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Detection of *Mycoplasma pneumoniae* and *Chlamydia trachomatis* in Iranian children with acute lower respiratory infections by polymerase chain reaction

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PEER REVIEW

Peer reviewer

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Comments

This study includes 102 children less than 5 years old diagnosed of CAP during a 14 months' period and attended in a tertiary care children's hospital in Teheran. In all patients, *M. pneumoniae* was investigated by PCR and Real-Time PCR. In the subgroup of 69 children less than 1 year old, *C. trachomatis* was also investigated by a molecular technique.
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ABSTRACT

Objective: To determine the frequency of *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydia trachomatis* (*C. trachomatis*) in young children with community acquired pneumonia (CAP) and detect *C. trachomatis* in the subgroup of infants under 1 year of age in Tehran, Iran.

Methods: This cross-sectional study was designed to detect *M. pneumoniae* from all children (<5 years of age) presenting with CAP, admitted to a tertiary care children's hospital affiliated with the Shaheed Beheshti University of Medical Sciences in Tehran during a period of 14 months, from November 2010 to December 2011. Nasopharyngeal and oropharyngeal swabs were collected from 102 children during the study period. Pathogens were detected using polymerase chain reaction and confirmed with real-time polymerase chain reaction.

Results: Only one case of *M. pneumoniae* was isolated from 102 children (1%). *C. trachomatis* was not detected in any of the 69 infants (<1 year of age).

Conclusions: According to our findings, *M. pneumoniae* is an uncommon cause of CAP in children under 5 years old and *C. trachomatis* could not be listed as causing CAP in infants in our study population. However, more studies with a larger sample size are needed to confirm this observation.

KEYWORDS

Acute lower respiratory infections, Iran, Community acquired pneumonia, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*

1. Introduction

Community acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide especially in young children, resulting in more than 4 million deaths annually[1]. Determining the etiology of these infections is very important

for optimal management which would decrease the financial and emotional cost to patient's family and the burden on the health care system. Two of the more common organisms causing respiratory tract infections are *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydia trachomatis* (*C. trachomatis*). As *mycoplasma* lacks a cell wall, thereby making

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them resistant to β -lactam antibiotics, a definite diagnosis would prevent unnecessary use of the antibiotics that are commonly prescribed for respiratory tract infections.

Different techniques have been used to detect *M. pneumoniae* and *C. trachomatis* from the respiratory tract of children presenting with CAP. Most recent of these are conventional polymerase chain reaction (PCR), real time PCR, nucleic acid sequence-based amplification, and nested PCR[2–4].

C. trachomatis has been implicated as a cause of pneumonia in infants in various studies, with a frequency ranging from 2.4% to 30% from different parts of the world[5–11]. In Iran, only a few studies have been done for detecting *C. trachomatis* by the PCR technique. In a study, Naghipour *et al.* indicated that respiratory secretions from 261 children (<5 years old) presented with acute upper and lower respiratory tract infections; *C. trachomatis* was isolated from 4 cases[12].

Since there is a paucity of studies about the role of *M. pneumoniae* and *C. trachomatis* as the causative agents of RTIs in children under 5 years of age in our country, we have attempted to define the frequency of these two microorganisms in childhood pneumonia by using PCR which has a high sensitivity and specificity for detecting different microbes[13,14].

2. Materials and methods

This cross-sectional study was performed on children presenting with CAP to a tertiary care children's hospital affiliated with the Shaheed Beheshti University of Medical Sciences in Tehran during a period of 14 months, from November 2010 to December 2011. This study was done to isolate *M. pneumoniae* from all patients (<5 years old); also, in the subgroup of infants (<1 year of age), we tried to detect *C. trachomatis* as well. Inclusion and exclusion criteria were based on guiding principles provided in previous researches[15–17]. Inclusion criteria: All children in the specified range were included if they had both of the following criteria.

1: At least 2 of the clinical manifestations given below:

- a. Temperature ≥ 38.3 °C.
- b. Tachypnea *i.e.* respiratory rates of ≥ 60 /minute, ≥ 50 /min and ≥ 40 /min; at ages of ≤ 1 months, between 1–12 months and 1–5 years, respectively.
- c. Cough for ≥ 24 h.
- d. Abnormal breath sounds (rales/wheeze/ronchi/decreased breath sounds).
- e. Respiratory distress (intercostal/subcostal/subclavicular retractions and/or nasal flaring).
- f. Chest pain (only for *Mycoplasma*).

PLUS

2: Positive findings on chest X-ray: Paracardiac/ perihilar/ peribronchial infiltration (reticulonodular/reticular/alveolar pattern); lobar/segmental/sub-segmental consolidation and/or hilar lymphadenopathy and/or pleural effusion.

Exclusion criteria: Children with any of the following conditions were excluded.

1. Immune deficiency.
2. Chronic co-morbidity.
3. Hospital acquired infection.
4. Cough lasting for ≥ 4 weeks.

After obtaining written parental consent, all children included

in the study were examined by members of the pediatric faculty or pediatric residents; diagnosis of CAP was confirmed by chest radiology. Children were treated as outpatients or hospitalized according to their general condition.

Trained investigators collected nasopharyngeal or oropharyngeal swabs from all patients. Dacron swabs were used for sample collection as these are better than cotton-tipped swabs[14]. Samples were inoculated immediately into thioglycolate culture media and transferred to the laboratory in the Pediatric Infections Research Center. All samples were tested for the presence of *mycoplasma* while isolation of *C. trachomatis* was attempted only in samples obtained from infants (<1 year of age). A short description of the technical logistics is given below:

All samples were incubated at 37 °C for 24 h then frozen at -70 °C till they were thawed for genome extraction; intron (DNA extraction mini kit, South Korea) was used. Primers used for detection of *Mycoplasma* were MP5-1 and MP5-2[18,19]. The sequences were:

MP5-1 primer: 5'-GAA GCT TAT GGT ACA GGT TCG-3'

MP5-2 primer: 5'-ATT ACC ATC CTT GTT GTA AGG-3'

Because of low yield, the process of detection of *Mycoplasma* by PCR was repeated twice. At this step, *Mycoplasma* was detected from one specimen so the whole process was repeated a third time. In addition, 27 samples from patients with clinical manifestations compatible with *Mycoplasma* infection were tested by real time PCR by using the MP5-4 DNA probing with the sequence CGT AAG CTA TCA GCT ACA[19].

For detection of *C. trachomatis* P1 and OMP2 primers were used with the following sequences[20]:

P1: 5'-ATG AAA AAA CTC TTG AAA TCG G-3'

OMP2: 5'-ACT GTA ACT GCG TAT TTG TCT G-3'

Since *C. trachomatis* was not detected in any of the samples, a rigorous search was started to isolate this microorganism. Initial steps for PCR were repeated using different set-ups and on failing to isolate *C. trachomatis* 15 samples from infants with clinical manifestations of atypical pneumonia were sent to the microbiology laboratory at the university.

All results were documented. No specific statistical analysis was done.

3. Results

One hundred and two infants and children with CAP were included in the study. Age range was 0.5 to 60 months with a mean age of (15.8 \pm 13.8) months; 69 infants were ≤ 1 year of age and 63 patients (61.8%) were male.

Most common clinical manifestations were: cough in 99 cases (97.1%), coryza in 59 cases (57.8%), wet cough in 55 cases (53.9%) and fever in 46 cases (45.1%). Signs on physical examination included: abnormal breath sounds in 85 cases (83.3%), tachypnea in 64 patients (45.1%), and pharyngeal inflammation in 9 children (8.8%). Thirty patients (29.4%) were hospitalized with respiratory distress and classified as suffering from moderate to severe disease. Most common finding revealed on chest X-ray was hyperaeration seen in 46%, following by bilateral paracardiac, paratracheal and peribronchial infiltration in 39% of children.

Mycoplasma was isolated from a 54-month-old boy who presented with wet cough without fever; chest auscultation

revealed fine rales on the right side and infiltration was evident in the right lower lobe (Figure 1).

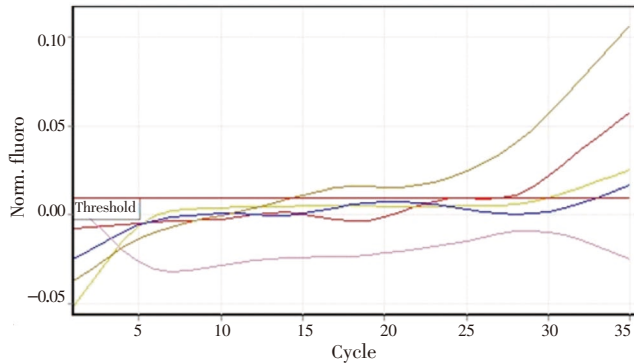


Figure 1. Real-time PCR for *M. pneumoniae* isolated from 1 patient in our study.

C. trachomatis was not isolated from any of the specimens taken from infants (<1 year old).

4. Discussion

We detected only one case of *M. pneumoniae* in children under 5 years old presenting with CAP and no *C. trachomatis* in infants with pneumonia. *C. trachomatis* infection is a perinatal infection and thus in older population its incidence can be dramatically low. These results are similar to reports from other parts of Iran as well^[12]. Prevalence of *Mycoplasma* in subjects with CAP has ranged from 10%–20% in different studies^[15]. *Mycoplasma* were isolated from 29.2% of children with CAP in Chile^[21], from 27.5% in Greece^[22] and from 23.2% in Japan^[23]. However, reports from the Netherlands quote figures of 2.4%^[24], and 7.1% in 0–5 years old from China^[25]. In reports from adults (>age of 18 years), *Mycoplasma* had been detected from 12.9% of patients in Belgium^[26], from 1.3% in the UK^[27] and from none of the cases of CAP in the Netherlands^[28]. In all these studies, molecular techniques (PCR) had been used to detect the organism. Studies from the UK report a frequency of 1.3% for isolation of *Mycoplasma* in adults with CAP^[27] and of 0% from the Netherlands^[28]. These figures are widely different from the study done on CAP in children (<5 years old) by Liu *et al.* that shows a frequency of *Mycoplasma* as 7.1%^[25]. Studies from Iran about the prevalence of *Mycoplasma* in respiratory tract infections in children is limited and ranged from 0.4% to 10%^[29]. In a Survey by Hadi *et al.* for detection prevalence of *M. pneumoniae* in Iranian children with acute lower respiratory tract infections, the rate of infection was 10%^[29]. This finding was not in agreement with our finding. In another study which carried out by Naghipour *et al.* in children under 5 years old, in which out of 267 patients presenting with upper and lower respiratory tract infection, *Mycoplasma* were isolated from only 2 youngsters (0.8%) and only one of these (0.4%) had infection^[12]. We had a so low incidence of *M. Pneumoniae*, probably due to the fact that the population in our study was very young. Since at this age, virus are the most frequent etiological agents.

Since the frequency of *M. pneumoniae* varies according to age, being more common in school-age children, difference in reports from different studies could be explained by the difference in the age of patients studied. Other confounding factors could be the season of year and the technique used for

specimen collection and /or isolation of the microorganism. It is expected that sample collection at different periods during all 4 seasons of the year, utilizing nasopharyngeal or Oropharyngeal swabbing with dacron rather than cotton-tipped swabs for obtaining the specimens, and utilizing real-time PCR for detection of the microbe would result in the highest yield of microorganisms^[30]. Another important factor that would interfere with the detection of the microorganism would be prior use of antibiotics. Various other factors including race, climate or socio-economic conditions could account for the wide discrepancies in the frequency of *M. pneumoniae* noticed in studies from different parts of the world done on similar age groups and by using similar isolation techniques (PCR), with a rate of 2.4% from the Netherlands and of 18.7% from Japan in youngsters under the age of 15 years^[3,24].

We could not detect *C. trachomatis* in any of our infants presenting with CAP despite repeated testing; this finding is different from the results from Thailand where they found a frequency of 2.4% in infants aged 1 to 24 months with CAP and from Turkey where *C. trachomatis* was isolated from 3% of children between the ages of 3 to 12 months with CAP^[5,31]. It has been estimated that approximately 50% of neonates delivered vaginally from mothers with chlamydial infection of genital tract would acquire the infection; approximately 25% of these will develop chlamydial conjunctivitis and between 5%–20% will go on to develop chlamydial pneumonia^[32]. If the delivery is through a cesarean section (CS), with intact membranes then these figures are significantly lower. One important factor that could account for this diversity in the isolation of *C. trachomatis* in various studies could be the differences in modes of delivery (vaginal delivery or CS), or the disparity in the frequency of *C. trachomatis* infection in pregnant women in different parts of the world. According to WHO, the acceptable rate of CS is between 10%–15%, while the rate of CS in Iran is much higher, a nation-wide study has shown the rate of CS in private hospitals in Tehran to be 76.8% of all deliveries and in public hospitals as 42.6%^[33]. As far as the rate of chlamydial infection in women is concerned, a report published by WHO about sexually transmitted diseases in the late 1990s, rates of chlamydial infection of the urogenital tract in asymptomatic women in European countries quoted figures that ranged from 2.7% to 8%; in pregnant women in the Western Pacific region, these rates were between 5.7% in Thailand to almost 26% in Papua New Guinea^[34]. Chlamydia surveillance report published by CDC in the United States has shown chlamydia positivity rates in females (15 to 24 years old) attending family planning clinics to be 6.6% in 2010^[35]. A search for the prevalence of chlamydial infection in Iran revealed an incidence of 6.4% to 11.6% in women of childbearing age from 2 studies in Tehran and in Babol from the north of Iran^[36,37]. Although we did not record the mode of delivery in our patients, figures from the studies cited above support the assumption that the scarcity of *C. trachomatis* in our study could be because of the high rate of CS and not due to a lower rate of *C. trachomatis* infection in mothers. A study from Iran on 2500 hospitalized neonates found conjunctivitis in approximately 10% of the infants and *C. trachomatis* was isolated from 16.6% of these neonates^[38]. Since the incidence of chlamydial pneumonia in infants is much lower than conjunctivitis, it is not surprising that we could not detect even a single case of *C. trachomatis* infection in the

69 infants with pneumonia in our study. This study should be considered as a preliminary report and should be expanded to including more children and during a longer period of time in order to draw more definite conclusions.

In our study *M. pneumoniae* was found to be an uncommon cause of RTIs in children under 5 years old and *C. trachomatis* could not be listed as causing CAP in infants. In other countries and studied populations, the evidences can be different. Antibiotic treatment should be adapted to every media. More studies with a larger sample size are needed to confirm this observation.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Respiratory tract infections are a major cause of morbidity and mortality worldwide especially in young children. *M. pneumoniae* and chlamydia trachomatis can be responsible for CAP by which the authors have performed a cross-sectional study for determining the incidence of both organisms in children presenting with CAP attended in a tertiary care hospital in Teheran during a 14 months' period.

Research frontiers

Just one single case of *M. pneumoniae* infection was detected as a cause of CAP among 102 Iranian children under 5 years old attended in a tertiary hospital. On the other hand, *C. trachomatis* was not detected in the subgroup of 69 patients under 1 year old in which this organism was screened.

Related reports

There are many published studies reporting the incidence of *M. pneumoniae* and *C. trachomatis* in CAP in children and adults. In fact, the authors refer up to 20 references dealing with this subject. However, the references should be limited to studies in children, mainly within the same range of age.

Innovations & breakthroughs

Data on prevalence of *M. pneumoniae* and *C. trachomatis* as cause of CPA in Iranian children are scarce. There are just a few papers dealing with this subject but mainly published in Persian. This study shows the low prevalence

of *M. pneumoniae* (just one case) and *C. trachomatis* (no case demonstrated) among 102 young children diagnosed of CAP.

Applications

The results may have some epidemiological interest and can also be useful for choosing the empirical treatment of CAP in young children, as the organisms screened could be considered as rare pathogens for the select group of patients studied.

Peer review

This study includes 102 children less than 5 years old diagnosed of CAP during a 14 months' period and attended in a tertiary care children's hospital in Teheran. In all patients, *M. pneumoniae* was investigated by PCR and real-time PCR. In the subgroup of 69 children less than 1 year old, *C. trachomatis* was also investigated by a molecular technique.

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