Global Journals LaTeX JournalKaleidoscope™
Artificial Intelligence formulated this projection for compatibility purposes from the original article published at Global Journals. However, this technology is currently in beta. *Therefore, kindly ignore odd layouts, missed formulae, text, tables, or figures.*

Comparative Study of Techniques in the Diagnosis of Mycoplasma Pneumonia among the Patients of Respiratory Tract Infections in Northern Indian Population

Dr. Abhineet Mehrotra[1]

[1] Geetanjaly Medical College

Received: 12 December 2014 Accepted: 2 January 2015 Published: 15 January 2015

Abstract

Background: In the present study an attempt was made to compare the different diagnosis of mycoplasma pneumonia among the patients of respiratory tract infections in northern Indian population. Commonly used techniques in the diagnosis of mycoplasma pneumonia are culture, serology and PCR and different studies assure the different sensitivity and specificity. In the present study author also aim to find the common risk factors of mycoplasma pneumonia. Material Methods: The present study was undertaken at Career Institute of Medical Science, Lucknow. The total number of study subjects were 193. The total number of male subjects were 115 (59.6

Index terms— mycoplasma pneumonia, culture, serology,

I. Introduction ycoplasmas (mushroom form) are eubacteria included within the class Mollicutes (from latin mollis = "soft," cutis = "skin"), which comprises the smallest and simplest self-replicating, pleotrophic bacteria that lack cells wall thus, betalactam antibiotics, such as penicillin and cephalosporins, are ineffective. The name Mycoplasma, from the Greek ?????, mykes (fungus) and ??????, plasma (formed), was first used by Albert Bernhard Frank in 1889 1 . Mycoplasmas are primarily mucosal pathogens, living a parasitic existence in close association with epithelial cells of their host, usually in the respiratory or urogenital tracts. M. pneumoniae exclusively parasitizes humans, whereas some of the other human mycoplasmas have also been recovered from nonhuman primates 2 . Mycoplasma pneumoniae is an important cause of respiratory tract infection and is increasingly being associated with other diseases such as asthma and extra-pulmonary complications. Considerable cross-reactivity is known to exist between the whole cell antigens used in the commercial serological testing assays. Usually, mycoplasma infection is a mild illness characterized by fever, cough, bronchitis, sore throat, and headache. In very rare cases, mycoplasma can cause serious illness such as encephalitis (an inflammation of the brain) or meningitis (inflammation of the lining of the brain and spinal cord).

As the diagnosis the clinical symptommatology of a mycoplasma infection does not show pathogenspecific characteristics, the diagnostic differentiation from other pathogens such as viruses and gram-positive bacteria is decisive for appropriate therapy 3 . The diagnosis can be based on direct detection and serology. Detection of the pathogen has been regarded as efficient diagnosis at acute, early stages of disease. Isolation of the pathogen by culture has been considered the reference method. However, it is too insensitive and time-consuming (6-14 days). A good quality pathogen DNA detection system (PCR) is not yet commercially available. To date, serology has been considered the method of choice for diagnosis of infections. The complement fixation test (CFT) represents the classic antibody detection. The CFT cannot discriminate between antibody isotypes. Agglutination tests cannot discriminate between antibody classes either. Both test systems detect mainly the IgM antibody response. In reinfections both CFT and agglutination tests provide predominantly negative results. Nevertheless these test systems currently dominate the ELISA technology 4 . By using ELISA, IgG, IgA, and IgM, antibodies can be differentiated. The crucial factor for a specific and sensitive ELISA is the antigen. 5 In the present study we try

to evaluate the effective method in the diagnosis of mycoplasma pneumonia among three techniques i.e. Serology, Culture & PCR and an attempt was made to explore the socio demographic and clinical characteristics.

1 II. Material & Methods

The present study was undertaken at Career Institute of Medical Science, Lucknow. The total number of study subjects were 286. The total number of male subjects were 115 (59.6 %) while 78 (40.4 %) were females. The present study protocol was approved by institutional ethical committee.

2 a) Culture, Serology and PCR

The expectorated sputum (throat swabs) and blood was collected from all patients. The M. pneumoniae standard strain was revived according to ATCC (American Type Culture Collection) 15531? guidelines (www.atcc.org) which is commercially available in market. In brief, the lyophilized culture was resuspended in 6 ml pleropnemonia like organism (PPLO) broth. A single drop was used to inoculate PPLO agar; 3 ml suspension was used to prepare glycerol stocks and stored at -70°C. The remaining 3ml suspension was incubated at 37°C and 5 per cent CO2 incubator till growth was observed with change in color from red to yellow. Mycoplasma pneumoniae strains are very slow growing and produce a very light turbidity. Growth in broth is best observed after 10 to 14 days of incubation. Usually it takes at least seven days for the first tubes to start showing growth. Growth is easily recognized by an indicator change from red to orange to yellow. The cells are best transferred when the medium is orange. After medium changes to yellow, cells have started to die. All the patients whose culture was found to be positive were taken for further consideration. The patients having positive culture were marked and further followed for serology. Only serum part which was separated from whole blood of those whose culture was positive. Serum was stored at -20 o C till assayed. ELISA (Enzyme-Linked Immunosorbent Assay) kit is used for the accurate qualitative measurement of IgM class antibodies against Mycoplasma pneumoniae in Human serum and plasma. The clinical measurements were recorded. After serological test those patients were again used for follow up (for the purpose of PCR) whose culture as well as serology was found to be positive.as well as all other samples of culture and serology negative were also processed for PCR.

The sputum samples were collected in PBS were centrifuged at 1957 x g for 10 min. Supernatant was decanted and the pellet was resuspended in 0.5 ml PBS and stored at -70oC till further processing. The positive control consisted of relevant purified mycoplasma DNA. The extraction of DNA was done using the organic methods described by Das et al [5]. A 543 bp section of the P1 protein gene of M.pneumoniae was selected for amplification. The primers (Bangalore Genei Pvt. Ltd., Banglore, India) were: Primer1: 5'CAAGCCAAACACGAGCTCCGGCC-3', which is complimentary to the P1 gene negative strand residues 3666-3688, and Primer 2 : 5'CCAGTGTCAGCTGTTTGTCCTTCCCC-3', which is complimentary to the P1 gene positive strand residues 4208-4183.

Amplification was done according to the guidelines of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Banglore, India). Amplified PCR products were subjected to electrophoresis on 3 ethidium bromide stained agarose gel, along with a molecular weight marker. A mixture of 2 µl genomic DNA, 2 µl of 6 X BPB and 8 µl of distilled water was loaded 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 bp was taken to be a positive result. The PCR of those patients whose culture and serology was positive was carried out.

3 III. Results

Table 1 represents the age wise distribution of study subjects. The total number of study subjects was 286. The total number of male subjects were 207 (72.4 %) while 79 (27.6 %) were females. Highest number of subject's i.e. 32.9 % subjects were less than 30 years while second highest number i.e. 30.4 % of study subjects was more than 45 years. Table 2 Gender wise distribution of study subjects. Table 3 shows the test result by different diagnosis technique of mycoplasma pneumonia. Out of 286 patients 168 (58.7 %) samples were found positive in culture and 98 (34.3%) samples were found to be positive in serology test. PCR testing shows only 80 (28 %) samples were found to be positive.

4 b) Sample size calculation

Sample size was calculated by the sample size calculation formula for sing proportion by n=4pq/d 2 , Where n: sample size, p: prevalence q: 1-p and d is specified absolute precision by assuming prevalence 21 %, with specified absolute precision 0.06 and 10 % data loss, be. Calculated sample size is 260. Adding 10% non-response, the final sample size is 286.

Sample size is 260. Adding 10% non-response, the final sample size is 286.

i. Inclusion criteria Patients clinically diagnosed clinical and radiological proven of atypical pneumonia will be included in the study with, 1. Community acquired pneumonia (CAP): 2. Presence of a new pulmonary infiltrate/ shadow on chest X-ray suggestive of pneumonia at/ within 24 h of hospitalization. 3. Patient residing in community.

ii. Exclusion criteria 1. Hospital acquired pneumonia i.e. pneumonia that developed 72 h after hospitalization or within 7 days of discharge. 2. Pulmonary shadow due to a cause other than pneumonia. 3. The serious

patients having other disease with atypical pneumonia will be excluded A written informed consent was taken from the parent/legal guardian of the children before them being enrolled in the study.

Clinical data from the patients were collected using a questionnaire developed and validated and a detailed examination was performed. Routine laboratory investigations were done in all subjects. In the present study culture, serology and PCR technique is used to diagnose the mycoplasma pneumonia.

5 IV. Discussion

A variety of techniques are used to assess M. pneumoniae, each with different advantages and disadvantages. In this study, PCR, ELISA, and culture methods were employed for the detection of M. pneumoniae infection in 286 suspected patients. The culture method for the isolation of M. pneumoniae requires 2-4 weeks, which limits itsclinical usefulness. Moreover, culturing of M. pneumoniae is expensive and time-consuming (7-10). Serological methods are more extensively used than culture, because they are easier to carry out and more affordable (11-13).However, they are generally nonspecific, retrospective, and need 2 samples for titration 7 . It should be noted that in our study, the most reliable result was obtained by the PCR method, which proved to be highly sensitive, specific, and faster than other methods. The specificity of the culture method was 100%, while its sensitivity barely reached 33%, relative to the results of the PCR method as a gold standard. All of the culture and IgM ELISA test positive patients were PCR-positive, too. The PCR method is more sensitive and is the gold standard currently being used for diagnosis of this organism in some laboratories 14

6 V. Conclusion

In the present study it is concluded that only one technique may not be sufficient to diagnose the mycoplasma pneumonia as these techniques do not show higher sensitivity and specificity as many results suggest the same [6]. However, serology and culture is done commonly.

7 VI. Acknowledgment

The authors acknowledge the help provided by various faculties, technicians and paramedical staffs of Career Institute of Medical Sciences, Lucknow, for providing patient samples. Authors would like to acknowledge the Dean/Director of the institute to provide great support. [1]

3

Figure 1: Figure 3 a

[1]Volume XV Issue IV Version IYear 2 015 © 2015 Global Journals Inc. (US)

7 VI. ACKNOWLEDGMENT

1

Age Group (years)	Sample N (%)	
<3	15	5.2%
3-5	21	7.3%
6-10	33	11.5%
11-15	19	6.6 %
16-30	94	32.9 %
31-45	87	30.4%
46-60	9	3.1%
>60	8	28 %

Figure 2: Table 1 :

2

Gender	N	%
Male	207	72.4
Female	79	27.6
	Figure 2	

Figure 3: Table 2 :

3

Test Result	Culture (n=286)	Serology (n=286)	PCR (n=286)
Positive Result	168(58.7%)	98 (34.3%)	80(28%)
Negative result	118(41.3%)	188 (65.7%)	206(72%)

Figure 4: Table 3 :

[Waites ()] , Ken B Waites , DeborahF . *Talkington Clin. Microbiol. Rev. October* 2004. 17 (4) p. .

[(October 200)] , 10.1128/CMR.17.4.697-728.2004. October 200.

[Talkington et al. ()] 'Analysis of Eight Commercial Enzyme Immunoassay Tests for Detection of Antibodies to Mycoplasma pneumoniae in Human Serum'. D F Talkington , S Shott , M T Fallon , S B Schwartz , W L Thacker . doi: 10. 1128/CDLI.11.5.862-867. *Clinical and Diagnostic Laboratory Immunology* 2004. 2004. 11 (5) p. .

[Shin et al. ()] 'Crystal structure of the DUF16 domain of MPN010 from Mycoplasma pneumoniae'. D H Shin , J-S Kim , H Yokota , R Kim , Kim S-H . 10.1110/ps.051993506. *Protein Science : A Publication of the Protein Society* 2006. 15 (4) p. .

[Sasaki et al. ()] 'Epidemiological study of Mycoplasma pneumoniae infections in Japan based on PCR-restriction fragment length polymorphism of the P1 cytadhesin gene'. T Sasaki , T Kenri , N Okazaki , M Iseki , R Yamashita , M Shintani . *J Clin Microbiol* 1996. 34 p. .

[Atkinson et al. ()] 'Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections'. T P Atkinson , M F Balish , K B Waites . *FEMS Microbiol Rev* 2008. 32 p. .

[Beersma et al. ()] 'Evaluation of 12 commercial tests and the complement fixation test for Mycoplasma pneumoniae-specific immuneglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard'. M F Beersma , K Dirven , A P Van Dam , K E Templeton , E C Claas , H Goossens . *J Clin Microbiol* 2005. 43 p. .

[gnostics Specific and sensitive diagnosis Mycoplasma pneumoniae IgG] `http://www.medac.de/medac_international/data/dia` *gnostics Specific and sensitive diagnosis Mycoplasma pneumoniae IgG*, (IgA-, IgM-ELISA) (nb)

[Daxboeck et al. ()] 'Laboratory diagnosis of Mycoplasma pneumoniae infection'. F Daxboeck , R Krause , C Wenissch . *Clin Microbiol Infect* 2003. 9 p. .

[Waites and Talkington ()] 'Mycoplasma pneumoniae and its role as a human pathogen'. K B Waites , D F Talkington . *Clin Microbiol Rev* 2004. 17 p. .

[Zhang et al. ()] 'PCR versus serology for diagnosing Mycoplasma pneumoniae infection: A systematic review & metaanalysis'. L Zhang , Z-Y Zong , Y-B Liu , H Ye , X-J Lv . *The Indian Journal of Medical Research* 2011. 134 (3) p. .

[Dunn et al. ()] 'Rapid detection of Mycoplasma pneumoniae IgM antibodies in pediatric patients using ImmunoCard Mycoplasma compared to conventional enzyme immunoassays'. J J Dunn , A K Malan , J Evans , C M Litwin . *Eur J Clin Microbiol Infect Dis* 2004. 23 p. .

[Liu et al. ()] 'Rapid diagnosis of Mycoplasma pneumoniae infection in children by polymerase chain reaction'. F C Liu , P Y Chen , F L Huang , C R Tasai , C Y Lwee , L C Wang . *J Microbiol Immunol Infect* 2007. 40 p. .

[Ma et al. ()] 'Rapid Mycoplasma culture for the early diagnosis of Mycoplasma pneumoniae infection'. L D Ma , M Chen , Y Dong , J Fan , L Xia , S Wang . *J Clin Lab Anal* 2010. 24 p. .

[Kashyap et al. (2008)] 'Saigal Comparison of PCR, culture & serological tests for the diagnosis of Mycoplasma pneumoniae in community-acquired lower respiratory tract infections in children Indian'. Bineeta Kashyap , Surinder Kumar , G R Sethi , * , B C R Das** & S . *J Med Res* August 2008. 128 p. .