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Detection of *Mycoplasma pneumoniae* in Pediatric Patients with Community-Acquired Pneumonia in Western Iran

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ABSTRACT

Background: *Mycoplasma pneumoniae* strains are among the main causes of community-acquired pneumonia (CAP) in humans. Early detection of this microorganism is important to improve treatment efficiency. This study aimed to detect *M. pneumoniae* (MP)-specific immunoglobulin M (IgM) and MP DNA among pediatric patients with CAP during one week after admission. .

Materials & Methods: From September 2019 to February 2020, 56 CAP patients aged 5 to 15 years were investigated for the presence of MP. Throat swabs for molecular detection of MP and blood samples for detection of cold agglutinins and MP-specific IgG and IgM antibodies were collected at admission. Blood and throat samples were taken again 6 days after admission. Macrolide resistance due to mutations in the 23S rRNA gene was also investigated

Findings: MP-specific IgM was found in 19.6%, IgG in 16.1%, and cold agglutinins in 26.8% of CAP patients. The combination of IgM+IgG was not found. Tachypnea and the need for intensive care were more common in IgM-positive than in IgM-negative patients. Only four patients were positive for MP DNA, of whom two patients carried macrolide-resistant isolates. One isolate had an A2063G mutation and the other had an A2064C mutation.

Conclusion: To the best of our knowledge, there are no data on the epidemiology of MP in 5-15-year-old patients with CAP in Kurdistan, western Iran. The possibility of false-positive or -negative reactions and co-presence with other microorganisms could not be excluded.

Keywords: Respiratory tract infection, *Mycoplasma pneumoniae*, Serology, Molecular diagnostic techniques, Macrolides, Drug resistance .

CITATION LINKS

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Introduction

Mycoplasma pneumoniae is one of the main causes of respiratory tract infections, including tracheobronchitis, bronchiolitis, and community-acquired pneumonia (CAP) [1, 2]. About 10-20% of CAP cases are caused by this cell wall-deficient pathogen [3], with a higher frequency in autumn and winter than in other seasons [4, 5]. Significantly, in school-aged children aged 5 to 15 years, *M. pneumoniae* (MP) is responsible for up to 40% of CAP cases [6].

The presumptive diagnosis of suspected MP pneumonia is often made based on non-specific clinical and radiological findings [7, 8]; however, mycoplasmas are not susceptible to beta-lactam antibiotics used for empirical therapy of pneumonia, which highlights the importance of a specific diagnostic tool [1]. Respiratory infections caused by MP are currently diagnosed by serological methods and/or detection of bacterial DNA in upper respiratory tract samples as advised by the British Thoracic Society and the Infectious Diseases Society of America [9, 10]. Serodiagnostic tests, especially enzyme-linked immunosorbent assays (ELISAs), are the most commonly used methods to detect MP [1, 2]. Measurement of MP-specific immunoglobulin M (IgM) titers in serum is widely used to diagnose MP, especially in children. IgM antibodies appear during the first week of the disease and could be detected in the early phase of MP infection. Detection of a four-fold or greater increase in immunoglobulin G (IgG) levels is also used for MP diagnosis; however, the need for paired sera makes it less useful in the management of pediatric patients [2, 11]. Before the development of more advanced serological methods, cold agglutinin detection was regarded as a valuable tool for MP diagnosis. Cold agglutinins often appear at the onset or in the first week of MP infection [12]. Although this test has several drawbacks, it is still used, especially in low-income countries [13]. Nucleic acid amplification techniques have also been increasingly used for the identification of respiratory bacteria, including MP. Since there are regions with a high degree of sequence conservation in the *P1* gene (encoding P1 adhesin) of MP, its amplification has been reported to be a sensitive method [14].

Effective management of MP respiratory infections is usually achieved with macrolides [15]; however, the widespread use of these antimicrobials has led to an increase in the prevalence of macrolide-resistant MP strains, especially in Asian countries [16, 17]. Macrolide resistance in MP is caused by point mutations in the V domain of the 23S rRNA gene. An A to C transversion at position 2064 or an A to G transition at position 2063 of this gene may confer a high level of macrolide resistance. Low-level macrolide resistance is induced by a C to G/A transversion at position 2617 and an A to G transition at position 2067 [18].Early detection of MP is necessary to improve treatment efficiency and avoid further complications and unnecessary use of antimicrobials. To the best of our knowledge, there are no data on the epidemiology of MP in CAP patients aged 5 to 15 years in western Iran.

Objectives: This study aimed to detect MP-specific IgM and MP DNA in pediatric CAP patients aged 5 to 15 years during one week after admission.

Materials and Methods

Ethical statement: This study was approved by the Ethics Committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1398.160). Written informed consent was obtained from parents or legal guardians of all pediatric patients before enrollment in this study.

Study design: This cross-sectional study was conducted from September 2019

to February 2020 on 5-15-year-old CAP patients attending hospitals affiliated to Kurdistan University of Medical Sciences in Sanandaj city (the capital of Kurdistan province), western Iran.

Demographic and clinical data were collected from all patients, including information about age, cough, fever, difficulty breathing, need for ventilator, and history of hospital admission due to pneumonia within the past four months. Clinical examination, chest radiography, and C-reactive protein (CRP) test were conducted on all patients on admission.

Inclusion criteria for patients were as follows: the presence of fever and/or acute respiratory symptoms (cough, chest pain, tachypnea, difficulty breathing, and sputum production) and the presence of infiltrate/shadow and pulmonary consolidation on chest radiography. Patients with the following criteria were excluded from the study: hospital-acquired pneumonia; immune deficiency and chronic diseases such as cystic fibrosis, cancer, and tuberculosis; pulmonary shadow due to causes other than pneumonia; and history of lung transplantation or immunosuppressive therapy. The study patients received no antibiotics during the 72 hours before sample collection.

Samples: Respiratory tract specimens (throat swabs) for molecular detection of MP and blood specimens for detection of cold agglutinins and MP-specific IgM and IgG antibodies were collected at admission from patients diagnosed with CAP. Blood and throat samples were taken again 6 days after admission.

Serological testing: Venous blood samples were used to measure cold agglutinins and IgM and IgG antibodies by ELISA. For serological tests, serum was separated from blood by centrifugation and stored at -20 °C until use.

The cold agglutinin test was performed as described previously [19]. Briefly, two-fold serial serum dilutions were made, and an equal volume of a 1% suspension of washed red blood cells was added to each serum dilution. After incubation at 4 °C, erythrocyte agglutination was investigated. A titer of ≥1:32 was considered as positive.

Detection of MP-specific IgM and IgG antibodies was performed and interpreted by ELISA using commercially available kits (Serion, Germany) according to the manufacturer's recommendations.

Molecular identification: PCR test was performed on throat swab samples to detect MP. Swabs were gently rubbed over the pharyngeal tonsil, the arch of the soft palate and uvula, the other pharyngeal tonsil, and then the posterior pharyngeal wall. The swab samples were placed into phosphate-buffered saline and immediately subjected to DNA extraction. Genomic DNA was extracted from 200 µL of samples using a QIAamp DNA mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The DNA samples were stored at -20 °C until analysis. DNA concentration was determined by measuring the absorbance at 260 nm, and the ratio of absorbance at 260 and 280 nm was used to measure the purity of DNA extracts. A range of ~1.6-2.0 was considered optimal.

To identify MP, a conserved 345-bp fragment of the *P1* gene (encoding P1 adhesin) was amplified by PCR using the following primers as previously described: 5'AGGCTCAGGTCAATCTGGCGTGGA3' and 5' GGATCAAACAGATCGGTGACTGGG3' [20]. The final volume of the reaction was 20 µL containing Taq PCR Master Mix (Ampliqon, Denmark; Taq DNA polymerase in reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP), 1 µM of each forward and reverse primer, and 1 µg of template DNA. The amplification was performed in a thermal cycler (Eppendorf,

Germany) under the following conditions: one cycle at 94 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplified products were electrophoresed on a safe stain–stained 1% agarose gel along with a 100-bp DNA ladder. The standard strain MP ATCC 29342 was used as a positive control.

Macrolide resistance detection: To identify macrolide resistance genotypes, a 1498-bp fragment of the 23S rRNA gene in the genome of MP was amplified using the primers 5' GGACAACAGGTTAATATTCCTG 3' and 5' CAATAAGTCCTCGAGCAATTAG 3' and sequenced as described previously [21]. This assay detects point mutations at four positions within the gene, namely 2063, 2064, 2067, and 2617. The cycling program was as follows: one cycle at 94 °C for 5 min, then 30 cycles at 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. The nucleotide sequences were determined with an ABI 3730XL DNA sequencer (Macrogen, Korea). The sequence data were aligned with the sequence of the macrolide-sensitive standard strain M129 registered at NCBI (http://www.ncbi.nlm.nih.gov) using BioEdit software Version 7.05.3.

Data analysis: Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) (Ver. 21; IBM Corporation, USA). Variables were compared using Pearson Chi-square and Fisher's exact tests (when appropriate). The level of significance was $p< .05$.

Findings

Characteristics of patients: In this study, 56 CAP patients aged 5 to 15 years were investigated for the presence of MP-specific IgM antibodies during one week after admission. All 56 patients were hospitalized based on the physician's decision.

Table 1 shows the characteristics of patients enrolled in this study. The mean age of the study patients was 7.94±2.48 years (mean ± standard deviation); 27 (48.2%) patients were 5-7 years old; therefore, 29 (51.8%) patients were 8-15 years old. Out of the 56 patients, five (8.9%) patients required critical care in the intensive care unit (ICU). Most of the patients were from low-income families (n=25, 44.6%), and 18 patients (32.1%) were exposed to passive smoking. Non-productive (dry) cough (n=38, 67.9%) and tachypnea (n=29, 51.8%) were the most prevalent symptoms. Hospital admission due to pneumonia within the past four months was found in 18 (32.1%) patients. CRP test was negative in 20 patients (35.7%); therefore, 36 (64.3%) patients showed positive reaction.

Radiological findings were also investigated and classified as interstitial infiltration, lobar infiltration, multi-lobar infiltration, and pleural effusion. All 56 cases (100%) showed lung involvement, and approximately half of them (48.2%) had interstitial infiltration. Pleural effusion had the lowest prevalence (n=5, 8.9%).

Moreover, the characteristics of 5-7-year-old and 8-15-year-old patients were compared (Table 2). Children above 7 years of age normally develop more complex sentences than younger children. They speak better and are more able to communicate with their classmates and other people, which might affect the frequency of symptoms in this group. In this study, tachypnea, cough (productive and non-productive), and ICU admission were more common in older patients, though it was not significant. Cough was present in 77.8% of 5-7-year-old patients and 93.1% of older patients. The need for intensive care and tachypnea were recorded in 3.7 and 44.4% of the younger group and lower than 13.8 and 58.6% in the older group, respectively. CRP was positive in

Table 1) Demographic, clinical, and radiological characteristics of 5-15-year-old pediatric patients with community-acquired pneumonia

Parameter		Number (N=56)	Percent
Age (years)	5-7	27	48.2
	8-15	29	51.8
Residency place	Village	22	39.3
	City	34	60.7
Income	High	12	21.4
	Middle	19	33.9
	Low	25	44.6
Passive smoking		18	32.1
Cough	Non-productive	38	67.9
	Productive	10	17.9
	Negative	8	14.3
Tachypnea		29	51.8
History of hospitalization	Once	17	30.4
	Twice	1	1.8
	Negative	38	67.9
Need for ventilation		26	46.4
ICU admission		5	8.9
Radiological findings	Lobar infiltration	7	12.5
	Multi-lobar infiltration	17	30.4
	Interstitial infiltration	27	48.2
	Pleural effusion	5	8.9

ICU: intensive care unit

70.4% (n=19) of the 5-7-year-old group and 58.6% (n=17) of the older group (p= .36). Among the demographic characteristics, passive smoking was more common in the younger group.

Multi-lobar infiltration was the most common radiological finding among the older group (37.9%), while 5-7-year-old patients mostly presented interstitial infiltration (63%). However, these differences were not significant between the two groups (p > .05).

Serology: Out of the 56 patients, MP-specific IgM antibodies were found in 11 (19.6%) patients, IgG antibodies were detected in nine (16.1%) patients, and 15 cases (26.8%) had positive CAT. Out of 11 IgM-positive patients, eight cases were CAT positive, and none of them were IgG-positive.

The characteristics of patients in the IgM-positive and IgM-negative groups were further analyzed (Table 3). Tachypnea, passive smoking, and the need for intensive care were more common in the positive group than in the negative group. In the positive group, 72.7% of patients were exposed to passive smoking, and 27.3% needed intensive care, while in the negative group, these values were 22.2 and 4.4%, respectively. Furthermore, 81.8% of positive patients had tachypnea, while this value was lower in the negative group (44.4%). As shown in Table 3, IgM-positive patients were older, and the prevalence of IgM in low-income patients was higher than in high- and middle-income patients. Positive CRP test was more common in the IgM-positive group than in the IgM-negative group (10 of 11, 90.9% vs. 26 of 45, 57.8%, respectively).

Table 2) Comparison of characteristics between 5-7-year-old and 8-15-year-old patients with community-acquired pneumonia

Parameter		*Age		*P* Value	Total, N=56
		5-7 years, N =27 N (%)	8-15 years N=29 N (%)		
Passive smoking		12 (44.4)	6 (20.7)	.06	18
Income	Low	10 (37)	15 (51.7)	.51	25
	Middle	10 (37)	9 (31)		19
	High	7 (25.9)	5 (17.2)		12
Radiological findings	Lobar infiltration	3 (11.1)	4 (13.8)	.18	7
	Multi-lobar infiltration	6 (22.2)	11 (37.9)		17
	Interstitial infiltration	17 (63)	10 (34.5)		27
	Pleural effusion	1 (3.7)	4 (13.8)		5
Cough	Negative	6 (22.2)	2 (6.9)	.19	8
	Non-productive	18 (66.7)	20 (69)		38
	Productive	3 (11.1)	7 (24.1)		10
History of hospitalization	Negative	21 (77.8)	17 (58.6)	.12	38
	Once	5 (18.5)	12 (41.4)		17
	Twice	1 (3.7)	0		1
ICU admission		1 (3.7)	4 (13.8)	.35	5
Tachypnea		12 (44.4)	17 (58.6)	.28	29
Need for ventilation		13 (48.1)	13 (44.8)	.8	26

*Data are presented as numbers (%). ICU: intensive care unit. Variables were compared using Pearson Chi-square or Fisher's exact tests.

In both IgM-positive and IgM-negative groups, the frequency of non-productive cough was more than productive cough. Interstitial infiltration, pleural effusion, lobar infiltration, and multi-lobar infiltration were numerically comparable in the positive and negative groups (Table 3). Interstitial infiltration was more common in patients than other lung involvement types, regardless of IgM positivity or negativity (45.5 and 48.9%, respectively). Furthermore, 18.2% of IgM-positive patients showed pleural effusion compared to 6.7% of IgM-negative patients.

Molecular detection of MP and macrolide resistance: DNA extracted from throat swabs was used for molecular detection of MP by amplifying a conserved 345-bp fragment of the *P1* gene (encoding P1 adhesin). Out of the 56 patients, only four (7.1%) patients had a positive PCR test. Furthermore, in order to identify resistance to macrolides, a 1498-bp fragment of the 23S rRNA gene in the genome of MP was amplified and sequenced. Of the four MP isolates, two isolates were macrolide-resistant. One isolate had an A-G transition at nucleotide 2063 in the 23S rRNA gene, and the other isolate had an A2064C mutation. No point mutations were found at other positions relevant to macrolide resistance in the 23SrRNA gene. Four PCR-positive patients were also positive in IgM serology test.

Discussion

Respiratory tract infections with MP are common in most regions of the world, with outbreaks occurring at intervals of 3-7 years [6]. In this study, the presence of MP-specific IgM antibodies and MP DNA in pediatric patients with CAP was investigated within one week after admission.

Atypical pneumonia could be distinguished from typical pneumonia by its mild clinical

Table 3) Comparison of *M. pneumoniae*-specific IgM-positive and -negative patients with community-acquired pneumonia

Variable		IgM positive, N=11 N (%)	IgM negative, N=45 N (%)
Passive smoking		8 (72.7)	10 (22.2)
Tachypnea		9 (81.8)	20 (44.4)
ICU admission		3 (27.3)	2 (4.4)
Age (years)	5-7	4 (36.4)	23 (51.1)
	8-15	7 (63.6)	22 (48.9)
Income	Low	6 (54.5)	19 (42.2)
	Middle	2 (18.2)	17 (37.8)
	High	3 (27.3)	9 (20)
Need for ventilation		7 (63.6)	19 (42.2)
Cough	Negative	2 (18.2)	6 (13.3)
	Non-productive	5 (45.5)	33 (73.3)
	Productive	4 (36.4)	6 (13.3)
History of hospitalization	Negative	7 (63.6)	31 (68.9)
	Once	4 (36.4)	13 (28.9)
	Twice	0	1 (2.2)
Radiological findings	Lobar infiltration	2 (18.2)	5 (11.1)
	Multi-lobar infiltration	2 (18.2)	15 (33.3)
	Interstitial infiltration	5 (45.5)	22 (48.9)
	Pleural effusion	2 (18.2)	3 (6.7)

*Data are presented as numbers (%); ICU: intensive care unit

course [3, 22]. Tachypnea and non-productive cough were the most common symptoms in the studied patients, in agreement with previous reports [3, 4]. Furthermore, cough, difficulty breathing, and tachypnea were more common in patients aged 8 to 15 years compared to 5-7-year-old patients. These observations showed that patients in the older group required more intensive care in the ICU and had a more severe disease compared to the 5-7-year-old group. Children older than 7 years are normally more able to communicate with their classmates and other people than younger children, which might explain the higher frequency of symptoms in this group.

The role of MP in respiratory infections has been studied in different regions, but the prevalence of MP varies considerably between studies. Among the patients investigated for this atypical pathogen in this study, 19.6% were positive for IgM antibodies. A study in Tehran, Iran found MP in 21.8% of samples [16]. Also, 19.4% of clinical samples in a study in India were found to be MP positive [23], and in another study in China, 25.5% of samples were positive for MP [17]. Although geographical regions could alter the prevalence of MP in respiratory infections, heterogeneity in epidemiology, diversity in clinical samples, sampling time, study population, and applied diagnostic methods could influence the results of studies. The frequency of MP is markedly higher during outbreaks. In a study conducted by Thurman et al. (2009) on 97 patients during two CAP outbreaks in the USA, about 81% of patients yielded positive results for MP [24].

When the characteristics of IgM-positive and IgM-negative patients were compared, smoking and tachypnea were more frequent in patients with IgM. A higher prevalence of IgM was observed in 8-15-year-old patients,

which might be due to more communication of patients in this group with others compared to 5-7-year-old children. Furthermore, the clinical manifestations of patients with IgM were featured by non-productive cough with no or little sputum. Coughing is of prime importance for transmitting infection from patients in the acute stages of MP infection[25]. The nonspecific presentation of MP infections highlights the importance of effective laboratory diagnostic tools. Many bacterial and viral infections could cause similar symptoms which are difficult to distinguish clinically [7, 8]. A sensitive and effective method for early detection of these agents is required to improve treatment efficiency and avoid unnecessary antibiotic use and further complications. The current standard test for diagnosing MP involves the detection of specific IgM or a ≥4-fold rise in IgG titer; however, convalescent-phase sera are not always available, and a rise in IgG titers is not helpful for early treatment of patients. Identification of MP-specific IgM titers in serum is widely used to diagnose MP, especially in children. Specific IgM antibodies almost emerge within 7 days after the onset of the disease and could be detected in the acute stage of the disease [2]. Before the development of advanced serological methods, cold agglutinin detection was regarded as a valuable tool for MP diagnosis. In this study, 26.8% of patients were positive for cold agglutinins. Cold agglutinin formation is the first humoral immune response to MP [12]. Although CAT has been identified as a test that should be interpreted with caution, it continues to be used, particularly in low-income countries, and optimization of cold agglutinin measurement for diagnosing MP is in progress [26].

Compared to conventional serum IgM antibody determination, nucleic acid amplification techniques such as PCR approaches have progressively been explored for early detection of MP in respiratory tract specimens [1]. There are differences between reports regarding the prevalence of MP by PCR. In a study in India, MP was positive in 16 (6.5%) patients by PCR: nine (56.2%) patients had serologically proven, and seven (43.7%) patients had serologically unproven MP infection [1]. In another study in Spain, MP was detected in 16.8% of cases by PCR, with an overall agreement with serology result of 76% [7]. In a study in Iran, among 270 specimens, MP was detected in 25.2% of samples using PCR [16]. In another study in China, among 835 clinical samples collected from pediatric patients with suspected MP infection, 25.5% tested positive for MP by PCR [17]. In the Netherlands, of 4390 specimens collected from patients with respiratory infections, 2.6% were positive for MP using PCR assay [27]. This heterogeneity between studies regarding the prevalence of MP by PCR could be due to different PCR types, different samples, or different sampling times. In this study, amplification of the *P1* gene was positive in four (7.1%) out of 56 samples tested. These four PCR-positive patients were also positive in IgM serology test. Therefore, seven IgM-positive patients showed negative results by PCR. Serologically positive cases with negative PCR results may be due to inhibitors or other problems in the assay, or may be due to low levels of MP load or DNA in some specimens[4, 17].

There are heterogeneities between studies regarding the diagnosis of MP by PCR and serology, and the diagnosis of MP-induced CAP could not be based exclusively on the detection of DNA in respiratory samples. Zhang et al. (2011) conducted a systematic review and meta-analysis on the diagnosis of MP by PCR and serology and reported inconsistent results and significant heterogeneity between the reviewed studies

[28]. Medjo et al. (2014) found that the sensitivity of IgM serology and PCR tests was equal (81.82%) for the detection of MP in children aged between 1 and 15 years [4]. Zhao et al. (2020) found that of 92 cases that were positive for MP DNA, 80 patients were also positive for IgM antibody [25]. However, Menendez et al. (1999) reported that PCR was less sensitive than serological methods for detecting MP in throat swab samples: 11.4% of patients were positive by serology, while only 1.6% were positive by PCR [29]. Chang et al. (2014) reported that compared to IgM serology as a gold standard, the sensitivity and specificity of PCR were 52.3 and 89.9%, respectively [30]. In a study by Rivaya et al. (2020), the sensitivity and specificity of PCR compared to serology were 55 and 92%, respectively [7]. In another study by Kumar and colleagues (2019), the sensitivity and specificity values of PCR were 16.18 and 95.48%, respectively [1]. Therefore, the results of MP diagnostic tests should be interpreted with caution.

MP infections could be controlled by macrolide therapy; however, the prevalence of macrolide-resistant strains has strikingly increased. Since macrolide resistance is induced by point mutations in the 23S rRNA gene, it is useful to apply molecular methods to detect these mutations [18]. Recent reports have documented high levels of macrolide resistance in MP isolates, with prevalence ranging from 0 to 15% in Europe and the USA and up to 90-100% in Asia [15, 16]. In a study in Iran, approximately 56.9% of MP isolates from CAP patients were resistant to macrolides [16]. In another study in China, among 223 MP-positive samples from six cities, 186 specimens were successfully tested for mutations in the 23S rRNA gene due to low levels of DNA in some specimens. Of them, 76.3% had mutations associated with macrolide resistance. The resistance rate in different cities ranged from 20 to 86.7% [17]. In the present study, out of four isolates detected by PCR, two isolates showed resistance to macrolides with an A-G transition at position 2063 and an A2064C mutation in the 23S rRNA gene. The presence of MP DNA with the A2063G mutation seems to be associated with severe disease [31]. Therefore, macrolide resistance needs to be investigated so that therapeutic strategies could be applied rapidly.

Limitations and conclusion

There are limitations in this study. The small number of samples was one of the limitations of this study. Due to the outbreak of COVID-19, we were unable to continue sampling. A larger sample size may provide a better understanding of the epidemiology of MP. Furthermore, the possibility of false-positive or -negative reactions and co-presence with other bacterial or viral microorganisms in some patients could not be excluded.

In conclusion, this study demonstrated that a relatively high proportion of 5-15-year-old CAP patients in western Iran were positive for MP-specific IgM antibodies. Caution should be taken to evaluate MP diagnostic tests. Improving diagnostic quality may contribute to reducing the use of macrolides and therefore preventing macrolide resistance. To the best of our knowledge, this study is the first report about the prevalence of MP in 5-15-year-old CAP patients in Kurdistan, western Iran. Further studies with multicenter designs and larger sample sizes as well as more sensitive methods such as quantitative techniques may help confirm our findings.

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from the Research Ethics Committee (REC), Faculty of Medicine, Kurdistan University of Medical Sciences (Ethical code: IR.MUK.REC.1398.160). An informed consent was obtained from all patients. All methods were carried out in accordance with relevant guidelines and regulations.

Authors' contributions: R.A. performed the study. S.D. analyzed the data. M.A. designed the study. All authors approved the manuscript.

Conflicts of interests: The authors declare that they have no conflict of interest.

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