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# Spectrum of Respiratory Pathogens Diagnosed by Multiplex-PCR in Suspected Cases of Atypical Pneumonia in a Tertiary Care Hospital of Bangladesh

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**Abstract**

**Background**

Pneumonia is one of the most common causes of death worldwide. The urgent and clinical heterogeneity of this infection results in dilemma of diagnosis and treatment. There is inconsistency in the definition of the group of microorganisms that cause "atypical pneumonia." Nevertheless, the use of this term in the medical and radiologic literature is common. Among the causes of community acquired pneumonia, atypical bacteria are responsible for approximately 15% of cases. Zoonotic and nonzoonotic bacteria, as well as viruses, have been considered among the causes of atypical pneumonia in a patient who is immunocompetent and have been associated with major community outbreaks of respiratory infection, with relevant implications in public health policies.

**Methods**

A total of 114 samples from Combined Military Hospital, Dhaka Cantonment was tested at Armed Forces Institute of Pathology, Dhaka Cantonment during the period of January to December 2022. Sputum, bronchial wash, nasal and throat swab was collected and tested by QIAstat-Dx Multiplex-PCR system.

**Results**

Among 114 samples 27 (23.65%) was found positive for various respiratory pathogen. Total 34 pathogen was detected as 22 samples found positive with single organism and 5 samples found positive with multiple organism. Rhinovirus was detected as the most prevalent organism (20.58%) followed by Influenza A $H_3$ (14.70%), Parainfluenza, Enterovirus and Adenovirus (11.76%), Coronavirus 229E, SARS-CoV-2 and Human Metapneumovirus A/B (5.88%), Coronavirus OC43, Respiratory Syncytial Virus A/B and Bocavirus (2.94%). Mycoplasma pneumoniae (2.94%) was found only bacteria among collected samples.

**Conclusion**

Multiplex real-time RT-PCR system is an excellent tool for rapid diagnosis of large number of respiratory pathogen and provides detail genetic information to clinician to start the management immediately. The assay exhibited improvements to sensitivity, specificity, time and cost-effectiveness compared to previous assays utilised for clinical and epidemiological applications.

**Keywords:** Acute Respiratory Infections, Atypical Pneumonia, Multiplex- PCR

## 1. Introduction

Acute respiratory infections (ARI) possess one of the most serious threats to global public health and are established to be a significant cause of morbidity and mortality in children and immunocompromised adults [1, 2]. Epidemiological data suggested lower respiratory tract infections were responsible for 2.7 million deaths in 2015, ranking it third in terms of disease burden [3].

A wide range of pathogens are involved in ARI. Among them a certain amount of bacteria and viruses causes atypical pneumonia. It is named as such because these organisms are hard to detect by conventional culture and serology. The microorganisms included in the atypical pneumonia group are characterized by constitutional symptoms and upper and lower respiratory tract involvement and can have a protracted clinical course with gradual resolution. Infections with *M pneumoniae*, *C pneumoniae*, and *Legionella pneumoniae* are the most common causes of atypical bacterial pneumonias. Respiratory viruses that most commonly affect humans include Adenovirus, Influenza A and B viruses, Parainfluenza virus, RSV, Human metapneumovirus and Coronaviruses [4]. In short, atypical pneumonia represent a category of infectious disease caused by

multiple pathogenic agents, this increases the difficulty of diagnosis and complicates treatment strategies due to the diversity and complexity of the infectious pathogens [5].
Therefore, an accurate and rapid diagnosis of the causative pathogens of infections is crucial to the selection of the appropriate therapeutics [6]. This will minimize the use of unnecessary antibiotics and ensure the swift implementation of the appropriate treatment [7].
Polymerase chain reaction (PCR) has proven to be fast, low-cost, and sensitive method of utilizing nucleic acid for the detection of various microorganisms [8]. QIAstat-Dx multiplex RT-PCR system was developed, which enabled the simultaneous detection of 21 different acute respiratory infection causing microorganisms in single tubes; these included bacteria and RNA and DNA based viruses [9].

## 2. Materials and Methods

This is a prospective study. It was conducted from January 1, 2022 to December 31, 2022 at the Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment. Irrespective of age and sex total 114 samples were tested who were admitted at Combined Military Hospital (CMH), Dhaka Cantonment with respiratory symptoms and whose diagnosis of causative organism was not possible by conventional culture and serological methods.
All data were entered into sheet containing epidemiological, clinical, paraclinical data, and clinical evaluation of patient. The samples like e.g., Sputum, bronchial wash, nasal and throat swab were collected aseptically with caution and transferred from CMH to Microbiology department, AFIP. The samples were analyzed according to the manufacturer's instructions by using the QIAstat-Dx Multiplex-PCR respiratory panel. This assay can detect and differentiate simultaneously 21 respiratory pathogens: 18 viruses (influenza viruses A and B, influenza virus A H1, H1N1 and H3, respiratory syncytial viruses (RSV) A/B, parainfluenza viruses 1, 2, 3 and 4, rhinovirus (RV)/enterovirus, coronaviruses (Cor) (229E, NL63, OC43, and HKU1), human metapneumovirus (hMPV)), adenovirus (ADV), bocavirus (BoV), and 3 bacteria (*Legionella pneumophila*, *Bordetella pertussis* and *Mycoplasma pneumoniae*) [9].
Statistical analysis was performed with the Package for the Social Sciences (SPSS, version 20)
Ethical clearance was taken from patients and Director General of Medical Services (DGMS).

## 3. Results

A total 114 samples like e.g., Sputum, bronchial wash, nasal and throat swab was collected. Among them 27 samples (23.68%) were found positive.

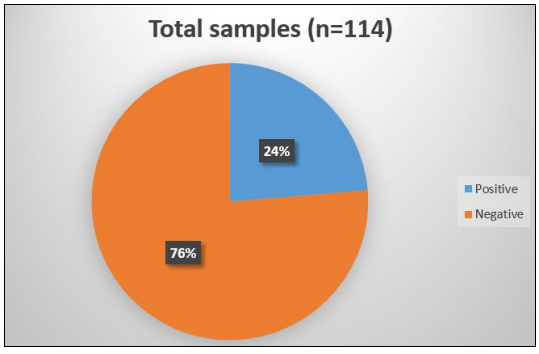


**Chart 1:** Total positive cases among total samples

Age group of above 50 years patients found mostly positive among positive samples.

**Table 1:** Age and sex distribution of positive patients (n=27)

| Age | No. of patient | Male | Female |
|---|---|---|---|
| 0-10 | 5 | 3 | 2 |
| 11-20 | 4 | 3 | 1 |
| 21-30 | 4 | 4 | 0 |
| 31-40 | 4 | 3 | 1 |
| 41-50 | 3 | 3 | 0 |
| 51-60 | 7 | 4 | 3 |
| Total | 27 | 20 | 07 |

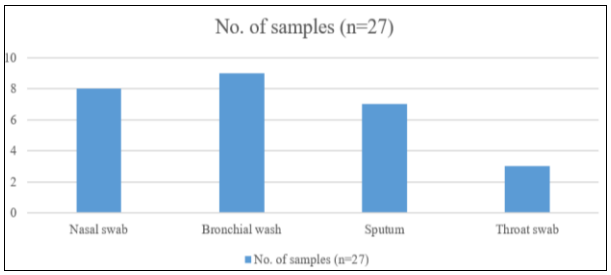


**Chart 2:** Number of different samples

Total 27 samples were found positive for various respiratory pathogen. Total 34 pathogen was detected as 22 samples found positive with single organism and 5 samples found positive with multiple organism. Rhinovirus was detected as the most prevalent organism (20.58%) followed by Influenza A $H_3$ (14.70%), Parainfluenza, Enterovirus and Adenovirus (11.76%), Coronavirus 229E, SARS Cov-2 and Human Metapneumovirus A/B (5.88%), Coronavirus OC43, Respiratory Syncytial Virus A/B and Bocavirus (2.94%). Mycoplasma pneumoniae (2.94%) was found only bacteria among collected samples.

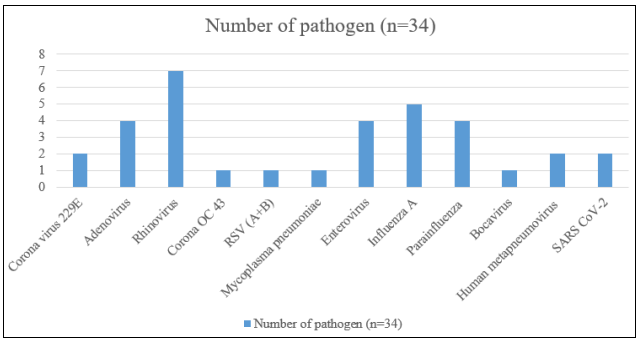


**Chart 3:** Number of different pathogens detected in positive samples

## 4. Discussion

Acute respiratory infections are caused by a complex array of pathogens, most commonly viruses and bacteria, as well as microorganisms such as *Mycoplasma* and *Chlamydia* [10, 11]. Furthermore, clinical diagnosis is complicated with co-infection of several pathogens leading to acute respiratory infection [12].
Thus, the development of a comprehensive, easy to implement and rapid molecular diagnostic tool capable of detecting and distinguishing various types of causative pathogens in clinical samples,
is invaluable for epidemiological surveillance, progress prediction and therapeutic strategies selection [2, 13, 14].

With its sensitivity and specificity regarding pathogen detection, RT-PCR has become a promising tool when looking to perform the rapid screening and identification of multiple pathogens simultaneously [15, 16].

This method targets pathogen-specific genetic material, rather than viral or bacterial antigens or antibodies, thus outperforming other traditional chemical immunoassay-based test procedures. Multiplex RT-PCR assays display a variety of benefits, including a significant reduction in the turnaround time of the assay compared to the use of multiple assays [12].

Viral infection accounts for appropriate 80% of acute respiratory infections, with influenza virus [17], respiratory syncytial virus (RSV) [18], and respiratory adenovirus [19] being the most common pathogens.

In this study Rhinovirus was detected as most common pathogen which is similar with another study conducted by Virology department, IEDCR, Dhaka [20].

In the current study, Mycoplasma pneumoniae infection was detected in 2.94 % of positive cases, which is relatively low. Higher frequencies of detection of Mycoplasma pneumoniae were reported by Defilippi *et al*. and Hadi *et al*. who reported 12% and 10% detection rate, respectively [21, 22].

## 5. Conclusion

The clinical spectrum of respiratory infections is complex and often nonspecific. Therefore, early and rapid detection of related causative agents is crucial. Multiplex RT-PCR assays for respiratory infections are not used as routine diagnostic test in Bangladesh. Their use in the near future will help physicians to choose an appropriate treatment, reduce the overall use of unnecessary antibiotics, initiate antiviral therapy and preserve intestinal flora, and help to decrease nosocomial infection by reducing the length of hospitalization. It will also decrease management costs. Multiplex RT-PCR assays for respiratory infections are likely to be the subject of future studies on larger samples and different age groups.

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