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Genotyping and macrolide resistance of *Mycoplasma pneumoniae* identified in children with community-acquired pneumonia in Medellín, Colombia

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**A B S T R A C T**

Objectives: The aim of this study was to describe the genotypes and the main characteristics of community-acquired pneumonia (CAP) caused by *Mycoplasma pneumoniae* in hospitalized children in Medellín and neighboring municipalities during the period 2011–2012.

Methods: The *M. pneumoniae* genotype was determined by PCR and sequencing of the p1 and 23S rRNA genes from induced sputum samples and nasopharyngeal swabs (NPS). Samples were obtained from children with CAP who were hospitalized in 13 healthcare centers. In addition, a spatio-temporal analysis was performed to identify the potential risk areas and clustering of the cases over time.

Results: A variant of type 2 was the dominant genotype in the induced sputum (96.1%) and NPS (89.3%) samples; the type 1 variant was identified in 3.9% and 10.7% of these samples, respectively. No strains with mutations in the 23S rRNA gene associated with macrolide resistance were found. The cases in Medellín were mainly concentrated in the northeastern areas and western districts. However, no temporal relationship was found among these cases.

Conclusions: A variant of type 2 of *M. pneumoniae* prevailed among children with CAP during the study period. No strains with mutations associated with macrolide resistance were found.

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**Introduction**

*Mycoplasma pneumoniae* is a major cause of community-acquired pneumonia (CAP) in children and adults. In both groups, *M. pneumoniae* explains 4–20% of cases, of which approximately 18% require hospitalization (Waites et al., 2008; Waites et al., 2017). The occurrence of *M. pneumoniae* CAP varies according to age: it is more common in school-age children and adolescents, but can occur at any age. The pneumonia is generally relatively mild, but it can be severe, or associated with extrapulmonary complications affecting the central nervous system (encephalitis, Guillain–Barré syndrome), mucosa (urticaria, Stevens–Johnson syndrome), and other organs (Waites et al., 2017; Waites and Talkington, 2004; Yimencioğlu et al., 2014; Mondaca et al., 2014).

*M. pneumoniae* strains can be classified into two main types – type 1 and type 2 – based on the sequence of the repetitive regions RepMP2/3 and RepMP4 of the p1 gene (Cousin-Allery et al., 2000; Dumke et al., 2003; Dallo et al., 1990). At the same time, these regions are recombined with similar regions outside the p1 gene, thereby generating the V1, V2a, V2b, V2c, and V2d variants. It is possible that changes in the p1 gene are associated with antigenic variation of the cellular surface of *M. pneumoniae*, which can favor evasion from the immune response and generate cyclic pattern in the infections, as well as triggering outbreaks (Razin et al., 1998; Kenri et al., 1999).

Despite the high frequency of infections caused by *M. pneumoniae*, knowledge of its epidemiology and the events related to the recombination and detection of variants is insufficient. Microbiological confirmation is not usually performed due to the absence of rapid and reliable techniques for the diagnosis of this infection, which, among other reasons, contributes to this lack of
knowledge. In addition, the typing techniques are only used at reference centers, limiting the knowledge of genotypes circulating during outbreaks of respiratory infections (Diaz et al., 2015a; Kawai et al., 2013).

The excessive use of macrolides for the management of infections of both the upper and the lower respiratory tract results in the selection of resistant strains of *M. pneumoniae* with mutations in the 23S rRNA gene. Mutations that occur at positions A2063G and A2064G of the gene are associated with high minimum inhibitory concentrations (MICs) of macrolides (Saraya et al., 2014; Bébéar et al., 2011; Morozumi et al., 2010). Although the real impact of resistant strains on the severity of the disease is unknown, in certain cases, these resistant strains can increase the duration of signs and symptoms, and they may require a change in the antimicrobial therapy (Suzuki et al., 2006; Matsubara et al., 2009; Zhou et al., 2014). The proportion of strains resistant to macrolides varies among countries, from 0% in the Netherlands and Thailand, to 8.2–12% in the USA and Canada, and up to 100% in China (Diaz et al., 2015a; Spuesens et al., 2012; Whistler et al., 2017; Eshghi et al., 2013; Zhou et al., 2015).

Due to the scarcity of information available on the genotypes of *M. pneumoniae* circulating in Colombia and Latin America, the aim of this study was to describe the genotypes and the proportions of strains with mutations associated with resistance in a group of children hospitalized with CAP between 2011 and 2012. In addition, it was aimed to explore the spatial and temporal distribution of the cases in the municipality of Medellín.

**Methods**

**Study population**

Children included in this study were aged between 1 month and 17 years and had *M. pneumoniae* demonstrated as the etiological agent of CAP; this subpopulation belonged to a cohort of 525 pediatric patients with CAP hospitalized in 13 hospitals of Medellín. CAP was defined by the presence of any recent opacity (alveolar or interstitial) on chest X-ray, plus one of the following signs or symptoms: (1) axillary temperature ≥38.3 °C; (2) tachypnea (in children <2 months, >60/min; in those aged 2–11 months, >50/min; in those 1–4 years, >40/min; in those 5–12 years, >30/min; and in those >12 years, >20/min); and (3) the presence of rhonchi, crackles, or wheezing on pulmonary auscultation. Patients who had received treatment with antibiotics for more than 72 h at the time of enrollment in the study and those with an innate or acquired immunodeficiency, cystic fibrosis, neurological or psychiatric disorders, bronchiolitis obliterans, or hematological malignancies were excluded. A diagnosis of CAP caused by *M. pneumoniae* was based on a positive PCR from the induced sputum samples (Seeplex Pneumobacter; Seegen, Songpa-gu, Korea), or the quadrupling of titers (IgM and/or IgG) of paired serology; the first sample was collected at the time of enrolment and the second sample between 24 days and 8 weeks later (Vircell; Vircell S.L., Granada, Spain).

**Processing of clinical samples**

All patients provided a sample of induced sputum and a nasopharyngeal swab (NPS) sample. Both types of sample were collected by trained staff. Samples were then stored at −80 °C until processing.

The DNA extraction was performed from 500 μL of the saline solution containing the NPS, and from 500 μL of the sputum sample that had previously been treated with N-acetyl-L-cysteine–NaOH at 2%, following the DNAeasy Blood and Tissue protocol (Qiagen, Hilden, Germany).

**Molecular typing of *M. pneumoniae* with the p1 gene**

The induced sputum and NPS samples were genotyped if the patient had a positive PCR for *M. pneumoniae* in induced sputum and/or positive paired serology. Genotyping of the samples was performed with the nested PCR protocol described by Dumke et al. (2006), which was standardized. The RepMP2/3 region was amplified in the nested PCR to differentiate between type 1 and type 2 and between the respective variants (V1, V2a, and V2b). The concentrations of MgCl2 (2 mM and 2.5 mM, respectively) and deoxynucleotide triphosphates (dNTPs) were modified in both rounds (0.2 mM dNTPs) to obtain a higher-quality band on the agarose gel.

**Description of mutations in the 23S rRNA gene**

The conventional PCR described by Chironna et al. (2011) was standardized for genotyping. This PCR amplifies a region of 217 bp; this region includes positions 2063 and 2064, which contain 95% of the mutations present in this region of the gene. In contrast to the protocol described by the author, the annealing temperature of the primers used in the present study was 58.2 °C.

All products of the PCR reactions were developed by 2.5% agarose gel electrophoresis (Amresco, Solon, OH, USA) stained with EZ-VISION (Amresco), in a run for 60 min at 70 V. The size of the band observed was confirmed to correspond approximately to the size of the expected fragment.

The intra- and inter-assay reproducibility of the PCR protocols was assessed. The presence of inhibitors was excluded for PCR reactions yielding negative results by the amplification of a fragment of the human glyceraldehydes-3-phosphate dehydrogenase (GAPDH) using the Inveraid First Strand cDNA Synthesis Kit (Thermo Scientific).

**Analysis of the sequences**

Both chains of the amplified region were sequenced. To determine the genotype according to the classification proposed by Dumke et al. (2010a), the consensus sequences were obtained from each sample and compared to the sequences of the p1 gene of the reference strains of *M. pneumoniae* M129 (type 1) (GenBank accession number M18639) and FH (type 2) (GenBank accession number AF299002.1) and the variants V1 (GenBank accession number AF299000.1), V2a (GenBank accession number AB024618.1), V2b (GenBank accession number DQ383277.1), and V2c (GenBank accession number JN048895). The V2c (GenBank accession number JN048895) and V2d (GenBank accession number EF656612) variants could not be analyzed because changes in the p1 gene are located in the repetitive region RepMP4, which was not amplified by the PCR used in this study (Xiao et al., 2014). The detection of mutations at positions 2063 and 2064 of the 23S rRNA gene was performed by comparing the consensus sequences with the sequence of the 23S rRNA gene (GenBank accession number X68422) in a multiple alignment.

**Georeferencing of the cases of CAP caused by *M. pneumoniae***

The addresses of the houses of the cases living in the urban area of Medellín were located on a map of the city using ArcGIS 10.2 software. Georeferencing of cases living outside the city could not be performed, because at the time of the study, the software did not have the information required to spatially locate the addresses of these sites. Global (Moran’s I and Getis-Ord G*) and local (Moran’s I and Getis-Ord G*) spatial statistics were used to identify spatial clusters of the cases at different distances and to graphically locate the neighborhoods with the larger numbers of cases. In addition,
the monthly distribution of cases was explored to determine whether there was any increase in the number of cases during a specific time across the study period.

**Results**

**Clinical characteristics of children with CAP caused by M. pneumoniae**

Of the 525 children with CAP included in the project “Clinical and etiologic characterization of the CAP in children hospitalized in the metropolitan area of Medellin, 2011–2013”, 73 cases showed evidence of recent *M. pneumoniae* infection detected by PCR, paired serology, or both methods. Eighteen of the 73 cases (24.7%) were positive using both techniques, 40 (54.8%) were positive with any of the PCR protocols used (Seeplex Seegene or genotyping), and 15 (20.5%) were positive only by paired serology. Table 1 describes the clinical and socio-demographic data of the group of 73 children in whom *M. pneumoniae* was detected, discriminating between the group of 58 children showing a positive result for any of the PCRs used for detection and the group of 15 children who were only positive for the paired serology. Seventy-five percent of the affected children were under 4 years of age, and there was a slight male predominance. The most frequent comorbidity was asthma, present in approximately 25% of the cases. There were no statistical differences in clinical or socio-demographic characteristics between the groups of children positive by any method, children with positive PCR in induced sputum or NPS, and children only positive by paired serology.

**Genotypic characterization of patients using the p1 gene in the studied samples**

Fifty-three of the 73 children with CAP caused by *M. pneumoniae* were positive for the p1 gene based on the PCR; 30 children showed positive PCR results for the induced sputum and the NPS sample, 22 children were positive only for the induced sputum, and one child was positive only for the NPS sample. The 52 available sequences of the induced sputum samples were analyzed. Twenty-eight of the 31 NPS samples were also analyzed. A poor quality sequence was obtained from the three remaining NPS samples. A variant of type 2 of *M. pneumoniae* was identified in 96.1% (50/52) of the induced sputum samples and in 89.3% of the NPS samples (25/28) (Figure 1). A discordant genotype (variant of type 2 in the induced sputum and type 1 in the NPS) was found for one of the 28 patients for whom the genotype was available for analysis from both samples.

**Detection of mutations in the 23S rRNA gene**

Amplification was achieved for 42 patients: six in NPS (8.2%), 24 in induced sputum (32.8%), and 12 in both samples. After the analysis of all DNA sequences from the 36 induced sputum samples and 17 of the 18 NPS samples (one did not yield a sequence of good

### Table 1

Clinical and socio-demographic characteristics of the children with community-acquired pneumonia who were positive for *Mycoplasma pneumoniae*.

| Characteristic                        | Children positive by any method (n = 73) | Children with positive PCR in induced sputum or NPS (n = 58) | Children only positive by paired serology (n = 15) | p-Value |
|--------------------------------------|----------------------------------------|-------------------------------------------------------------|-------------------------------------------------|---------|
| Age in years, median (IQR)           | 2.0 (1–4)                              | 3 (1–4)                                                     | 2 (0–4)                                        | 0.105   |
| Male, n (%)                          | 41 (56.2)                              | 31 (53.4)                                                   | 10 (66.7)                                      | 0.267   |
| Passive smoking*, n (%)              | 32 (43.8)                              | 24 (41.3)                                                   | 8 (53.3)                                       | 0.507   |
| Number of people who live with the child, median (IQR) | 5 (4–6)                                | 5 (4–6)                                                     | 5 (4–6)                                        | 0.777   |
| Attending a kindergarten, n (%)       | 50 (68.5)                              | 40 (69.0)                                                   | 10 (66.7)                                      | 0.547   |
| Days of symptoms, median (IQR)       | 3 (2–6)                                | 6 (4–8)                                                     | 3 (2–8)                                        | 0.083   |
| Days of hospitalization, median (IQR) | 4 (3–7)                                | 4 (3–6)                                                     | 4 (2–8)                                        | 0.181   |
| Comorbidities, n (%)                 | 17 (23.3)                              | 14 (24.1)                                                   | 3 (20.0)                                       | 0.517   |
| Asthma                               | 71 (97.3)                              | 56 (96.6)                                                   | 15 (100)                                       | 0.629   |
| Fever                                | 68 (93.2)                              | 54 (93.1)                                                   | 14 (93.3)                                      | 0.729   |
| Dyspnea                              | 53 (72.6)                              | 42 (72.4)                                                   | 11 (73.3)                                      | 0.610   |
| Expectoration                        | 31 (42.4)                              | 22 (37.9)                                                   | 9 (60)                                         | 0.254   |
| Signs, n (%)                         | 1 (1.4)                                | 1 (1.7)                                                     | 0 (0)                                          | 0.795   |
| Alterations in level of consciousness | 4 (5.5)                                | 3 (5.2)                                                     | 1 (6.6)                                        | 0.60    |
| Nasal flaring                        | 3 (4.1)                                | 3 (5.2)                                                     | 0 (0)                                          | 0.496   |
| Pleural effusion                     | 2 (2.7)                                | 1 (1.7)                                                     | 1 (6.7)                                        | 0.371   |
| Severity of the pneumonia*, n (%)    | 56 (76.7)                              | 45 (77.6)                                                   | 11 (73.3)                                      | 0.785   |
| Mild                                 | 16 (21.9)                              | 12 (20.7)                                                   | 4 (26.7)                                       | 0.083   |
| Severe                               | 1 (1.4)                                | 1 (1.7)                                                     | 0 (0)                                          |         |
| Co-infection with other respiratory pathogens, n (%) | 55 (75.3)                              | 45 (77.5)                                                   | 12 (80)                                        | 0.461   |
| Mycobacterium tuberculosis           | 2 (2.7)                                | 1 (1.7)                                                     | 1 (6.6)                                        | 0.371   |
| Respiratory virus*                   | 49 (67.1)                              | 38 (65.5)                                                   | 11 (73.3)                                      | 0.77    |
| Pyogenic bacteria                    | 20 (27.4)                              | 15 (25.8)                                                   | 5 (33.3)                                       | 0.619   |
| Other atypical bacteria*             | 9 (12.3)                               | 6 (10.3)                                                    | 3 (20.0)                                       | 0.269   |

IQR, interquartile range; NPS, nasopharyngeal swab.

* Child living with an adult smoker.

b The severity of the pneumonia was determined according to the classification criteria of the World Health Organization (WHO, 2014).

c Parainfluenza virus 1, 2, 3, or 4, influenza virus A or B, respiratory syncytial virus, human metapneumovirus, adenovirus, human bocavirus, rhinovirus, coronavirus.

d *Chlamydia pneumoniae, Legionella pneumophila, Bordetella pertussis.*
quality), no strains with mutations associated with macrolide resistance were found.

**Sensitivity of the PCR used in the detection of M. pneumoniae**

The sensitivity of each PCR used was determined, with the diagnosis of CAP caused by *M. pneumoniae*, as defined in this study, being considered the reference. The sensitivity of the Seeplex Pneumobacter PCR was 73.8% (54/73) in the induced sputum samples. For the PCR of the *p1* gene used for the typing, the sensitivity was 71.2% (52/73) in the induced sputum samples and 42.4% (31/73) in the NPS samples. Finally, when used to amplify the 23S rRNA gene as a molecular marker, the PCR showed a sensitivity of 49.3% (36/73) in the induced sputum samples and 24.6% (18/73) in the NPS samples.

**Spatial and temporal distribution of the cases of CAP caused by M. pneumoniae**

Georeferencing could be performed for the 48 children who had available home addresses and lived in the urban area of Medellin. The clustering of cases was evident at two sites that were considered of higher risk: the neighborhoods of the northeastern area and District 13 to the west of the city (Figure 2). No significant increase in CAP cases caused by *M. pneumoniae* was found at a specific time point during the study period (Figure 3).

**Discussion**

The results of this study showed that a variant of type 2 *M. pneumoniae* was the dominant genotype among the children with CAP, and that the genotypes associated with macrolide resistance, which were not found in the population studied, do not seem to be present in this cohort.

Since the first description in 1999 (Kenri et al., 1999), variants of type 2 have been reported in different regions. It is believed that variants are generated by homologous recombination between repetitive regions of the *p1* gene and similar regions that are distributed across the bacterial genome (Kenri et al., 1999; Zhao et al., 2011). The present results differ from those obtained by Martinez et al. in Chile, who observed the circulation of type 1 (78.3%) and type 2 (21.7%) strains of *M. pneumoniae*, and a small proportion (7.1%) of variants of type 2 during the 1-year study period (Martinez et al., 2010).

This study is the first to report genotypes of *M. pneumoniae* in Colombia, which means that the genotypic evolution of *M. pneumoniae* in the city of Medellin is unknown. Whether the high frequency of the variant of type 2 identified in these children with CAP is usual in Medellin or is only a result of a type-change phenomenon requires elucidation in further studies.

Although the discriminating power of *p1* genotyping used in this study appears to be lower than the power of other techniques such as multilocus variable-number tandem-repeat analysis (VNTR-MLVA) with four loci, the genotyping used did allow clustering of the *M. pneumoniae* strains in a similar way. Furthermore, the studies of Diaz, Pereyre, Waller, Dumke, and their collaborators described similarities in the distribution of the MLVA types and the types detected with the *p1* gene (Diaz et al., 2015a; Pereyre et al., 2012; Waller et al., 2014). These authors found that MLVA type 4572 is distributed similarly to type 1 of the *p1* gene, and that MLVA types 3562 and 3662 are distributed similarly to type 2 of the *p1* gene and its variants.

The association of a specific *p1* genotype with clinical characteristics or the severity of infection caused by *M. pneumoniae* has not been demonstrated to date. However, some authors have found an association between MLVA typing and the severity of the disease or macrolide resistance (Pereyre et al., 2012; Waller et al., 2014), while others have not found such an association (Qu et al., 2013; Ho et al., 2015).

The development of new typing techniques and/or the refining of existing techniques have allowed the identification of *M. pneumoniae* with greater success. Spuesens et al. performed whole genome sequencing (WGS) of 20 strains of *M. pneumoniae* obtained from persons with and without symptoms, and did not find a specific genotype of *M. pneumoniae* associated with virulence (Spuesens et al., 2016). More recently, Diaz et al. performed WGS analysis on 107 strains of *M. pneumoniae* and found six distinct subtypes (three subtypes in type 1 and three in type 2), although they did not report information regarding the genetic features associated with the clinical outcomes (Diaz et al., 2017). The authors concluded that WGS will improve the ability to monitor the circulation of various *M. pneumoniae* types, as well as identify the emergence of new variants or genetic features that may impact transmission or virulence.

No mutations associated with macrolide resistance were found in the sequences of the 23S rRNA gene obtained in this study. This finding is consistent with those of studies reported from countries such as Denmark (1–3%) and Germany (1–3%), as well as the Netherlands and Thailand, where the resistance rate is zero (Spuesens et al., 2012; Jacobs, 2012; Dumke et al., 2010b). However, the results are in contrast to those reported from certain Asian countries, where the percentage of macrolide-resistant *M. pneumoniae* has been high, with rates above 90% in some studies (Zhao et al., 2013). In those countries, the rates of Streptococcus pneumoniae isolates with lower sensitivity to penicillin are also high, which could be a consequence of the excessive use of

![Figure 1. Distribution of genotypes in the respiratory samples assessed: genotypes and variants of *Mycoplasma pneumoniae* identified through typing of the *p1* gene in the respiratory samples of children with community-acquired pneumonia collected in Medellin, Bello, Envigado, and Itagui, Colombia, from August 2011 to September 2012.](image-url)
antibiotics as empirical treatment for respiratory infections, mainly in children (Kawai et al., 2013; Saraya et al., 2014; Bébéar et al., 2011; Zhao et al., 2013). The relationship between previous exposure to macrolides and the development of resistance to these antibiotics in *M. pneumoniae* has been addressed by several authors. Winchell et al. reported that the administration of macrolides during the 13 days prior to study enrollment was higher in patients with resistant strains (71%) than in patients with sensitive strains (23%) (Díaz et al., 2015b), results that were similar to those found by Kawai et al. (2013). In the present study, the prior administration of antibiotics for more than 72 h was an exclusion criterion for patient recruitment; therefore, the children included in this study were not significantly exposed to macrolides, which could explain, in part, the absence of mutations associated with

Figure 2. Spatial distribution of the cases of community-acquired pneumonia (CAP) caused by *Mycoplasma pneumoniae*: mapping in Medellín of the spatial distribution of the 48 cases of CAP associated with *M. pneumoniae*, according to the scale of the Getis–Ord Gi* index. Two risk areas were found in the city based on the scale of colors determined by the index.
macrolide resistance. Indeed, the percentage of those receiving macrolides during the week before enrollment did not exceed 4.5% (1/22).

Three aspects of this study should be highlighted. First, the results showed the superiority of the induced sputum sample over the NPS sample (78.0%, 57/73 vs. 45.2%, 33/73) for obtaining a positive PCR result for the diagnosis of *M. pneumoniae*, results very similar to those of other authors (Herrera et al., 2016; Collier and Clyde, 1974; Cho et al., 2012; Rátý et al., 2005). Second, the variability of the sensitivity of the different PCR protocols used on induced sputum was notable. These differences could have been due to the diversity of the amplified regions in the different genes, or to the different conditions under which the PCR was performed. In the case of the 23S rRNA gene, the sensitivity varied between 72.6% and 90.7% (Spuesens et al., 2012; Eshaghi et al., 2013; Yamada et al., 2012), and for the p1 gene, the variation was much higher, from 21% up to 90% (Thurman et al., 2009; Ratliff et al., 2014). Third, a significant number of cases were diagnosed only by paired serology (n = 15); all PCR assays were negative. This can be explained by problems in the sensitivity of the PCR protocols or because many of these infections were subclinical. In these cases, the *M. pneumoniae* infection could have been added to the real causative agent of the CAP (another respiratory pathogen, such as a virus or other bacterium), although the percentages of mixed infection were similar in this group and in the patients showing any positive PCR. Considering the aspects mentioned regarding the PCR techniques for *M. pneumoniae*, especially as the paired serology could have detected cases that were not found by PCR (15/73 = 20.5%), and because the serological approach is not practical since it requires two measurements (one in the acute phase and one in the convalescent phase of the disease), the design of better diagnostic tests, the redesign of current tests, or the identification of new molecular targets is imperative. This should be done through appropriately validated studies in diverse groups of patients and with different clinical and epidemiological conditions.

Another aspect that should be evaluated is the possibility that the same patient was a carrier of different genotypes in the respiratory samples assessed. Although the genotype was consistent in both samples in 98% of the patients in this study, two different genotypes could be detected for at least one patient. This suggests the possibility that infections by this microbe are compartmentalized in different sites of the respiratory tract, similar to the situation described for other agents such as *Pneumocystis jirovecii* (Helweg-Larsen et al., 2001). Thus, further studies are needed to determine the clinical and epidemiological implications of this finding.

The findings of the spatial analysis indicated the presence of two risk areas in the city where the concentration of cases was higher than in the surrounding areas. This distribution is similar to the distribution previously reported in a spatial analysis of tuberculosis cases in the city (Molina, 2012), suggesting the likelihood of the presence of certain factors (social, environmental, geographical, and health) that favor the acquisition and dissemination of respiratory pathogens in these zones. These factors should be investigated further in future studies.

The absence of *M. pneumoniae* cultures from the different respiratory samples could have been a limitation of this study. However, culture of *M. pneumoniae* is not practical because it is cumbersome, lacks sensitivity, and is expensive. Furthermore, the short time period of patient recruitment – only 14 months (from August 2011 to September 2012) – limits the epidemiological information on *M. pneumoniae* in this study. Time-series studies over periods of at least 4 years would be necessary to allow a confident assessment of the changes in circulation of the different genotypes.

Finally the *M. pneumoniae* strains associated with these children with CAP belonged to a variant of type 2, and only a small proportion of the samples were identified as belonging to type 1. Currently, no strains associated with resistance are circulating in this environment.

**Ethical approval and consent to participate**

This study had the approval of the Ethics Committee of the School of Medicine at the University of Antioquia (proceeding number 001 of the year 2015). The previous study, during which the children were recruited, was approved by the same committee (proceeding number 017 of the year 2011) and at each one of the 13 participating institutions. Written informed consent was obtained from the parents or guardians of each child, and informed consent was obtained from children older than 7 years. Furthermore,
additional consent to store the samples and use them in subsequent research was obtained. Only patients who authorized the use of their samples were included.

Conflict of interest

The authors declare that they have no competing interests.

Author contribution

All of the authors participated in the study design and interpretation of the data, contributed to the intellectual content, revised the manuscript, and approved the final version.

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