Original Research Article

Comparative analysis of community acquired pneumonia due to *Mycoplasma pneumoniae* with pneumonia due to non-*Mycoplasma pneumoniae* agents.

ABSTRACT:

Background:

Community Acquired Pneumonia (CAP) has been one of the most important causes of morbidity and mortility. *Mycoplasma pneumoniae* being one of the most important etiological agents amongst many others has been evaluated in the study.

MATERIALS AND METHODS:

A Hospital based study in a tertiary care center was conducted from August 2014 to February 2016. 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. A multiplex PCR was performed for the simultaneous detection of Mycoplasma pneumoniae and Legionella pneumophila by targeting P1 gene and mip(macrophage infectivity potentiator) gene respectively.

RESULT:

All included cases (n=140) had mean age of 57 years and mean hospital stay of 7 days, with 67.6% being males and 32.4% being females . Amongst all the cases of CAP that were included in the study, *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. In 58.4% of patients with COPD, mixed infections with *M.pneumoniae* was observed, so did the mortality (12.5%), as compared to *M.pneumoniae* alone, which was statistically significant (p=<0.001).

CONCLUSION:

Since the treatment for CAP largely depends upon the etiology, the timely and meticulous diagnosis for *M.pneumoniae* with other agents can reduce the inconsistency in the usage of

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antibiotics and the therapy targeting the specific organism can be initiated, thus making the process of patient care even better.

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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

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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 underdiagnosed. Hence, in this research, apart from typical agents, we have focussed 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*.

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.

MATERIALS AND METHODS:

Study Design: A prospective, observational study was carried out in a span of eighteen months (August 2014 to February 2016). Ethics approval was obtained from the Institutional Ethics Committee.

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:

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$n = Z^2 + P(1-P)/D^2$

n= sample size

Z^2 = confidence interval

P= estimated proportion

D^2= desired precision

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.

Inclusion criteria:

- Cough
- Expectoration
- Temperature $>38^0$C or $<35^0$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

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)

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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 work up were taken.

LABORATORY PROCEEDINGS:

Samples collected for the laboratory work up were- Sputum, Endotracheal Aspirate, Broncheoalveolar 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 <10 epithelial cells/LPF. In other samples: For other samples routine Gram's stain is done.

Sampleinoculationandculture: 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 35^0C 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.

Moleculardiagnostics: 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

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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'CAAGCCAAACACGAGCTCCGGCC 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 $MgCl_2$, 200 μM dNTPs (MBI Fermentas), 10 pM of each primer, 1 U of Taq-polymerase and 5 μL of extracted DNA. PCR thermal profile consisted of Initial activation – 94ºC, 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 .

RESULTS

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 table1.

SAMPLESCOLLECTED	Non-*Mycoplasma pneumoniae (116)*	*Mycoplasma pneumoniae (23)*
1. TYPE OF SPECIMEN		
• Sputum	90 (77.6%)	10 (41.7%)

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• ET aspirate	5 (4.3%)	8 (33.3%)
• BAL	10 (8.6%)	5 (20.8%)

Table1:Tableshowingthetypeandnumberofsamplescollectedforthediagnosisof CAP

PerformanceofGramStainindeterminingtheetiology: 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 figure 1.

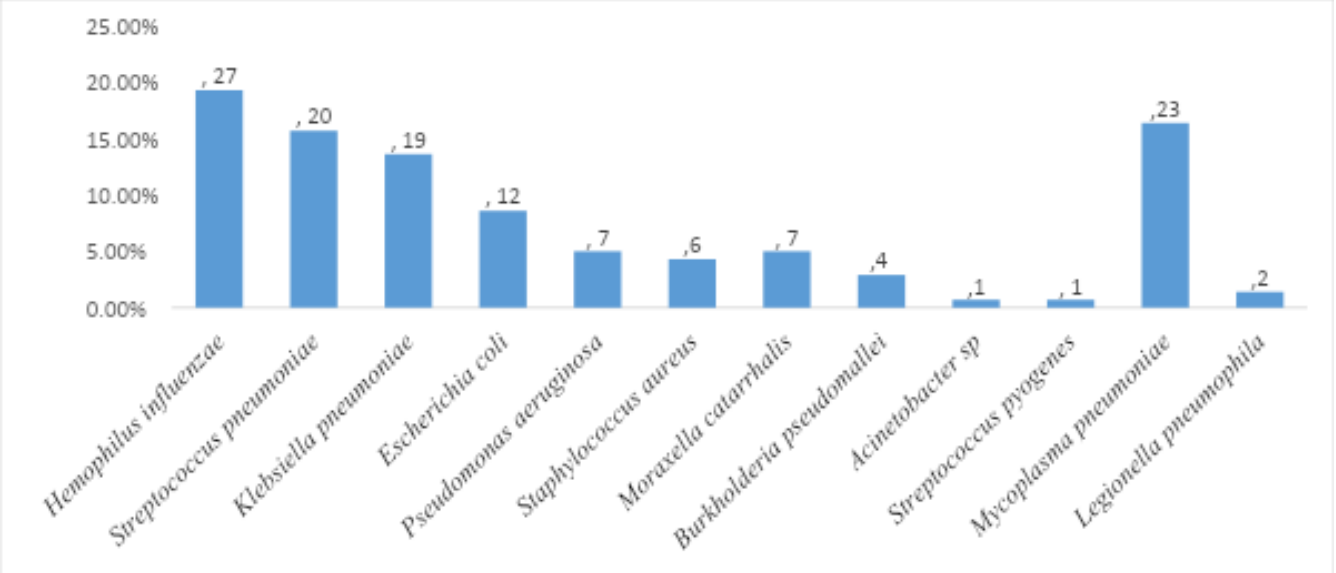

Figure1:BargraphshowingetiologicalagentsinthecasesofCAP

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 figure 2.

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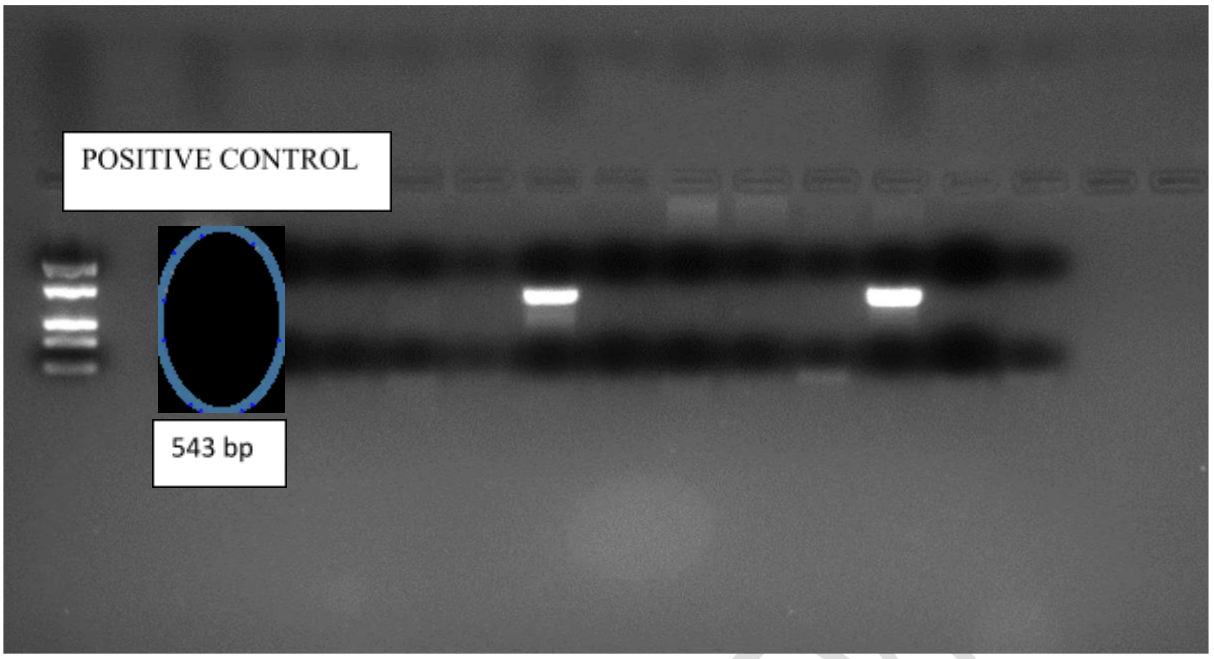

Figure 2: Figure showing the PCR for *Mycoplasma pneumoniae* targeting P1 gene

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.

	NON- *Mycoplasma pneumoniae* (116)	***Mycoplasma pneumoniae* (23)**	***p* value**
RISK FACTORS			

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SMOKING	44 (37.9%)	9 (37.5%)	0.983
ALCOHOLISM	21 (18.l%)	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 PAINS	23 (16.4%)	11 (45.8%)	0.002
EARACHE	2 (1.4%)	10 (41.7%)	0.001
NEUROLOGICAL SYMPTOMS	4	1	0.774
PAIN ABDOMEN	7	4	0.045
HEPATOMEGALY	4	3	0.039
ANAEMIA	52	16	0.027
ORGAN FAILURE	5	2	0.324
SEPSIS	25	13	0.002
SEPTIC SHOCK	5	2	0.234
LABORATORY PARAMETERS			
CONSOLIDATION ON CXR	73	12	0.026
RAISED TLC	90	18	0.638

RAISED CRP	63	12	0.969
PROCALCITONIN	33	10	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

Table2:Comparisonofcommunityacquiredpneumoniacasesduetomycoplasma pneumoniaeversusduetootherbacterialagents

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.

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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).

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 (11). Effect of Mycoplasma pneumoniae in COPD patients appears to be multifactorial and involves a complex integration of airway inflammation and IgE hypersensitivity (12). 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 (3).

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 non specific 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 (13). Miyashita et al reported that delaying of antibiotic administration specific to *Mycoplasma*

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pneumoniae due to lack of diagnostic measures can lead to fulminant respiratory failure in the cases of CAP (14).

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 (15). Non specific myalgias and arthralgias and renal manifestations such as acute glomerulonephritis, renal failure and IgA nephropathy maybe found in 14% of cases (16).

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.

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 (17).

The study, however, was not free of flaws. One being that *Mycoplasma pneumoniae* was diagnosed through a single test, that is, conventional PCR. Other tests such as culture

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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.

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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