INFLUENZA SURVEILLANCE IN PUNE, INDIA, 2003

BL Rao, LR Yeolekar, SS Kadam, MS Pawar, PB Kulkarni, BA More and MR Khude

National Institute of Virology, Pune, India

Abstract. Influenza surveillance was conducted in Pune, India in 2003. A total of 573 throat swabs/ nasal swabs (TS/NS) and 190 nasopharyngeal aspirates (NPA) were collected from 763 in- and outpatients who were mostly children aged 0-16 years. TS/NS (507/573) and NPA (42/190) specimens were processed in MDCK cell cultures and identified with the hemagglutination inhibition test (HI). A total of 37 influenza viruses was isolated: twenty-three type A (H3N2) and 14 type B of the Yamagata lineage were isolated from 29 children and 8 adults. Three type A (H3N2) isolates were characterized as being similar to A/Panama/2007/99 like, A/Korea/770/2000 like, and B/Sichuan/379/99 like strains.

INTRODUCTION

Influenza is a highly contagious acute respiratory illness recognized since ancient times. Influenza viruses are unique among respiratory viruses as they show antigenic variation in their surface glycoprotein antigens; hemagglutinin (HA) and neuraminidase (NA) and cause explosive epidemics. There are three immunological types of influenza virus: A, B, C. The type A virus is highly variable and shows continuous antigenic variation and is a major cause of frequent epidemics and periodic pandemics. It also infects animals and birds. Type B virus shows antigenic variation to a lesser degree which results in epidemics, whereas type C appears to be antigenically stable and causes sporadic upper respiratory tract illness. It is estimated that annually around 0.5-1 million people die and 600 to 1,200 million people become sick due to influenza epidemics worldwide (Layne *et al*, 2001). Thus the disease affects a large segment of the world population resulting in significant mortality, morbidity and economic loss. The World Health Organization has established a global network of 112 national influenza centers in 83 countries and regularly reports on the global influenza situation and recommends current updated strains for use in the influenza vaccine. Presently antigenic variant strains of influenza type A(H1N1), A(H3N2) and type B viruses are causing frequent epidemics in humans globally. Surveillance is essential for identifying the new variants of these types and subtypes for the selection of vaccine strains.

In 2003-2004, devastating avian influenza H5N1 outbreaks occurred in poultry in several countries of Asia. Some human cases of H5N1 infection with high mortality were reported from Vietnam and Thailand (WHO, 2004b). Therefore influenza surveillance has become very important to understand the local and regional epidemiology of influenza and circulating types of influenza virus strains.

As a part of this influenza program, a study was initiated at the National Institute of Virology (NIV) Pune City, Maharashtra State, India, since 1976 which has been recognized as the National Influenza Center since 1980 by the WHO. During the course of this continuous surveillance of influenza in Pune City between 1976 to 2002, NIV investigated several outbreaks of influenza and isolated 43 antigenic variant strains of influenza types A and B, which included many global epidemics strains (Rao *et al*, 1979, 1982; Rao and Banerjee 1993; Rao, 2003). The present communication reports the variant strains of influenza type A (H3N2) and type B isolated during influenza outbreaks in the year 2003 from Pune City.

MATERIALS AND METHODS

During influenza surveillance in 2003, respiratory specimens were collected from two hos-

Correspondence: BL Rao, National Institute of Virology, 20-A, Dr Ambedkar Road, PB No.11, Pune-411001, India.

pitals and five dispensaries located in different areas of Pune. Patients presenting with symptoms of fever, bodyache, headache, sore throat, running nose and cough attending outpatient departments (OPD) and some patients with pneumonia and bronchiolitis admitted to inpatient departments (IPD) were included in the study. Most of the patients were children. The age groups of the patients were 0-5 yrs (414), 6-10 yrs (122), 11-15 yrs (74), >16 years (153).

A total of 573 throat swabs/nasal swabs (TS/NS) and 190 nasopharyngeal aspirates (NPA) were collected from patients attending outpatient and inpatient departments. About 50% of NPA specimens were from patients admitted to the pediatric ward of the two hospitals. The method described by Kendal *et al* (1982) was followed for virus isolation in MDCK cell culture and identification with the hemagglutination inhibition (HI) test. Five hundred seven out of 573 TS/NS specimens and 42 out of 190 NPA specimens were processed in MDCK cell cultures grown in T25 flasks, incubated at 35ºC and observed daily for cytopathic effects (CPE). Those showing 3+ or 4+ CPE were transferred to 4ºC and those not showing CPE were incubated for a week. All the inoculated MDCK cultures were tested with a hemagglutination test (HA) with guinea pig and fowl red blood cells (RBC). HA positive specimens were tested by the HI test for identification of the virus isolates employing reference antisera of fowl and sheep. The sheep antisera were kindly supplied by the WHO Collaborating Center for influenza reference and research CDC, Atlanta, USA.

RESULTS

A total of 37 influenza virus isolates; 23 A(H3N2) and 14 type B of the Yamagata lineage were isolated and identified. Twenty-eight of these were isolated from TS/NS and 9 were from NPA specimens which included two pediatric patients with pneumonia and bronchiolitis (H3N2 strains isolated from these two cases) admitted to the ward. Twenty-nine of these isolates were from children and 8 from adults.

Two type A (H3N2) isolates in January and March 2003 and one type A(H3N2) and one type B isolate in August 2003 were sent to the WHO Influenza Collaborative Center, CDC, Atlanta, USA. They were characterized at the CDC as similar to A/Panama/2007/99 like (January, March H3N2 isolates), A/Korea/770/2002 like (August H3N2 isolate) and B/Sichuan/379/99 like strains.

DISCUSSION

During the year 2003, increases in influenza activity was noted in two peaks, March-April (10 H3N2 viruses isolated in the summer season) and July-August (11 H3N2 and 14 type B viruses isolated in the rainy season) with a maximum number of 35 isolates obtained during these peaks. There are not many reports on the seasonality of influenza outbreaks in the tropics. Our continuous surveillance in Pune (which has a tropical monsoon climate) shows that influenza outbreaks occurred predominantly during the rainy months. Increases in influenza activity were frequently noticed during the period February-April in Pune. These two seasonal peaks of activity have been frequently observed (Rao and Banerjee, 1993).

Influenza virus isolates were obtained from all the health centers included in the present study, which indicated influenza strains were circulating in different localities of Pune City as influenza outbreaks are characterized by rapid spread affecting a large segment of the population within a short span of time.

In the year 2003, A/Panama (H3N2) like strain was isolated during the first peak of influenza activity between March–April and A/Korea (H3N2) like strain (which is similar to A/Fujian/411/2002 vaccine strain) was isolated during the second peak of activity in July-September from Pune. A/Panama (H3N2) like strains were also isolated during the year 2002 from Pune. However, A/Caledonia/20/99 (H1N1) strain which was isolated in Pune in the year 2000 after a gap of 10 years, was not isolated between the years 2001-2003, although H3N2 and type B virus strains were isolated during this period. Current strains of H3N2 virus are known to cause severe illness and the highest hospitalization rates, H1N1 strains the lowest and influenza B outbreaks are of intermediate severity. Predomi-

Table 1
Influenza virus strains isolated during epidemics in Pune City, India, 1976-2003, identified by the WHO as similar to the reference strains shown.

Type A(H3N2) strains		Type A (H1N1) strains	Type B strains	
A/Victoria/3/75	A/Sichuan/2/87	A/USSR/90/77	B/Hong Kong/5/72	B/Hong Kong/22/89
A/Texas/1/77	A/Sichuan/68/89	A/Brazil/11/78	B/Singapore/222/79	B/Panama/45/90
A/Taiwan/1/79	A/Beijing/353/89	A/India/6263/80	B/USSR/100/83	B/Harbin/07/94
A/Bangkok/2/79	A/Shanghai/86/90	A/England/333/80	B/Hong Kong/8/83	B/Sichuan/379/99
A/Oregon/4/80	A/Johannesburg/33/94	A/New Caledonia/4/83	B/Kanagawa/2/84	B/Shizuoka/15/2001
A/Arizona/2/80	A/Wuhan/359/95	A/Singapore/6/86	B/Texas/1/84	B/Beijing/243/97
A/Shanghai/31/80	A/Sydney/05/97	A/S.Carolina/6/88	B/Ann Arbor/1/86	
A/Philippines/2/82	A/Panama/2007/99	A/Czechoslovakia/2/89	B/Victoria/2/87	
A/Taiwan/16/83	A/Korea/770/2002	A/New Caledonia/20/99	B/USSR/2/87	
A/Mississippi/1/85			B/Yamagata/16/88	

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nant circulation of H3N2 strains when compared to H1N1 strains have been reported from all over the world during this past 12 years (WHO, 1990, 1994, 1999).

Two antigenically and genetically distinct lineages of influenza B viruses (B/Victoria lineage and B/Yamagata lineage) have co-circulated globally since 1988 (Rota *et al,* 1990, 1992; Kaneagae *et al*, 1990). Since 1991, the majority of the influenza type B viruses isolated through out the world have been of the Yamagata lineage with intermittent isolation of influenza type B Victoria lineage reported only in East Asia. Reappearance of Victoria lineage viruses outside East Asia was reported during the influenza season of 2001 and 2002 (Shaw *et al*, 2002). However, during the second half of 2003, type B strain of B/Yamagata lineage was reported from most parts of the world (WHO, 2004a). Our continuous surveillance in Pune demonstrated a similar trend in influenza type B activity. Strains of influenza type B belonging to B/Victoria lineage were isolated at Pune between 1987 and 1989 and again in the year 2002 (Yeolekar *et al*, 2004). All the type B strains isolated in Pune between the years 1990 and 2001 and in the year 2003 belonged to the B/Yamagata lineage. Such global surveillance activities aid in the proper selection of these type B lineages for vaccine use.

NIV Pune is the main influenza center in India. During the course of continuous surveillance influenza in Pune City between 1976 to 2003, NIV isolated 44 antigenic variant strains of influenza types A and B during 26 outbreaks of influenza. They included several global epidemic strains and were found to be similar to the strains prevailing through out the world during the same period (Rao, 2003) (Table 1).

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