Original Research Paper

Volume-9 | Issue-5 | May-2019 | PRINT ISSN No 2249 - 555X

Microbiology

PREVALENCE OF *MYCOPLASMA PNEUMONIAE* IN PATIENTS OF ATYPICAL PNEUMONIA IN TERTIARY CARE HOSPITAL

Kanchan Gupta	Post Graduate student, Department of Microbiology, Gajra Raja Medical College, Gwalior, Madhya Pradesh
Vaibhav Misra*	Professor, Department of Microbiology, Gajra Raja Medical College, Gwalior, Madhya Pradesh *Corresponding Author
Shweta sahai	Associate Professor, Department of Medicine, Gajra Raja Medical College, Gwalior, Madhya Pradesh.

ABSTRACT

Introduction: *Mycoplasma pneumoniae* is an important cause of atypical pneumonia, whose sign and symptoms mimics other respiratory disease also.

Aim: The aim of this study is to evaluate the prevalence of *M. pneumoniae* among patients of atypical pneumonia, by isolating and identifying it.

Material and methods: We prospectively analysed 105 patients of atypical pneumonia in our tertiary care hospital, Gwalior. Respiratory samples were collected in sterile containers containing Pleura pneumonia like organism (PPLO) broth and processed and identified by standard laboratory technique.

Results: Out of 105 patients, 09 (8.57%) were positive for *M. pneumoniae* by culture method.

Conclusions: This pathogen are important cause of atypical pneumonia, but under looked due to lack of proper diagnostic methods. So we need to find out true prevalence rate.

KEYWORDS : *Mycoplasma pneumoniae*, Pleura pneumonia like organism broth, Pleura pneumonia like organism agar, Fried egg colony.

INTRODUCTION-

Mycoplasma pneumoniae is a common respiratory pathogen, produces diseases of varied severity ranging from mild upper respiratory tract infection to severe atypical pneumonia.[1] It accounts for up to 20% of all community-acquired pneumonias.[2] The prevalence being highest among school aged children. *M. pneumoniae* is transmitted through inhalational route. Common manifestation include fever, sore throat, cough, headache and general malaise.[3] Apart from respiratory tract infections, this organism is also responsible for producing a wide spectrum of non-pulmonary manifestations including neurological, hepatic, cardiac diseases, hemolytic anemia, polyarthritis and erythema multiform.[4]

Diagnosis of this disease not only based on clinical signs and symptoms, but also laboratory test for detecting *M. pneumoniae* is particularly important. Due to small size and volume of mycoplasmal cells, it cannot be detected by light microscopy. *Mycoplasma* have an extremely small genome, hence fastidious growth requirements complicate it's detection by culture method.[5] *M. pneumoniae* grows slowly (requiring 2 to 5 week for colonies to become visible), so culture is time consuming process. It ferments glucose, absorbs erythrocytes in the growing colonies, and reduces tetrazolium. All these characteristics have been used for identification.[6-11]

Serological assays are most widely used. But sensitivity of these assays depends on whether the first serum sample is collected early or late after the onset of disease and on the availability of paired serum samples collected with an interval of 2 to 3 weeks.[12,13]

Nucleic acid amplification techniques (NAATs) have the potential to generate rapid, sensitive and specific results, but proper validation and standardization are often lacking, and quality control studies have revealed frequent deficiencies resulting in both false negative and false positive results. Treatment of *M. pneumoniae* infections on depends upon case by case. Newer macrolides are usually preferred over erythromycin due to their greater tolerability, once- or twice-daily dosing requirements, and shorter treatment duration. Hence the present study was conducted to evaluate the prevalence of *M. pneumoniae* among the respiratory tract diseases and to isolate and identify it.[14]

MATERIALS AND METHODS-

Study design: This prospective study was conducted over a period of one year from April 2017 to March 2018 among respiratory disease patients of all age groups in Department of Microbiology, Medicine and Pediatrics, Gajra Raja Medical College, Gwalior a tertiary care center.

Inclusion Criteria: Patients presenting in OPD or IPD who were clinically suspected of having respiratory tract infection were included, provided they have at least two of the following clinical features (1) fever, (2) cough, (3) wheeze, (4) dyspnoea, and (5) chest pain.

Exclusion Criteria: Children less than 2 year of age and Co-morbid patients were excluded.

Sample: The specimens like nasopharyngeal swab, throat swab and cough obtained from the 105 respiratory disease patients after their written consent. Sample was collected in sterile containers containing Pleura pneumonia like organism (PPLO) broth. Sample were transported in PPLO broth to laboratory.[1]

Culture in PPLO broth: PPLO broth is used for enrichment and cultivation of *Mycoplasma* species. This medium was prepared using commercial PPLO broth base, yeast-extract, serum and glucose solution.Yeast extract provides nutrition to organisms and serum provides cholesterol. *Mycoplasma* metabolizes glucose and produces lactic acid, resulting in a shift to acidic pH. The Colour of broth changes from red to yellow due to phenol red indicator. Inoculated broth was incubated in 5% CO_2 at 35°C up to 30 days.

Examine the tubes once in two days for a change in pH. A pH shift will cause the medium to change from red color to yellow color due to growth of microorganism. As soon as a pH shift is noted, subculture PPLO broth to PPLO agar medium. Growth negative PPLO broth was discarded after 30 days of incubation

Culture in PPLO agar: PPLO agar base is used for isolation of colony of *Mycoplasma*. PPLO broth with growth was subcultured by taking 0.1ml (100 µl) broth on "Pleuropneumoniae like organism" agar surface. Incubate these plates in 5% CO_2 at 35ºC for up to 30 days. Observe for colonies under 40X and 100X of microscope organisms are recognized by a typical "fried egg" colonies or finely granular ("ground glass") colonies that penetrate the agar surface. Colonies can range from 20-300µm in size.

Isolates were identified by Diene's staining, Hemadsorption test, Tetrazolium Reduction Test and Glucose fermentation test.[15]

RESULTS -

A total of 105 patients were enrolled in this study, out of which maximum number 35 (33.34%) belong to age group 06-15 years and minimum number 10 (9.53%) belong to 2-5 years age group. In the present study 73(69.53%) cases were male and 32(30.47%) cases were female. Out of 105 suspected patients 61 (58.10%) patients were from OPD and 44(41.90%) patients were from IPD.

In the present study out of 105 suspected patients 09 (8.57%) were culture positive and 96 (91.43%) were culture negative [Figure 1]. Maximum prevalence of *M. Pneumoniae* infection was observed in 6-15 year age group i.e. 11.42% [Table 1]. Out of 9 culture positive cases, 7(77.78%) were male and 2(22.22%) were female. The maximum number of *M. pneumoniae* culture-positive patients was in OPD (66.67%) cases as compared to IPD (33.33%) Cases. In the present study, out of 9 *M. pneumoniae* culture positive cases, 8(88.89%) cases were associated with cough followed by fever (77.78%), wheezes (66.67%), dyspnea (55.56%) and chest pain (22.22%) [Table 2].

Figure 1 : Culture based distribution of suspected patients

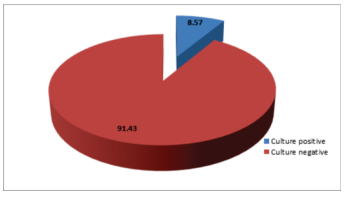

Table 1 :Age wise distribution of culture-positive patients

Age groups (in years)	Suspected cases	Culture-positive	
		No. of case	Percentages
2-5	10	00	00.00
6– 15	35	04	11.42
16– 25	20	02	10.00
25– 40	15	01	6.67
>40	25	02	8.00
Total	105	09	8.57

Table 2 : Clinical feature wise distribution of culture-positive patients

Clinical feature	Culture-positive	
	No. of cases	Percentages
Cough	08	88.89
Fever	07	77.78
Wheezes	06	66.67
Dyspnea	05	55.56
Chest pain	02	22.22

DISCUSSION

Atypical microorganisms are emerging as important causes of human respiratory tract disease in both adults and children. This study proposed that, the prevalence of *Mycoplasma pneumoniae* in respiratory disease patient by culture method is 8.57 %. This is in accordance to study of Rama Chaudhary *et al.*[2], who found that *Mycoplasma pneumoniae* was prevalent in 7 % patients of CAP by culture method. Difference in the prevalence of *M. pneumoniae* infections could be due to various reasons like methods of laboratory procedures, patient study group, age and sex distribution, and the predisposing factors.

Maximum suspected cases were belongs to 6-15 year age group. We found that, culture positivity was seen maximum among 6-15 years age group (11.42%) followed by 16-25 years age group (10%), > 40 years age group (8%), 25-40 year age group (6.67%), and 2-5 year age group (00%). These above findings are, in accordance with Rama Chaudhary *et al.*[2] who showed that prevalence is more in pediatrics patients than adults. In our study, it was seem that *M. pnemoniae* was more prevalent in males (77.78%) as compare to female (22.32%). It showed that 77.78 % male and 22.3 % female contributed to total positive patients. So it concludes that males were partially more susceptible for colonization as compare to female. According to Kashyap B *et al.*[16] reported 75% male and 25% female to have *M. pneumoniae* infection. So our study is in accordance with the above studies.

Most of the suspected patient in our study belongs to the OPD and maximum number of culture positive patients were in OPD (66.67%) cases. On the other hand IPD culture positive patients were 33.33 %. The probable reason for lower prevalence of IPD cases is early administration of antibiotics before collection of samples. This discrimination of prevalence between OPD and IPD justify the '*M. pneumoniae*' as a causative agent of 'walking pneumonia'.

On comparison of clinical features in *M. pneumoniae* culture positive patients, it reveals that cough, fever and wheezes are the most common (> 75 %) while dyspnea and chest pain are less common. Dyspnea in 55 % cases and chest pain less common (<25 %).Presenting symptoms in our study were comparable with the studies done by R. Chaudhary *et al* [2],Singhi *et al.*[17], Kabra *et al.*[18]and Kumar *et al.*[19]

Symptoms	R. Chaudhary *et al* [2]	Singhi *et al.*[17]	Kabra *et al.*[18]	Kumar *et al.*[19]	Our study
Cough	83.33 %	96.50%	92%	100%	88.89 %
Fever	77.77 %	97%	82%	88%	77.78 %

So, on comparison cough and fever are the predominant findings in culture positive patients. On chest auscultation wheezes are predominant finding and heard in 7 of 9 culture positive patients (77.78 %).

CONCLUSION-

The clinical presentation of Atypical pneumonia is similar, despite of having different pathogenic microorganism causing it. This leads to a blanket treatment in the clinical settings. So we are emphasizing on the relevant microbiological testing like culture method to prevent the development of drug resistance to β lactams antibiotics. In our study, the prevalence of *M. pneumoniae* is 8.57 % in respiratory disease patients by culture method, while prevalence ranges from 20-40 % by other diagnostic method like serological test and molecular method. However, future studies are always needed to a combination of two or three methods can be the most reliable approach for identification *M. pneumoniae* in respiratory disease patients.

REFERENCES-

1. Koneman, E.W., et al. Color Atlas and Textbook of Diagnostic Microbiology,7th edition p 70-75.
2. Bartlett J G. Is activity against atypical pathogens necessary in the treatment protocols for community – acquired pneumonia, issue with combination therapy. CID; 47. 232-35.
3. Chaudhry R,Sharma S, Javed S, Passi K, Dey AB, Malhotra P. Molecular detection of Mycoplasma pneumoniae by quantitative real-time PCR in patients with community acquired pneumonia. Indian J Med Res ; 2013.138: 244-251. |
4. Razin S,Yogev D, Naot Y. 1998. Molecular biology and pathogenicity of mycoplasmas. MicrobiolRev.;63:1094–156.
5. Shehabi AA, Baadran I. 1996. Microbial infection and antibiotic resistance patterns among Jordanian intensive care patients. Eastern Mediterranean Health Journal; 2:515-20.
6. Waites K.B., Talkington D.F. 2004. Mycoplasma pneumoniae and Its Role as a Human Pathogen. Clin Microbiol Rev;17:697-728.
7. Domingues D., Nogueira F., Tavira L., Exposto F. 2005. Mycoplasmas:What is the role in human infections? Acta Med Port;18:377-84.
8. Andreu L.M., Molinos A.S., Fernandez R.G. 2006. Serologic diagnosis of Mycoplasma pneumoniae infections. Enferm InfeccMicrobiol Clin;24 Suppl 1:19-23.
9. Ferwerda A., Moll H.A., de Groot R. 2001. Respiratory tract infections by Mycoplasma pneumoniae in children: a review of diagnostic and therapeutic measures. Eur J Pediatr;160:483-91.
10. Waites K.B. 2003. New concepts of Mycoplasma pneumoniae infections in children. Pediatr Pulmonol;36:267-78.
11. Baseman J.B., Tully J.G. 1997. Mycoplasmas: sophisticated, reemerging and burdened by their notoriety. Emerg Infect Dis;3:21-32.
12. Sillis M. 1999. Modern methods for diagnosis of Mycoplasma pneumoniae pneumonia. Rev Med Microbiol; 4 : 24-31.
13. Uldum SA, Jensen JS, Søndergård-Andersen J, Lind K. 1992. Enzyme immunoassay for detection of immunoglobulin M (IgM) and IgG antibodies to Mycoplasma pneumoniae. J Clin Microbiol; 30 : 1198-204.
14. Hyman HC, Yogev D, Razin S. 1987. DNA probes for detection and identification of Mycoplasma pneumoniae and Mycoplasma genitalium. J Clin Microbiol; 25 : 726-8.
15. Koneman, E.W., et al. Color Atlas and Textbook of Diagnostic Microbiology,7th edition, chapter 18. Mycoplasma and Ureoplasma p 1193-1195.
16. Kashyap B, Kumar S, Sethi GR, Das BC, Saigal SR. 2008. Comparison of PCR, culture & serological tests for the diagnosis of Mycoplasma pneumoniae in community-acquired lower respiratory tract infections in children. Indian J Med Res;128: 134-139.
17. Dr. Abhineet Mehrotra, Dr. S. K. Mehra, Dr. M E Siddque & Dr. Sushil Suri. 2015. Comparative Study of Techniques in the Diagnosis of Mycoplasma Pneumonia among the Patients of Respiratory Tract Infections in Northern Indian Population. Global Journal of Medical Research: 15; 4: 53-58.
18. Kabra SK., et al. 2003. Etiology of acute lower respiratory tract infection" Indian Journal of Pediatrics 70.1: 33-36.
19. Kumar N., et al. 2002.Clinical evaluation of acute respiratory distress and chest wheezing in infants. Indian Pediatric 39.5 :478-83.