# Comparison of Throat and Nasopharyngeal Swab Specimens for Molecular Diagnosis of Mycoplasma pneumoniae

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**Abstract:** *Mycoplasma pneumoniae is a common cause of respiratory infections in humans. Although it is usually associated with mild acute respiratory infections such as sore throat, pharyngitis, rhinitis and tracheobronchitis. The aim of this study was to evaluate the prevalence of M. pneumoniae among the patients of respiratory tract infection (RTI) in hospitalized patients. A total of 290 patients were included and one specimen of throat and Nasopharyngeal swabs from each patient was taken.  The mean age of the patients was  34.5 (±9.80) years and 57.2% were males. The prevalence of* Mycoplasma pneumoniae *was significant higher (15.5%) among NPS samples compare with throat (7.9%) samples (RR=0.51, 95%CI=0.32-0.82, p=0.005). The prevalence of* Mycoplasma pneumoniae *was found to be decreased with increase in the age of the patients among both throat and Nasopharyngeal swabs, however, this was statistically insignificant (p>0.05). The prevalence was higher among male patients compared with female among both throat and Nasopharyngeal swabs, and this was statistically significant (p<0.05). There was seasonal variations in the prevalence of* Mycoplasma pneumoniae. *The prevalence was higher in winter season than summer among both throat (Winter=5.9%, Summer=9.3%) and Nasopharyngeal (Winter=2.1%, Summer=6.2%) swabs.*
**Key words:** Mycoplasma pneumoniae, prevalence, *seasonal variations*

## I.    Introduction

Mycoplasma pneumoniae is a common cause of community-acquired pneumonia and is transmitted by aerosol or close contact. *Mycoplasma pneumoniae* infections occurs both endemically and epidemically worldwide, especially in children and young adults (Atkinson et al, 2008). While pneumonia may be the most typical manifestation, children may more commonly have symptoms like cough and wheezing, often accompanied by symptoms of upper respiratory tract infection, and the symptoms mimic those seen in viral respiratory syndromes (Othman et al, 2005).

Historically, the highest prevalence of M. pneumoniae infection among children is found in school-aged children and young adults, among whom the prevalence rises with higher age. Recent studies, however, suggest that the infection may be under-diagnosed in children under the age of five years (Gadsby et al, 2012; Defilippi et al, 2008).

*Mycoplasma pneumoniae* is a common cause of respiratory infections in humans. Although it is usually associated with mild acute respiratory infections such as sore throat, pharyngitis, rhinitis and tracheobronchitis, it can also cause more critical infections including pneumonia or lung abscess. *M. pneumoniae* is known to be responsible for 10 to 30 per cent of community-acquired pneumonia (CAP) cases and is also associated with acute exacerbations of asthma (Gil et al, 1993; Lieberman et al, 1996) and chronic obstructive pulmonary disease (COPD) (Varma-Basil et al, 2009), and even causing community outbreaks similar to influenza (Walter et al, 2008).

The aim of this study was to evaluate the prevalence of *M. pneumoniae* among the patients of respiratory tract infection (RTI) in hospitalized patients.

## II.       Material And Methods

In this cross-sectional study, we included the patients admitted in the out-patients clinic at western part of Uttar Pradesh, India. All infections were community-acquired. The decision to test for M. pneumoniae was based on the physician's clinical evaluation. The study was approved by the ethical committee of the hospital and consent was taken from each of the patients.

The throat swab specimen was collected with a viscose swab, which was placed in a transport medium. Specimens were analysed for the presence of M. pneumoniae by a real-time PCR using a hydrolysis probe and targeting the gene encoding adhesin P1. Nasopharyngeal swabs (NPS) samples were obtained by use of flocked swabs and trans-ported in universal transport medium. All specimens re-transported to the central laboratory at 4°C within 24 hr of collection. Demographic data included age, weight and sex.

For the PCR assay, a rapid DNA extraction protocol was used. In a sterile tube, 100 ml of the BAL and 300 ml of a suspension consisting of 15% (wt/vol) Chelex 100 resin (Bio-Rad Laboratories, Richmond, Calif.) in 10 mM Tris-HCl (pH 8.0)–0.1 mM EDTA–0.1% sodium azide were mixed vigorously with a vortex mixer for 30 s. The tube was then placed in a boiling water bath. After 10 min of incubation, the tube was removed and allowed to cool to room temperature. Following complete settlement of the resin, 20 ml of the supernatant was carefully removed and used for amplification directly, without further purification. For PCR, the upstream primer DD50B (59-biotin-GCAAA GTTATGGAAACATAATGGAGGTT-39 [positions 999 to 1026]) and the downstream primer DD54B (59-biotin-GGTT AGCAACACGTTTTTAAATATT-39 [positions 1401 to 1425]) from the published sequence of the gene encoding the small subunit rRNA (16S rRNA) of *M. pneumoniae* (GenBank accession no. M29061) were used. This set of primers, which was chosen from a region of the 16S rRNA gene which contains *M.pneumoniae*-specific sequences, allows amplification of a 427-bp fragment. PCR was done in 100-ml reaction mixtures containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3 mM MgCl2, 5% glycerol, 200 mM (each) deoxynucleoside triphosphates (including dUTP instead of dTTP), 50 pmol (each) oligonucleotide primer, 20 ml of extraction supernatant, 5 U of *Taq* polymerase (AmpliTaq; Perkin-Elmer, Langen, Germany), and 2 U of uracil-*N*-glycosylase (AmpErase; Perkin-Elmer) in a Perkin-Elmer System 9600 thermocycler (Perkin-Elmer, Norwalk, Conn.). After incubation at 50°C for 2 min, two cycles consisting of 20 s at 98°C, 20 s at 62°C, and 45 s at 72°C followed by 35 cycles consisting of 20 s at 94°C, 20 s at 62°C, and 45 s at 72°C were run. After the final cycle, the tubes were incubated for an additional 10 min at 72°C.

Univariate statistical analysis of the data was performed using $\chi^2$ test on categorical comparisons of two populations. All tests were two-tailed and p values below 0.05 were considered significant. 95% confidence intervals were used.

## III.       Results

A total of 290 patients were included and one specimen of throat and Nasopharyngeal swabs from each patient was taken.
The mean age of the patients was 34.5 (±9.80) years and 57.2% were males (Table-1).

The prevalence of *Mycoplasma pneumoniae* was significant higher (15.5%) among NPS samples compare with throat (7.9%) samples (RR=0.51, 95%CI=0.32-0.82, p=0.005) (Table-2).

The prevalence of *Mycoplasma pneumoniae* was found to be decreased with increase in the age of the patients among both throat and Nasopharyngeal swabs, however, this was statistically insignificant (p>0.05). The prevalence was higher among male patients compared with female among both throat and Nasopharyngeal swabs, and this was statistically significant (p<0.05) (Table-3).

There was seasonal variations in the prevalence of *Mycoplasma pneumoniae*. The prevalence was higher in winter season than summer among both throat (Winter=5.9%, Summer=9.3%) and Nasopharyngeal (Winter=2.1%, Summer=6.2%) swabs (Fig.1).

## IV.       Discussion

In our study, *M. pneumoniae* was the most frequent cause of respiratory tract infection in all age groups of outpatients . This result is in agreement with previously reported studies (Atmar et al, 1989; Ghosh,, 1992; Gnarpe et al, 1992). This high prevalence was certainly due to the use of a reliable, sensitive detection technique; PCR has proved its usefulness for accurate diagnosis and is now accepted as a clinically useful technique (Leng, et al, 1994). Our study also confirmed that *M. pneumoniae* can be responsible for epidemics.

This analysis of outpatients infected with *M. pneumoniae* emphasizes its pathogenic role but revealed no specific sign or symptoms. *M. pneumoniae* was responsible for rhinopharyngitis, bronchitis, and otitis. These symptoms were observed in the different age groups, unlike in previous studies (Dominguez 1996). Interestingly, no specific increase in severe atypical pneumonia was observed during winters with a high prevalence of *M. pneumoniae* infection.

We found that there was seasonal variations in the prevalence of *M. pneumoniae* being higher in winter season than summer. However, A study has shown that *M pneumoniae* infection did not show seasonal variations; (Foy et al, 1979) in contrast, however, recent studies have shown that *M pneumoniae* infection peaks in winter (Sidal et al, 2007) or spring (Lieberman, 1996), and this aetiology should be considered first when it occurs in the summer and autumn months (Mansel 1989). The clear cyclical and seasonal occurrence suggests that climatic factors could play a role. Moreover, another study has shown that the most important factor explaining the variance in CAP is the direct and indirect effects of meteorological variables (Lieberman, 1999). However, few quantitative studies have investigated the impact of weather factors and variability on the incidence of *M pneumoniae* pneumonia, allowing for the mutual confounding between weather factors and potential confounding by other seasonally varying factors

## V. Conclusion

*M. pneumoniae* is a frequent pathogen in upper respiratory tract infection, and since it can be cured by appropriate therapy, its detection should be systematically performed in patients presenting with ARI.

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### Table-1: Demographic distribution of the patients

| Demographic parameters | n=290 |
|---|---|
| Age in years, mean±sd | 34.5±9.80 |
| Male sex, no. (%) | 166 (57.2) |
| Weight in kg, mean±sd | 37.12±19.87 |

**Table-2: Comparison of prevalence of *Mycoplasma pneumoniae* among throat and Nasopharyngeal swabs (NPS)**

| Type of swab | No. (n=290) | % | RR (95%CI), p-value |
|---|---|---|---|
| Throat | 23 | 7.9 | 0.51 (0.32-0.82), 0.005* |
| Nasopharyngeal | 45 | 15.5 | |

RR-Relative Risk, CI-Confidence Interval, *Significant

**Table-3: Comparison of prevalence of *Mycoplasma pneumoniae* among throat and Nasopharyngeal swabs (NPS) by Age and Gender**

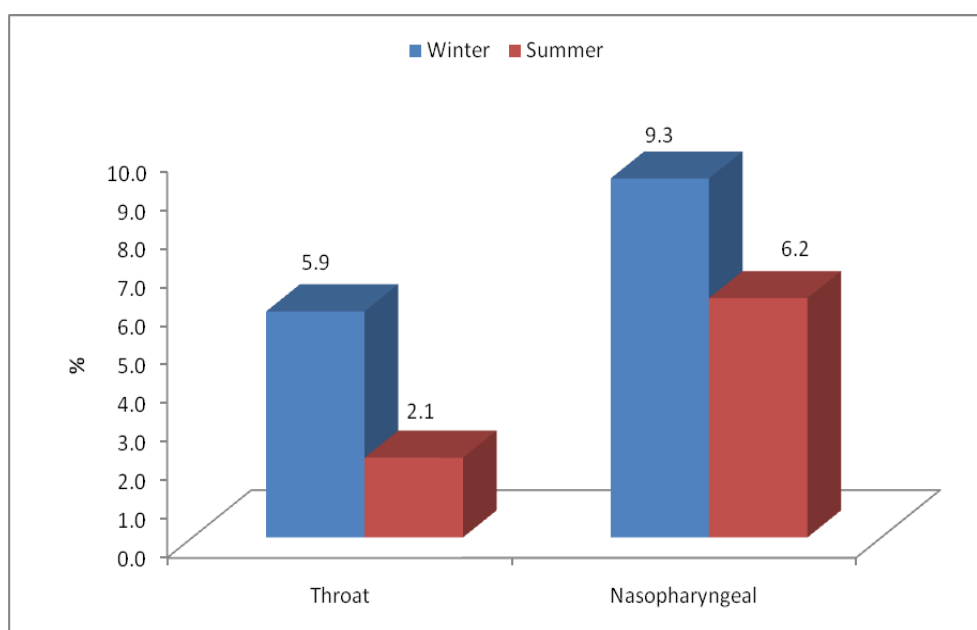| Age and Gender | No. of samples | Throat | | Nasopharyngeal | |
|---|---|---|---|---|---|
| | | No. with *M. pneumoniae* | % | No. with *M. pneumoniae* | % |
| Age in years* | | | | | |
| <4 | 94 | 9 | 9.6 | 18 | 19.1 |
| 4-14 | 78 | 7 | 9.0 | 14 | 17.9 |
| 15-29 | 36 | 3 | 8.3 | 5 | 13.9 |
| 30-44 | 31 | 2 | 6.5 | 4 | 12.9 |
| 45-59 | 24 | 1 | 4.2 | 2 | 8.3 |
| 60-65 | 19 | 1 | 5.3 | 1 | 5.3 |
| >65 | 8 | 0 | 0 | 1 | 12.5 |
| Gender** | | | | | |
| Male | 166 | 14 | 8.4 | 27 | 16.3 |
| Female | 124 | 9 | 7.3 | 18 | 14.5 |

*p>0.05,



**Fig.1: Seasonal variation in the prevalence of *Mycoplasma pneumoniae* in throat and Nasopharyngeal swabs**