

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.806.286>

## Detection of Respiratory Syncytial Virus Infection in Adults with Lower Respiratory Tract Infection Using Real-Time PCR Assay

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### ABSTRACT

#### Keywords

Respiratory syncytial virus, Adults, Lower respiratory tract infection, Real-Time PCR

#### Article Info

Accepted:  
18 May 2019  
Available Online:  
10 June 2019

Respiratory Syncytial Virus (RSV) causes pediatric pneumonia and lower respiratory tract infection (LRTI) in older adults and immunocompromised individuals. The Present study aims to determine the proportion of RSV infection in patients with acute LRTI. Two step quantitative reverse transcriptase real time PCR was carried out on 80 samples for detection of RSV. The proportion RSV infection in our patients was found to be 1.25%. Our study showed that RSV infection is not a common cause of LRTI in adults in this area. Early detection of RSV infection by qRT- PCR would help reduce morbidity & mortality in such patients.

### Introduction

Respiratory Syncytial Virus (RSV) causes pediatric pneumonia and lower respiratory tract infection (LRTI) in older adults and immunocompromised individuals. RSV infections in adults are generally represented as re-infection with mild to moderate severity; however, immunocompromised individuals are more susceptible to severe disease.

Epidemiological evidence indicates that RSV is second only to influenza as a significant viral pathogen in the elderly (Murata and Falsey, 2007). Illness due to RSV generally goes unrecognized as clinical features are indistinct and the inadequate sensitivity of available diagnostic modalities due to low viral load in the samples (Terry Nolan *et al.*, 2015). Therefore, a diagnostic test with better performance characteristics is needed for

timely management of patients having RSV infection. The reverse transcriptase Real-time PCR is a sensitive nucleic acid based modality which also quantifies the viral load in clinical samples (Kraupp and Aberlr, 2011).

The Present study aims to determine the proportion of RSV infection, using reverse transcriptase, real-time PCR, in patients with acute lower respiratory tract infection in a hospital outpatient and inpatient setting. The data so obtained could provide guidance for decisions regarding empirical management of patients with lower respiratory tract infection.

### **Materials and Methods**

The study was conducted from July 2015 to June 2016 among 80 adult patients who attended the Outpatient / Inpatient clinics of pulmonary medicine department of Bhopal Memorial Hospital and Research Centre Bhopal (M.P.). Patients having symptoms of lower respiratory tract Infection were included in the study after obtaining a written consent from the patients. Ethical approval was obtained from the Institutional Ethics Committee for conducting the study.

### **Specimen collection**

Nasopharyngeal swabs were collected in Hi- viral transport medium (HiMedia, Mumbai, India) and transported to the laboratory. Samples were stored at  $-80^{\circ}\text{C}$  until further use. Viral RNA was extracted using a commercially available kit (QIAamp viral RNA mini kit (Qiagen, Germany).

### **cDNA synthesis**

cDNA was synthesized from RNA using a commercially available kit (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems). It was performed by taking 10

$\mu\text{l}$  of viral RNA in a final volume of 20  $\mu\text{l}$ . The reaction mixture contained 10 $\times$  RT Buffer 2.0  $\mu\text{l}$ , 25 $\times$  dNTP Mix 0.8  $\mu\text{l}$ , 10 $\times$  RT Random Primers 2.0  $\mu\text{l}$ , MultiScribe™ Reverse Transcriptase 1.0  $\mu\text{l}$ , and Nuclease-free H<sub>2</sub>O 4.2  $\mu\text{l}$ . The PCR was carried out for 40 cycles with the following thermal conditions; 37° C for 120 min then 85° C for 05 min.

### **Real-time PCR**

The amplicons (cDNA) were further subjected to RT-PCR using a commercially available kit (Fast start essential DNA probe master, Roche Diagnostics, Meylan, France).

The primer and Probe sequences were synthesized from IDT (Integrated DNA Technologies) scientific system & chemicals (p) Ltd. And Invitrogen bioservices India Pvt. India, as published previously (Mentel *et al.*, 2003), given below. The Probe was labeled with 5' reporter dye 6- carboxy-fluorescein (FAM) and 3' quencher dye 6- carboxytetramethyl rhodamin (TAMRA). The experiment was performed on LC 480 II Light cycler (Roch Diagnostics, Meylan, France).

It was performed by taking 15  $\mu\text{l}$  PCR master mix and 5.0 $\mu\text{l}$  cDNA, making the final reaction volume of 20  $\mu\text{l}$ . The reaction mixture contained Nuclease free water 1.4  $\mu\text{l}$ , Reverse Primer 1.0  $\mu\text{l}$ , Forward Primer 1.0  $\mu\text{l}$ , Probe 1.6  $\mu\text{l}$ , RT-PCR Master Mix 10  $\mu\text{l}$ . The PCR was carried out for 45 cycles with the following thermal condition; 95° C for 10 min, 95° C for 15 Sec, 60° C for 60 min. and 72° C for 01 Sec.

### **Results and Discussion**

Out of a total of 80 patients 72.5 % were male & 27.5% were female. The average age of the patients was 62.96 years, of which males had an average age of 62 years and females had an

average age of 63 years. There were 56.25% patients included in the study who had at least one co-morbidity. The most common co-morbidities were: Chronic Obstructive Pulmonary Disease (22.5%), Hypertension (8.75%), Bronchial Asthma (5%), Coronary artery disease (5%), Diabetes mellitus type II with Coronary artery disease (3.75%), Chronic obstructive pulmonary disease with Hypertension (2.5%), Diabetes mellitus type II (2.5%), Bronchial Asthma with Hypertension (2.5%), Bronchial Asthma with Diabetes mellitus type II with Hypertension (1.25%), Diabetes mellitus with Hypertension (1.25%) and Hypertension with Coronary artery disease (1.25%). Respiratory Syncytial Virus was detected in one patient having chronic obstructive pulmonary disease (Table 1). This patient also had lung infiltrate in his X-ray findings. RSV infection in older adults is of concern, especially those with co-

morbidities and impaired immunity. Many studies have reported that in older adults co-morbid conditions like congestive heart failure and chronic pulmonary disease contribute to the higher risk of getting severe forms of RSV illness (Falsey *et al.*, 2005, Walsh *et al.*, 2004).

In India most of the studies have been conducted on children therefore there is paucity of available data on RSV infection in adult population. While early diagnosis of RSV infection among adults may help in clinical management of the disease, it is found that RSV infection is not ruled out in routine clinical practice in adult patients even among high risk individuals (Talbot and Falsey, 2010). This may probably be due to paucity of data regarding RSV infection in this population

**RSV primers and probe Sequences**

	Name	Use	Sequence(5'-3')	Region	Position
<b>Real-time RT-PCR</b>	RS-F1	Forward primer	AACAGATGTAAGCAGCTCCGTTATC	F	789-813
	RS-F2	Reverse primer	CGATTTTTATTGGATGCTGTACATTT	F	878-853
	RS-F3	Probe	TGCCATAGCATGACACAATGGCTCCT	F	822-847

\*Gen Bank accession no. AF067125

**Table.1** Patients associated with RSV Infection

S. No.	RSV Infection	Total	
		No	% n=80
1	Positive	01	1.25
2	Negative	79	98.75
	<b>Total</b>	<b>80</b>	<b>100</b>

In our study, we found that reverse transcriptase real-time PCR is a technique which requires minimal sample handling resulting in minimal cross-contamination.

It has high sensitivity and accuracy. For the detection of RSV, we collected nasopharyngeal swabs since it has a higher rate of sensitivity than any other clinical samples, eg.

oropharyngeal swabs (Lieberman *et al.*, 2009). The proportion RSV infection in our patients was found to be 1.25%. A study, conducted in three continents of the northern hemisphere, has reported 7.4% RSV infection with moderate to severe influenza like illness in older adults (False *et al.*, 2014).

Molosh R.E.*et al.*, carried out a study on middle-aged adults and found 7% RSV in hospitalized patients with highest prevalence among 50-64 years old patients (Malosh *et al.*, 2017).

Our study showed that RSV infection is not a common cause of LRTI in adults in this area. However, further studies with a larger sample size, including community based studies, would help come to a definitive conclusion regarding RSV infection among adults in this population.

Early detection of RSV infection by RT PCR would help reduce morbidity & mortality in such patients.

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### How to cite this article:

Rajkumari Ahirwar, Nikita Panwalkar, Lalit Kumar, Mahesh Rathore and Prabha Desikan. 2019. Detection of Respiratory Syncytial Virus Infection in Adults with Lower Respiratory Tract Infection Using Real-Time PCR Assay. *Int.J.Curr.Microbiol.App.Sci*. 8(06): 2404-2407. doi: <https://doi.org/10.20546/ijcmas.2019.806.286>