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Prevalence of *Mycoplasma Pneumoniae* in Patients

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ABSTRACT

Researchers have recently become interested in Mycoplasma pneumoniae. Although prevalence studies are conducted often in countries outside of India, they are rarely conducted in India. With this knowledge, researchers have attempted to characterize and elucidate the occurrence and prevalence of Mycoplasma pneumoniae infections. The majority of the available data on Mycoplasma pneumoniae infections comes from studies conducted in the United States, Europe, and Japan. This study is essential seroprevalence investigations that show the existence of antibodies to Mycoplasma pneumoniae and suggest that inhabitants in these areas have been infected with this organism.

1. Introduction

After the bacterium was identified as the cause of primary atypical pneumonia in the early 1960s, researchers began to investigate it. M. pneumoniae was found to be responsible for 15 to 20% of all occurrences of community-acquired pneumonia, or two instances per 1,000 people, in Seattle, Washington, between 1962 and 1975, according to antibody tests and culture. Additional retrospective serological studies in Denmark revealed a pattern of M. pneumoniae infections spanning a 50-year period from 1946 to 1995, with endemic disease transmission punctuated by cyclic epidemics every three to five years. These findings were similar to those made in the United States. Similar patterns have been found in investigations conducted in North America and Europe over the last three decades. The extensive use of PCR for M. pneumoniae investigations since the mid-1990s has substantially improved our understanding of M. pneumoniae diagnosis. When pure DNA is employed, the sophisticated molecular techniques have exceptionally high sensitivity, theoretically allowing them to detect a single organism or a single copy of the targeted gene. This is far higher than the culture detection threshold, which is around 100 to 1,000 cells under ideal conditions. Despite the fact that population-based research has provided us with a picture of the prevalence and incidence of M. pneumoniae infections around the world, the Indian situation is unclear. This is most likely owing to a lack of understanding of the severity of M. pneumoniae infections, as well as the misconception that they are minor and self-limiting. Until now, the majority of research in India have focused on children or those who have an underlying ailment such as asthma or chronic obstructive pulmonary disease (COPD) (COPD). The HIV-positive patients are the other study group. Only a few studies included suspected community-acquired pneumonia patients of all ages. The majority of these reports came from the north, with a handful from the south. The research in the South is seroprevalence studies, but the ones in the North are more in-depth investigations that use culture, serology, and molecular approaches. In this region of the country, however, there are limited reports of similar research on the prevalence of M. pneumoniae. This study aims to investigate the incidence of M. pneumoniae in a tertiary care centre in Navi Mumbai using dependable and user-friendly methods of growing the organism, molecular detection of its nucleic acid, and serological assays. To the best of our knowledge, there is currently no national surveillance system for Mycoplasma pneumoniae infections.

Aim & Objectives: To investigate the frequency of Mycoplasma pneumoniae in patients with a lower respiratory tract infection, as well as the relationship between age, sex, and predisposing factors, IPD/OPD.

2. Materials and Methods:

The study was undertaken in a Navi Mumbai tertiary care centre to determine the prevalence of Mycoplasma pneumoniae infections in this region of the country. The following were the criteria for including and excluding patients from the study, as well as the type of sample obtained and sample analysis procedures.

Inclusion and Exclusion Criteria of Patients:
Inclusion criteria:

- Clinical, radiological, and blood counts were used to diagnose community-acquired pneumonia.
- Clinical: Cough, sputum output, and a temperature more than 37.80°C
- Radiological: Pulmonary infiltration on chest X-ray suggests pneumonia
- Total leukocyte count (TLC) > 12,000/pL on blood counts

Exclusion criteria:

- Hospital-acquired pneumonia, defined as pneumonia that develops within 72 hours of admission or within 7 days of discharge.
- A pulmonary shadow caused by something other than pneumonia.

Sample collection and transport:

Respiratory specimen:

In sterile wide mouthed containers, endotracheal tube aspirate and sputum samples were collected. Microscopy, culture, and polymerase chain reaction were performed on the samples right away in the microbiology lab. If a processing delay was expected, the samples were kept at 40°C for no more than 24 hours.

1. Screening of Samples:

In the case of sputum samples, Gram's staining was used to grade them according to Bartlet's grading system (BGS). The presence of pus cells and the type (morphology) of bacteria present in the gramme stain-smears were analysed. Only samples with pus cells were included in the study. The sample was not processed if the BGS score was zero. After screening, a total of 150 samples (131 sputum and 19 endotracheal tube aspirate) were chosen for further analysis and research.

No. of Neutrophils per 10X Low-Power Field*	No. of Epithelial Cells per 10X Low-Power Field*	Grade
<10	-	0
10-25	-	+ 1
>25	-	+2
Presence of mucus	-	+ 1
-	10-25	-1
-	>25	-2

Table 1: Bartlet's Grading System

In around 20-30 different 10X microscopic fields, the average number of cells. The total was computed. A score of 0 or less implies that there is no active inflammation or saliva contamination. A repeat specimen should be sought in such circumstances.

2. Sample processing:

The equipment required Fluorescence Microscope (Nikon, Japan; NI-SSR930575). The reagents required FITC labeled antibodies, Bovine Serum Albumin, DAPI (4',6-diamidino-2-phenylindole), Phosphate buffer saline, TritonX100, Tween 20.

Controls used: Positive- PPLO broth inoculated with *Mycoplasma pneumoniae* FH strain (ATCC 15531), Negative-Sterile PPLO broth.

The method was standardized for directly staining sputum and endo tracheal tube aspirate specimens with FITC antibodies to *Mycoplasma pneumoniae*. The protocol was a modification of the methodology adopted by Bhartiya D et al.

The culture preparation equipments are CO_2 incubator, incubator, desiccator, Stereomicroscope, Biological safety Cabinet, Class II B2. The reagents are PPLO agar, PPLO broth, horse serum, yeast extract etc.

In a bio-safety cabinet, the respiratory samples were transferred into Pleuro pneumonia Like Organism (PPLO) broth under aseptic conditions (Class II B2). Only 200 pL of the sample was used to inoculate 2mL of PPLO broth. To remove any other microorganisms in the sample, the inoculation soup was filtered through a 0.22pm syringe filter. The inoculated PPLO broth bottles were stored in the incubator in desiccators (candle jars). The ideal conditions for Mycoplasma pneumoniae growth are 5 percent CO_2 and 370 degrees Celsius. Positive growth is indicated by a change in the colour of the broth to yellow. Before declaring the cultures negative, they are incubated for one month to observe colour change.

Whenever there is a change in colour in the PPLO broth, 100pL of the broth is inoculated on PPLO agar and stored at 370C in a CO_2 incubator with 5% CO_2. On PPLO agar, colonies that look like fried eggs can be seen.

Controls used: Positive- Mycoplasma pneumoniae FH strain (ATCC 15531) inoculated in PPLO broth, Negative-Sterile PPLO broth.

3. Serological Tests: ELISA for IgM and IgG

Serology specimen:

Venipuncture was used to obtain 2-3 mL of venous blood utilising appropriate sterile precautions. Blood was collected in plain tubes. Serum was separated and put into sterile eppen doff tubes after collection. After that, they were held at -800C until further processing.

The following procedure was adopted as per manufacturer's instructions

- **In case of IgM detection:**

Prior to IgM detection, serum samples were pretreated with rheumatoid factor-absorbents. Rheumatoid factor-absorbent was diluted 1:4 in this experiment by mixing 200 gL Rf-absorbent with 800 gL dilution buffer. After that, 10gL of patient serum was distributed into 1mL of the previously diluted Rf-absorbent, resulting in a 1:101 dilution. It was then vortexed thoroughly and incubated at room temperature for 15 minutes.

- **In case of IgG detection:**

By dispensing 10gL of serum into 1mL of dilution buffer, serum samples were diluted 1:101. The mixture was then thoroughly blended by vortexing.

4. Polymerase Chain Reaction:

Requirements procured: The following is a list of the equipment and reagents used in polymerase chain reaction.

By boiling 200pL of inoculated PPLO broth, genomic DNA was recovered. In a nutshell, 200 pL of PPLO broth was centrifuged for 15 minutes at 14,000 rpm. Phosphate buffer saline was used to wash the pellet twice (pH 7.2). The pellet was re-suspended in 100 pL of sterile water and cooked for 10 minutes in a boiling water bath. After that, 30 seconds of

centrifugation at 12,000 rpm was performed. The extracted DNA is found in the supernatant.

PCR for P1 adhesin gene:

A primer set specific for a 375bp segment of the Mycoplasma pneumoniae P1 cytadhesion gene was used in the PCR. Forward (5' CCG CGA AGA GCA ATG AAA AAC TCC 3') and reverse (5' TCG AGG CGG ATC ATT TGG GGA GGT 3') primers were employed. 189>190 The primers were examined and verified using the NCBI nucleotide BLAST programme, which is detailed later in this chapter. In the Mycoplasma pneumoniae FH strain, the target 375bp sequence amplified by the primer set is as follows:

5'**CCGCGAAGAGCAATGAAAAACTCC**AGGGCGATGAA TCCAAGTCTTCCAATGG

ATCTTCAAGCACTTCCACCACCACCCAACGTGGTTCG ACCAATTCCGACACCAAA

GTCAAGGCTTTAAAAATAGAGGTGAAAAAGAAATCGG ACTCGGAGGACAATGGT

CAGCTGCAGTTAGAAAAAAATGATCTCGCCAACGCTC CCATTAAGCGGGGTGAG

GAGTCGGGTCAGTCCGTCCAACTCAAGGCGGACGAT TTTGGTACTGCCCCTTCCA

GTTCGGGATCAGGCGGCAACTCCAACCCCGGTTCCC CCACCCCCTGAAGGCCGTG

GCTTGCGACTGAGCAAATTCACAAGG**ACCTCCCCAAA TGATCCGCCTCGA**3'

In *Mycoplasma pneumoniae* M129 strain the 375bp sequence amplified is below:

5'**CCGCGAAGAGCAATGAAAAACTCC**AGGGCGCTGA GGCCACTGGTTCTTCAAC

CACATCTGGATCTGGCCAATCCACCCAACGTGGGGGT TCGTCAGGGGACACCAA

AGTCAAGGCTTTAAAAATAGAGGTGAAAAAGAAATCG GACTCGGAGGACAATG

GTCAGCTGCAGTTAGAAAAAAATGATCTCGCCAACGC TCCCATTAAGCGGAGCG

AGGAGTCGGGTCAGTCCGTCCAACTCAAGGCGGACG ATTTTGGTACTGCCCTTTC

CAGTTCGGGATCAGGCGGCAACTCCAATCCCGGTTC CCCCACCCCCTGAAGGCCG

TGGCTTGCGACTGAGCAAATTCACAAGG**ACCTCCCCA AATGATCCGCCTCGA**3'

PCR Specifications: The PCR reaction constituted of the following :

Master mix (2X bioline, Allied Scientific): 12.5 µL

Molecular grade water (Allied Scientific): 5.5 µL

Forward primer (Genetix): 1 µL

Reverse primer (Genetix): 1 µL

Extracted genomic DNA: 5 µL

Final volume: 25 µL

PCR for 16S rRNA gene:

The set of primers used were Mycoplasma pneumoniae species specific primers which targets a 277bp fragment of the 16S rRNA gene. The primers were forward (5 ' AAG GAC CTG CAA GGG TTC GT 3 ') and reverse (5 ' CTC TAG CCA TTA CCT GCT AA 3 ').3,154,191 and were verified using NCBI nucleotide BLAST. The amplified target sequence of the 16S rRNA gene is below:

5' '

AAGGACCTGCAAGGGTTCGTTATTTGATGAGGGTGCGCCA TATCAGCTAGTT

GGTGGGGTAACGGCCTACCAAGGCAATGACGTGTAG CTATGCTGAGAAGTAGAA

TAGCCACAATGGGACTGAGACACGGCCCATACTCCTA CGGGAGGCAGCAGTAGG

GAATTTTTCACAATGAGCGAAAGCTTGATGGAGCAAT GCCGCGTGAACGATGAA

GGTCTTTAAGATTGTAAAGTTCTTTTATTTGGGAAGAA TGACTTTAGCAGGTAAT

GGCTAGAG 3 '

The 16SrRNA gene was amplified using the identical components of the PCR reaction mixture as the P1 gene.

Controls used: DNA from Mycoplasma pneumoniae FH strains as a positive control (ATCC 15531). Water was utilized instead of DNA in the reaction mixture as a negative template control.

Down streaming was performed on the amplified PCR results using a 1% agarose gel electrophoresis. The expected 375bp band for the P1 gene and 277bp band for the 16S rRNA gene were compared to a 100bp to 1000bp DNA ladder (Bioline, Allied Scientific).

Controls used: Positive- Mycoplasma pneumoniae FH type II strain PCR amplified products (ATCC 15531).

3. Result and Discussion:

The findings of a study done to determine the prevalence of Mycoplasma pneumoniae in a tertiary care centre:

Fluorescence Microscopy:

Fluorescence microscopy was used to examine the patient samples in this study (n=150). A total of 16 cases tested positive for M. pneumoniae, resulting in a prevalence rate of 10.66 percent using this approach.

Interpretation: The fact that the chi-square p-value is less than 0.05 suggests that the proportion of positive cases recorded in the population is not equal. The observed proportion of positives can be deemed to be substantial.

Culture:

All 150 patient samples were examined under a microscope to determine the type and number of bacteria and inflammatory cells present. After that, the samples were assessed using Bartlet's grading system (BGS). 15 The samples were put in culture to screen for the presence of Mycoplasma pneumoniae when the BGS score was greater than zero. All of the samples had a BSG score of one or higher.

Mycoplasma pneumoniae could be identified in culture from two of the 150 samples. By culture, the prevalence of Mycoplasma pneumoniae is found to be 4.66 percent (7 out of 150 cases).

BSG score of the two samples positive by culture:

Sample 1- BSG score 1 (Pus cell 10-25/low power field)

Sample 2- BSG score 2 (Pus cell >25/low power field)

Interpretation: Since the p-value for the chi-square is less than that of 0.05 indicates that the observed proportion of the positive and negative is not equal in the population. It can be concluded that the observed proportion of positive is significant.

Serological Tests: ELISA for IgM and IgG

IgM and IgG antibodies to Mycoplasma pneumoniae were tested in the 150 patients who took part in the trial. This screening was carried out using ELISA testing for IgM and IgG antibodies, which provided information on the organism's seroprevalence in the area. IgM antibodies to Mycoplasma pneumoniae were detected in 19 instances (12.66%), while IgG antibodies were detected in 24 cases (16 percent).

Interpretation: The fact that the chi-square p-value is less than 0.05 suggests that the observed proportion of positive and negative in the population is not equal. The observed proportion of positives can be deemed to be substantial.

- **Polymerase Chain Reaction:**

PCR revealed the presence of Mycoplasma pneumoniae in 26 of the 150 patients in the study. Two of these samples revealed bacterial growth in culture. The P1 cytadhesin gene as well as the 16S rRNA gene PCR were both positive in all 26 cases. On downstream processing with a 1% agarose gel, the expected 375bp and 277bp amplicons for the P1 gene and 16S rRNA gene, respectively, could be detected. Mycoplasma pneumoniae was discovered to be present in 17.33 percent of the patients, or 26 out of 150.

Interpretation: The fact that the chi-square p-value is less than 0.05 suggests that the observed proportion of positive and negative in the population is not equal. The observed proportion of positives can be deemed to be substantial.

Comparison of the methods used for detection of Mycoplasma pneumoniae.

Fluorescence microscopy, culture, ELISA, and PCR were used to compare the approaches for detecting Mycoplasma pneumoniae. The largest number of cases detected by PCR, i.e., 26 out of 150, was discovered.

PCR was determined to be the most sensitive and specific method of detecting Mycoplasma pneumoniae in this investigation. PCR also has the highest statistical proportion of positive. As a result, all subsequent analysis was done based on the PCR results.

Demographic Profile (Based on PCR positivity)

There were 95 men and 55 women among the 150 patients who were studied. M.pneumoniae was found in 16 of the 95 male patients, representing a 16.84 percent frequency. In the case of females, 10 out of 55 patients had positive results, resulting in an 18.18 percent prevalence.

The fact that the chi-square p-value is larger than 0.05 implies that there is no link between gender and positivity and negativity.

Age Range	No of Individual	No of Positive Individual	Percentage
1-10	5	1	20.00%
11-20	12	2	16.66%
21-30	20	3	15.00%
31-40	22	3	13.63%
41-50	21	3	14.28%
51-60	30	5	16.66%
61-70	23	5	21.73%
71-80	13	3	23.07%
81-90	4	1	25.00%

Table 2: Age wise distribution of patients

4. Conclusion:

Various laboratory procedures were performed on the respiratory samples of 150 patients, including fluorescence microscopy, culture, ELISA, and PCR. The study's findings are described further down.:

1. Fluorescence Microscopy:

In 16 of the 150 cases, fluorescence microscopy was able to detect Mycoplasma pneumoniae. Using this strategy, a prevalence percentage of 10.66% was discovered. When compared to PCR, fluorescence microscopy had a sensitivity of 61.73 percent and a specificity of 100 percent.

2. Culture:

Seven cases of Mycoplasma pneumoniae were isolated from 150 patients using microscopy and culture as the detection method. This approach revealed a 4.66 percent prevalence of Mycoplasma pneumoniae. In one of the Mycoplasma pneumoniae-infected cases, the BGS score was +1, whereas in the other, it was +2. This approach was shown to have a sensitivity of 26.92 percent and a specificity of 100 percent.

3. Serological Tests: ELISA for IgM and IgG

Mycoplasma pneumoniae IgM antibodies were identified in 19 of the 150 patients. This results in a prevalence rate of 12.66 percent. ELISA has a sensitivity of 73.08 percent and a specificity of 100 percent for IgM antibodies. IgG antibodies to Mycoplasma pneumoniae, on the other hand, were found in 24 out of 150 cases, or 16 percent. For IgG antibodies, ELISA had a sensitivity of 92.31 percent and a specificity of 100 percent.

4. Polymerase Chain Reaction:

Out of 150 patients, PCR was able to discover 26 Mycoplasma pneumoniae infected individuals. As a result, the prevalence of Mycoplasma pneumoniae detected by PCR was 17.33%.

Comparison of the methods used:

When all of the detection methods were compared, PCR was determined to be the most sensitive and specific. Culture

revealed a prevalence of 4.66 percent (7 cases), whereas fluorescence microscopy revealed a prevalence of 10.66 percent (16 cases), ELISA for IgM revealed a prevalence of 12.66 percent (19 cases), and IgG revealed a frequency of 16 percent (24 cases). The highest prevalence percentage discovered by PCR was 17.33 percent, or 26 instances (p-value 0.000373). The low percentage of M. pneumoniae isolation in culture could be related to patients receiving antibiotic therapy elsewhere before coming to our hospital. It can stop M. pneumoniae from growing in the culture medium.

The current study agrees with Kashyap B et al.148, who found Mycoplasma pneumoniae prevalence to be 5.33 percent (4 out of 75 cases) by culture and 17.3 percent by PCR (13 out of 75 cases). The ELISA prevalence was 21.3 percent (16 out of 75 cases), which is higher than the PCR prevalence. However, ELISA was positive in only 11 of the 13 PCR positive instances, showing that PCR is more sensitive, as this study discovered.

By using an ELISA test, Basil MV et al.193 discovered M. pneumoniae seroprevalence in 16 out of 100 patients (16%). The organism could not be isolated in any of the cases. By culture, Sahoo R et al.178 found a 17 percent prevalence of M. pneumoniae and a 37 percent prevalence by ELISA. Only 2% of instances had both culture and ELISA positivity, while 22% had only culture positivity and 22% had only ELISA positivity. These findings are similar to those of the current investigation, in which we discovered that ELISA is more sensitive than culture.

In their investigation, Ieven M et al. found M. pneumoniae in 8 out of 371 samples (2.15%) by culture and 13 out of 371 samples (3.50%) by PCR. Culture had a 61.5 percent sensitivity compared to PCR. Varshney AK et al.[21] discovered M. pneumoniae infection in 15 of 150 (10%) cases by PCR, but the organism could not be isolated in culture in any of the cases. 92 cases and 74 controls were investigated by Dorigo-Zetsma JW et al.183. By PCR (8 percent), they discovered 7 patients with M. pneumoniae infections, and by culture, they discovered 6 patients with M. pneumoniae infections (7 percent). The organism was not found in any of the control instances. According to Buck GE et al.[112], the considerably more sensitive PCR could detect 1 to 10 organisms, whereas culture detection required 103 CFU/mL organisms. All of these researchers' findings are consistent with the current study, which demonstrated that PCR is more sensitive than culture.

The methodology of laboratory techniques, patient study group, age and sex distribution, and predisposing variables may all play a role in the prevalence of M. pneumoniae infections among various employees in India and other countries. Droplet infections and formites are used to disseminate M. pneumoniae infections in the population. The capacity of M. pneumoniae to transmit from one patient to another may be influenced by environmental and climatic factors such as temperature, humidity, and season. Finally, variations in the prevalence may be due to variations in patient health education regarding illness prevention and implementation of aseptic procedures by health care staff and doctors.

Demographic profile:

There were 95 males and 55 females among the 150 patients in this study. M. pneumoniae was found in 16 of the 95 males and 10 of the 55 females tested. As a result, M. pneumoniae is found in 16.84 percent of males and 18.18 percent of females. However, no statistically significant (p-value 0.861) link between gender and M. pneumoniae positive was discovered.

In their investigation, Chaudhry R et al.[149] discovered M. pneumoniae infection in 27 of 92 (69%) males and 16 of 42 (38%) females. M. pneumoniae infection was found in 12 of 46 males (26.09%) and 4 of 29 females (13.79%), according to Kashyap B et al.148. The prior investigations indicated no statistically significant link between sex and M. pneumoniae infections, which is consistent with the current findings.

When the patients were divided into age groups, it was discovered that M. pneumoniae infections were most common in the age group 1 to 10 years (20%), followed by 61 to 70 years (21.73%), 71 to 80 years (23.07%), and 81 to 90 years (23.07%). (25 percent). However, no statistically significant (p-value 0.664) link between M. pneumoniae infection and age distribution was discovered.

M. pneumoniae infections were found in 6 of 37 (16%) juvenile (0-15 years) and 37 of 97 (38%) adult (16-90 years) cases reported by Chaudhry R et al.149. In the current study, it was also discovered that 2 of 11 (18.18 percent) of the participants were between the ages of 0 and 15, and 24 of 139 (17.26 percent) were between the ages of 16 and 90.

In the age category of 6 months to 12 years, Kashyap B et al.148 discovered that M. pneumoniae infection was detected in 18 of 75 cases (24 percent) by culture, serology, or PCR. Two of the eight instances (25%) in this study belonged to the age bracket of 6 months to 12 years. These studies similarly found no statistically significant link between age and M. pneumoniae infections, and hence support the findings of the current investigation.

Ward wise distribution profile:

The patients in this study came from both the OPD and the IPD. 97 patients had IPD, with 17 cases (17.52%) testing positive for M. pneumoniae, and 53 patients had OPD, with 9 instances testing positive for M. pneumoniae (16.98 percent). There was no statistically significant link between OPD, IPD, and M. pneumoniae infections (p-value 0.93624).

Patients from various wards were included, and it was discovered that the Respiratory Medicine department had the highest number of positive M. pneumoniae cases (16 out of 67). (23.88 percent). Statistically, the number of patients from the Respiratory Medicine department was found to be significantly higher (p-value 0.00007559).

A careful search of the available literature revealed no research linking Mycoplasma pneumoniae to OPD, IPD, or any particular department. However, many studies have been undertaken on individuals who have been admitted to the hospital due to respiratory tract infections. From time to time, studies focusing on outbreak investigation are also reported.

Specimen wise distribution of *M. pneumoniae:*

This study includes 150 respiratory specimens, including 131 sputum samples and 19 endotracheal tube aspirate samples. M. pneumoniae was found by PCR in 23 of the 131 sputum samples (17.55%), and three of the 19 endotracheal tube aspirate samples (15.78%) showed the presence of M. pneumoniae by PCR.

Radiological profile: Primary atypical pneumonia caused by M. pneumoniae has a wide range of symptoms and can resemble a variety of lung illnesses. The radiological profile of the 26 M. pneumoniae PCR positive patients revealed that 20 cases (76.92%) had significant X-ray findings and 6 cases (23.07%) had no significant X-ray findings. There was a statistically significant link between substantial X-ray results and M. pneumoniae infections (p-value 0.006). According to Chaudhry R et al.149 from Delhi, India, 42 of 43 M.pneumoniae positive cases had substantial X-ray findings (98 percent). M. pneumoniae infections were identified in 8 out of 13 individuals (61.53 percent) with bronchopneumonia, according to Kashyap B et al.148 from Delhi, India. Bilateral involvement may occur in roughly 20% of instances, according to Marrie TJ. 198 The findings of the previous investigations are consistent with the current study, since we identified significant X-ray findings in the majority of cases with M. pneumoniae infections. Predisposing conditions: When the 26 PCR positive M. pneumoniae cases were examined for any underlying predisposing factors, 5 cases (19.22%) were found to have them. There were three people with bronchial asthma and two people with COPD. It was discovered that the proportion of instances with these predisposing conditions was statistically significant (p-value 0.000001865). In a study of 150 asthma patients, Varshney AK et al.131 from Delhi, India discovered 33 (22%) incidences of M. pneumoniae infections. They discovered that instances of M. pneumoniae with a positive PCR were more common in severe asthma than in mild asthma (21.9 percent). In a study by Basil MV et al.193 from Delhi, India, 16 out of 100 (16%) COPD patients had serological evidence of M. pneumoniae infection. M. pneumoniae was found in 37 percent of asthma patients, according to Sahoo R et al.178 from Mangalore, India. COPD was found in two of the 26 M. pneumoniae positive cases (7.69%) and asthma in three of the 26 M. pneumoniae positive cases (7.69%) in this investigation (11.53 percent). Environmental factors such as climate, geographical location of the study site, and personal habits all have a part in the link between asthma and M. pneumoniae infections. The research that found a substantial link between the above predisposing cases are largely from India's north and south. The weather and climatic circumstances in this region of the country are significantly diverse, which could account for a large part of the variation in the current study's results. Furthermore, in this area, substantial studies on the relationship of COPD and asthma with M. pneumoniae infections can provide us with a better understanding.

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