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Surveillance and molecular characterization of human influenza B viruses during 2006–2010 revealed co-circulation of Yamagata-like and Victoria-like strains in eastern India

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ABSTRACT

Acute respiratory illness (ARI) is one of the major health problems in tropical countries of Asia, like India where approximately 0.5 million children in the age group of <5 years die annually. Previously we have reported the genetic characterization of influenza A (Inf-A) strains circulating in Kolkata, eastern India. This study was initiated to characterize the genetic diversity of the circulating influenza B (Inf-B) viruses. Of 3035 nasal/throat swabs, 494 (16.3%) samples were identified as influenza A/B positive by real time RT-PCR, of which 244 samples were confirmed having Inf-B infection. Comparison of nucleotide (nt) and amino acid (aa) sequences of HA and NA gene of Inf-B viruses revealed co-circulation of B/Yamagata and B/Victoria lineages. Of the 32 randomly selected Inf-B strains from Kolkata, seventeen strains possessed reassorted NA gene. There was a single Histidine to Asparagine substitution in the 131st position which is a part of 120 loop on HA1 region along with a deletion at position 178 in the Kolkata strains belonging to the Yamagata lineage. Amino acid substitution was observed at position 198 on NA gene in the strains B/Kol/542/2006, B/Kol/1373/2008, B/Kol/1880/2008, B/Kol/2044/2008 and in all the representative strains isolated during 2009 with respect to the circulating vaccine strains. This substitution is responsible for reduced sensitivity of neuraminidase inhibitors. The results highlight the importance of monitoring Inf-B viruses for development of antiviral resistance among circulating strains.

1. Introduction

In tropical countries of Asia, like India, approximately 0.5 million children of <5 years of age die annually due to acute respiratory illness (Reddaiah and Kapoor, 1988; Ahmad et al., 2000; Williams et al., 2002), though the etiology of pathogen remains largely unknown. Of common respiratory pathogens, influenza viruses (A and B) are associated with annual worldwide epidemics (World Health Organization, 2003). Due to the high frequency of antigenic drift and shift, Inf-A viruses result in occasional pandemics. Though Inf-B does not show antigenic shift, genetic variations, including insertions, deletions and reassortment among different lineages, are common (Air et al., 1990; Lindstrom et al., 1999; McCullers et al., 1999; Nerome et al., 1998; Chi et al., 2002; Shaw et al., 2002; Yamashita et al., 1988). It is also associated with Reye's syndrome among children (Reynolds et al., 1972). Inf-B virus belongs to the family *Orthomyxoviridae* and contains a single-stranded, negative sense, segmented genome (Neumann et al., 2004). The eight gene segments code for 11 proteins namely PB1 (segments 1), PB2 (segment 2), PA (segment 3), HA (segment 4), NP (segment 5), NA and NB (segment 6), M1 and BM2 (segment 7), NS1 and NS2 (segment 8) (Lamb and Choppin, 1983). Inf-B viruses were grouped together as represented by strain B/Lee/40 before the divergence into the two genetic lineages, namely B/Victoria/2/87 and B/Yamagata/16/88. These two lineages have diverged since 1983 (Rota et al., 1992; Hay et al., 2001). Due to the probability of inter-lineage reassortment among Inf-B viruses, continuous global surveillance of both Inf-A and Inf-B strains is recommended for monitoring drug resistance, selecting vaccine strains and for early detection of epidemics.

Influenza surveillance network was initiated in eastern India in 2005, with the support from Centers for Disease Control and Prevention, USA to fill up the lacunae in information on influenza virus strains and disease burden in Indian subcontinent. Influenza

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activity has been reported from other parts of the country (Rao et al., 2001, 2003; Ramamurty et al., 2005). Prevalence and correlation of Inf-A infection with rainy season in Kolkata and introduction of oseltamivir resistant Inf-A (H1N1) strains during 2009 in eastern India has been reported by our group earlier (Agrawal et al., 2009, 2010). In the present study we have analyzed the HA and NA genes of Inf-B viruses identified during 2006–2010 and compared them with the concurrent strains circulating worldwide.

2. Materials and methods

2.1. Sample collection and transportation

All samples (Nasal and/or throat swabs) used in the present study, were collected from outpatient department of hospitals in Kolkata, West Bengal; with influenza like illness (sudden onset of fever, nasal discharge, sore throat/cough, fatigue/body ache/difficulty in breathing) as described earlier (Agrawal et al., 2009). In addition, large numbers of hospitalized cases were referred from different hospitals in eastern India during the pandemic period (June 2009–December 2010) (Mukherjee et al., 2010). The informed consent forms and detailed case histories were recorded before collection of sample. The study was approved by the Institutional Ethical Committees of the involved institutes.

2.2. RNA extraction and screening of clinical samples for influenza virus

Viral RNA was extracted using commercially available QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) as per manufacturer's instructions. For initial detection of influenza viruses, amplification of matrix (M) gene of Inf-A and B viruses was carried out by real time RT-PCR as described previously by our group (Agrawal et al., 2009).

2.3. Full length amplification of HA and NA genes of influenza B

For sequencing, HA and NA genes were amplified using gene specific primers for Inf-B (Table 1). Briefly RNA was transcribed using 1 mM of universal primer (Uni12), as described earlier (Hoffmann et al., 2001). The amplification reaction was carried out in a thermocycler (ABI 9700 Applied Biosystems, Foster City, CA,USA) as 95 °C for 5 min; 35 cycles, each consisting of 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min; and final elongation at 72 °C for 7 min. The PCR products were purified using PCR purification kit (Qiagen GmbH, Hilden, Germany) as per manufacturer's protocols.

2.4. Sequencing and sequence analysis

Nucleotide (nt) sequencing was carried out in an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) using gene specific forward and reverse primers for HA and NA genes. Nucleotide and protein sequence BLAST search was performed using the National Centre for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) Basic Local Alignment Search Tool (BLAST) server on GenBank database release 143.0 (Schäffer et al., 2001).

2.5. Phylogenetic analysis

Pairwise sequence alignments were performed using LALIGN software. Multiple alignments were done with Clustal W program which is available at DDBJ software. Phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using the MEGA program, version 4.1. HA gene sequence of A/chicken/Assam/142007/2008 and NA gene sequences of A/swine/North Carolina/44837/2009 were used as out groups for phylogenetic analysis of HA and NA genes respectively.

Table 1
Primer pairs used for amplification of HA and NA genes of influenza B strains in Kolkata, eastern India.

Gene	Primer name	Sequence (5′–3′)	Position	Product size (bp)
HA	B/HA-F	(+) ATC CAC AAA ATG AAG GCA ATA	1–21	
	B/HA-PR	(−) TGC AGG AGG TCT ATA TTG G	1077–1059	1077
	B/HA-PF	(+) ACC AAA TAT AGA CCT CCT GC	1057–1076	
	B/HA-R	(−) TTA ACT TYC CTT ATA GAC AGA T	1777–1755	721
NA	B/NA-F	(+) AAT GAA CAA TGC TAC CTT CAA	1–21	
	B/NA-R	(−) CAG AAA CAA TTA AGT CCA GTA AGG	1488–1465	1488

2.6. Gene bank accession numbers

The nucleotide sequence data of the Inf-B strains were submitted to the GenBank under the accession numbers JF693236–JF693251 and JF965340–JF965355 for HA gene, JF693252–JF693267 and JF965356–JF965371 for NA gene.

3. Results

3.1. Screening of influenza viruses

During January, 2006 through December, 2010, a total of 3035 samples with ILI were tested for influenza viruses. Real time PCR analysis revealed that 494 (16.3%) out of 3035 samples were positive for influenza viruses (A & B); of which 244 samples (8%) were confirmed as Inf-B (Table 2A). During the pandemic (pH1N1) period in 2009–2010, additional 2964 samples from hospitalized patients were tested of which 535 samples (18%) were influenza positive, and among them 69 samples (2.3%) were sub typed as Inf-B (Table 2B). Year wise frequency of Inf-B among ARI samples, varied between 2% and 6% during 2006–2009, but in 2010, Inf-B infection predominated throughout the year with 17.7% prevalence rate. Age-wise distribution of positive cases revealed that, maxi-

Table 2
(A) Year wise distribution of influenza B positivity from 2006 to 2010 in Kolkata, eastern India. (B) Distribution of influenza B virus during the 2009–2010 pandemic periods in eastern India.

Year	Total no. of patients	Total no. of positive cases	Total no. positive for Inf B
(*A*)			
2006	611	45	22
2007	500	70	19
2008	591	103	35
2009	437	68	9
2010	896	208	159
Total	3035	494 (16.3%)	244 (8%)
(*B*)			
2009	2110	270	17
2010	854	265	52
Total	2964	535 (18%)	69 (2.3%)