

# Detection of Mycoplasma Pneumonia in Blood Specimen in Mycoplasma Pneumonia PCR Positive Clinical Specimen of the Patients in a Medical college of North India

Abhineet Mehrotra<sup>1</sup>, S.K. Mehra<sup>2</sup>, M.E. Siddique<sup>3</sup>

## ABSTRACT

**Introduction:** Current study was aimed at detecting Mycoplasma pneumonia in blood in those patients who are already PCR positive through clinical specimen. All the patient who are Mycoplasma Pneumonia PCR positive have this disease as primary infection and not as secondary infection. This study was a part of a larger study of comparing techniques to detect Mycoplasma pneumonia by culture, serology and PCR.

**Material and Methods:** The present study was undertaken at Career Institute of Medical Science, Lucknow. The total number of study subjects were 80 which were Mycoplasma Pneumonia PCR positive by clinical specimen (nasopharyngeal swab, throat swab and expectorated sputum) which is the part of larger study of total 286 samples processed for PCR. Conventional PCR with GEL.DOC were used for the processing of the samples.

**Results:** Non of the 80 PCR positive samples ran were found to be positive in blood

**Conclusion:** This concludes that detection of Mycoplasma Pneumonia as a primary infection of a person(as detected in clinical specimen by conventional PCR) is not detected in blood specimen by conventional PCR

**Keywords:** Mycoplasma Pneumonia, Clinical Specimen, conventional PCR, blood specimen

## INTRODUCTION

Mycoplasma Pneumonia is devoid of cellwall. Mycoplasma pneumonia are chiefly mucosal organism living in a parasitic manner on the epithelial wall of their hosts. Mycoplasma Pneumoniae being an important cause of respiratory tract infection and are also found to be associated with extra pulmonary complications. In spite of the fact that Mycoplasmas resides on mucosal surface; in the genitourinary tract, oral cavity and gut as commensal flora in humans, but when certain Mycoplasmas penetrate into the blood, organ; and tissues they can cause acute and chronic signs and symptoms.

Although it has been suggested that Mycoplasma bacteremia infection is not unusual<sup>2</sup>, few reports have presented direct evidence of hematogenous dissemination by means of isolation of this organism from blood.<sup>3,4</sup> This is due presumably to the fact that culture of clinical samples is rather insensitive in comparison with antigen or gene detection methods<sup>5-7</sup>, although positive results with the latter methods not always certifies the viability of the organisms. Webster et al. suggested that immunocompetent patients would produce enough antibody at the mucosa to prevent systemic spread of the infection.<sup>8</sup> Because of this it is inferred that if the host immune response against the organism is strong enough to elicit pneumonia, the organism can no longer penetrate into blood or may be eliminated rapidly from blood. Extra pulmonary manifestation

of Mycoplasma pneumonia is a notable aspect and are seen in up to 25% of infected persons.<sup>9-11</sup> This has come to notice that high prevalence of M. pneumonia infection in maximum populations is due to irrelevant events reporting as though they were part of the disease.<sup>10</sup> and its so especially for single case reports which were diagnosed only by serology testing.<sup>9,10</sup>

The characteristic of Mycoplasma suggested that this organism could adhere to erythrocytes during extrapulmonary dissemination and such adherence could contribute to pathogenesis.

First, M. pneumonia has been cultured from extrapulmonary infection sites such as synovial fluid and pericardial fluid.<sup>9-11</sup> Hence on reaching such sites it concludes that Mycoplasma Pneumonia is able to have entry in blood stream. Secondly, its known that M. pneumonia adheres to human erythrocytes in vitro<sup>12,13</sup> and under the electron microscope it shows that it does not only adhere to the erythrocytes but also deform their surface as well.<sup>13</sup> Third, M. pneumonia belongs to the same phylogenetic group that contains the hemotropic mycoplasma; these uncultivated Mycoplasma inhabitates erythrocytes of mammalian hosts and manifests acute and chronic blood infections alongwith hemolytic anemia and other illness.<sup>14</sup>

As these were study in patients suffering from Mycoplasma Pneumonia as a secondary disease, Our study was to find bacteremia of mycoplasma pneumonia in patients suffering from Mycoplasma pneumonia as primary disease.

Since mycoplasma pneumonia was found in the clinical specimen, it was more further thought of detecting in blood with the aim that if found in blood as a primary disease and tested by a basic conventional PCR, it would be more easy to collect blood from the patients rather than to collect this from clinical specimen (sputum, throat swab, nasopharynx) adequacy of which is many times questionable and for this patients are also sometimes educated (especially for sputum and for nasopharynx aspirates as well).

<sup>1</sup>Assistant Profesor, Department of Microbiology, T.S. Mishra Medical College, <sup>3</sup>Ex H.O.D., Department of Microbiology, Career Institute of Medical College and Hospital, Lucknow, UP, <sup>2</sup>Ex H.O.D., Department of microbiology, Geetanjali Medical College and Hospital Udaipur, Rajasthan, India

**Corresponding author:** Dr. Abhineet Mehrotra, B6/2 Nirala Nagar, Lucknow, U.P., India

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### MATERIAL AND METHODS

The cross-sectional study, on PCR positive Mycoplasma Pneumonia patients of clinical specimen, was carried out at Career Institute of medical sciences and Hospital in the department of microbiology from 2013-15, for molecular characterization of Mycoplasma Pneumoniae and comparison of techniques in its detection namely PCR, serology and Culture. For this around 286 samples were taken as M. pneumoniae DNA was detected in 21% in a study.<sup>15</sup> On the basis of this prevalence, the sample size is calculated by using the formula with 80% power and 5% significance level:

$n = \frac{4pq}{d^2}$  where n: sample size p: prevalence (21%), q: 1-p. The calculated sample size is 260. Adding 10% non-response, the final sample size is 286. Out 286 PCR samples 80 were positive in its clinical specimen (i.e. expectorated sputum, nasopharyngeal aspirate and throat swab). Of these 80 samples their corresponding blood were taken and PCR run to detect the same Mycoplasma pneumonia in blood.

**Inclusion Criteria:** All PCR positive samples out of 286 clinical samples processed by PCR

**Exclusion Criteria:** All PCR negative samples out of 286 clinical samples processed by PCR

The testing of Mycoplasma Pneumonia in blood of the patient was a part of bigger study conducted in which 286 clinical samples were processed for PCR and out of these 80 samples which were positive, their corresponding blood samples were run by conventional PCR. A whole-blood sample was routinely centrifuged at 3,000g for 15 minutes to remove clots (this gravity is generally considered not to precipitate cell debris or free mycoplasmas), and the serum was stored at -20°C until use. The serum samples, usually 1-3 ml, were then centrifuged at 13,000g for 30 minutes to precipitate cell debris as well as free mycoplasma; in most cases, this resulted in the formation of a pellet 0.5-1.0 mm in size. The pellet was dissolved in 120 µL of a proteinase solution, DNA was extracted and purified through phenol extraction and ethanol precipitation. The PCR procedures were performed A 543 base pair section of the P1 protein gene of M. pneumonia was selected among many other genes for amplification. The primers (Bangalore Genei Pvt. Ltd., Bangalore, India) were

*Primer 1:* 5'CAAGCCAAACACGAGCTCCGGCC-3', which is complimentary to the P1 adhesin gene negative strand residues 3666-3688, and

*Primer 2:* 5'CCAGTGTTCAGCTGTTTGTCTTCCCC-3', which is complimentary to the P1 adhesin gene positive strand residues 4208-4183.

Amplification was done according to the guidelines of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). Amplification of PCR products were undergone electrophoresis on 3 ethidium bromide stained agarose gel, along with a molecular weight marker. A mixture containing 2 µl genomic DNA, 2 µl of 6 X BPB and 8 µl of distilled water was added into the 1 per cent agarose gel. The electrophoresis was carried out at a constant voltage of 50 V for 1 h, and a band at 543 base pair was taken as a positive result. The PCR of those patients whose culture and serology was positive was carried out.

### STATISTICAL ANALYSIS

Statistical analysis was done with the help of SPSS version

21. Descriptive statistics like mean and percentages were used to interpret the data. Chi square test was used for gender wise comparison.

### RESULT

In our study, all the 80 clinical samples which were Mycoplasma Pneumonia PCR samples, None was positive when their corresponding blood samples were run by PCR. A study of comparison of techniques in detection of Mycoplasma Pneumoniae were undertaken as a bigger study as its sample size was 286 and the clinical specimen (expectorated sputum, throat swab, and nasopharyngeal aspirate) were run for PCR and Culture and serum was used for serology detection. Out of these 286 samples 80 samples were there which were PCR positive, 98 were seropositive with Igm and 168 were culture positive. Since PCR positivity is considered as most sensitive and specific hence 80 samples of the patients with PCR positive in their clinical specimen were run with their corresponding blood. But the result this time was all negative and this proves that the patients which were PCR positive and which had mycoplasma pneumonia no bacteremia is detected when the sample is run on conventional PCR. Of the total 286 samples which was run for comparison of techniques by PCR, culture and serology, 80 samples were PCR positive. Of these 15 (18.8% i.e. 15/80) were male and 65 (81.3% i.e. 65/80) were female (Table-1). Of all the 80 samples 15 of male and 65 of female which were PCR positive in their clinical specimen NONE were found to have Mycoplasma Pneumoniae in blood when PCR of their blood samples were done (Table-2).

### DISCUSSION

PCR have demonstrated M. pneumonia DNA in serum.<sup>16-19</sup> In some cases M. pneumonia DNA was found for periods even exceeding 20 days, suggesting of bacteremia.<sup>14</sup> In about 15 out of 29 seropositive patients were found to have M. Pneumoniae DNA isolated by real time PCR study.<sup>20</sup> Detection of M. Pneumonia DNA in interior tissue in these findings and also in other PCR reports concludes the presence of organism as thought by many investigators, then its not might be rare to have blood entry of M. Pneumonia. But Webster et al. suggested that immunocompetent patients would produce enough antibody at the mucosa to prevent systemic spread of the infection.<sup>21</sup>

Gender	Positive (n=80)		Negative (n=206)	
	N	%	N	%
Male	15	18.8%	64	31.1%
Female	65	81.3%	142	68.9%

χ<sup>2</sup> value =4.373; p\_value=0.037; consider significant.

**Table-1:** Gender wise diagnostic positivity in patients according to PCR test (n=286).

Gender	PCR Positive Clinical Specimen (n=80)	PCR Negative Blood specimen
	N (%)	N (%)
Male	15(18.75)	0%
Female	65(81.25)	0%

**Table-2:** Genderwise comparison of the results of PCR positive of clinical specimen with PCR negative result of the same patients with their corresponding blood

From this point of view, it can be speculated that if the host immune response against the organism is strong enough to elicit pneumonia, the organism can no longer penetrate into blood or maybe eliminated rapidly from blood. More studies of a prospective nature are needed to characterize patients without pneumonia to further our understanding of the pathophysiology of extrapulmonary complications associated with mycoplasmal infection. Such studies will provide information useful for the treatment of patients, such as which patients should be treated with antibiotics and which with corticosteroids.<sup>18</sup> As there were evidence of mycoplasmal pneumonia bacteremia<sup>16</sup> this study was conducted to find in patients of Mycoplasma Pneumoniae as a primary infection.

## CONCLUSION

The study concludes that there is no Mycoplasma Pneumonia bacteremia in patients in whom this organism was detected in clinical specimens and where these patients have pneumonia by Mycoplasma as a primary disease and the method of detecting the organism is conventional PCR.

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