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## ORIGINAL ARTICLE

# *MYCOPLASMA PNEUMONIAE* INFECTION IN CHILDREN WITH ACUTE EXACERBATION OF BRONCHIAL ASTHMA: CLINICAL, LABORATORY AND RADIOLOGICAL EVALUATION

**By**

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**Background:** ***Mycoplasma pneumoniae (M. pneumoniae) causes many respiratory syndromes that clinically mimic viral respiratory infections. Only a few studies have associated M. pneumoniae infection with acute exacerbation of bronchial asthma (AEBA) in children. Due to the fastidious nature of the organism, Culture is of no practical value and the diagnosis is challenging. This study was undertaken to delineate the extent of involvement of M. pneumoniae in children with AEBA in children, correlates between laboratory, clinical and radiological findings and M. pneumoniae infection, and to evaluate PCR, IgM ELISA, and direct antigen enzyme immunoassay (EIA) as rapid methods for diagnosis of M. pneumoniae infection.***

**Methods:** ***We prospectively evaluated 37 children (27 males and 10 females; age, 6 months-15 years) admitted with AEBA. They underwent clinical, radiological and laboratory examinations. M. pneumoniae infection was diagnosed by PCR, IgM ELISA, and direct antigen EIA.***

**Results:** ***Among studied 37 cases, M. pneumoniae infection was diagnosed in 7/37 (18.9%) cases. Among 7 cases positive for M. pneumoniae, IgM was positive in 5 cases (71.43%), Antigen detection was positive in one case (14.29%), and PCR was positive in 5 cases (71.43%). There were no statistically significant difference (P>0.05) in age groups or sex between M. pneumoniae positive and negative cases. Absence of coryza and presence of fine rales on chest auscultation were noted significantly (P<0.05) more in M. pneumoniae positive asthmatic patients than in M. pneumoniae negative cases. M. pneumoniae infection was associated with thrombocytosis although it was not statistical significant. Among 33 cases that were not associated with co-morbidity, M. pneumoniae infection was diagnosed in 5 cases (15.33%). On the other hand, among 4 cases with co-morbidity (chronic renal failure (CRF), congenital heart disease (CHD), bone marrow transplantation, and leukemia), M. pneumoniae infection was diagnosed in 2 cases (50%) (P= 0.042).***

**Conclusion:** ***Our data suggests an association between acute Mycoplasma pneumoniae infection and AEBA in children. Absence of coryza and presence of fine rales on chest auscultation in addition to the presence of thrombocytosis may implicate M. pneumoniae associated with AEBA, which may be suggestive to start additional antibiotic treatment for M. pneumoniae especially for children with comorbidities (immunocompromised).***

**Keywords:** ***Mycoplasma pneumoniae, asthma, acute exacerbation, children.***

## INTRODUCTION

Viral respiratory infections have been commonly associated with exacerbations of asthma in children.[43] The potential role of atypical bacterial infection in the pathogenesis of asthma is a subject of continuing debate. Their exact contribution to asthma development and/or persistence remains to be determined.[37,5] Only a few studies have associated *Mycoplasma pneumoniae* (*M. pneumoniae*) infection with acute exacerbation of wheezing in the asthmatic patients.[16,34,18,39] M. pneumoniae is a major respiratory pathogen that can cause clinical disease ranging from mild upper respiratory tract infection (URTI) to severe, occasionally fatal pneumonia.[28] Primary *M. pneumoniae* infection can persist for weeks, and data from both human and animal models of disease suggest that M. pneumoniae infection exacerbates chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD).[34,25] An accurate estimate of the extent of *M. pneumoniae* infection in the general population is lacking, and infection is probably more extensive than currently accepted due to difficulties in culturing the organism from clinical samples and unreliable diagnostic laboratory tests.[38] A rapid and specific diagnosis of *M. pneumoniae* is important because treatment of such infection by currently used β lactam antibiotics is ineffective. It has been found that usage of macrolides or fluoroquinolones decrease morbidity, mortality and reduce complications.[32] An early laboratory diagnosis of *M. pneumoniae* infection has a clinical relevance as it would help the clinician to decide upon the choice and initiation of an appropriate antimicrobial treatment as β-Lactam antibiotics are not effective against this microorganism.[33,29] It has been our belief, as well as others, that recent mycoplasma infections play a much greater role in the pathophysiology of asthma and acute exacerbation of bronchial asthma (AEBA) than is readily recognized. We also suspected that infections with mycoplasma were often overlooked and not treated, and often times the infection was termed a "viral infection." This study was an attempt to assess the incidence of associated mycoplasma infections in children with AEBA for those who were admitted to hospital and to correlate their clinical, and radiological findings to M. pneumoniae infection laboratory findings. And thus may find clinical or radiological signs which may propose the prescribtion of early antibiotic treatment against *M. pneumoniae* in children with AEBA.

## PATIENTS AND METHOD

The study was performed on 37 children with AEBA (27 males and 10 females; age, 6 months-15 years), Admitted toinpatients wards of Chest and Allergy Unit of Pediatric department of Mansoura University Children hospital, Al-Mansoura, Egypt, from September 2008 to December 2009. AEBA was defined as episodes of rapidly progressive increase in shortness of breath, cough, wheezing, chest tightness, or some combination of these symptoms necessitating a non-scheduled visit, and associated to a decrease of respiratory airflow.[5]

***Inclusion criteria:*** The presence of typical asthma symptoms, improvement in the prebronchodilator FEV1 of ≥12% after administration of salbutamol (200μg), and positive skin prick test. The clinical severity of asthma was defined according to established guidelines of Global Strategy for Asthma Management and Prevention.[12] Written informed consent was obtained from the parents or guardians of all cases before enrollment in the study. The study was approved by the local Institutional Review Board of Mansoura University, Egypt.

Patients were subjected to complete history-taking, plain radiographs to exclude associated abnormalities, pulmonary function tests using computerized spirometry, and basic laboratory investigations including CBC and CRP.

Respiratory samples were collected for PCR and direct antigen EIA; morning sputum samples were obtained from 11 (29.73%) patient and nasopharyngeal aspirate (NPA) from 26 (70.27%) patients. Selection of respiratory sample was according to age and clinical condition of the patient. Blood samples were obtained aseptically from each patient for serologic study; the serum was separated and stored frozen at –70°C for IgM ELISA.

Direct PCR for Sputum Samples: sputum and NPA were thawed and an equal volume of sputasol solution (Oxoid) were added to the thawed sputum, NPA samples. The mixture was shaked well, placed in 37oC water bath with periodic shaking, until liquefaction is complete. DNA was extracted from 200μl of each respiratory sample using QIAamp DNA blood mini kit (QIAgen, Hilden, Germany) (cod nº 51104) according to the manufacturer's instructions. We then amplified the extracted DNA using primer 1 (5'CAAGCCAAACACGAGCTCCGGCC3') and primer 2 (5'CCAGTGTCAGCTGTTTGTCCTTCCCC3') for p1 adhesion gene. Samples to be amplified were incubated in a 50-μL reaction volume containing 10 μL of extracted DNA, 40 μL of premixed primers and optimized PCR buffer, and 0.2 μL of Taq DNA polymerase. Samples were amplified for 35 cycles by using a PCR processor (GeneAmp PCR system 2700, Applied Biosystems, Foster City, CA), starting with a 1-minute predenaturing step at 96°C. In the optimized PCR, each cycle consisted of the following steps: denaturation at 94°C for 1 minute; annealing at 60°C for 1 minute; and elongation at 72°C for 1 minute. After 35 cycles, a temperature-delay step of 10 minutes at 72°C was done to complete the elongation. Next, 10 μL of the PCR product was separated by electrophoresis on a 2% agarose gel. A band of 543 base pairs stained with ethidium bromide under UV light was considered a positive result.

***M. pneumoniae direct antigen detection by ELISA:***

SERION ELISA antigen kit for *M. pneumoniae* (Serion Virion) (Cat. No. A127) according to the manufacturer's instructions.

***Mycoplasma pneumoniae IgM Antibodies detection by ELISA:*** serum IgM antibody against *M. pneumoniae* was measured by ELISA (RIDA SCREEN *M. pneumoniae* IgM) (R- Biopharm, Germany, K4331) according to the manufacturer's instructions.

***Statistical analysis:*** Data were statistically analyzed using SPSS 12 (Statistical Package for Social Science version 12) program. Qualitative data were expressed as numbers and percentages. Prevalence rate was determined from the number of identified cases at the time of the study divided by all patients examined. Chi square was used as a test of significance for the qualitative data, P value ≤0.05 was considered to be statistically significant. Student's t test was used to determine whether there are differences between two means or between a target value and a calculated mean (quantitative data).

## RESULTS

Our study included thirty seven asthmatic children with AEBA, with the age range from 6 months to 15 years (Mean±SD: 5.38±3.91), including 27 males (72.97%) and 10 females (27.03%). The main presenting symptoms were dry cough in 24 cases (64.86%), productive in 13 cases (35.14%), dyspnea in all cases (100%), fever in 26 cases (70.27%), chest pain in 4 cases (10.81%), and coryza in 17 cases (45.95%). Physical examination revealed: wheezes in all cases (100%), rales in 10 cases (27.03%) and retraction in 7 cases (18.92%). Only 4 cases (10.81%) were associated with co-morbidity (chronic renal failure (CRF), congenital heart disease (CHD), bone marrow transplantation, and leukemia).

*M. pneumoniae* infection was diagnosed in 7 cases (18.9%). Among 7 cases positive for *M. pneumoniae*, IgM was positive in 5 cases (71.43%), Antigen detection was positive in one case (14.29%), and PCR was positive in 5 cases (71.43%). *M. pneumoniae* was diagnosed in all 3 age groups with high incidence of diagnosis in age group (6 month-3 year) (25%), but there is no statistical significant difference between 3 age groups regarding *M. pneumoniae* infection (P>0.05).

*M. pneumoniae* infection was higher in females than in males without statistically insignificane (P>0.05).

Absence of coryza and presence of fine rales on chest auscultation were noted to be significant difference (P<0.05) more in M. pneumoniae positive asthmatic patients than in *M. pneumoniae* negative cases.

With or without *M. pneumoniae* infection, there was no difference in laboratory, radiological or pulmonary function test results. However *M. pneumoniae* infected cases were associated with thrombocytosis although it was not statistical significant.

Among 33 cases that were not associated with co-morbidity, M. pneumoniae infection was diagnosed in 5 cases (15.33%). On the other hand, among 4 cases with co-morbidity (CRF, CHD, BM transplantation, and leukemia), M. pneumoniae infection was diagnosed in 2 cases (50%). (P= 0.042).

**Table 1. Demographic and clinical data of all studied patients.**

| | | | Patients(n=37) |
|---|---|---|---|
| **Age** | | | |
| | Mean±SD | | 5.38 ± 3.91 year |
| **Sex** | | | |
| | Male / female | | 27 (72.9%) / 10 (27.03%) |
| **Symptoms** | | | |
| | Fever | Present | 26 (70.27%) |
| | | Absent | 11 (29.73%) |
| | Cough | Dry | 24 (64.86%) |
| | | Productive | 13 (35.14%) |
| | Chest pain | Present | 4 (10.81%) |
| | | Absent | 33 (89.19%) |
| | Dyspnea | Present | 37 (100.0%) |
| | | Absent | - |
| | Coryza | Present | 17 (45.95%) |
| | | Absent | 20 (54.05) |
| **Physical examination** | | | |
| | Rales | Present | 10 (27.03%) |
| | | Absent | 27 (72.97%) |
| | Wheezes | Present | 37 (100.0%) |
| | | Absent | - |
| | Retraction | Present | 7 (18.92%) |
| | | Absent | 30 (81.08%) |
| **Co- Morbidity** | | | |
| | Present | | 4 (10.81%) |
| | Absent | | 33 (89.19%) |

**Table 2. Radiological and laboratory data of studied patients.**

| | Patients (n=37) | |
|---|---|---|
| **Radiological findings** | | |
| Chest X-ray | Patchy infilteration | 6 (16.22 %) |
| | hyperinflation | 21 (56.76 %) |
| | Others | 10 (27.02 %) |
| **Pulmonary function tests** | | |
| | Obstructive | 25 (67.57%) |
| | Restrictive | 8 (21.62%) |
| | Mixed | 4 (10.81%) |
| **Laboratory findings** | | |
| **CBC** | | |
| Platelets (K/u L) | Range | 73.0 - 796.0 |
| | Mean ± SD | 379.08 ± 165.66 |
| RBCs (M/ u L) | Range | 3.50 - 7.50 |
| | Mean ± SD | 4.76 ± 0.72 |
| WBCs ( K/ u L) | Range | 4.70 - 28.0 |
| | Mean ± SD | 11.21 ± 5.03 |
| Lymphocytes % | Range | 11.1 - 76.9 |
| | Mean ± SD | 39.18 ± 15.42 |
| Granulocytes % | Range | 10.7 - 84.5 |
| | Mean ± SD | 51.36 ± 17.25 |
| **CRP** | positive | 15 (40.54 %) |
| | negative | 22 (59.46 %) |

**Table 3. Evaluation of diagnostic tests for *Mycoplasma pneumoniae*.**

| ***M. pneumoniae* positive cases No=37** | **Diagnostic tests** | | | | | |
|---|---|---|---|---|---|---|
| | **Positive IgM** | | **Positive Ag detection** | | **Positive PCR** | |
| | no | % | no | % | no | % |
| 7 (18.92%) | 5 | 71.43 | 1 | 14.29 | 5 | 71.43 |

**Table 4. Relation of the reported clinical data to *Mycoplasma pneumoniae* infection (Chi- square test).**

| | | M. pneumoniae positive (n=7) | M. pneumoniae negative (n=30) | P |
|---|---|---|---|---|
| **Symptoms** | | | | |
| Fever | | 6 (85.7%) | 20 (66.7%) | 0.37 |
| Cough | Productive | 3 (42.9%) | 10 (33.3%) | 0.64 |
| | Dry | 4 (57.1%) | 20 (66.7%) | |
| Chest pain | | 1 (14.3%) | 3 (10%) | 0.74 |
| Dyspnea | | 7 (100%) | 30 (100%) | - |
| Coryza | | 0 | 17 (56.7%) | 0.007* |
| **Physical examination** | | | | |
| Fine rales | | 5 (71.4%) | 5 (16.7%) | 0.003* |
| Wheezes | | 7 (100%) | 30 (100%) | - |
| Retraction | | 3 (42.9%) | 4 (13.3%) | 0.073 |

*: Significant difference $p<0.05$.

**Table 5. Relation of laboratory and radiological data to *M. pneumoniae* infection.**

| | | *M. pneumoniae* positive (n=7) | *M. pneumoniae* negative (n=30) | P |
|---|---|---|---|---|
| **Laboratory findings** | | | | |
| CBC | | | | |
| Platelets (K / u L) | Mean ± SD | 462.14 ± 189.45 | 359.7 ± 156.81 | 0.14 |
| RBCs (M / u L) | Mean ± SD | 4.94 ± 0.41 | 4.72 ± 0.77 | 0.47 |
| WBCs (K / u L) | Mean ± SD | 8.1 ± 2.83 | 11.94 ± 5.18 | 0.068 |
| Lymphocytes % | Mean ± SD | 43.49 ± 12.67 | 38.18 ± 16.01 | 0.42 |
| Granulocytes % | Mean ± SD | 45.86 ± 13.76 | 52.64 ± 17.92 | 0.36 |
| Positive CRP | | 5 (71.4%) | 10 (33.3%) | 0.065 |
| **Pulmonary function tests** | | | | |
| | Obstructive | 5 (71.4%) | 20 (66.7%) | |
| | Restrictive | 1 (14.3%) | 7 (23.3%) | |
| | Mixed | 1 (14.3%) | 3 (10%) | |
| **Radiology** | | | | |
| Chest X-ray | Patchy infiltration | 2 (28.6%) | 4 (13.3%) | 0.18 |
| | Hyperinflation | 5 (14.3%) | 16 (53.3%) | |
| | Others | 0 | 10 (33.3%) | |

## DISCUSSION

The relationship between respiratory infections and asthma exacerbation has been observed since the '80s in both children and adults. First reports analysed the role of respiratory viruses, whereas later on several observations pointed out the possible involvement of atypical bacteria in asthma, particularly *M. pneumoniae* and *C. pneumoniae* both in chronic stable asthma and in acute exacerbations.[27,8,23]

Clinical presentation of *M. pneumoniae* is variable and diagnosis confirmation is a challenge to even the most experienced clinicians [35]. It is difficult to set up criteria for the 'gold standard' to detect acute or remote infections.[31,13]

In our study, out of 37 children having AEBA; 7 (18.9%) cases were found to be positive for *M. pneumoniae* infection. IgM was positive in 5 (13.5%) cases, PCR was positive in 5 (13.5%) cases, and *M. pneumoniae* specific Ag was detected in one case (2.7%). Our results were in accordance to Yossef et al.[42] who investigated the relationship between *M. pneumoniae* infection and bronchial asthma (BA) in 120 Egyptian children and they reported that the incidence of *M. pneumoniae* in BA in Egyptian children was 17.5%. Six patients (5%) had a positive ELISA test for serum specific IgG *M. pneumoniae* Ab and 15 (12.5%) had positive anti M. pneumoniae IgM Ab.

However, Gil et al.[11] reported evidence of *M. pneumoniae* in 24.7% of asthmatics. Kondo et al. [20] demonstrated a higher percentage of seroconversion to Mycoplasma infection in association with acute asthma exacerbation. Similarly, Varsheny et al.[39] conducted a study on 50 children with AEBA and found that 19 (38%) cases were positive by IgM ELISA and 11(22%) cases were positive by P1 PCR. Of these PCR- positive cases, 8 patients were positive by both IgM ELISA and PCR.

Cunningham et al.[6] had a lower percentage of Mycoplasma infection as it was only found in (0.7%) of asthmatic samples. Annagür et al.[1] demonstrated positive Mycoplasma IgM in 8.1% of asthmatic children. Also, Silva et al.[36] found a lower prevalence of *M. pneumoniae* (16.4%) in BA cases.

Our data suggest an association between *M. pneumoniae* infection and AEBA and emphasizes the importance of laboratory diagnosis of *M. pneumoniae* with its reflection in therapeutic methods in AEBA.

In our study, it was noted that infection with M. pneumoniae was higher (25%) in children aged between 6 months-3 years. But with no significant difference between other age groups and infection. This is in disagreement with Waites and Talkington;[41] Vervloet et al.[40] who reported that *M. pneumoniae* causes infection in all age group studied, being less frequent and more severe in children under the age of five and the variation in incidence of infection in different age groups occurs in epidemics.

Other previous studies reported that *M. pneumoniae* infection is most frequent in primary- school children and among their parents.[21,7,9] They explained this fact by hypersensitivity reaction in older children while there is local acquired immunity from past infection developed in older age.

Therefore, we suggest that *M. pneumoniae* infection is not rare in children age <5 years. These data provide an indication for whether to treat patients aged <5 years with lower RTIs with anti-Mycoplasma agents such as macrolides.

Esposito et al.[8] Higashigawa et al.[15] found no specific clinical or laboratory observations permit a definite diagnosis of Mycoplasma infections.

***In our study:*** only the absence of coryza and presence of fine rales on chest auscultation were noted significantly more often in *M. pneumoniae* positive cases than in M. pneumoniae negative cases. This finding shows the difficulty in discriminating *M. pneumoniae* from other viral pathogens causing AEBA on clinical parameters only and emphasizes the need for laboratory confirmation.

Similarly, Foy et al.[10] reported that the absence of coryza was the only parameter that correlated with M. pneumoniae infection. Chang et al.[3] Ou et al.[30] reported that; fever, cough and rales on chest auscultation are the most common symptoms and signs of acute M. pneumoniae infection in BA.

Routine laboratory studies are not likely to distinguish between respiratory tract infection due to *M. pneumoniae* and to viruses. The peripheral WBCs count is normal or slightly elevated.[13]

*M. pneumoniae* infection has more commonly been associated with thrombocytopenia related to the production of anti-platelet antibodies.[4] But in the present study *M. pneumoniae* infection correlated with the presence of thrombocytosis > 400.000x106 / L.

The present finding of thrombocytosis is an unusual finding that had been reported once in elderly adults[24] and in children.[29]

In our study, we observed that *M. pneumoniae* infection was associated with positive CRP in but without significant statistical difference. In confirmation to our

results, Higashigawa et al.[15] found that CRP was significantly elevated in patients with pneumonia and BA groups. Other studies showed that non-specific parameters such as WBCs, CRP, and platelete were not correlated
to *M. pneumoniae* infection[17,22] and thus they were not significantly useful for therapeutic decision making.

In the current study; the pattern of presentation of *M. pneumoniae* on chest radiography are non-specific, consisting of patchy or interstitial areas of infiltration, hyperinflation, or other non-specific radiographic patterns with no significant statistical difference between M. pneumoniae-positive and *M. pneumoniae*-negative cases. This finding is in agreement with Hanhan et al.[14] who reported that the presence of infiltrates on chest X-ray in status asthmaticus warrants tests for *M. pneumoniae*. Also, Miyashital et al.[26] reported non-specific chest radiographic patterns of M. pneumoniae pneumonia that vary between patchy areas of airspace consolidation, reticular interstitial infiltrates or both and hence they suggested that CT images can more accurately provide detailed information about the lung parenchyma than routine chest radiography.

From our study we found that *M. pneumoniae* infection was significantly associated with the presence of co-morbidity; among 4 cases with underlying disease; M. pneumoniae was diagnosed in 2 cases (50%). The 2 cases were diagnosed by PCR and were serologically negative (considered immunocompromised).

This finding is in agreement with Kim et al.[19] they found that children diagnosed PCR positive but serologically negative were more immunocompromised than seropositive children. They concluded that serological diagnosis may be of reduced diagnostic value in young children and in immunocompromised patients.

## CONCLUSION

Our study revealed that, *M. pneumoniae* is a common bacterial pathogen associated with AEBA in children, absence of coryza and presence of fine rales in breathing sounds on chest auscultation in addition to the presence of thrombocytosis >400.000/ml implicate *M. pneumoniae* infection might be associated with AEBA, which may be suggestive to start additional antibiotic treatment for *M. pneumoniae* especially for children with comorbidities (immunocompromised).

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