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DIRECT FLUORESCENCE MICROSCOPY: EFFECTIVE METHOD OF *MYCOPLASMA PNEUMONIAE* IDENTIFICATION IN CLINICAL SAMPLES.

Bhattacharjee Minakshi[1,2*], Urhekar A. D.[1] and Sharma Revati[1,2]

[1]Department of Microbiology, MGM Medical College and Hospital, Kamothe, Sector1, Navi Mumbai-410209.

[2]Central Research Laboratory, MGM Medical College and Hospital, Kamothe, Sector1, Navi Mumbai-410209.

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***Corresponding Author**
Dr. Bhattacharjee Minakshi
Department of Microbiology, MGM Medical College and Hospital, Kamothe, Sector1, Navi Mumbai-410209.

ABSTRACT

The study aims at the detection of *Mycoplasma pneumoniae*, a leading causative organism of community acquired pneumonia. For this an in-house method of direct fluorescence microscopy using FITC labeled antibodies to the organism was standardized. The results obtained showed a 10.66% prevalence of *Mycoplasma pneumoniae* in this area. These findings are promising. Augmented studies on larger population could be up taken to obtain a better picture of the prevalence of the organism in this part of the country.

KEYWORDS: *Mycoplasma pneumoniae*, Direct Fluorescence microscopy, FITC labeled antibodies.

INTRODUCTION

The etiological agents of community acquired pneumonia include *Mycoplasma pneumoniae* at the second place after *Streptococcus pneumoniae*.[1] The lower respiratory tract infections attributed by this organism are not rare but highly under studied. The reason for this is the lack of proper diagnostic facilities. This is to a great extent due to its delicate and fastidious nature. Western countries have data on the organism, obtained from reports of various population based studies. There are no national surveillance studies going on currently worldwide.[2] The Indian picture is even more distorted. Only few studies from Delhi and Chennai are available. Disease burden obtained from these available data in USA is 30.6% of

all community acquired pneumonia. It is responsible for more than one lakh hospitalizations each year in America.[3] Studies from India mentions an average disease burden of 28.6% pneumonia cases.[4] The Indian studies are mostly from Delhi and Chennai.[5,6,7,8] The present study makes an effort to investigate and understand the application of an indigenous direct fluorescence microscopy method in detection of *Mycoplasma pneumoniae*. The method is based on the principle that a single primary antibody is chemically linked to a fluorophore. The primary antibody recognizes the target molecule (antigen) and binds to a specific region called the epitope. The attached fluorophore can be detected via fluorescence microscopy, which, depending on the messenger used, will emit a specific wavelength of light once excited.[9] An attempt to screen the organism's prevalence in this particular area of the country was also made.

METHODOLOGY

The method was standardized for directly staining sputum and endo tracheal tube aspirate specimens with FITC antibodies to *Mycoplasma pneumoniae*. The protocol was adopted from Bhartiya D et al.[10] A total of 150 samples, consisting of 19 endotracheal tube aspirate samples and 131 sputum samples were processed. Following is the detailed procedure: Preparation of smear- smear was fixed on the slide with 4% para-formaldehyde. Preparation of blocking agent- 5% bovine serum albumin (BSA, HiMedia, India) was used as blocking agent. This was made by adding 0.5mg BSA to 1ml 0.02% PBST. 300µL of the above blocking agent was used for blocking. The slide was kept in a moist chamber for1 hour at room temperature to complete the blocking step. Preparation of Antibody- FITC labelled antibody (Thermo Fisher Scientific India Pvt Ltd.) was diluted 1:100 times. Blocking agent was used as the diluent. 50µL to 100µL of diluted FITC labelled antibody was added to the sample. This was followed by overnight incubation at 4^0C in a moist chamber. After incubation the slide was washed by flooding with 0.02% PBST for 5 minutes. Counter staining with DAPI- Immediately 500µL of DAPI (4',6-diamidino-2-phenylindole) counter staining was added and left for 15 minutes at room temperature. Concentration of DAPI (HiMedia, India) used was 300nM. Two quick washes with 0.02% PBST was given. A third wash was given for 5 minutes. Addition of Mounting solution: A drop of Vectashield was used for mounting. Sealing of the slide-The slide was then covered with a cover slip, sealed with DPX (Dibutyl Pthalate Xylene) and observed under a fluorescence microscope (Nikon, Japan). Controls used: Positive conrol- PPLO broth inoculated with *Mycoplasma pneumoniae* FH strain (ATCC 15531). Negative control- Sterile PPLO broth.

RESULTS

Initially, positive PPLO culture broth was treated with FITC labeled antibodies to *M. pneumoniae* and was used to standardize the direct fluorescence microscopy method (Fig1). The method was then applied to samples of sputum and endo tracheal tube aspirate. Any positive sample obtained by direct fluorescence microscopy was co-related with the PCR results for confirmation. The patient samples included in this study subjected to fluorescence microscopy were 150. A total of 16 cases showed the presence of *M. pneumoniae* and the prevalence percentage hence found by this method is 10.66%. p-value was 5.70808E-22 i.e. < 0.05, it can be concluded that the observed proportion of positive is significant statistically.

DISCUSSIONS

The diagnostics tests available for the detection of *M. pneumoniae* are sparse and those available are time consuming and laborious. Another reason for the lack of proper laboratory setups is the slow generation time of this bacterium, around 6hrs.[2] The culture shows positive growth only after two to three days. The pleomorphic nature of *M. pneumoniae* attributed due to its lack of cell wall adds on to the difficulties. Gram's staining is not preferred, due to the absence of cell wall. They are better stained by Giemsa stain but hard to identify.

Fluorescence microscopy is an advanced, highly sensitive and specific method, wherein few cells per cubic micrometer can be detected. The dark background facilitates the easy detection of the bright target objects.

The indirect fluorescence microscopy technique is comparatively invasive as blood samples are required for analysis. The method detects the presence of antibodies to *M. pneumoniae* in the test sample. It is more costly as well.

The direct fluorescence microscopy method standardized in the present study can be applied straight away on the patient samples. It is non invasive, as sputum sample is sufficient for the test. It is cost effective in comparison to most of the available methods. Though the availability of a fluorescent microscope can be a problem in rural areas, the nearest reference centre can be sought for sample analysis. The method thus sows the seed of easier preliminary diagnosis of M. pneumonia on routine basis.

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