Mycoplasma pneumoniae Infections in a Tertiary Care Hospital

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

Background: *Mycoplasma pneumoniae* is a common causative agent of community-acquired and atypical pneumonia. *M. pneumoniae*-associated respiratory tract infections are most often mild and self-limiting in nature, and severe courses of disease are not common.
Aims and Objectives: We aimed to analyze respiratory samples of hospitalized patients by real-time (RT) PCR for *M. pneumoniae* infections.
Methodology: In this study, hospitalized patients with respiratory symptoms including nasal congestion, rhinorrhea, nasal discharge, wheezing, sore throat and cough, were tested for *Mycoplasma pneumoniae* between April 2018 and March 2020 and the results of these patients were analyzed retrospectively.
Results: We retrospectively investigated the results of 1245 nasopharyngeal swab samples. Records of 693 (55.7%) male and 552 (44.3%) female patients between 0-93 years were investigated.All of the patients were below 26 years. There were 37 *M. pneumoniae* positive patients and the highest *M. pneumoniae* positivity was in 2-5 age group, with 16 children. *M. pneumoniae* positivity rate was highest in the 0-1 age group with 8.3%. There were 19 co-infections with viruses and rhino/enterovirus (n=12) was the most frequent accompanying virus.
Conclusion: *M. pneumonia* infections are found in about 3% of respiratory samples. *M. pneumoniae* is usually a part of co-infections accompanied by bacteria and viruses. Patients with respiratory infection symptoms should be diagnosed with multiplex rapid PCR panels including *M. pneumoniae*.

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1. INTRODUCTION

Mycoplasma pneumoniae is a common causative agent of community-acquired and atypical pneumonia [1].The most common atypical pneumonia causative agents are *Mycoplasma pneumoniae, Chlamydia psittaci (psittacosis), Francisella tularensis (tularemia), Coxiella burnetii (Q fever), Chlamydia pneumoniae, and Legionella* [2,3]. *M. pneumoniae* is responsible for about 10%–40% of the community acquired pneumonia (CAP) in children annually [4]. The highest incidence rates for *Mycoplasma pneumoniae* is seen in children and young adults, and it is typically characterized by a slowly progressing onset, followed by a persisting cough [5,6]. *M. pneumoniae*-associated respiratory tract infections are most often mild and self-limiting in nature, and severe courses of disease are not common [7,8]. Although *M. pneumoniae* infection is often considered a self-limiting disease, some cases become refractory or fulminant and these cases can have serious extrapulmonary complications, even becoming life threatening in children [9]. Therefore, the early and rapid detection of *M. pneumoniae* is important for timely clinical treatment [10].

Methodologies for detection of *M. pneumoniae* include nucleic acid amplification tests (NAAT), serology and culture with varying sensitivities and specificities. There is no international standard material for quality control detection in assays, although external quality control schema exist for some methodologies. There are no internationally defined guidelines on the requirements for surveillance of *M. pneumoniae*, macrolide resistance testing and surveillance, reference system structure, routine testing and bacterial strain discrimination. However, a few countries such as France and the United States (US) have surveillance within specific regions and national surveillance is seen in countries such as Denmark and Japan, the latter of which has maintained an active surveillance system for this pathogen for some time [11,12].

In this retrospective study, our aim was to analyze respiratory samples of hospitalized patients by real-time (RT) PCR for *M. pneumonia* infections between April 2018 and March 2020 in our tertiary care hospital.

2. MATERIALS AND METHODS

Bursa Uludag University Hospital is a tertiary care hospital which is located in the Northwest of Turkey with a population of about six million. In this study, hospitalized patients with respiratory symptoms including nasal congestion, rhinorrhea, nasal discharge, wheezing, sore throat and cough, were tested for *Mycoplasma pneumoniae* between April 2018 and March 2020 and the results of these patients were analyzed retrospectively. Inclusion criteria is adequate sampling and transporting of nasopharyngeal samples of the patients with respiratory symptoms. Exclusion criteria is inadequate sampling from the nasopharynx of the patients with respiratory symptoms and inappropriate transfer of the samples.

Results of nasopharyngeal swab PCR between 04.01.2018 and 03.31.2020 were analyzed. FLOQ Swabs (Copan, Italy) were used for nasopharyngeal sampling and placed in a transport media (UTM-RT, Copan, Italy) and stored at -80°C. The samples were analyzed within four days.

In our laboratory, we used Fast Track Diagnostics (FTD) respiratory pathogens 33 (Fast-track Diagnostics, Malta) kitfor RT-PCR, in Rotor-Gene Q (Qiagen, Germany) between April 2018 and December 2019. We used QIAStat Dx Respiratory panel(Qiagen, Germany) in QIAStat Dx (Qiagen, Germany) for detection of *Mycoplasma pneumoniae* between January 2020 and March 2020. QIAStat Dx (Qiagen, Germany) combines nucleic acid extraction and RT-PCR in the same device by a cartridge. Both of the kits use a panel for respiratory tract viruses and bacteria including *Mycoplasma pneumoniae*.

We included the results of the samples including *M. pneumoniae* positive respiratory samples. The results of FTD respiratory pathogens 33 (Fast-track Diagnostics, Malta) kit were interpreted according to the manufacturer's instructions; if the cycle threshold(Ct) was ≤35 in the presence of a sigmoid curve the result was positive. PCR results were analyzed according to MIQE guidelines key criteria chart [13]. QIAStat Dx Respiratory panel (Qiagen, Germany) tests were analyzed by the device software and results were reported according to the instructions of the manufacturer. Patients were divided into four groups according to their ages.

IBM SPSS Statistics v20 programme (SPSS INC, Chicago, USA) was used for statistical analysis. We used chi-square test on SPSS for statistical analysis. A p value of $P \leq 0.05$ was accepted as significant.

3. RESULTS AND DISCUSSION

We retrospectively investigated the results of 1245 nasopharyngeal swab samples. There were 693 (55.7%) male and 552 (44.3%) female patients between 0-93 years with a mean age of 18.2 years. Patients were divided into four groups according to their ages and most of the patients were in the 2-5 years group (Table 1). There were 37 *M. pneumoniae* positive patients and the highest *M. pneumoniae* positivity was in 2-5 age group, with 16 children (Table 1). While 18 patients were male, 19 were female. *M. pneumoniae* positivity rate was highest in the 0-1 age group with 8.3% which declined to 0.4% in the adult group. Positivity rate declined from childhood to adulthood among the age (Table 1). Mean age of the patients was 5. The oldest patient was 25 year old.

We investigated the number of samples and *M. pneumoniae* positives according to months. Although the highest number of samples were sent in January (293 samples), the highest number of *M. pneumoniae* positives were in October, which was followed by December (Table 2). 20 of 37 *M. pneumoniae* samples were detected in the autumn period for Turkey between October to December (Fig. 1).

There were 19 co-infections with viruses (Table 3). Rhino/enterovirus (n=12) was the most frequent accompanying virus. Rhino/enterovirus co-infections were seen in every season of the year. Rhino/enterovirus co-infections were seen in all the groups of pediatric patients. There were 5 bocavirus co-infections which were between October and February in the autumn and winter period of the Northern Hemisphere. All of the co-infections were seen in the pediatric age groups.

Although there are different techniques for detection of *M. pneumoniae*, nucleic acid amplification techniques are gaining importance and widely used for diagnosis [12]. COVID-19 pandemic has showed the importance of rapid diagnosis of viral infections by Polymerase Chain Reaction (PCR) [14,15,16]. As *M. pneumoniae* is a hard to cultivate bacteria, nucleic acid amplification techniques such as PCR can be used more in the diagnosis of atypical bacteria in

the near future [12,13]. Denmark and Israel is widely using PCR for diagnosis [12]. In addition respiratory panel tests including most common viruses and bacteria are gaining importance for the diagnosis of respiratory infections and most of the panels include *M. Pneumoniae* [14,16,17]. These panels can determine the causative agents in the respiratory specimens of patients only in a few hours which can be useful for the specific and rapid antimicrobial treatment. For the treatment of *M. pneumoniae,* macrolide antibiotics, such as azithromycin, are used as the first-line of treatment in many countries. Tetracyclines and fluoroquinolones are the other choices for the treatment of *M. pneumonia* [18-20].

We investigated the patients according to sex but there was no statistical difference for *M.pneumoniae* infections between males and females. *M. pneumoniae* positivity was statistically higher in the 0-1 age group among all other age groups. Although the highest number of positivity was in the 2-5 years group, 0-1 age group had the highest rate of positivity. Most of the patients were in the pediatric age group and only 1 of 37 patients were 25 years old, which means that *M. pneumoniae* infections are usually seen in children and young adults which is concordant with the literature [1,7,12]. In our laboratory we have been testing *M. pneumoniae* since 2015 and we had detected 17 *Mycoplasma pneumoniae/Chlamydia pneumoniae* positive patients in 311 respiratory samples [16]. Our previous kit was able to detect *Mycoplasma pneumoniae/Chlamydia pneumonia* not in alone but our newer kits provided the data that we detect *M. pneumoniae* much more frequent than *C. pneumoniae*. We detected only one *C. pneumoniae* between April 2018 and March 2020 which means that most of the *Mycoplasma pneumoniae/Chlamydia pneumonia* positive samples were *M. pneumoniae* between December 2015 and March 2018. Positivity of samples for *M. pneumoniae* was statistically higher than April 2018 and March 2020 which may be due to the seasonal variation of the disease and the common peak seen in 2015 in the European countries [12]. *M. pneumoniae* has infection peaks within 4 to 7 years in moderate climates. Little is understood about the transmission of *M. pneumoniae* within populations and several factors have been postulated to account for transmission dynamics, including the immunity level of the population, the bacterial population based on the P1 adhesin type, the age and extent of mixing of children in

educational settings [12]. Our study highlights that the seasonality and age groups are in accordance with the literature [12,21,22]. Seasonality of *M. pneumoniae* is changing among countries. In this study we found that most of the infections were in October, November and December. Eurosurveillence data shows that *M. pneumoniae* infections have peaks between weeks 27 and 39 [12]. Our peaks were in 45th week of 2018 and 41th week of 2019. Additionally an outbreak of *M. pneumoniae* was recorded in Finland in 2017 and 2018 and Kurkela et al. [23] found that the peak was in 44th week.These data show that our findings are concordant with the literatüre and this means that climate conditions of Turkey is suitable for *M. pneumoniae* infections in later weeks of the year.

There is plenty of data about *M. pneumoniae* co-infections with viruses. Zhao et al. [24] found that 38% of *M. pneumonia* were a part of co-

infections which is lower than our ratio (58%). Zhao et al. [24] showed the most common co-infections were with rhinoviruses, followed by parainfluenza viruses. Our data about the most common co-infection is concordant with the data of Zhao et al.[24]. In addition, *M. pneumoniae* and *Streptococcus pneumonia*e co-infections are common [24,25]. Zhang et al. [26] found that *M. pneumoniae* was a part of co-infections in 27% of infections;most of the co-infections were with *S. pneumoniae* among bacteria and bocavirus and rhinovirus among viruses which is concordant with our data. As being a part of co-infections diagnosis of respiratory infections should be done by multiplex PCR panels including most common viruses and *M. pneumoniae* for rapid diagnosis and specific antimicrobial treatment [27,28].

This study has a limitation; data was collected from single center.

Table 1. Number of test requests and *M. pneumoniae* positivity according to age groups

| Age	Test request	*M. pneumoniae* (+)	Positivity rate %
0-1	109	9	8.3
2-5	520	16	3.1
6-17	364	11	3.0
≥18	252	1	0.4
Total	1245	37	3.0

Table 2. Number of requests and *M. Pneumoniae* positive samples according to months.

Months	Total samples	Positive samples (%)
January	293	2(0.7)
February	182	4(2.2)
March	160	3(1.9)
April	70	3(4.3)
May	90	1(1.1)
June	19	1(5.3)
July	29	1(3.4)
August	56	0(0.0)
September	53	2(3.8)
October	95	8(8.4)
November	86	7(8.1)
December	112	5(4.5)

Table 3. Mycoplasma pneumoniae co-infections with viruses

Virus	Number
Rhino/enterovirus	12
Bocavirus	5
RSV	2
Parainfluenza virus	2

Abbrevations: RSV respiratory syncytial virus
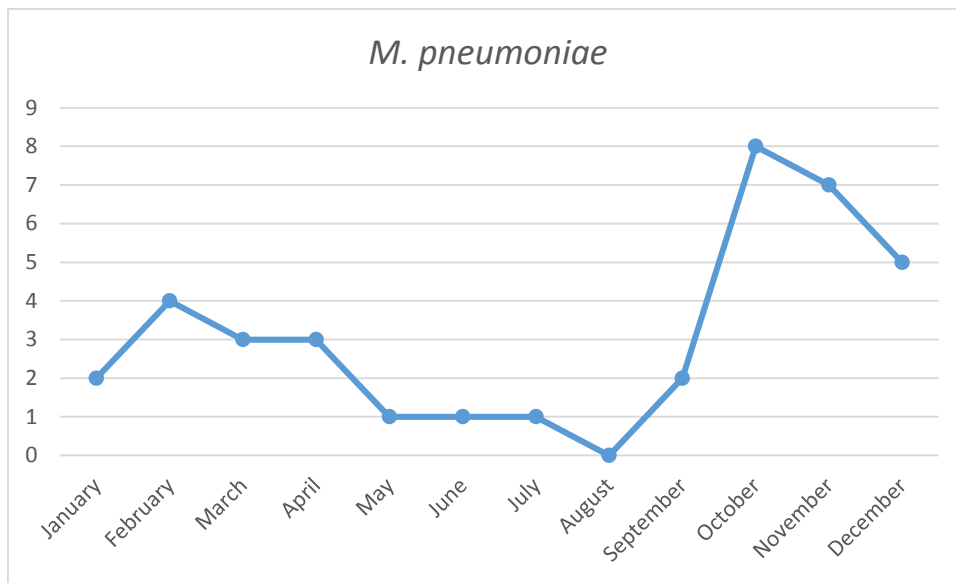
Fig. 1. Number of *M. pneumoniae* positive samples according to months
(Vertical line: Number, horizontal line months)

4. CONCLUSION

M. pneumoniae is an important agent for respiratory infections. Our study revealed that *M. pneumonia* infections are found in about 3% of respiratory samples. *M. pneumoniae* is usually a part of co-infections accompanied by bacteria and viruses. Rhinovirus is the most common co-infecting virus. Patients with respiratory infection symptoms should be diagnosed with multiplex rapid PCR panels including *M. pneumoniae*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patient's consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that the study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75938