Role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in Children with Community-Acquired Lower Respiratory Tract Infections

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In order to evaluate the role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, we studied 613 children aged 2–14 years who were hospitalized for community-acquired lower respiratory tract infections (LRTIs). The patients were enrolled in the study by 21 centers in different regions of Italy from May 1998 through April 1999. Paired serum samples were obtained on admission and after 4–6 weeks to assay the titers of *M. pneumoniae* and *C. pneumoniae* antibodies. Nasopharyngeal aspirates for the detection of *M. pneumoniae* and *C. pneumoniae* were obtained on admission. Acute *M. pneumoniae* infections in 210 patients (34.3%) and acute *C. pneumoniae* infections in 87 (14.1%) were diagnosed. Fifteen of the 18 children with *M. pneumoniae* and/or *C. pneumoniae* infections whose treatments were considered clinical failures 4–6 weeks after enrollment had not been treated with macrolides. Our study confirms that *M. pneumoniae* and/or *C. pneumoniae* plays a significant role in community-acquired LRTIs in children of all ages and that such infections have a more complicated course when not treated with adequate antimicrobial agents.

Lower respiratory tract infections (LRTIs) are a common cause of morbidity and mortality among children [1, 2], and a large number of studies over the past decade have addressed the problems of their diagnosis and management. Determining the etiology of these clinical manifestations is a challenge, because the diagnostic tests of respiratory samples that are noninvasively obtained are insufficiently sensitive to identify the causative pathogen [3, 4]. Empirical therapy is therefore adopted in most cases [5, 6].

On the basis of the limited published data, pediatricians have developed a functional algorithm with regard to the most important organism to treat against in different age groups. These causative organisms are believed to be mainly respiratory viruses, *Streptococcus pneumoniae* and *Haemophilus influenzae* in young children and *S. pneumoniae* and *Mycoplasma pneumoniae* in older children [7, 8]. Few data are available with regard to *Chlamydia pneumoniae* in pediatric age groups [9, 10].

Some studies have recently shown that *M. pneumoniae* and *C. pneumoniae* seem to play a more significant role than previously thought as causes of LRTIs

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Appropriate informed consent was obtained and the research was conducted in accordance with the guidelines for human experimentation specified by the authors' institutions.

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Table 1. Incidence rates of acute *Mycoplasma pneumoniae* infection on the basis of serological and PCR findings.

Positive rest result, diagnosis	Proportion (%) of patients with infection, by age group			
	2–4 y	5–7 y	>7 y	All
Serological				
Acute bronchitis	12/62 (19.3)	8/34 (23.5)	9/17 (52.9)	29/113 (25.6)
Wheezing	12/53 (22.6)	4/15 (26.6)	7/14 (50.0)	23/82 (28.0)
Pneumonia	42/209 (20.0)	53/123 (43.0)	47/86 (54.6)	142/418 (33.9)
All	66/324 (20.4)	65/172 (37.8)	63/117 (53.8)	194/613 (31.6)
PCR				
Acute bronchitis	8/62 (12.9)	6/34 (17.6)	6/17 (35.3)	20/113 (17.7)
Wheezing	7/53 (13.2)	3/15 (20.0)	4/14 (28.6)	14/82 (17.1)
Pneumonia	33/209 (15.8)	36/123 (29.3)	39/86 (45.3)	108/418 (25.8)
All	48/324 (14.8)	45/172 (26.2)	49/117 (41.9)	142/613 (23.2)
Serological and/or PCR				
Acute bronchitis	14/62 (22.5)	10/34 (29.4)	12/17 (70.5)	36/113 (31.8)
Wheezing	12/53 (22.6)	5/15 (33.3)	7/14 (50.0)	24/82 (29.2)
Pneumonia	43/209 (20.5)	56/123 (45.5)	51/86 (59.3)	150/418 (35.8)
All	69/324 (21.3)	71/172 (41.3)	70/117 (59.8)	210/613 (34.3)

in children of all ages [11–14]. A specific diagnosis is important, because β-lactam antibiotic treatment of infections due to these atypical pathogens is ineffective, whereas the use of antibiotics such as macrolides can markedly reduce the duration of the illnesses [15, 16].

Since it is difficult to detect *M. pneumoniae* and *C. pneumoniae,* in clinical practice specific etiologic diagnoses are established in only a minority of cases. The detection of antibodies in paired serum samples has been considered the standard laboratory diagnostic method [17, 18], but PCR has recently been found to be useful for rapidly detecting these pathogens in respiratory secretions [19–21].

The aim of this study was to investigate the role of *M. pneumoniae* and *C. pneumoniae* in community-acquired LRTIs in children, with use of both serological tests and PCR analysis.

PATIENTS AND METHODS

Study design. This was a prospective, multicenter trial designed to evaluate the incidence of *M. pneumoniae* and *C. pneumoniae* infection in hospitalized children with community-acquired LRTIs. The patients were enrolled by 21 centers in different regions of Italy from May 1998 through April 1999. The study protocol was approved by the institutional review boards of each center.

Inclusion/exclusion criteria. Previously healthy male and female children aged 2–14 years who had been hospitalized for signs and/or symptoms of community-acquired LRTI were considered eligible for inclusion. The exclusion criteria included severe concomitant diseases (neoplasia, kidney or liver disease, immunodepression, cardiovascular disease, malabsorption syndrome), nosocomial infections, and the use of antibiotics in the 48 h that preceded enrollment. A parent or legal guardian was required to provide written informed consent, and older children were asked for their assent before being enrolled in the study.

Enrollment and evaluation of patients. At the time of admission, systematic recordings were made of each patient's medical history, including the date of onset of the current illness, the underlying respiratory symptoms, and the presence of fever (temperature ⩾37.8°C). After a complete physical examination, chest radiographs were obtained and centrally reviewed by an experienced radiologist who did not know the patient's clinical history or laboratory data. On the basis of both clinical and radiological findings, the children were classified into 3 disease groups: (1) acute bronchitis, cough, and/or rhonchi, with a normal chest radiograph; (2) wheezing, cough, and/or dyspnea with expiratory rales and/or wheezes unrelated to any known specific sensitization, with a normal chest radiograph or hyperinflation; and (3) pneumonia, with diffuse or lobar pulmonary infiltration evident on the chest radiograph [22].

The laboratory samples taken at enrollment included venous blood specimens for hematologic and blood chemistry tests (hemochromocytometric, leukocyte formula, erythrocyte sedimentation rate, and C-reactive protein tests), serum for assaying for antibodies to *M. pneumoniae* and *C. pneumoniae,* and nasopharyngeal aspirates for the detection of *M. pneumoniae* and *C. pneumoniae* DNA.

The children were treated according to the judgment of their

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Table 2. Incidence rates of acute *Chlamydia pneumoniae* infection on the basis of serological and PCR findings.

Positive test result, diagnosis	Proportion (%) of patients with infection, by age group			
	2–4 y	5–7 y	>7 y	All
Serological				
Acute bronchitis	2/62 (3.2)	5/34 (14.7)	4/17 (23.5)	11/113 (9.7)
Wheezing	4/53 (7.5)	4/15 (26.6)	3/14 (21.4)	11/82 (13.4)
Pneumonia	11/209 (5.2)	8/123 (6.5)	11/86 (12.7)	30/418 (7.1)
All	17/324 (5.2)	17/172 (9.9)	18/117 (15.4)	52/613 (8.5)
PCR				
Acute bronchitis	3/62 (4.8)	7/34 (20.6)	2/17 (11.7)	12/113 (10.6)
Wheezing	5/53 (9.4)	3/15 (20.0)	3/14 (21.4)	11/82 (13.4)
Pneumonia	10/209 (4.8)	6/123 (4.9)	9/86 (10.4)	25/418 (5.9)
All	18/324 (5.5)	16/172 (9.3)	14/117 (11.9)	48/613 (7.8)
Serological and/or PCR				
Acute bronchitis	5/62 (8.0)	12/34 (35.2)	5/17 (29.4)	22/113 (19.4)
Wheezing	8/53 (15.0)	7/15 (46.6)	4/14 (28.5)	19/82 (23.1)
Pneumonia	18/209 (8.6)	13/123 (10.5)	15/86 (17.4)	46/418 (11.0)
All	31/324 (9.6)	32/172 (18.6)	24/117 (20.5)	87/613 (14.1)

attending pediatricians, in the absence of serological and PCR results. During hospitalization, the results of a detailed physical examination of the respiratory apparatus, any changes in clinical symptoms, and the clinical response to therapy were recorded every day. The patients were discharged from the hospital if they were afebrile and their clinical condition had been stable for 48 h. After hospitalization, the children were asked to return immediately to the study center for evaluation if they experienced any recurrent or worsening signs and symptoms.

The medical history, general physical condition, and clinical symptoms of each child were reevaluated 4–6 weeks after enrollment, and, at the same time, a second serum sample was obtained to assay for convalescent *M. pneumoniae* and *C. pneumoniae* antibody titers. During this evaluation, the clinical response to therapy was defined as "cure" (complete resolution of signs and symptoms of LRTI), "improvement" (incomplete resolution of signs and symptoms of LRTI), or "failure" (the persistence or progression of signs and symptoms of LRTI after 3 days of therapy or the development of new clinical findings consistent with active infection) [23]. The antibiotic treatment was considered evaluable if it had been administered in accordance with the recommended posology and duration [24].

Evaluation of infections. The serum samples were stored at −20°C and later assayed in the central laboratories. Each sample was tested for IgM and IgG antibodies to *M. pneumoniae* (ELISA, Pantec) and for IgM, IgA, and IgG antibodies to *C. pneumoniae* (microimmunofluorescence; Labsystems). The serological tests for *M. pneumoniae* were performed according to the manufacturers' instructions and have a sensitivity limit of 1:100 for both IgM and IgG antibodies. No cross-reactions between *M. pneumoniae* and *Mycoplasma genitalium* with use of the Pantec kit have been described. The serological tests for *C. pneumoniae* were performed according to the technique developed by Wang et al. [25, 26]; a titer of 1:64 was used as the cutoff level for IgG, and 1:16 was used for IgA and IgM.

The nasopharyngeal aspirates were placed in a sucrose-phosphate-glutamate transport medium and stored at −70°C before being sent in dry ice to the central laboratories for *M. pneu-*

Table 3. Distribution of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections by geographic area.

Area	No. (%) of infections			
	M. pneumoniae	*C. pneumoniae*	Both	Neither
Northern Italy ($n = 306$)	77 (25.2)	24 (7.8)	25 (8.2)	180 (58.8)
Central Italy ($n = 151$)	35 (23.2)	14 (9.3)	5 (3.3)	97 (64.2)
Southern Italy ($n = 156$)	56 (35.9)	7 (4.5)	12 (7.7)	81 (51.9)

NOTE. No significant difference was observed in relation to the geographic distribution of either etiologic agent.

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moniae and *C. pneumoniae* DNA detection. Nested PCR was performed for both pathogens by means of validated methods as described elsewhere [27, 28]. The nested PCR for *M. pneumoniae* was developed by Abele-Horn et al. [27], and that for *C. pneumoniae* was developed by Tong et al. [29]; both techniques have been found to correlate well with cultures [27, 29].

Sample preparation, PCR amplification, and product analysis were performed in separate rooms, to avoid the risk of contamination. Positive and negative controls were included in each assay. The negative controls contained all of the PCR reagents and sterile distilled water; in the case of *M. pneumoniae,* we also used DNA from the reference strains *Mycoplasma orale* T519, *Mycoplasma salivarium* A889, and *M. genitalium* G37c, as described elsewhere [27]. Serial dilutions of purified *M. pneumoniae* and *C. pneumoniae* were used as positive controls for all of the runs to ensure successful nucleic acid amplification. All of the PCR-negative samples were analyzed by PCR for the presence of β-actin DNA in order to confirm the presence of DNA in the samples.

The MP-1 and MP-2 primer set was used for *M. pneumoniae*–specific amplification [27]. The reaction volumes for the first and second rounds of amplification were 50 μL, with 0.1 μM of each primer [27]. Amplification was carried out for 40 cycles, each consisting of 20 s at 95°C, 2 min at 63°C, and 1 min at 72°C [24]. For *M. pneumoniae* nested PCR, MUH-1 and MUH-2 primers were used [27]. The nested amplification was performed with 5 μL of a 1:10 dilution of the PCR product (5 μL in 45 μL of sterile water) from the first round of amplification under identical conditions [27].

Touchdown nested PCR for the detection of *C. pneumoniae* DNA was performed with use of primers designed to detect the major outer-membrane protein [28, 29]. Extracted DNA solution (10 μL in a total volume of 50 μL) was used in the first PCR round, and then 5 μL of the PCR products amplified by the outer primers was transferred to a new 50-μL PCR reaction mix for a second amplification using the inner primers [28]. The first round consisted of 40 cycles and the second round of 35 cycles [28].

Acute *M. pneumoniae* and/or *C. pneumoniae* infection was diagnosed if the patient had a significant antibody response to one of the pathogens in paired serum samples (*M. pneumoniae:* IgM specific antibody $\geqslant$1:100, IgG specific antibody $\geqslant$1:400, or a 4-fold increase in IgG antibody titer; *C. pneumoniae:*IgM specific antibody $\geqslant$1:16, IgG specific antibody $\geqslant$1:512, or a 4-fold increase in IgG antibody titer) and/or if the nasopharyngeal aspirates were PCR-positive. Past *M. pneumoniae* infection was diagnosed if the patient had an IgG antibody titer $\geqslant$1:100 but $<$1:400 without a 4-fold increase in paired serum samples; past *C. pneumoniae* infection was diagnosed if the patient had an IgG antibody titer $\geqslant$1:16 but $<$1:512 without a 4-fold increase in paired serum samples and/or an IgA specific antibody titer of $\geqslant$1:16. The serological evidence of positivity was based on criteria established elsewhere [11, 23, 26].

The reference laboratories were the Pediatric Department I at the University of Milan (Milan, Italy) for *M. pneumoniae* serological tests and PCR analysis, and the Institute of Respiratory Diseases of IRCCS Maggiore Hospital, also at the University of Milan, for *C. pneumoniae* serological tests and PCR analysis.

Statistical analysis. The data were analyzed with SAS Windows (version 12; SAS Institute). All of the patients were included in the analysis. For all of the statistical tests, $P < .05$ was considered statistically significant. Parametric data were compared using analysis of variance with terms for treatment and tests for multiple comparisons. When the data were not normally distributed or were nonparametric, the Kruskal-Wallace test was used. Categorical data were analyzed with contingency table analysis and the χ^2 test or Fisher's exact test.

RESULTS

Demographics. A total of 613 children were enrolled (310 males and 303 females; mean age, 5.16 ± 3.06 years): 324 (52.8%) were aged 2–4 years, 172 (28.1%) were aged 5–7 years, and 117 (19.1%) were aged 8–14 years. On the basis of the disease classification, 113 (18.4%) had acute bronchitis, 82 (13.4%) had wheezing, and 418 (68.2%) had pneumonia.

Acute* M. pneumoniae *and* C. pneumoniae *infection. Ta-

Table 4. Incidence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections at different times of the year.

Months	No. (%) of infections			
	M. pneumoniae	*C. pneumoniae*	Both	Neither
May–July (n = 132)	56 (42.4)	3 (2.3)	6 (4.5)	67 (50.8)
August–October (n = 107)	18 (16.8)	10 (9.3)	4 (3.7)	75 (70.1)
November–January (n = 201)	49 (24.4)	11 (5.5)	25 (12.4)	116 (57.7)
February–April (n = 173)	45 (26.0)	21 (12.2)	7 (4.0)	100 (57.8)

NOTE. No significant seasonal difference was observed for either etiologic agent.

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Table 5. Clinical signs and symptoms of children with acute bronchitis at enrollment.

Characteristic	Children infected with indicated microorganism, %			
	M. pneumoniae ($n = 27$)	*C. pneumoniae* ($n = 13$)	Both ($n = 9$)	Neither ($n = 64$)
Onset				
Gradual	77.7	69.2	38.9	72.5
Acute	22.3	30.8	61.1	27.5
Similar illness within family	3.7	15.3	0	6.2
Cough	44.4	61.5	22.2	59.3
Rhinitis	7.4	15.3	11.1	14.0
Tachypnea	18.5	7.6	11.1	14.0
Fever	81.4	61.5	66.6	75.0
Rales	77.7	61.5	55.5	79.6
Wheezing	11.1	15.3	11.1	14.0
Rhonchi	66.6	84.6	85.5	70.3
Duration (d), mean ± SD				
Of illness	15.19 ± 8.86[a]	12.17 ± 8.27	16.25 ± 13.48[a]	10.37 ± 7.49
Of hospitalization	5.77 ± 2.82[a]	5.50 ± 2.78	6.00 ± 5.12[a]	4.35 ± 2.19

NOTE. *C. pneumoniae, Chlamydia pneumoniae; M. pneumoniae, Mycoplasma pneumoniae.*

[a] $P < .05$ versus no *M. pneumoniae* or *C. pneumoniae* infection; no other difference was significant.

ble 1 shows the incidence of acute *M. pneumoniae* infection in the study population, according to serological and PCR findings. Serological evidence of acute infection was observed in 194 patients: a specific IgM titer ⩾1:100 was detected in 79 patients (40.7%); specific IgM titer ⩾1:100 and specific IgG titer ⩾1:400 in 43 patients (22.2%); IgM titer ⩾1:100 and a ⩾4-fold rise in IgG titer in 33 patients (17.0%); specific IgG titer ⩾1:400 alone in 32 patients (16.5%); and a ⩾4-fold rise in IgG titer alone in 7 patients (3.6%). In 126 of these children (64.9%), *M. pneumoniae* DNA was also detected. In 16 more children, PCR for *M. pneumoniae* was positive without serological evidence of acute infection but with evidence of past infection; 3 of these subjects were aged 2–4 years, 6 were aged 5–7 years, and 7 were aged 8–14 years.

Table 2 summarizes the incidence of acute *C. pneumoniae* infection on the basis of serological and PCR findings. Serological evidence of acute infection was observed in 52 children (⩾4-fold increase in IgG in 36 patients [69.2%] and an IgG antibody titer ⩾1:512 in 16 patients [30.7%]; none were positive for IgM) and confirmed by PCR in 13 patients (25.0%). *C. pneumoniae* DNA was detected in 35 more patients without any serological evidence of acute infection: 14 were aged 2–4 years, 15 were aged 5–7 years, and 6 were aged 8–14 years. IgA specific antibodies were also detected in 9 of the 35 children.

Forty-two of the patients (6.8%) had *M. pneumoniae* and *C. pneumoniae* coinfection: 9 had acute bronchitis, 7 had wheezing, and 26 had pneumonia. The incidence of coinfections was age-dependent (4.3% of patients aged 2–4 years, 6.3% of those aged 5–7 years, and 14.5% of those aged 8–14 years).

Tables 3 and 4 show the geographic and seasonal distribution, respectively, of the infections: some minor variations were detected, but none was significant.

Clinical findings. Tables 5–7 summarize the clinical signs and symptoms of the study population at enrollment by diagnostic classification. Regardless of the diagnosis, it was noted that disease onset, presence of a similar illness in the family, and signs and symptoms at enrollment were similar for the children with acute *M. pneumoniae* or *C. pneumoniae* infection, for those with *M. pneumoniae* and *C. pneumoniae* coinfection, and for those with no evidence of either infection. In the presence of acute bronchitis, the duration of the illness and hospitalization were significantly longer for the children with *M. pneumoniae* infection or *M. pneumoniae* and *C. pneumoniae* coinfection than for those with no evidence of either infection, although there was a large overlap; in the presence of wheezing or pneumonia, there was no significant difference in duration of either the illness or hospitalization in the different groups.

Laboratory evaluation. Table 8 shows the laboratory data. There were no significant differences in total and differential WBC counts, erythrocyte sedimentation rate, or C-reactive protein value for the children with acute *M. pneumoniae* or *C. pneumoniae* infection, those with *M. pneumoniae* and *C. pneumoniae* coinfection, and those with no evidence of either infection. There also was no significant difference between the clinical presentations.

Clinical response 4–6 weeks after enrollment. The patients received treatment with a wide range of antimicrobial regimens, which were always chosen without reference to the laboratory

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diagnostic data. A total of 146 (57.2%) of 255 children with acute *M. pneumoniae* and/or *C. pneumoniae* infection received inappropriate antibiotics: 82 (56.2%) had been treated with antimicrobials that were inactive against atypical bacteria, and 64 (43.8%) had received macrolides for too short a duration (58 children) or in inadequate doses (6 children). These 64 children were considered unevaluable in the analysis of clinical response.

Among the 191 children with acute *M. pneumoniae* and/or *C. pneumoniae* infection who were evaluated, 106 (97.2%) of 109 children treated with macrolides and only 67 (81.7%) of 82 children treated with other antibiotics were considered cured or improved after 4–6 weeks ($P < .05$). Of the 15 clinical failures (18.3% of 82) in the absence of appropriate therapy, 9 involved new clinical findings consistent with active infection, 3 involved progression, and 3 involved persistent signs and symptoms of LRTI. The findings relating to the 3 clinical failures after receipt of macrolides were consistent with active infection. Eighteen clinical failures followed treatment with these antibiotics: amoxicillin (7 clinical failures), amoxicillin + clavulanate (4), cefaclor (2), cefotaxime (2), clarithromycin (1), roxithromycin (1), and erythromycin (1).

DISCUSSION

Atypical bacteria are emerging as important causes of human respiratory tract disease in both adults and children. Recent reports indicate a high incidence of infections in patients with community-acquired pneumonia (6%–40%) and that these infections play a considerable role in closed communities [7, 8, 12, 30]. Furthermore, the same pathogens are involved in upper respiratory tract infections (pharyngitis, sinusitis, and otitis), acute bronchitis, and exacerbations of asthma [31–36].

The results of this large, prospective multicenter study of pediatric LRTIs show that nearly one-half of the cases of community-acquired acute bronchitis, wheezing, and pneumonia involve *M. pneumoniae* and/or *C. pneumoniae* infections and that macrolide antibiotics may lead to their clinical cure. The percentage of cases of infection attributed to these atypical bacteria in our population was similar to that reported by Block et al. [12] and Harris et al. [23], despite the fact that our study was conducted in a different location and at a different time. Moreover, as others have observed elsewhere [12], we found an age-dependent increase in the incidence of *M. pneumoniae* and/or *C. pneumoniae* infection, with atypical bacterial infections being frequent in children aged <5 years.

Recent reports have associated *M. pneumoniae* and *C. pneumoniae* with the initiation and promotion of asthma in both adults and children [13, 14, 32, 34, 35], and we found an interesting correlation between wheezing on initial examination and acute infection due to these pathogens. This intriguing finding suggests that atypical pathogens may play a role in the exacerbation of childhood asthma. It is likely that *M. pneumoniae* and *C. pneumoniae* trigger the "wheezing process" or act as cofactors in genetically predisposed subjects.

M. pneumoniae and *C. pneumoniae* coinfection was not rare in our population, but its clinical implications are not clear. This phenomenon has been increasingly recognized over recent

Table 6. Clinical signs and symptoms of children with wheezing at enrollment.

	Children infected with indicated microorganism, %			
Characteristic	*M. pneumoniae* (*n* = 17)	*C. pneumoniae* (*n* = 12)	Both (*n* = 7)	Neither (*n* = 46)
Onset				
Gradual	38.7	50.0	57.2	54.4
Acute	61.3	50.0	42.8	45.6
Similar illness within family	11.7	0	0	4.3
Cough	47.0	33.3	28.5	36.9
Rhinitis	35.2	41.6	14.2	17.3
Tachypnea	64.7	83.3	28.5	50.0
Fever	47.0	33.3	42.8	54.3
Rales	47.0	25.0	28.5	45.6
Wheezing	82.3	75.0	100.0	73.9
Rhonchi	47.0	75.0	42.8	41.3
Duration (d), mean ± SD				
Of illness	9.56 ± 6.19	9.73 ± 9.94	9.71 ± 5.22	9.00 ± 5.60
Of hospitalization	5.56 ± 2.94	4.17 ± 2.17	4.86 ± 2.91	4.79 ± 2.43

NOTE. None of the differences were significant. *C. pneumoniae, Chlamydia pneumoniae; M. pneumoniae, Mycoplasma pneumoniae.*

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Table 7. Clinical signs and symptoms of children with pneumonia at enrollment.

Characteristic	Children infected with indicated microorganism, %			
	M. pneumoniae (*n* = 124)	*C. pneumoniae* (*n* = 20)	Both (*n* = 26)	Neither (*n* = 248)
Onset				
Gradual	59.7	75.0	53.8	56.1
Acute	39.3	25.0	46.2	43.9
Similar illness within family	6.4	5.0	0	5.2
Cough	60.4	50.0	46.1	53.2
Rhinitis	14.5	30.0	3.8	13.7
Tachypnea	14.5	25.0	23.0	20.9
Fever	86.2	80.0	84.6	83.0
Rales	84.6	85.0	84.6	77.8
Wheezing	12.9	20.0	7.6	15.3
Rhonchi	11.2	15.0	7.6	18.9
Duration (d), mean ± SD				
Of illness	12.74 ± 7.60	13.11 ± 8.54	12.96 ± 6.29	12.01 ± 10.40
Of hospitalization	6.31 ± 2.82	5.95 ± 2.34	6.63 ± 3.25	5.68 ± 3.63

NOTE. None of the differences were significant. *C. pneumoniae, Chlamydia pneumoniae; M. pneumoniae, Mycoplasma pneumoniae.*

years, therefore demonstrating the complex etiology of childhood LRTIs [7, 37]. As has been pointed out elsewhere [37], it is not possible to identify the initiating pathogen that allows a secondary invader to gain access to the respiratory tract. The important unanswered question in this regard is whether one pathogen simply facilitates the penetration of the other or whether both cause the respiratory tract infection. It is also unknown whether the combination of *M. pneumoniae* and *C. pneumoniae* leads to a more severe clinical illness.

One limitation of this study was the absence of testing for *Bordetella pertussis* and respiratory viruses. It is possible that *B. pertussis* and respiratory viruses act as cofactors in rendering subjects more susceptible to other stimuli such as atypical bacteria, and concurrent outbreaks of *M. pneumoniae* and *B. pertussis* infections have been reported elsewhere [38, 39]. However, >90% of our patients had received 3 doses of whole-cell or acellular *B. pertussis* vaccine during the first year of life; consequently, we think that *B. pertussis* could have played only a minor role. Furthermore, the seasonal patterns of respiratory virus infections show peaks from September through February and from April through May [40, 41], and given that we found no geographic or seasonal difference in the incidence of *M. pneumoniae* and *C. pneumoniae* infections, it seems that atypical bacterial infections are not related to clear local outbreaks and are not associated with respiratory viruses.

It is common practice to attribute specific clinical and laboratory characteristics to a particular etiologic agent [5], but we found that the clinical and laboratory findings for the children we studied were insufficient to distinguish precisely one infection from another. None of the signs, symptoms, or laboratory parameters we considered seem to be unique to atypical bacterial infections (which suggests that they are not useful in therapeutic decision-making), and it was not possible to predict their presence purely on the basis of the initial clinical and laboratory findings. Our findings support those of previous studies that have cast doubts on the specificity of clinical and laboratory features in predicting the microbial cause of LRTIs [30, 42].

Sixteen children positive for *M. pneumoniae* DNA and 35 positive for *C. pneumoniae* DNA had no serological evidence of acute infection and might simply be considered carriers. However, the number of patients with a significant serological response to *M. pneumoniae* was so high that these 16 patients did not affect the conclusions of the study; furthermore, it is well known that the lack of an immunological response in children after *C. pneumoniae* infection may be caused by an immature ability to produce a specific humoral response or poor antigenic stimulation [32, 43].

The significant difference in the efficacy of the antibiotics used to treat the children with atypical bacterial infections highlights the fact that, in the case of *M. pneumoniae* and/or *C. pneumoniae* infections, the use of a macrolide is associated with a better clinical outcome [44–47].

In conclusion, our data underline the role of *M. pneumoniae* and *C. pneumoniae* in children with community-acquired LRTIs, even in children aged <5 years. These atypical bacterial infections also seem to be related to wheezing and may present

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Table 8. Laboratory data gathered from the study population.

Parameter	Value (mean ± SD) for children infected with indicated microorganism			
	M. pneumoniae (n = 168)	*C. pneumoniae* (n = 45)	Both (n = 42)	Neither (n = 358)
WBC count, cells/μL	12,225 ± 7093	12,859 ± 7120	10,994 ± 5751	12,383 ± 6553
Neutrophils, %	64 ± 17	63 ± 19	62 ± 15	62 ± 17
Lymphocytes, %	26 ± 15	27 ± 17	27 ± 13	27 ± 16
Monocytes, %	7 ± 4	7 ± 4	10 ± 13	8 ± 6
Eosinophils, %	2 ± 3	1 ± 2	2 ± 2	2 ± 2
Basophils, %	0.4 ± 0.6	0.4 ± 0.5	0.4 ± 0.4	0.5 ± 0.7
ESR, mm/1 h	44 ± 30	39 ± 28	45 ± 28	46 ± 35
CRP, μg/dL	48 ± 76	56 ± 83	46 ± 76	59 ± 93

NOTE. None of the differences were significant. *C. pneumoniae, Chlamydia pneumoniae;* CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; *M. pneumoniae, Mycoplasma pneumoniae.*

a more complicated course when not treated with adequate antimicrobial agents.

MOWGLI STUDY GROUP

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