Community Acquired Pneumonia Due to *Mycoplasma pneumoniae* versus Non-Mycoplasma Pneumoniae: A Comparative Analysis from a Tertiary Care Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

**Aims:** The study aims to compare the clinical and microbiological profile in adult, hospitalised patients of community acquired pneumonia due to Mycoplasma pneumoniae (MP) vs other bacterial agents.

**Study Design:** Prospective, observational study.

**Place and Duration of Study:** Study was carried out in Department of Microbiology, Kasturba Medical College, Manipal in a span of eighteen months (August 2014 to February 2016).

**Methodology:** A Hospital based study in a tertiary care center was conducted. Adult hospitalised patients suspected of community acquired pneumonia (according to IDSA guidelines) were included in the study. Cases with immunosuppression and prior hospital admission were excluded. Respiratory samples were collected and cultured for all the studied cases. PCR was performed for the detection of *Mycoplasma pneumoniae* by targeting P1 gene.

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**Results:** The study cases (n=140) had mean age of 57 years and mean hospital stay of 7 days, comprising 67.6% males and 32.4% females. Amongst all the cases of CAP that were included in the study, *Mycoplasma pneumoniae* was detected in 23(16.4%) cases with 12 (52.2%) cases due to MP alone and 11 cases (47.8%) had multiple bacterial etiology. Symptoms such as chest pain (91.7%), joint pains (45.8%), earache (41.7%) and sepsis (56.5%) were significantly higher (p<0.005) when Mycoplasma pneumonia was the detected pathogen. Moreover worsening of clinical condition and mortality was also observed higher in this group.

**Conclusion:** Association of higher morbidity and mortality, as observed in current study, highlights the importance of early and timely diagnosis of *Mycoplasma pneumoniae* in hospitalized patients with community-acquired pneumonia.

Keywords: *Mycoplasma pneumoniae*; pneumonia; CAP; P1; multiplex PCR.

1. **INTRODUCTION**

Community acquired pneumonia (CAP) is one of the most common acute infections necessitating hospitalization resulting in a considerable clinical and economic burden. In Asia, CAP is estimated to cause almost one million adult deaths per year. Many of these deaths occur in the elderly, but a large number occur in those with good life expectancy, including 160 000 among those aged 15–59 years [1]. Etiological agents are broadly divided into typical including *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and atypical agents being *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydophila pneumoniae*, *Coxiella burnetti* and Respiratory Viruses such as Influenza virus A and B, Human rhinovirus, Adenovirus, Human metapneumovirus, Parainfluenza virus type 1,2 and 3, Enterovirus and Respiratory syncytial virus type A and B.

*Mycoplasma pneumoniae* as a pathogen is known to cause both epidemics and endemics of respiratory tract infections. Although most mycoplasma infections occur among outpatients (hence the colloquial term “walking pneumonia”), *M. pneumoniae* is a significant cause of bacterial pneumonia in adults requiring hospitalization in the United States. Marston et al. [2] reported that *M. pneumoniae* was definitely responsible for 5.4% and possibly responsible for 32.5% of 2,776 cases of community-acquired pneumonia in hospitalized adults in a two-county region of Ohio, using CF antibody determinations for detection [3]. In India, the etiological agent of CAP varies with geographical distribution e.g., *Streptococcus pneumoniae* predominates as etiological agent of CAP in Shimla and Delhi [4], while *Pseudomonas aeruginosa* predominates in Ludhiana [5]. A study done in 2010 concluded that as many as 15% of CAP was due to *Mycoplasma pneumoniae* [4]. The above studies, however, only looked for atypical agents. Chaudhry et al reported serological positivity of 27.4% in their study [6].

More often than not, CAP due to typical pathogens can easily be diagnosed by routine microbiological tests such as culture and biochemical reactions, but, atypical agents such as *Mycoplasma pneumoniae* agents, being not easy to isolate in culture, largely remains under diagnosed. Hence, in this research, apart from typical agents, we have focused on the detection of *Mycoplasma pneumoniae* in the cases of CAP and compared the outcomes between the two groups i.e. non-*Mycoplasma pneumoniae* pneumonia and pneumonia due to *Mycoplasma pneumoniae*.

2. **METHODOLOGY**

2.1 **Aims and Objectives**

The study aims to compare the clinical and microbiological profile in adult, hospitalised patients of community acquired pneumonia due to *Mycoplasma pneumoniae* vs other bacterial agents.

The objectives for the study were a) To look for bacterial etiology in the cases of community acquired pneumonia. b) To observe the detection rate of *Mycoplasma pneumoniae* by Polymerase Chain Reaction. c) To compare the prognosis of cases with CAP due to *Mycoplasma pneumoniae* with CAP due to other pathogens.

2.2 **Study Design**

A prospective, observational study was carried out in a span of eighteen months (August 2014 to February 2016).
2.3 Sample Size

Anticipating 10% of CAP to be of atypical type with 90% sensitivity of PCR and 5% precision at 95% Confidence Interval, 140 cases were screened using formula:

\[ n = \frac{Z^2 + P (1-P)}{D^2} \]

\( n \) = sample size
\( Z^2 \) = confidence interval
\( P \) = estimated proportion
\( D^2 \) = desired precision

2.4 Target Population

All adult patients admitted in Medicine ward (including all the units) and Pulmonary Medicine ward of Kasturba Hospital, Manipal fulfilling the following criteria for Community Acquired Pneumonia.

2.4.1 Inclusion criteria

- Cough
- Expectoration
- Temperature >38°C or <35°C

And one or both of the following:

1) Consistent auscultatory findings
2) New pulmonary infiltrates on Chest X-Ray at the time of presentation

2.4.2 Exclusion criteria

- There should be no history of prior hospital admission within two weeks during the time of presentation.
- There should be no history of antibiotic consumption within two weeks during the time of presentation.
- Immunosuppressed patients (Patients with carcinoma, HIV or on chemotherapy).

2.5 Clinical Work Up

The case were identified for Community Acquired Pneumonia as per the inclusion and exclusion criteria. The cases fulfilling the inclusion criteria were taken under study and details of their clinical workup were taken.

2.6 Laboratory Proceedings

Samples collected for the laboratory work up were- Sputum, Endotracheal Aspirate, Bronchoalveolar Lavage, Blood (wherever possible) and Urine (for Legionella urinary antigen test).

Microscopy: In sputum sample: Microscopy was done by Gram’s staining and the results of the Gram stain done with the sputum sample formed the basis of the acceptance/rejection of the sample. Criteria for the acceptance of the sputum sample: >25 pus cells/LPF, <5 epithelial cells/LPF. In other samples: For other samples routine Gram’s stain is done.

Sample inoculation and culture: Collected samples are then inoculated in four types of agar: 5% Sheep Blood agar, MacConkey agar, Sheep Chocolate agar. The inoculated samples were aerobically cultured at 35°C overnight and the growth was observed and colony characteristics were identified.

Antibiotic Susceptibility Testing: AST was done by modified Kirby Bauer method of disc diffusion using Mueller Hilton agar.

Molecular diagnostics: Polymerase Chain Reaction was used to detect *Mycoplasma pneumoniae* and *Legionella pneumophila*.

Standardisation of the PCR: Positive control for *Legionella pneumophila* was obtained from Himedia Laboratories Pvt. Ltd. Positive control DNA for *M. pneumoniae* was provided by All India Institute of Medical Sciences, New Delhi and the standardisation was done with the study by keeping Williamson J et al. [7] as reference study. Primers for the same are as follows: (Primers reconstituted in 100 microlitre of nuclease free water): Gene- P1 with forward primer as FP- 5’ CAAAGCCCAACACGACGGCTCCGGCC 3’ and reverse primer being RP- 5’ GGGGAAGGACAAACAGCTGACACTGG 3’. Amplicon size being 543 bp, the final volume of the PCR mixture (50 μL) contained 1 × PCR buffer, 1.5 mM MgCl₂, 200 μM dNTPs (MBI Fermentas), 10 μM of each primer, 1 U of Taq-polymerase and 5 μL of extracted DNA. PCR thermal profile consisted of Initial activation – 94°C, 2 min, Denaturation - 94°C, 1 min Annealing - 55°C, 1 min Extension - 72°C, 2 min, Final Extension - 72°C, 10 min, for 35 cycles.

3. RESULTS AND DISCUSSION

Total number of 140 cases were enrolled in the study. The elderly population predominated as
cases for CAP with 44 (31.4%) of subjects being 50 years of age or above. Young adults with age ranging from 18 to 25 years were the least in the study with 19 (13.5%). In the study, males were found to be affected by CAP more than females with ratio of male to female being 2.1:1. 95 (67.8%) of males presented with CAP while it was observed in 45 (32.2%) cases. Sample collection of various samples is shown in Table 1.

**Performance of Gram Stain in determining the etiology:** Causative agents were determined in 67 out of 140 samples and the sensitivity of the Gram stain (taking culture as gold standard) was found to be 52.3%.

Bacterial etiology (with culture and sensitivity) was found in 124 (91.2%) admitted patients of CAP, with 24 (16.4%) being due to *Mycoplasma pneumoniae* and 116 cases (83.6%) caused by other bacterial agents, as depicted in Fig. 1.

*Mycoplasma pneumoniae* was detected by polymerase chain reaction in 23 (16.4%) of cases with 12 cases due to *Mycoplasma pneumoniae* alone and 11 cases had *Mycoplasma pneumoniae* with other non-*Mycoplasma pneumoniae* agents, as seen in Fig. 2.

As can we observed in the table given below, most of the risk factors were not found to be significant in causing *Mycoplasma pneumoniae* infection, except in COPD patients ($p$=0.001). Also, as seen in clinical features, respiratory signs and symptoms such as chest pain, empyema and respiratory failure were also seen more with *Mycoplasma pneumoniae* cases as compared to the cases with non-*Mycoplasma pneumoniae* agents. Prognosis was also found to be worse in infections with *Mycoplasma pneumoniae* with 20.8% patients having complications due to CAP and mortality was seen in 12.5% cases of *Mycoplasma pneumoniae*, as opposed to 1.4% of non-*Mycoplasma pneumoniae* cases. The comparative evaluation of such cases is given in Table 2.

**Table 1. Table showing the type and number of samples collected for the diagnosis of CAP**

| Samples collected | Non- *Mycoplasma pneumoniae* (116) | *Mycoplasma pneumoniae* (23) |
|-------------------|-----------------------------------|------------------------------|
| Type of specimen  |                                   |                              |
| Sputum            | 90 (77.6%)                        | 10 (41.7%)                   |
| ET aspirate       | 5 (4.3%)                          | 8 (33.3%)                    |
| BAL               | 10 (8.6%)                         | 5 (20.8%)                    |

![Chart](chart.png)

**Fig. 1. Bar graph showing etiological agents in the cases of CAP**
Table 2. Comparison of community acquired pneumonia cases due to *Mycoplasma pneumoniae* versus due to other bacterial agents

| Risk factors                  | Non-Myco (116) | Myco (23) | p value |
|-------------------------------|----------------|-----------|---------|
| Smoking                       | 44 (37.9%)     | 9 (37.5%) | 0.983   |
| Alcoholism                    | 21 (18.1%)     | 7 (29.2%) | 0.211   |
| Asthma                        | 28 (24.1%)     | 3 (12.5%) | 0.277   |
| COPD                          | 28 (24.1%)     | 14 (58.4%)| 0.001   |
| Hypertension                  | 34 (29.3%)     | 5 (20.8%) | 0.243   |
| Diabetes mellitus             | 32 (27.6%)     | 1 (4.2%)  | 0.257   |
| Contact                       | 35 (30.2%)     | 10 (41.7%)| 0.430   |

| Clinical features             |                |           |         |
|-------------------------------|----------------|-----------|---------|
| Fever                         | 103 (73.6%)    | 19 (79.2%)| 0.899   |
| Breathlessness                | 92 (65.7%)     | 17 (70.8%)| 0.920   |
| Chest pain                    | 67 (47.9%)     | 22 (91.7%)| 0.001   |
| Empyema                       | 1 (0.7%)       | 2 (8.3%)  | 0.013   |
| Respiratory failure           | 6 (4.3%)       | 8 (33.3%) | 0.001   |
| Hypotension                   | 5 (3.6%)       | 2 (8.3%)  | 0.324   |
| Oliguria                      | 6 (4.3%)       | 4 (16.7%) | 0.026   |
| Joint pain                    | 23 (16.4%)     | 11 (45.8%)| 0.002   |
| Earache                       | 2 (1.4%)       | 10 (41.7%)| 0.001   |
| Neurological symptoms         | 4 (2.8%)       | 1 (4.2%)  | 0.774   |
| Pain abdomen                  | 7 (5%)         | 4 (16.7%) | 0.045   |
| Hepatomegaly                  | 4 (2.8%)       | 3 (13.1%) | 0.039   |
| Anaemia                       | 52 (37.1%)     | 16 (69.5%)| 0.027   |
| Organ failure                 | 5 (3.6%)       | 2 (8.7%)  | 0.324   |
| Sepsis                        | 25 (17.8%)     | 13 (56.5%)| 0.002   |
| Septic shock                  | 5 (3.6%)       | 2 (8.3%)  | 0.234   |

| Laboratory parameters         |                |           |         |
|-------------------------------|----------------|-----------|---------|
| Consolidation on CXR          | 73 (52.1%)     | 12 (52.1%)| 0.026   |
| Raised TLC                    | 90 (64.3%)     | 18 (78.3%)| 0.638   |
| Raised CRP                    | 63 (45%)       | 12 (52.1%)| 0.969   |
| Procalcitonin                 | 33 (26.9%)     | 10 (41.7%)| 0.175   |

| Prognosis                     |                |           |         |
|-------------------------------|----------------|-----------|---------|
| Treatment change              | 66 (47.1%)     | 11 (45.8%)| 0.670   |
| Cured                         | 107 (76.4%)    | 12 (50%)  | 0.921   |
| Worsened                      | 5 (3.6%)       | 5 (20.8%) | 0.001   |
| Expired                       | 2 (1.4%)       | 3 (12.5%) | 0.001   |

### 3.1 Discussion

The study was directed to observe comprehensive differences between pneumonia due to *Mycoplasma pneumoniae* and pneumonia due to other bacterial agents. As seen above, majority of the patients presenting with CAP aged 50 years and above with male population being twice that of female. The following data agrees with the results by N. J. Gadsby et al who found median age of their patients to be 67 years with 54.8% patients being male [8]. The cause of preponderance in older age is explained by decrease in immune response with age as well as weakening of respiratory functions such as alterations in normal respiratory flora.

*Mycoplasma pneumoniae* as a causative agent of CAP was isolated from 16.4% of patients through conventional PCR targeting P1 gene. This was again seen in study by R. Chaudhary et al in 2013 who found 19% of their cases with *Mycoplasma pneumoniae*, confirmed by quantitative real time PCR [9]. Arnold et al in 2007, proved *Mycoplasma pneumoniae* to be the most common atypical etiological agent of CAP with 11-15% of cases occurring due to it [10]. In Europe, *M. pneumoniae* may be responsible for approximately 11% of CAP cases and, in Italy, up to 17% of the hospitalised adult cases of CAP [11].

Amongst the risk factors observed, patients having COPD were significantly at risk of having...
Mycoplasma pneumoniae infection ($p=0.001$) as compared to the patients without COPD. Mycoplasma pneumoniae, a known coloniser in such cases, thus can be implicated in causing CAP. A study conducted in a tertiary care center in New Delhi, India, observed as much as 40% of seropositivity in the patients with CAP with COPD [12]. Effect of Mycoplasma pneumoniae in COPD patients appears to be multifactorial and involves a complex integration of airway inflammation and IgE hypersensitivity [13]. The release of proinflammatory cytokines in association with M. pneumoniae infection has also been implicated as a possible mechanism leading to or exacerbating underlying chronic pulmonary diseases [14].

Clinical manifestations due to Mycoplasma pneumoniae alone seems to be milder and can be responsible for both upper respiratory and lower respiratory tract infections. Pneumonia due to M. pneumoniae causes nonspecific respiratory symptoms such as laryngitis, cough, wheezing, slight fever. Complications might occur either in untreated cases or due to coinfection with another agent of CAP. In our study, complications such as empyema, respiratory failure and sepsis were significantly higher in the cases of Mycoplasma pneumoniae infections. Nilsson et al in 2010 reported that complications in the cases of Mycoplasma pneumoniae is mainly due to bacterial load more than due to the genotype [15]. Miyashita et al reported that delaying of antibiotic administration specific to Mycoplasma pneumoniae due to lack of diagnostic measures can lead to fulminant respiratory failure in the cases of CAP [16].

Extra pulmonary manifestations such as oliguria, joint pains, earache, pain abdomen and anaemia were also seen more in Mycoplasma pneumoniae CAP. According to DF Talkington et al, as much as 25% of patients with Mycoplasma pneumoniae can present with extra pulmonary manifestations, autoimmune reactions being implicated in the pathogenesis of the same [17].

Non specific myalgias and arthralgias and renal manifestations such as acute glomerulonephritis, renal failure and IgA nephropathy maybe found in 14% of cases [18].

Prognosis, as observed in the cases of Mycoplasma pneumoniae were found to be worse as compared to other agents (1.4%), with 12.5% patients of CAP due to Mycoplasma pneumoniae eventually worsening or dying due to complications. Worsening could be seen in the patients with COPD or other lung associated conditions such as bronchial asthma more than in previously healthy individuals. Furthermore, co-infection of Mycoplasma pneumoniae with other atypical or typical agent can lead to the debilitation of the patient, thus, increasing the need for hospitalisation or ICU admissions.

![Figure showing the PCR for Mycoplasma pneumoniae targeting P1 gene](image_url)
The severity in *Mycoplasma pneumoniae* can be due to many reasons, most important being cytoadhesion mediated damage to ciliated epithelium of lung. Another mechanism is cytotoxicity as mediated by Community Acquired Respiratory Distress (CARD) toxin as well as oxidative damage to the lung leading to release of cytokines, neutrophils and recruitment of other mediators of inflammation. These mechanisms are exaggerated in the presence of other organisms such as respiratory viruses as well as other typical pathogens [18].

Occurrence of Macrolide Resistant *Mycoplasma Pneumoniae* (MRMP) is another reason why *Mycoplasma pneumoniae* must be diagnosed on time. Continuous selective pressure of routinely used antibiotic drugs and high population density can possibly explain the emergence of MRMP. The extent of *Mycoplasma pneumoniae* infection simultaneously increased with rising resistance, further resulting in increased consumption of ineffective antibiotic drugs and rise in need for hospitalisation [19].

The study, however, was not free of limitations. One being that *Mycoplasma pneumoniae* was diagnosed through a single test, that is, conventional PCR. Other tests such as culture isolation and serology were not evaluated for the same. Also, other non-bacterial atypical agents such as viral etiology was not ruled out in our study.

**4. CONCLUSION**

Hence, in this study cases due to *Mycoplasma pneumoniae* was found to be more severe as compared to other cases which makes the timely diagnosis even more important. Timely diagnosis, therefore, can help clinicians in streamlining therapy accordingly.

**CONSENT**

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper.

**ETHICAL APPROVAL**

Ethics approval was obtained from the Institutional Ethics Committee.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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