Microbiology Research Journal International

30(6): 1-10, 2020; Article no.MRJI.57809
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Seroprevalence of IgM Antibody in Atypical Pneumonia Causing Pathogens by Pneumoslide, IFA

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PS and SM designed the study. Author PS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SM and VL managed the analyses of the study. Author PS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i630223
Editor(s):
(1) Prof. Essam Hussein Abdel-Shakour, Al-Azhar University, Egypt.
Reviewers:
(1) Gulshan Umbreen, University in Lahore, Pakistan.
(2) Shana Priscila Coutinho Barroso, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57809

Original Research Article

Received 13 April 2020
Accepted 18 June 2020
Published 04 July 2020

ABSTRACT

Background: Atypical bacterial and viral pathogens play an important role in atypical pneumonia are responsible for one of the leading causes of morbidity and mortality, particularly in developing countries.
Objective: The purpose of this study to determine the prevalence of bacterial and viral pathogens causing acute atypical pneumonia in different age groups and seasonality patterns of prevalence in India.
Methods: This retrospective study was conducted on 680 samples tested during December 2018 to August 2019, performed at Microbiology department of *Dr. Lal Path Labs.* Serum samples were used for Pneumoslide IgM test diagnose 9 Atypical bacterial & viral pathogens: *Legionella pneumophila (LP), Mycoplasma pneumoniae (MP), Coxiella burnetti (COX), Chlamydophila pneumonia (CP) Adenovirus (ADV), Respiratory syncytial virus (RSV) Influenza A (INFA), Influenza B (INFB), Parainfluenza serotype*s 1,2 &3(PIVs).
Results: Of a total 477(70.1%) samples were positive for atypical pneumonia pathogens. Atypical pneumonia was seen in extremes of age ie: <=5 years and >60 elderly adults without much of a

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gender bias. Co infections was seen in 62.1%. *Legionella pneumophila* (42.5%) was the dominant pathogen followed by *Influenza B* (41.7%) *Mycoplasma pneumoniae* (33.4%), *Parainfluenza serotype*s 1,2 &3 (29.4%) respectively. Atypical pneumonia has a spring predominance that is peaking in March.

Conclusion: Among six predominant atypical pathogens, *Legionella pneumophila* and *Influenza B* was most predominant pathogens, as a causative agent of atypical pneumonia followed by *Mycoplasma pneumoniae* seen mostly in young (0-5 years) comparison to all age groups. Hence, Pneumoslide IgM as a multi panel test needed to ensure initiation of targeted therapy. Pneumoslide IgM, by IFA is a rapid, cost effective easy to identify & classify atypical pneumonia causing pathogens.

Keywords: Pneumoslide IgM; atypical pathogens; Legionella pneumophila; Mycoplasma pneumonia; influenza A; influenza B; parainfluenza serotypes 1,2 &3(PIVs).

1. INTRODUCTION

Atypical bacterial and viral pathogens play an important role in atypical pneumonia are responsible for one of the leading causes of morbidity and mortality, especially in children. Almost 2 million children die from atypical pneumonia each year from developing countries. [1,2] Our study using the Pneumoslide IgM test to investigate the atypical pneumonia by atypical pathogens on the basis of different age groups and seasonility patterns. The incidence of atypical pneumonia higher in children of 0-5 years of age caused by atypical bacteria and viral pathogens including *Legionella pneumophila, Mycoplasma pneumoniae, Coxiella burnetti (COX), Chlamydophila pneumonia (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV) Influenza A(INFA), Influenza B(INFB), Parainfluenza serotype*s 1,2 &3(PIVs). The prevalence of atypical pathogens varied from country to country and could be due to differences in seasons and geographic areas [1-10]. Atypical bacteria and viruses pathogens were frequently diagnosed through Pneumoslide IgM antibodies against nine pathogens because the cell cultures for viral and atypical bacterial isolation are usually take a time to results may be as long as 14 days. Recently PCR technique reported as rapid method for detection, but PCR assay need specialized equipment and reagents are expensive yet it is not widely available due to its expensive initial cost [4]. Pneumoslide IgM, IFA Test found to be sensitive, highly specific, easy, rapid and cost effective technique for detection of atypical pathogens causing mild to life threatening atypical pneumonia in developing countries.

2. MATERIALS AND METHODS

This retrospective study performed on 680 serum sample tested between December 2018 to August 2019, at Microbiology department of *Dr Lal Path Labs,* Delhi.

Pneumoslide IgM, which is an indirect immune fluorescent assay kit (VIRCELL PNEUMOSLIDE, Spain) for the simultaneous diagnose the respiratory tract atypical pathogens. Antibodies to *Legionella pneumophila serogroup (LP), Mycoplasma pneumoniae (MP), Coxiella burnetti (COX), Chlamydophila pneumoniae(CP) Adenovirus (ADV), Respiratory syncytial virus (RSV) Influenza A(INFA), Influenza B(INFB), Parainfluenza serotype*s 1,2 &3(PIVs) are used in this kit.

Each slide has 10 wells, each containing one of the above agent antigens and cell control. Serum samples were diluted 1:1 with Phosphate Buffered Saline (PBS) then treated with anti-human IgG sorbent. The sorbent treated diluted serum was incubated 90 min at 37°C with the 10 slide wells. The slide washed twice with PBS. A fluorescent secondary IgM antibody (Anti-human IgM/FITC) added to the wells and incubated at 37°C for 30 min then washed twice with PBS. If positive, an IgM response (greenish yellow fluorescence) obtained. Moreover, patients in whom any one of the targeted pathogens detected using the above methods regarded as positive. Cases in which a single pathogen detected referred to as mono-infections; cases of two or more pathogens referred to as co-infections or multiple infections, respectively. Most of our samples collected from different states of North India including Delhi and Kolkata.

2.1 Statistical Analysis

General data presented as a percentage, we divided the data into several groups according to the pathogen, month and age group, and Statistical analysis performed using Microsoft Excel 2010.

3. RESULTS

Of a 680 serum samples tested, 477(70.1%) samples were positive for Bacteria, Viruses and Mixed infection. Among the positive cases (n=477), 22.4% (107/477) were bacterial-infections of the cases while 15.5% (74/477) were viral infections and 62.1% (296/477) were co infection or multiple bacterial and viral infections with more than one etiologic agent (Fig 1). *Legionella pneumophila* (42.5%) was the dominant pathogen followed by *INF B* (41.7%) *MP* (33.4%) *PIVs* (29.4%) respectively. Among all of the pathogens, *Coxiella burnetti* (1.2%) and *Chlamydophila pneumoniae*(2.8%) infections were detected low in our study (Fig. 2).

The positive percentages of *Legionella pneumophila serogroup (LP), Mycoplasma pneumoniae (MP), Coxiella burnetti (COX), Chlamydophila pneumonia (CP) Adenovirus (ADV), Respiratory syncytial virus (RSV) Influenza A(INFA), Influenza B(INFB), Parainfluenza serotype*s 1,2 &3(PIVs) found to be associated with age. This study male predominance seen with 361(53.1%) isolates while in female it was isolated in 319 (46.9%) cases. The infection is most common in extremes of age i e: 30.8% (0-5 Y) and 23.2% (>=60 Y). The incidence of atypical pathogens infection was higher among male patients in every age group except 21-30 years age group (Fig. 3).

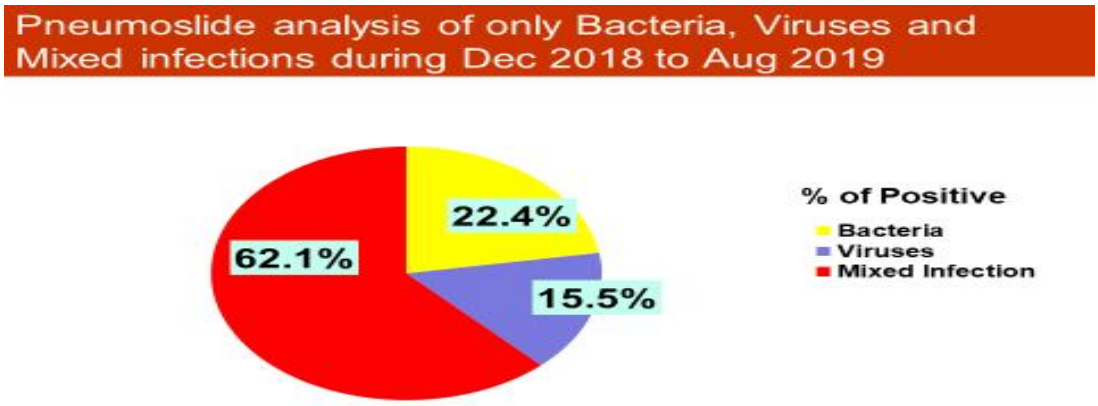

Fig. 1. The total percentages of atypical pathogens with Pneumoslide IgM

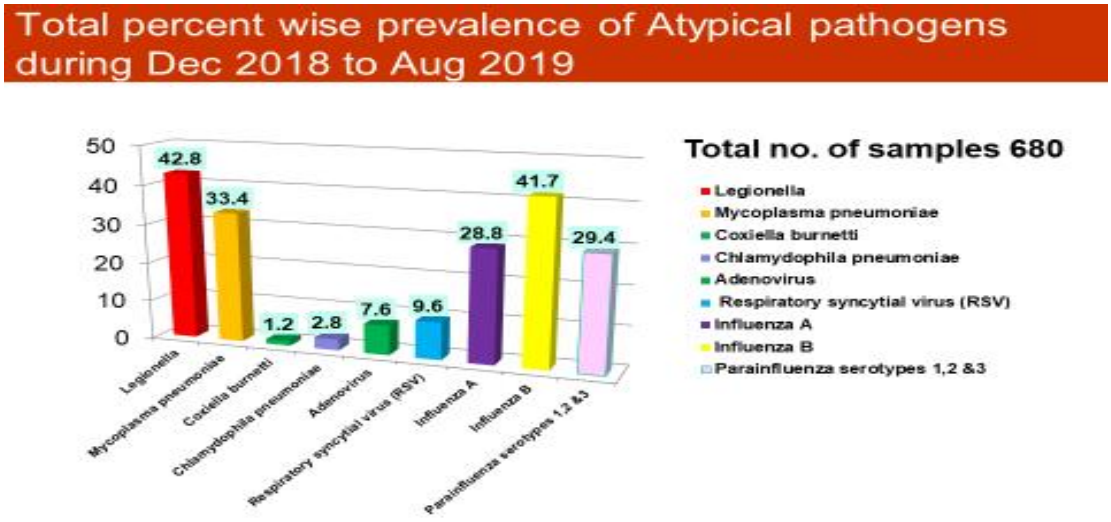

Fig. 2. The percentages of different respiratory bacterial and viral pathogens

*Mycoplasma pneumoniae, Coxiella burnetti, Chlamydophila pneumoniae, Adenovirus, Respiratory syncytial virus (RSV) Influenza A, Influenza B, Parainfluenza serotype*s 1,2 &3) was found higher in (0-5 years) age group and predominant atypical bacteria *Legionella pneumophila* were the major cause of atypical pneumonia in young children and elderly adults (Table 1).

Among the mono infections *Legionella pneumophila*was the leading causative agent (15.9%, n=76) followed by *Mycoplasma pneumoniae* 7.5% and other pathogens (Table 2).

Legionella pneumophila was the most frequent pathogen among Co infections and multiple infections (Table 3). The spectrum of mixed infections of diverse pathogens in all age group including *Legionella+ Mycoplasma pneumonia + Influenza A and Influenza B* was the highest dominant pathogen 13.8% followed by *Influenza A + Influenza B +PIVs* (8.8%), *Legionella pneumophila + Mycoplasma pneumoniae* (7.1%), and others respectively (Table 3).

477 (70.1%) were positive for at least one viral or bacterial pathogen among whom the predominant bacteria were *Legionella, Mycoplasma* and viruses were *Influenza A* and B. the common respiratory atypical pathogens detected peaked in the March month but Mycoplasma detection peaked in April. Analysis of the monthly distribution of atypical pneumonia revealed that *Legionella pneumophila, Mycoplasma pneumoniae, Influenza A, Influenza B* and *Parainfluenza serotype*s 1,2 &3) detected continuously increasing throughout the winter to spring and suddenly decreased in summer month. RSV infection was more common in February and March with a very small peak occurring in April. *Chlamydophila pneumoniae* occurred sporadically in February with a small peak (Table 4).

Disease has a late winter and spring predominance that is peaking in March. Following are our seasonal findings (Table 4).

Legionella pneumophila was prevalent throughout nearly the entire study period, with two small peaks in February and March. *Legionella pneumophila, Mycoplasma pneumoniae, Influenza A, Influenza B* & *Parainfluenza serotype*s 1, 2 &3 infection was more prevalent in late winter and spring, with peaks in February, March and April (Fig. 4).

4. DISCUSSION

The use of specific Pneumoslide IgM against atypical pathogens causing atypical pneumonia led to identifying IgM antibodies against at least one organism in 70.1% of the patients in similar with worldwide studies El Seify et al.,(65.5%) and Chen et al. (51.8%) respectively [6,8].

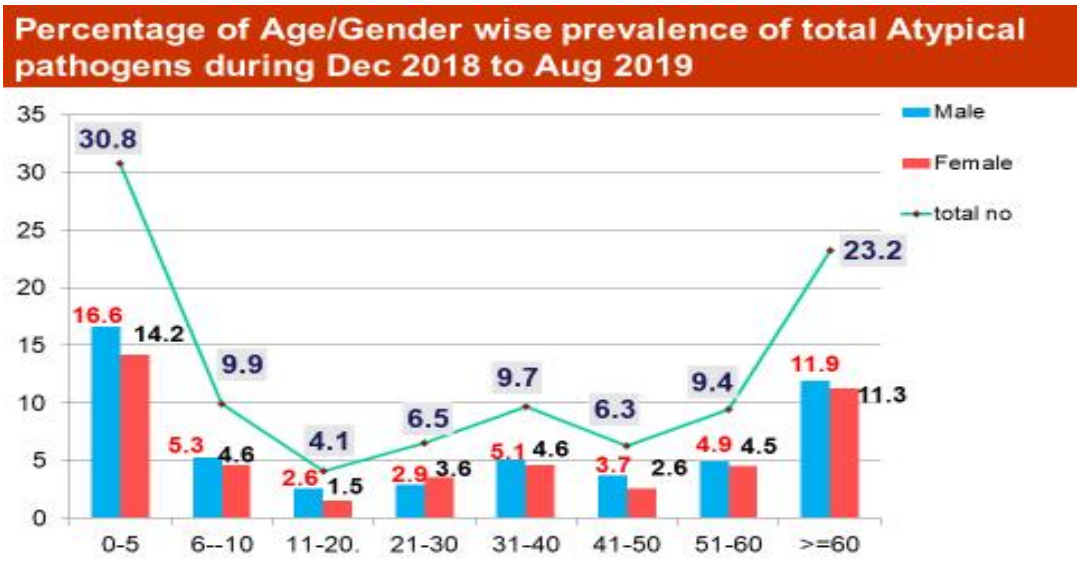

Fig. 3. The percentages of age/gender wise distribution of total Pneumoslide positive samples

Table 1. The prevalence of Atypical pathogens isolated from different age groups

Age	*Legionella pneumophila* N=289 (%)	*Mycoplasma Pneumonia* N= 227 (%)	*Coxiella burnetti* N=7(%)	*Chlamydophila pneumoniae* N= 19 (%)	Adenovirus N=52 (%)	Respiratory syncytial virus (RSV) N=65 (%)	Influenza A N=196 (%)	Influenza B N=284 (%)	Parainfluenza serotypes 1,2 & 3 N=200 (%)
0-5	73 (25.3)	85 (37.4)	3 (42.8)	11 (57.9)	28 (53.8)	30 (46.2)	72 (36.7)	107 (37.7)	77 (38.5)
6-10	39 (13.5)	30 (13.2)	1 (14.3)	3 (15.8)	4 (7.7)	3 (4.6)	23 (11.7)	35 (12.3)	27 (13.5)
11-20	15 (5.2)	13 (5.7)	--	--	1 (1.9)	1 (1.5)	8 (4.1)	12 (4.2)	6 (3)
21-30	15 (5.2)	11 (4.8)	1 (14.3)	1 (5.2)	1 (1.9)	4 (6.2)	7 (3.6)	13 (4.5)	10 (5)
31-40	32 (11.1)	26 (11.4)	--	2 (10.5)	1 (1.9)	4 (6.2)	28 (14.3)	36 (12.7)	17 (8.5)
41-50	23 (7.9)	9 (3.9)	1 (14.3)	--	4 (7.7)	2 (3.1)	12 (6.1)	16 (5.6)	11 (5.5)
51-60	30 (10.4)	18 (7.9)	1 (14.3)	1 (5.2)	5 (9.6)	8 (12.3)	19 (9.7)	23 (8.1)	17 (8.5)
>=60	62 (21.5)	35 (15.4)	--	1 (5.2)	8 (15.4)	13 (20)	27 (13.8)	42 (14.8)	28 (14)

Table 2. Total number and percentages of atypical pathogens causing mono-infection

Legionella	*Mycoplasma pneumoniae*	*Coxiella burnetti*	*Chlamydophila pneumoniae*	Adenovirus	Respiratory syncytial virus (RSV)	Influenza A	Influenza B	Parainfluenza serotypes 1,2 &3
76	36	0	3	3	5	4	11	2
15.9%	7.5%	0%	0.6%	0.6%	1.1%	0.8%	2.3%	0.4%

Table 3. Total number of atypical pathogens causing co- infection and multiple infection of different bacteria and viruses

Co-infection (n=477)	Positive	% of positive
Legionella pneumophila + *Mycoplasma pneumoniae*	34	7.1
Legionella+ Mycoplasma pneumoniae+ Inf A+ Inf B+ Para Inf 1,2,3	25	5.2
Legionella+ Mycoplasma pneumoniae+ Inf A+ Inf B	66	13.8
Legionella+ Mycoplasma pneumonia + RSV +Inf A + Inf B+ Para Inf 1,2,3	20	4.2
Legionella+ Mycoplasma pneumoniae+Adenovirus+ RSV +Inf A+ Inf B+ Para Inf 1,2,3	13	2.7
Legionella+ Mycoplasma pneumonia +Coxiella burnetti +Inf A + Inf B+ Para Inf 1,2,3	3	0.6
Legionella+ Mycoplasma pneumonia +Cox+ Adeno +RSV +Inf A +Inf B + Para Inf 1,2,3	2	0.4
Legionella+ Mycoplasma pneumonia + Chlamydiae pneumophila+Inf A+Inf B+ Para Inf 1,2,3	7	1.5
Mycoplasma pneumonia + Inf A+ Inf B+ Para Inf1,2,3	21	4.4
Mycoplasma pneumonia + Inf A+ Inf B	6	1.2
Mycoplasma pneumonia + Chlamydiae pneumophila +Inf A+ Inf B	3	0.6
Coxiella burnetti + Mycoplasma pneumonia	1	0.2
RSV +Inf A+ Inf B+ Para Inf 1,2,3	12	2.5
RSV+ Adenovirus +Influenza A + Influenza B+ Para Inf 1,2, and 3 serotypes	7	1.5
Influenza A + Influenza B	34	7.1
Influenza A+Influenza B+ Para Inf 1,2, and 3 serotypes	42	8.8
Influenza B+ Para Influenza 1,2, and 3 serotypes	23	4.8

This study demonstrated common respiratory atypical pathogens were the major cause of atypical respiratory infection in young children and elderly adults [1].

As observed in our study, there were six predominant atypical bacterial and viral pathogens causing atypical pneumonia whereas *Legionella pneumophila* (42.8%) was most predominant pathogens causes' mono infection and mixed infection among these nine pathogens. Previous studies have focused that 90% of cases of Legionnaires disease in United States and Europe [10-14] and have reported 0-25% of cases in other Asian countries [1,3,2,5,8]. In contrast, our data indicates that *Legionella pneumophila sero group*1 were predominant in Indian population. An important finding of this study were that the predominant Legionella infection in India differ from that found in other developed and developing countries [1,2-7] but similar with New Zealand with about 40% of cases due to *Legionella pneumophila* [9]. The positive percentage of *Legionella pneumophila* was high throughout the seasons compared to the other eight pathogens. Furthermore, the seasonal distribution of Legionella, which reached its high peak from March to May, very similar with previous study. [9] although multiple years of seasonal factors data needed to established the periodicity of disease.

The results of our study also demonstrate *Mycoplasma pneumoniae* as being frequently isolated after *Legionella pneumophila*. Age is one of the factors that might aid in indicating the aetiology of pneumonia. Among children, we found that the incidence of both *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* was highest among patients aged <5 years and second highest among >=60 years aged. In the majority of studies, along with our study *Chlamydophila pneumoniae and Mycoplasma pneumoniae* found in all age groups with a higher prevalence in children, aged 0-5 years [1,2,8]. In adulthood, the incidence of both *Chlamydophila pneumoniae and Mycoplasma pneumoniae* is highest among the elderly adults. In addition, adult pneumonia caused by *Mycoplasma pneumonia* (5-8%) in our studies was similar with some European studies [10,11,12]. The detection rate for infection with *Chlamydophila pneumoniae* using Pneumoslide IgM Test was low in our study (2.8%) similar with other studies [10,11].

Table 4. Monthly prevalence of atypical pneumonia causing atypical pathogens during December 2018- August 2019

Month N=477	*Legionella pneumophila (%)*	*Mycoplasma pneumoniae (%)*	*Coxiella burnetti (%)*	*Chlamydophila pneumoniae (%)*	Adenovirus (%)	Respiratory syncytial virus (RSV)(%)	Influenza A (%)	Influenza B (%)	Parainfluenza serotypes 1,2 & 3 (%)
December 2018	31 (6.5)	14 (2.9)	1 (0.2)	1 (0.2)	2 (0.4)	2 (0.4)	2 (0.4)	20 (4.2)	21 (4.4)
January 2019	35 (7.3)	19 (3.9)	--	--	3 (0.6)	6 (1.3)	16 (3.4)	26 (5.5)	20 (4.2)
February	47 (9.9)	43 (9.1)	--	9 (1.9)	12 (2.5)	16 (3.4)	40 (8.4)	56 (11.7)	47 (9.9)
March	67 (14.1)	46 (9.6)	3 (0.6)	4 (0.8)	17 (3.6)	15 (3.1)	58 (12.2)	67 (14)	48 (10.1)
April	42 (8.8)	48 (10.1)	1 (0.2)	1 (0.2)	9 (1.9)	17 (3.6)	42 (8.8)	57 (11.9)	39 (8.6)
May	38 (7.9)	44 (9.2)	2 (0.4)	2 (0.4)	7 (1.5)	9 (1.9)	25 (5.2)	37 (7.8)	18 (3.8)
June	12 (2.5)	9 (1.9)	--	2 (0.4)	1 (0.2)	--	7 (1.5)	8 (1.7)	4 (0.8)
July	9 (1.9)	1 (0.2)	--	--	--	--	3 (0.6)	8 (1.7)	2 (0.4)
August	8 (1.7)	3 (0.6)	--	--	1 (0.2)	----	3 (0.6)	5 (1.0)	1 (0.2)

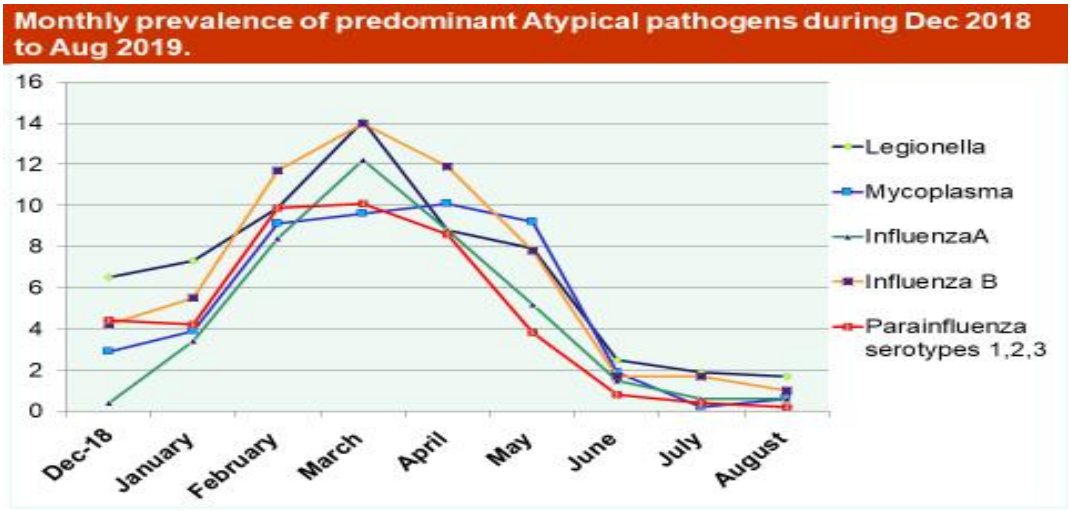

Fig. 4. Prevalence of most dominant pathogens isolated from different months

Coxiellaburnetii, a zoonotic bacterial pathogen transmitted to humans through direct contact with milk, urine, feaces, or semen from infected animals as well as inhalation of aerosolized particles from animal placentas, and environmental dust. While infection by *Coxiella burnetii* in humans known as Q fever varies geographically, can present as hepatitis and pneumonia [15]. However, the serological data in this study reveal only 1.2% cases of *Coxiella burnetii*. It prevalence worldwide except in New Zealand [16,15].

The prevalence of viral pathogen varied from country to country and could be due to differences in the geographic areas or due to different diagnostic procedures used in different studies. *Influenza B* reported to be the more frequent agent and this is similar with other findings [2,6,8,9,17].

The commonest viral aetiology was Influenza B among these 9 pathogens commonly occur as Co pathogens in mixed infections, which is associated with particularly high mortality. This study demonstrated most dominant common respiratory viruses responsible for atypical pneumonia in children and adults were *Influenza A*, *Influenza B* and *Parainfluenza serotype*s 1, 2 &3. However, these viruses as the major prevalent agent caused acute severe atypical pneumonia in children results were concordant with other studies [2,5,17,18]' in contrast, *Respiratory syncytial virus*re ported to be the more frequent agent through the world [7,19,20].

Our results show *Respiratory syncytial virus (RSV)* was the most common viral infections in children especially younger than 5 years, and incidence of low in adults and the positive percentage of *RSV* were higher in spring season this is very similar to other findings [2,5,8].

In our study suggested that *Adenovirus* involved in co-infections in children were high as compared to other age group and the positive percentage of *Adenovirus* were higher in March month this is very similar with Europe and Taiwanwhere *Adenovirus* is most frequently involved in co-infections [17,18].

In contrast, studies from the other Asian countries, United States and Europe found that incidence of mixed infection reported vary greatly, ranging from two to 50% [1,5,6,8,18-20]. Some reports showed that the clinical spectra for co infections were more severe than single infections [2,8]. However, the present study *Legionella pneumophila*, *Mycoplasma pneumoniae* and *Influenza B* often highly participated in mixed infection, was not agreement with previous findings but some reports were similar with our study [2].

Data were analysed to describe the prevalence and seasonality results in the study, the predominant bacteria were *Legionella pneumophila*, *Mycoplasma pneumoniae* and viruses were *Influenza A* and *Influenza B*. Common atypical pathogens detected peaked in the March month. Thus, our findings demonstrated late winter and spring season were

highly sensitive to atypical pneumonia caused by atypical pathogens in India.

5. CONCLUSION

Among six predominant atypical pathogens, *Legionella pneumophila* and *Influenza B* was most predominant pathogens, as a causative agent of atypical pneumonia followed by *Mycoplasma pneumoniae* seen mostly in young (0-5 years) comparison to all age groups. These findings provide a better understanding of viruses and bacterial infection in all age group stratification by gender and seasonality, all of which will contribute to therapeutic approaches and development of effective prevention strategies for this respiratory infection. Hence, Pneumoslide IgM as a multi panel test needed to ensure initiation of targeted therapy. Pneumoslide IgM, by IFA is a rapid, cost effective easy to identify & classify atypical pneumonia causing pathogens, which can play decise role in deciding the treatment line thus avoiding unnecessary usage of antibiotics.

ACKNOWLEDGEMENTS

We are thankful to Dr Reena Nakra, Lab director operations National Reference Laboratory, Dr Lal Path Labs, Delhi for providing us operational support and team Microbiology for technical assistance in conducting the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57809