Background & objectives: Respiratory viral infections have a major impact on public health. Acute respiratory infections largely caused by viruses, are the most common illnesses experienced by otherwise healthy adults and children. Among the respiratory viruses, influenza viruses are known to cause outbreaks globally. Information on the activity of influenza virus in our country is limited and none from Chennai. The present study was carried out to isolate and identify the influenza virus serotypes causing acute respiratory infection in children attending a tertiary care centre at Chennai.

Methods: During January to December 2002, 240 children with acute respiratory infection attending the out patient clinic of Institute of Child Health were included by convenient sampling. Throat swabs were collected from 4 to 5 cases every week. Isolation of influenza virus was attempted by inoculating the sample in Madin Darby Canine Kidney (MDCK) cell line. The isolates were typed by haemagglutination inhibition test and confirmed by immunoflorescence assay.

Results: Virus isolation was positive in 30 (12.5%) of the 240 samples. Influenza A/H3N2/Panama/2000/99 was the predominant serotype isolated accounting for 24 (80%) of the 30 isolates. Influenza B/Sichuan/379/99 was isolated in 4 (13.33%) and a combination of Influenza A/H3N2 and B/Sichuan in 2 (6.6%) of the isolates.

Interpretation & conclusion: Isolation of influenza A and B viruses indicated a significant activity of these viruses in Chennai. Peak activity was observed during and after the first spell of rain. The predominance of A/H3N2/ Panama is an indication that the Indian scenario is similar to the global picture of influenza activity.

Key words: Acute respiratory infection - children - influenza A and B viruses

Acute viral respiratory infections causing significant morbidity are the most common illnesses experienced by otherwise healthy adults and children. Mortality due to acute viral respiratory illness in economically developed countries is less compared to the developing countries. The mortality rate due to acute respiratory tract infections was estimated to be approximately 2707/1,00,000 children (under 1 yr) in rural India. Monthly incidence of respiratory infections is 23 per cent in urban area and 17.65 per
cent in rural area\(^4\). Outbreaks of respiratory tract infections are also common in tertiary care centres for the aged\(^5\).

Influenza viruses are known to cause frequent epidemics and periodic pandemics, and are unique with regard to their antigenic variability, seasonality and impact on general population. Though children are mainly affected during epidemics\(^6\), these are also responsible for substantial mortality in the aged and chronically ill persons\(^7\).

Studies on the aetiology of acute respiratory infections have been carried out in many parts of India\(^8,^9\). Seroepidemiological studies have been conducted during 1997-1999 in Pune\(^10\). Spectrum of influenza activity is not known in southern states of India except for a seroprevalence study conducted in Vellore\(^11\). Influenza viral activity in Chennai, a coastal city with high humidity is not known. The present study was therefore undertaken to identify the influenza serotypes responsible for acute respiratory infection in children at Chennai.

**Material & Methods**

Sample collection: Institute of Child Health and Hospital, a premier tertiary care hospital for children, Chennai, was identified as a sentinel site for sample collection. Samples (4-5 in number) were collected every week from cases that met the clinical case definition of sudden onset of fever >38°C, cough or sore throat and/or running nose in the absence of other diagnoses. One sample was collected per child. Convenient sampling was adopted to avoid bias. The study was approved by the institutional ethical committee and informed consent was obtained from the parent/guardian of the child before recruitment.

A total of 240 children in the age group of 6 months to 12 yr (mean age of 3.25±1.08 SD) with acute respiratory infection attending outpatient clinic were included in the study during January to December 2002. Samples from 152 cases were collected between 1 to 4 days of onset of symptoms and 88 were collected between days 5-7. Throat swabs were collected into the viral transport medium - Hanks balanced salt solution (HBSS) with antibiotics and bovine serum albumin (BSA) fraction V (1%), and transported to the laboratory maintaining cold chain.

**Virus isolation and identification:** This was performed at the Virology Department, King Institute of Preventive Medicine, Guindy, Chennai.

Samples were processed as per standard CDC protocol\(^12\), and inoculated onto a 48 h monolayer culture of MDCK (Madin Darby Canine Kidney) cells in tubes. The isolates were typed by haemagglutination inhibition (HAI) test\(^13\) with the reference antisera and were further confirmed by indirect immunofluorescence (IIF) assay\(^14\).

The reference antisera and mouse monoclonal antibodies were obtained from Influenza Division, Centers for Disease Control, Atlanta, fluorescent and iso thio cyanate conjugate was procured from Dako Corporation, Denmark. MDCK cell line obtained from National Center for Cell Sciences, Pune, was maintained in the cell bank of the department of virology at King Institute of Preventive Medicine, Chennai. All other reagents were obtained from Hi-Media, Mumbai, India.

**Results & Discussion**

Of the 240 infants and children with acute respiratory infections, 30 (12.5 \%) were positive for influenza virus.

Influenza/A/H3N2 Panama was the predominant isolate accounting for 24 of the 30 isolates (80\%). The H3N2 isolates were antigenically related to A/Panama/2007/99. A/H3N2/Panama has been documented to be involved in widespread outbreaks and sporadic cases in 2002 from the Americas, Europe and Australia\(^15\)-\(^18\). These were closely related to the A/Panama/2007/99 and A/Moscow/10/99 viruses\(^19\). Thus, findings of our study corroborated with the global picture of influenza activity.

During the same period, A/H1N1 activity was reported from Chile, South Africa, Hong Kong, New Zealand, Africa, United States, Indonesia, Europe and Australia\(^19\). However, A/ H1N1 was not detected in Chennai in the present study.

Among the influenza B serotypes, influenza/B/Sichuan accounted for 4 (13.33\%) of the isolates and was antigenically similar to the B/Sichuan/379/99 viruses. Influenza B/Sichuan/379 was isolated in 2002 from many parts of the world\(^20\)-\(^24\). There are also
reports of isolation of B/Hong Kong from Canada, Hong Kong, Japan, Oman, Philippines, Italy, Netherlands, Singapore and Thailand during the same period. However, B/Hong Kong was not detected in our study.

Two of the isolates were a combination of A/H3N2 and B/Sichuan. This was observed during the peak influenza season and may be due to co-circulation of both viruses. This is unique to our study and has not been reported before.

Of the 152 samples collected within 4 days of onset, 26 (17%) were positive, while only 4 (4.5%) of the 88 samples collected between day 4-7 were positive, indicating high isolation rate if samples are collected early in the acute phase of illness.

Analysis of the monthly data (Fig.) showed that influenza activity in Chennai, commenced with a sporadic case in February and continued till November, peaking in June coinciding with the onset of the Southwest Monsoon. The activity observed from September to November coincides with the Northeast Monsoon. Influenza activity was not seen in the months of March-May and during December and January. Surveillance studies have pointed out that influenza outbreaks occurred predominantly during the monsoon months. In countries with temperate climate, influenza outbreaks occur in winter.

Studies have demonstrated that relative humidity, rainfall and differences in temperatures influence the outbreaks of Influenza. Influenza /A/H3N2/Panama was detected between June to November, whereas B/Sichuan was detected in February and for a short period between June and July (Fig).

In conclusion, our findings showed the co-circulation of influenza A and B types in Chennai with peak activity during and after the first spell of rain. Continuous monitoring would be required for early detection of any antigenic variants, to understand the seasonality and analyze the factors like temperature, rainfall and humidity in the transmission of influenza viruses.

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