

Respiratory Viruses in Acute Respiratory Tract Infections in Western India

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ABSTRACT

Objective. To study the circulation pattern of respiratory viruses in out patients department (OPD) and hospitalized children with acute respiratory tract infection.

Methods. Nasopharyngeal aspirates were collected from 385 children with acute respiratory tract infections attending the OPD (n=199, 51.7%) and admitted to pediatric ward (n=186, 43.2%). Specimens were screened for seven respiratory viruses by immunofluorescence test (IFT) using Respiratory panel 1 screening and identification kit.

Results. Viral antigens were detected in 57 (28.6%) and 86 (46.2%) patients from OPD and admitted cases respectively, giving an overall positivity of 143 (37.1%) for respiratory viruses. Of the six respiratory viruses, the most common was respiratory syncytial virus (RSV) in 100 (26%) patients, followed by influenza viruses in 21 (5.4%), parainfluenza in 8 (2.07%), adenovirus in 3 (0.8%). One patient had mixed infection of RSV and adenovirus. RSV was most frequently detected in the hospitalized children (39.8%).

Conclusion. RSV appeared to be the most common respiratory viral infection in the age group 0-1 year causing hospitalization. [Indian J Pediatr 2008; 75 (4) : 341-345] E-mail: rgdamle2000@yahoo.co.in.

Key words : Respiratory; Circulation; IPD; OPD; Seasonality

Acute respiratory infections (ARI) are the leading causes of morbidity and mortality throughout the world particularly in developing countries.¹ Viral pathogens account for 30-40% of ARI cases.^{2,3} The viruses most frequently involved are influenza A and B, respiratory syncytial virus (RSV), parainfluenza (PIV) 1, 2, 3 and adenoviruses. Various patterns of seasonality in the appearance of RSV epidemics across continents, within the countries and regions have been reported.⁴ In India, marked rise in the number of respiratory infections during the rainy season and a second peak in the winter season occur every year with substantial morbidity.^{2,5} However, there are no reports on the current status of circulating respiratory virus infections from Western India.

From India, except for a three-year study conducted in Vellore, Southern India² most of the reports on respiratory viral infections are based on studies of severely ill hospitalized children.^{6,7} The present study was conducted

to determine the prevalence of lower respiratory tract infection due to respiratory viruses in pediatric hospitalized and outpatient cases in Pune for a period of three years, 2002-2004.

MATERIAL AND METHODS

The study was conducted in King Edward Memorial (KEM) Hospital, Pune.

Case definition: The specimens were randomly obtained twice a week from children visiting out-patient department (OPD) the complaint of respiratory tract infection and all patients admitted to the hospital in in-patient department (IPD) with complaints of respiratory tract infections.

Sample collection: Nasopharyngeal aspirates (NPAs) were collected from the pediatric patients with respiratory infection under supervision of the attending doctor using a sterile disposable suction catheter with a mucus trap (Romsons, New Delhi, India) connected to a hand suction pump (Nalgene, USA). The specimens were transported immediately to the laboratory or were stored at 4°C for a

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maximum of 48 hrs before processing. The institutional ethical committee approved the study and parental consent was obtained prior to collection of specimens. A total of 385 NPA, 199 from OPD and 186 from IPD were collected between February 2002 and December 2004.

Sample processing: The mucous was washed into the trap with 3 ml of transport medium (Hanks balanced salt solution with 0.1% bovine serum albumin and antibiotics). Samples were aliquoted and to 1.0 ml of specimen 2.0 ml mucolytic agent (0.5% N-acetyl L-cysteine, Sigma in 1.5% sodium citrate) was added, vortexed vigorously and incubated at room temperature (RT) for 20 min.⁸ After every five minutes the vials were vortexed for 20-30 sec. Specimens were centrifuged at 1500x g for 10 min at RT. Supernatant was discarded and cell pellet was again subjected to the same mucolytic treatment till all the mucous was completely removed. Cells were then washed with and suspended in PBS (0.01M, pH 7.2) and coated on multislot slides, air-dried and fixed with chilled acetone.

Immunofluorescence test (IFT): IFT was performed *as per* manufacturer's instructions using Respiratory panel 1 viral screening and identification kit (Chemicon International, USA) which contains monoclonal antibodies (MAbs) to influenza A and B, RSV, PIV 1, 2, 3 and adenoviruses. Specimens containing only few cells, insufficient for screening individual viruses were screened using viral screening antibodies (VSA), which contained pool of MAbs against all seven viruses. Normal mouse serum was supplied as a negative control. Briefly, different MAbs were allowed to react with each specimen by incubating at 37°C for 1 hr in humidified chamber. This was followed by three washes with PBS containing 0.2% Tween 20. The bound MAbs were detected with anti-mouse IgG-FITC conjugate. Slides were washed, mounted and observed under the fluorescence microscope (Leica, Germany).

Specimens positive for influenza virus were further processed for virus isolation in MDCK cells and the

strains were identified by hemagglutination inhibition test (HI).⁹

RESULTS

Of the 385 NPA, 143 (37.1%) were positive for respiratory viral antigens; 133 for one of the 7 individual respiratory viruses and 10 for VSA in IFT. The proportion of positives in IPD (86/186, 48.3%) was significantly higher than OPD (57/199, 28.6%), $p < 0.01$. Age-wise distribution of positive cases is presented in Table 1.

Respiratory syncytial virus infections: Of the 186 IPD cases 73 (39.2%) had RSV infection. This was significantly higher than the RSV detection in OPD patients (27/199, 13.6%) (RSV IPD *vs* OPD, $p < 0.01$). Overall incidence of RSV infections in 0-12 month age group (80/102) was also significantly higher ($p < 0.01$) than in the age groups > 1 yr (20/41) (Table 1).

Of the 27 RSV positive cases from the OPD, 22 had fever, 25 had cough and all were suffering with cold (Table 2). Bronchiolitis was predominant diagnosis (45/73, 61.6%) in IPD cases with pneumonia ($n=26$), and UTRI ($n=2$) also being diagnosed among RSV positive IPD patients (Table 3). One case of RSV with pneumonia had fatal outcome.

The majority of the RSV infections were detected in rainy months of July-September in 2002 and 2004. However, during the same season in 2003, RSV circulation was not detected though the number of specimens collected during these months in 2003 ($n=69$ from July-Sept) was comparable to those collected in 2002 ($n=53$) and in 2004 ($n=66$) (Fig 1).

Influenza virus infections: Of the 385 NPAs tested 21 (5.45%) were positive for influenza A ($n=14$) and B ($n=7$) viruses. These patients were attending the OPD and did not require hospitalization. Influenza B virus was detected in February 2002 but this was subsequently

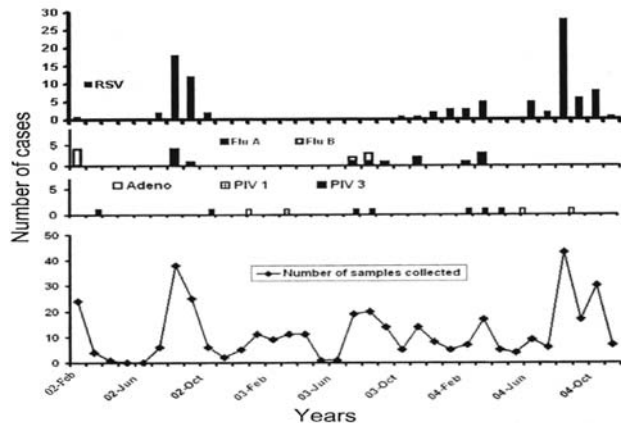
TABLE 1. Distribution of Respiratory Viruses by Age

Age/ Virus	0-3 months		3-6 months		6-9 months		9-12 months		1-3 years		3-6 years		>6 years		Total
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	
Positive Flu A+B for virus	1	0	1	2	0	1	6	0	5	0	4	0	1	0	21
antigen RSV	3	28	4	15	1	11	8	10	6	7	4	1	1	1	100
PIV	0	1	0	1	1	0	0	1	2	2	0	0	0	0	8
Adeno	0	0	0	0	0	0	0	1	0	2	0	0	0	0	3
RSV+Adeno	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
VSA	1	0	0	1	0	0	4	0	3	0	1	0	0	0	10
Total	5	29	5	19	2	12	18	12	16	12	9	1	2	1	143
Virus -ve	6	23	12	28	4	6	30	10	66	16	17	10	7	7	242
Total	11	52	17	47	6	18	48	22	82	28	26	11	7	8	385

Flu A: Influenza A virus; Flu B: Influenza B virus; RSV: Human respiratory syncytial virus
PIV: Parainfluenza virus; Adeno: Adeno virus; VSA: Viral screening antibody pool

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replaced by influenza A virus in August – September 2002. During July and August 2003 both, influenza A and B viruses were co-circulating. Influenza A viruses were detected only in the months of February-March of the year 2004 (Fig. 1). Influenza virus infections were detected in significantly higher proportion in age groups >1 yr (10/41, 24%) than in age groups <1 yr (11/221, 5%) (Table 1).



Flu A: Influenza A virus
 Flu B: Influenza B virus
 RSV : Human respiratory syncytial virus
 PIV : Parainfluenza virus
 Adeno: Adeno virus

Fig. 1. Temporal pattern of different respiratory virus infections

TABLE 2. Association of Respiratory Symptoms with Virus Positivity in OPD Patients

Virus	Cold		Cough		Fever	
	+	-	+	-	+	-
Flu A+B positive	18	0	16	2	18	0
RSV	27	0	25	2	22	5
PIV	3	0	3	0	3	0
Adeno	0	0	0	0	0	0
VSA	9	0	9	0	6	3
Total	57	0	53	4	49	8
Virus -ve	137	5	124	18	102	40

Cold, cough and fever were the common symptoms among the influenza positive OPD patients (Table 2). Only three cases, two with influenza A and one with influenza B virus infection required hospitalization and were diagnosed with bronchiolitis (Table 3).

All 21 IFT positive specimens were positive for virus isolation in MDCK cells. The influenza B strains isolated in the month of February 2002 (n=4) were identified as similar to B/Beijing/243/97 of the Victoria lineage and isolates of August-September 2003 were similar to B/Sichuan/379/99 of the Yamagata lineage.

The influenza A strains isolated in the month of July-August 2002 (n=5) and January-May 2003 (n=4) were of A(H3N2) subtype, similar to A/Panama/2007/99. An antigenic drift was detected in the month of August 2003

TABLE 3. Diagnosis of Respiratory Viral Infections in IPD Patients

	Total positive	Bronchiolitis	Pneumonia	Croup	URTI
Flu A	2	2			
Flu B	1	1			
PIV 1	1		1		
PIV 3	4	1	2	1	
RSV	73	45	26		2
RSV/Adeno	1		1		
Adeno	3		3		
VSA	1	1			
Total	86	50	33	1	2

Flu A: Influenza A virus
 Flu B: Influenza B virus
 RSV : Human respiratory syncytial virus
 PIV : Parainfluenza virus
 Adeno: Adeno virus
 VSA: Viral screening antibody pool
 URTI: Upper respiratory tract infection

and one A(H3N2) strain isolated during this period was identified as similar to A/Korea/770/2002. A second antigenic drift was identified in the year 2004 when all the A(H3N2) strains (n=4) were identified as similar to A/California/07/2004.

Parainfluenza and adenovirus infections: Eight cases (2.1%) of PIV infections were detected during the course of this study. Of these three were from OPD and five from IPD patients. One PIV-1 case was in March 2003; the remaining seven were of PIV-3. Though the incidence of PIV was significantly lower among the pediatric population, the sporadic cases were detected in all the three years of study. The only PIV-1 case admitted to the hospital with a diagnosis of pneumonia had fatal outcome.

Adenovirus infection was detected in 3 (0.08%) cases, one in year 2003 and two in 2004. All three cases were classified as pneumonia and died.

DISCUSSION

This study was undertaken with the aim to determine the prevalence of respiratory tract infection due to 7 respiratory viruses in Pune. Of the 385 samples collected respiratory viral antigens were detected in 37.1% of the specimens tested. Total case positivity of 37.1% was within the range of 22.0 to 51.9% as reported earlier from other countries¹⁰ and India.^{6, 11-15} A three-year systematic study conducted at Vellore (Southern India) indicated viral etiology in 49% and 38% of lower and upper respiratory tract infection cases, respectively.² A recent study by Legg *et al*¹⁶ to understand the viral etiology of respiratory infection in infants during their first winter indicated 46% positivity for picornaviruses. Our rate of detection of respiratory viruses of 37.1% could be an

under-estimate since these viruses were not included in our study.

Considering the detection of different viruses in OPD and IPD in this study it is apparent that both influenza and RSV are detected to similar extent in OPD but RSV appears to be responsible for severe infection leading to hospitalization where bronchiolitis was in higher proportion. Predominance of RSV has been reported in most of the previous studies conducted in hospitalized children in India and significant influenza activity in OPD.^{15,17-20} During peak RSV activity in the month of August 2002 and 2004 detection of RSV was as high as 78% in hospitalized children. Chattopadhyaya, *et al*²⁰ also reported 70% positivity in cases with lower respiratory tract infection during the period of suspected epidemic of RSV. Interestingly, we rarely detected influenza viruses in admitted cases but could detect influenza in OPD. This could be either because influenza may not be causing severe infection or by the time the patient develops severe symptoms virus load may be dropping to levels below the sensitivity of the detection test used. Low detection of influenza viruses in pneumonia cases has also been reported earlier.¹⁰

Respiratory infections in children between age group 0-1 year were mainly due to RSV and influenza infections were noted only after 1 year of age. Our observation was that during peak RSV activity infants as young as 22 days old had infection with RSV. Studies at Vellore indicated 30% infections in the age group <1 year due to RSV.² A study conducted in Madras with 400 infants hospitalized for severe respiratory infection showed 25.8% isolation rate for RSV in age group 7-12 months.¹³

In our study, seasonal pattern of RSV varied in all three years. In the year 2002 a sudden outbreak of RSV was detected from July-October. No RSV activity was detected from January-November of the year 2003. RSV was detected in all the months in 2004 except June with a small peak in February to April and a larger peak in August. Year round or annual episodes of RSV occurring during winter or rainy season have been reported earlier.^{4,10,15} Lack of set pattern in RSV circulation in our study calls for more extensive surveillance in this region.

Influenza viruses are detected all the year round, more commonly in children with mild infections. Three antigenic changes (drift) were observed in A(H3N2) and 2 in type B influenza virus. These changes were similar to those recorded world over highlighting the need for continuous monitor of influenza viruses.

Data from Vellore (Southern India), Chandigarh and New Delhi (North India) and our study from Western India highlights that RSV is one of the commonest agents detected in children with mild to severe respiratory tract infection. The age group affected is <1 yr and the pattern of RSV circulation varies from place to place^{2,6,17}. All these

studies also underscore that an epidemiological approach to acute respiratory infections in children can improve the understanding of the disease and help prepare the ground for effective control.

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