).

Approximately 53.7% of the patients had a single radiological pattern, whereas the remainder had mixed radiological patterns. The interstitial-bronchopneumonia combination and the interstitial-overdistension combination were the most frequent (21.1% each) (Table 6).

**Table 4** Clinical signs in patients with CAP

Signs	Total, n=154 (%)	<i>M. pneumoniae</i> , n=41 (%)	P-value
Tachycardia	48 (31.2)	13 (31.7)	0.931
Bradycardia	1 (0.6)	1 (2.4)	0.096
Tachypnea	118 (76.6)	31 (75.6)	0.858
Oxygen desaturation ( $SpO_2 < 92\%$ )	148 (96.1)	39 (95.1)	0.704
Respiratory distress	131 (85.1)	34 (82.9)	0.654
Median days of respiratory distress (range)	2 (1–10)	2 (1–10)	0.598
Nasal flaring	61 (39.6)	14 (34.1)	0.404
Intercostal retractions	127 (82.5)	32 (78)	0.385
Subcostal retractions	44 (28.6)	9 (22)	0.273
Xiphoid retractions	72 (46.8)	17 (41.5)	0.428
Thoracoabdominal dissociation	21 (13.6)	6 (14.6)	0.828
Wheezing	63 (40.9)	18 (43.9)	0.649
Cyanosis	30 (19.5)	6 (14.6)	0.360
Crackles	115 (74.7)	27 (65.9)	0.129
Hypoventilation (breath sounds)	53 (34.4)	12 (29.3)	0.418
Clinical syndrome of pleural effusion	8 (5.2)	2 (4.9)	0.915

Abbreviation: CAP, community-acquired pneumonia.

**Table 5** Hematological results of patients with CAP

Parameters	Total, n=142 (%)	<i>M. pneumoniae</i> , n=37 (%)	P-value
Median hemoglobin in g/dL (range)	12.2 (3.7–19.1)	12.8 (7.3–17.1)	0.205
Anemia	31 (21.8)	5 (13.5)	0.154
Leukocytosis	28 (19.7)	7 (18.9)	0.884
Leukopenia	26 (18.3)	7 (18.9)	0.911
Neutrophilia	39 (27.5)	11 (29.7)	0.720
Neutropenia	5 (3.5)	0	0.017
Lymphocytosis	1 (0.7)	0	0.551
Lymphopenia	79 (55.6)	21 (56.8)	0.873
Eosinophilia	9 (6.3)	2 (5.4)	0.787
Monocytosis	72 (50.7)	22 (59.5)	0.215
Thrombocytosis*	44 (31)	3 (8.1)	<0.001
Thrombocytopenia	19 (13.4)	8 (21.6)	0.083

Note: \* $P < 0.05$  statistically significant.

Abbreviation: CAP, community-acquired pneumonia.

## Complications

In total, 26.8% of the patients in the group of *M. pneumoniae* had complications, required mechanical

ventilation and were admitted to the ICU (Table 7), and 4.9% of the patients died. However, none of these complications were statistically significant.

## Discussion

This study reports the frequency of *M. pneumoniae* in pediatric patients diagnosed with CAP in Mexico. *M. pneumoniae* was identified in 26.6% of the patients, which is very similar to several reports in Asian countries.<sup>34–36</sup> However, other studies report lower frequencies; for instance, a multicenter study conducted by the Centers for Disease Control and Prevention in three US hospitals found *M. pneumoniae* in 8% of the patients.<sup>17</sup> Other authors report lower frequencies ranging from 0.74% (Ecuador) to 6% (Austria).<sup>25,37–40</sup>

We measured the analytical sensibility of the real-time PCR method with  $Ct \leq 38.8$  for *pl* gene and  $Ct \leq 38.7$  for *CARDS* gene. In 26 respiratory samples, the *pl* gene was

**Table 6** Radiological findings of patients with CAP

Pattern	Total, n=154 (%)	<i>M.pneumoniae</i> , n=41 (%)	P-value
Single pattern	82 (53.2)	22 (53.7)	0.951
Mixed pattern	72 (46.8)	19 (46.3)	0.951
Interstitial	112 (72.7)	32 (78)	0.372
Consolidation	20 (13)	3 (7.3)	0.207
Bronchopneumonia	52 (33.8)	12 (29.3)	0.477
Multifocal*	11 (7.1)	7 (17.1)	0.004
Atelectasis	8 (5.2)	2 (4.9)	0.915
Effusion	9 (5.8)	3 (7.3)	0.639
Overdistention	22 (14.3)	4 (9.8)	0.333

Note: \* $P < 0.05$  statistically significant.

Abbreviation: CAP, community-acquired pneumonia.

**Table 7** Complications in patients with CAP

Type of complications	Total, n=154 (%)	<i>M. pneumoniae</i> , n=41 (%)	P-value
Complications	47 (30.5)	11 (26.8)	0.483
Pleural effusion	9 (5.8)	3 (7.3)	0.639
Pericarditis	1 (0.6)	1 (2.4)	0.096
Admission to PICU	40 (26)	11 (26.8)	0.884
Median duration of PICU stay in days (range)	9.5 (3–60)	9 (3–60)	0.867
Mechanical ventilation	51 (33.1)	11 (26.8)	0.318
Median days of mechanical ventilation (range)	9 (3–32)	10 (3–25)	0.582

Abbreviations: PICU, pediatric intensive care unit; CAP, community-acquired pneumonia.

positive and *CARDS* gene was not detected. *pl* gene is the most used target in other reports; however, we decided to use both genes in order to improve *M. pneumoniae* detection. Two single nucleotide polymorphisms have been described in *CARDS* gene,<sup>41</sup> one of them is located in the segment where probe is aligned. This could be the reason why *CARDS* gene was not detected.

*M. pneumoniae* infection occurs throughout the year, but there may be seasonal variation. In this study, the highest number of cases of CAP due to *M. pneumoniae* was detected in spring (39%) ( $P<0.001$ ), similar to one study in Poland (32.3%).<sup>42</sup> In Serbia and Korea, a higher frequency was found in autumn (33.3% and 48.4%, respectively).<sup>9,43</sup> The first of two independent studies conducted in China detected the highest number of cases in summer and autumn,<sup>44</sup> whereas the second study showed a peak during the autumn and winter.<sup>45</sup> These results indicate that the seasonal behavior of *M. pneumoniae* may be affected by the geographic region.

In this study, no statistical significance by gender was observed. Among patients with *M. pneumoniae*, 51.2% were female. Similar frequencies have been reported in other studies<sup>36,46</sup>

Of the children who had *M. pneumoniae* ( $n=41/154$ ), 53.7% were under 6 years and 46.3% were over 6 years ( $P<0.001$ ). Previous studies from Korea have reported that 70.9% of the infections occurred in children between 1 and 6 years.<sup>43</sup> In China, a frequency of 80% was reported in children under 7 years,<sup>44</sup> while in Taiwan, *M. pneumoniae* was found in 48% of the children under 5 years.<sup>34</sup> In contrast, a study conducted in the US reported that 73.3% of the pediatric patients with CAP due to *M. pneumoniae* were over 5 years.<sup>4</sup> Children of school age (over 6 years) may be more susceptible to infection with *M. pneumoniae*, as school attendance favors overcrowding and increases the risk of infection.

In total, 83% of the patients with *M. pneumoniae* had an underlying disease ( $P=0.024$ ). Cardiac and respiratory malformations have been reported as risk factors for the development of CAP due to *M. pneumoniae*.<sup>35</sup> In Taiwanese children ( $n=492$ ) with *M. pneumoniae*, 3.1% had an underlying disease (neurological disease, cardiac disease or asthma).<sup>34</sup> Because the INP is a reference hospital, this study offers information about CAP due to *M. pneumoniae* in a pediatric population group with underlying diseases. Most of the reports focus on the description of the clinical and epidemiological characteristics of CAP

due to *M. pneumoniae* in previously healthy pediatric population.<sup>9,17,43</sup>

Of the underlying diseases observed in our patient population, renal diseases, primary immunodeficiencies and autoimmune disease showed a statistically significant association with *M. pneumoniae* ( $P<0.001$ ). Among the seven patients with immunodeficiency and *M. pneumoniae*, there were three patients with AIDS, two cases of hypogammaglobulinemia, one case of agammaglobulinemia and one case of hyper-IgE syndrome. In a study in India that included 90 patients with AIDS, *M. pneumoniae* was detected in 32.3% of this population.<sup>47</sup> A case of a patient with pneumonia due to *M. pneumoniae* who had hyper-IgM syndrome has been previously reported.<sup>48</sup> Our results indicate that in patients with immunodeficiencies who present with CAP, *M. pneumoniae* should be suspected as the etiologic agent.

Associations between *M. pneumoniae* and asthma have been described in previous studies. In France, *M. pneumoniae* was detected in 13.6% of the children with chronic asthma.<sup>49</sup> In Paraguay, in a study of 56 pediatric patients with asthma, *M. pneumoniae* was detected more frequently in children with severe asthma (22.2%) compared to children with stable asthma (6.9%).<sup>50</sup> In this study, of the 16 patients with asthma and CAP, *M. pneumoniae* was detected only in one patient (6.3%). Regarding clinical presentation, fever (80.5%;  $P=0.103$ ) and cough (95.1%;  $P=0.758$ ) were the most frequent symptoms, as reported by other authors.<sup>8,24,34,46</sup> The association of *M. pneumoniae* with arthritis/arthralgias was statistically significant ( $P=0.004$ ), which has not been previously reported. Other studies have suggested an association of headache and wheezing with infection by *M. pneumoniae* ( $P<0.05$ ).<sup>9,51</sup> In this study, 43.9% of the patients had wheezing and 4.9% had headaches, but there was no statistically significant association; however, some of the children were not old enough to report this symptom. Other symptoms such as vomiting (22%), abdominal pain (9.8%) and diarrhea (4.9%) were found in patients with *M. pneumoniae*. Similarly, in Taiwanese children with *M. pneumoniae*, 20.5% had vomiting, 15.7% had abdominal pain and 11.8% had diarrhea.<sup>34</sup> With respect to the clinical signs, 82.9% of the patients presented some degree of respiratory distress, a higher prevalence than reported in a study from Colombia (73%),<sup>52</sup> Poland (48%)<sup>42</sup> and Serbia (8.3%).<sup>9</sup> In these studies, children were previously healthy, which could explain this difference. We found that 65.9% of the patients had crackles,

greater than reported in Korean (51.5%) and Chinese patients (38.6%).<sup>36,53</sup> None of the clinical signs evaluated in this study showed statistically significant associations with *M. pneumoniae*. Other studies found no relationship between clinical presentation and CAP due to *M. pneumoniae*.<sup>42,54</sup> These findings show that the diagnosis of CAP due to *M. pneumoniae* can rarely be established based on the clinical presentation alone.

Regarding the laboratory data, only 18.9% of the patients had leukocytosis, a prevalence lower than that reported in a study in China (24.3%).<sup>55</sup> Other study carried out in the same country reported that 58.8% of the patients had leukocytosis.<sup>36</sup> In Korean patients, there was no difference in the count of leukocytes, but the authors noted that lymphopenia could occur with *M. pneumoniae* infection.<sup>56</sup> In our study, 56.8% of the patients ( $P=0.873$ ) had lymphopenia. An inverse association was observed between thrombocytosis and the presence of *M. pneumoniae* (8.1%;  $P<0.001$ ). The frequency of thrombocytosis has been reported to range from 8% to 58%.<sup>56,57</sup> In a study conducted in Serbia, there was no difference in the leukocyte and platelet counts with *M. pneumoniae* infection.<sup>9</sup> Pneumonia caused by *Streptococcus pneumoniae* could increase the number of leukocytes and neutrophils and in viral CAP lymphocytosis is more common. In this study, there was not a distinct hematological finding associated with *M. pneumoniae*.

Approximately 81.3% of the patients with *M. pneumoniae* had high levels of CRP ( $>0.82$  mg/L;  $P=0.223$ ). One study found that in Chinese children, 62.9% of the patients had a CRP level  $>8$  mg/L,<sup>55</sup> but other study reported only 26.6% with high values.<sup>36</sup> In Serbian children, there was no difference in CRP levels with *M. pneumoniae*.<sup>9</sup> This suggests that CRP is not a good marker for the presence of *M. pneumoniae*.

Of the 41 patients who tested positive for *M. pneumoniae* by real-time PCR, IgM and IgG were positive in 1.9% and 14%, respectively. Some studies that used real-time PCR as the gold standard found that the ranges of sensitivity and specificity of serology were 62.2–80% and 85.5–98.6%, respectively.<sup>8,9</sup> In Chinese children, *M. pneumoniae* was identified by real-time PCR in more patients (15.6%) compared to serology (IgM) (12.1%).<sup>58</sup> In our study, the samples that had positive real-time PCR and negative serology, the presence of immunodeficiencies in some patients could have affected the production of antibodies. In other patients who did not have an underlying disease, the difference in days between

the onset of symptoms and blood sampling might not have allowed the development of an immune response. The diagnosis of *M. pneumoniae* must be accompanied by an analysis of the clinical history, the underlying disease previous antimicrobial treatments and epidemiological factors, as the choice of the best technique for detection of *M. pneumoniae* will depend on this assessment and its interpretation.

Regarding the radiological findings, we found that the interstitial pattern was the most frequent in both groups (78%;  $P=0.372$ ), as has been reported in Poland (85.8%).<sup>42</sup> In Japan, the interstitial pattern was documented only in 28.2% of the patients, whereas the consolidation pattern was the most common radiological finding (87.3%), followed by bronchopneumonia (60.6%).<sup>59</sup> In this study, bronchopneumonia was observed in 29.3% of the patients and consolidation only in 7.3%, other studies have reported consolidation as the most common radiological finding.<sup>36,60</sup> The multifocal pattern was statistically significantly associated with *M. pneumoniae* (17.1%;  $P=0.004$ ). This radiological pattern was reported also in 38.6% of the Chinese children.<sup>55,61</sup> In contrast, in Serbian children, no association was found between radiological findings and infection with *M. pneumoniae*.<sup>9</sup> These data indicate that the presence of *M. pneumoniae* has not a characteristic radiological pattern.

Patients with *M. pneumoniae* pneumonia presented complications in 26.8%. The most frequent complications in our patients were admission to the pediatric ICU (PICU;  $P=0.884$ ) and mechanical ventilation ( $P=0.318$ ). In Taiwan, 26% of the patients had complications, the frequency of admission to the PICU was 22.1% and the use of mechanical ventilation occurred in 5.5% of the patients.<sup>34</sup> Other studies report lower frequencies: in the US, it was reported that 10.8% of the patients were admitted to the PICU and 1.7% required mechanical ventilation.<sup>4</sup> In Israel, admission to the PICU was reported in 4.6% ( $n=8$ ) of the patients, of whom two died.<sup>61</sup> In this study, one of the child who died required admission to PICU and mechanical ventilation.

The mortality in this study was 4.9% ( $n=2$ ). One of the patients had an underlying disease (AIDS, congenital heart disease and gastroesophageal reflux) and the second child was previously healthy. Infection with *M. pneumoniae* could contribute to both deaths. In Taiwan, the death of six children with *M. pneumoniae* CAP was reported; of these, half had an underlying disease.<sup>62</sup> In Texas, an outbreak of this pathogen was reported in five previously



healthy siblings, two of whom died of respiratory complications.<sup>63</sup> It is important to mention that up to 25% of the people infected with *M. pneumoniae* may have extrapulmonary complications,<sup>64</sup> which can lead to increased mortality.

## Conclusions

This study demonstrated the involvement of *M. pneumoniae* as a cause of CAP in Mexican pediatric patients. The frequency was 26.6%. Classically, *M. pneumoniae* pneumonia is referred more frequently in patients over 5 years, we found that 53.7% were under 6 years. The course of CAP due to *M. pneumoniae* usually is self-limited; however, we found that 26.8% of the patients have come complications and 4.6% died. Most of the reported studies of *M. pneumoniae* CAP have been reported in previously healthy children; in this study, 83% of the patients had underlying diseases, so this report offers important data regarding *M. pneumoniae* pneumonia in this group of patients. The use of molecular techniques was more efficient in the detection of *M. pneumoniae* than serological tests. Because the treatment of *M. pneumoniae* in children is different from other CAP-causing microorganisms, timely detection of this pathogen could reduce the development of complications, the presence of extrapulmonary manifestations and patient mortality.

## Abbreviations list

CAP, community-acquired pneumonia; RT-PCR, real-time polymerase chain reaction; CRP, C-reactive protein; INP, Instituto Nacional de Pediatría; CARDS, Community-Acquired Respiratory Distress Syndrome; SNPs, single nucleotide polymorphisms; PICU, pediatric intensive care unit; AIDS, acquired immune deficiency syndrome.

## Ethics

This study was approved by the Research and Ethics Committees of the INP (Institutional Review Board: 00008064, reference number 14/058), following the guidelines of the Declaration of Helsinki. Written informed consent was obtained from a parent or guardian of each child enrolled in the study.

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## Author contributions

Jocelin Merida Vieyra performed the acquisition, analysis and interpretation of data, and drafted the initial manuscript. Alejandra Aquino Andrade was responsible for the conception and design, the acquisition, analysis and interpretation of data, acquisition of funding, and drafting the initial manuscript. Deborah Palacios Reyes contributed to obtaining informed consent, the acquisition, analysis and interpretation of data, and review and editing of the manuscript. Chiharu Murata performed the analysis and interpretation of data and review and editing of the manuscript. Rosa Maria Ribas-Aparicio analyzed the data and reviewed and edited the manuscript. Agustin De Colsa Ranero was responsible for the conception and design, the analysis and interpretation of data, acquisition of funding, and review and editing of the manuscript. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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