RESEARCH NOTE

ANTIGENIC AND GENETIC CHARACTERIZATION OF INFLUENZA B VIRUSES IN 2012 FROM SLUMS, DHAKA, BANGLADESH

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Abstract. Nasal and throat swab samples were collected from 400 subjects with influenza-like illness during June to September, 2012 from two heavily crowded slums, Rayerbazar and Hazaribagh, situated southeast of Dhaka, Bangladesh. Forty-one samples were positive for influenza B virus using quantitative RT- PCR, but no influenza A virus was detected. Antigenic characterization revealed that the influenza B viruses were of Yamagata and Victoria lineages, which was confirmed from genetic analysis of hemagglutinin (HA) and neuraminidase (NA) genes. Co-circulation of influenza B viruses of both Yamagata and Victoria lineages in the slums of Dhaka indicates that introduction of a tetravalent vaccine formulation that includes both of these influenza B virus lineages would be more effective in this population.

Keywords: influenza virus B, influenza-like illness, slum, Bangladesh

INTRODUCTION

Influenza viruses can cause highly contagious respiratory illnesses. From 5% to 15% of the global population are infected each year by influenza viruses (Carrat *et al*, 2006), but data of its burden among the low-income heavily crowded populations in tropical countries such as Bangladesh are limited. Influenza-like illness is a frequent cause of visits to out-patient clinics in Bangladesh. Children aged <5 years are hospitalized for influenza in greater proportion than children in other age groups. The estimated prevalence of severe acute respiratory infections associated with influenza in children <5 years old is 6.7, 4.4 and 6.5 per 1,000 person-years during the 2008, 2009 and 2010 influenza outbreaks, respectively (Azziz-Baumgartner *et al*, 2012).The 2009 pandemic influenza A virus caused approximately 6,000 deaths, which costs 6.1 million US dollars (Homaira *et al*, 2012).

There are three types of influenza viruses; A, B, and C, among which influenza type A has most variable antigenicity and is mainly responsible for epidemic

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influenza. Influenza type B also exhibits antigenic variations and can on occasions cause epidemics (McCullers *et al*, 1999). Whereas influenza A virus infects humans and a wide variety of mammalian and avian species, influenza B virus infects humans exclusively (and perhaps seals) (Osterhaus *et al*, 2000).

Bangladesh has experienced one of the highest urban population growth rates (7% per year) mainly due to migration from rural areas. Dhaka, the capital of Bangladesh, has approximately 4,500 slums of highly dense population estimated at 3.4 million with inadequate health and hygiene support systems (Democracy Watch, 2002; Angeles *et al*, 2008). Typically slum houses are 75-100 feet^2 in size and consist of a single room. Very high population density, poor environmental conditions and low socioeconomic status are ubiquitous characteristics promoting various types of infectious diseases in this population. The present study reports the prevalence and characteristics of influenza viruses in two slums of Dhaka.

MATERIALS AND METHODS

Study populations and sample collection

Rayerbazar and Hazaribagh are two of the biggest slums in Dhaka located in the southeastern periphery of the city and are populated with > 9,000 people, approximately one third of whom are children. Nasal and throat swab samples were collected from dwellers with any of the following symptoms: cough, fever, running nose, sore throat, headache, and/or malaise. The study was carried out during June to September, 2012.

Quantitative (q)RT-PCR

Viral RNA was extracted using QIAamp® MiniElute® Virus Spin Kit (QIAGEN, Hilden, Germany) according to manufacturer's instruction. Influenza virus was detected from the extracted viral RNA by qRT-PCR using AgPath one step RT-PCR kit and oligonucleotide primers and probes as previously described (WHO, 2011).

Antigenic characterization

Random qRT-PCR positive samples (n = 8, 20%) were selected for tissue culturing regardless of C_T value/viral load. Tissue culture was performed in Madin-Darby Canine Kidney (MDCK) cell line. Culture supernatant was used for antigenic characterization of influenza B virus by hemaglutination inhibition (HI) assay using WHO recommended kit.

Nucleotide sequencing

M13-tagged oligonucleotide primers were used for amplification of hemagglutinin (HA) and neuraminidase (NA) gene segments. HA and NA genes of five influenza B viruses were sequenced according to procedures described previously (WHO, 2009). In brief, PCR amplicons were purified using ExoSAP-IT (USB, Cleveland, OH) and sequenced using ABI PRISM®BigDye Terminator Cycle Sequencing Reaction Kit (Foster City, CA) employing M13 forward primer in ABI PRISM® 3500xL Genetic Analyzer. Nucleotide sequences were analyzed using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD, USA) and compared with GenBank database. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5.0 software package (Tamura *et al*, 2011).

RESULTS

Among nasal and throat swab samples collected from 400 subjects with

influenza-like signs and symptoms during June to September 2012 from Rayerbazar and Hazaribagh slums, Dhaka, 41 (10%) were positive for influenza B virus, of whom 27 (6%) were females and 14 (4%) males, and > 50% of the positive cases were children < 5 years of age (data not shown). No influenza A virus was detected.

Influenza B viruses were isolated by culture assay from eight randomly selected qRT-PCR-positive samples. Antigenic characterization revealed that six viruses belonged to Yamagata lineage and two were of Victoria lineage (data not shown). Both HA and NA genes of five influenza B viruses were partially amplified (450 bp) and sequenced, which indicated that the isolates were genetically related to Yamagata and Victoria lineages (data not shown). This genetic analysis also was confirmed by HI assay (data not shown). Four isolates constituted Bangladesh/Dhaka109/2012, Bangladesh/Dhaka146/2012, Bangladesh/Dhaka243/2012, and Bangladesh/Dhaka388/2012. These isolates showed more than 99% sequence identity with NA and HA gene sequences with WHO recommended vaccine strains for northern hemisphere of 2012-2013 season (Wisconsin/01/2010-Yamagata lineage). Phylogenetic analysis that included Bangladesh/Dhaka243/2012 strain confirmed that it had high similarity with the vaccine strain of northern hemisphere of 2012-2013 season based on both partial HA and NA gene sequences (Fig 1). The other isolate, Bangladesh/Dhaka79/2012, showed 100 and 98.5% identity with NA and HA gene, respectively of WHO recommended vaccine strains, B/Brisbane/60/2008, had high antigenic similarity to Victoria lineage and was phylogenetically clustered with the strains belonging to influenza B Victoria lineage (Fig 1).

DISCUSSION

The findings of this study suggest that an outbreak of influenza B virus among slum dwellers in Dhaka, Bangladesh occurred during summer of 2012. This is consistent with the Hospital Based Influenza Surveillance (HBIS) data (June-September, 2012) that showed in hospitalized patients with influenza-like illnesses a concurrent occurrence of influenza viruses, with 5% and 95% of the specimens being influenza virus A and B, respectively (IEDCR, 2012).

Similar to other South Asian countries, we found co-circulation of both influenza B virus Yamagata and Victoria lineages in the slums of Dhaka (Roy *et al*, 2011). Since the early 1980s, the seasonal flu vaccine has been trivalent (a three-component vaccine) to protect against the three main groups of influenza viruses circulating in humans. Selecting the proper influenza B virus strain to include in the vaccine is particularly difficult, because two lineages of the influenza B viruses co-circulate. Therefore, it has already been proposed for a tetravalent influenza vaccine, which could include two influenza A virus strains and both lineages of influenza B strains (WHO, 2012). Continuous monitoring of the genetic and antigenic characteristics of influenza strains in circulation is thus an essential activity. The recommended vaccine strain for northern hemisphere during 2012-2013 might be effective against the influenza B viruses of the Yamagata lineage in Bangladesh, but tetravalent vaccine that includes both influenza B virus lineages should be more effective. Although our study might not represent the total slum populations in Bangladesh, it does provide pertinent information on the frequency of influenza B infection in slums of Dhaka, strain diversity and concurrence of strains with those

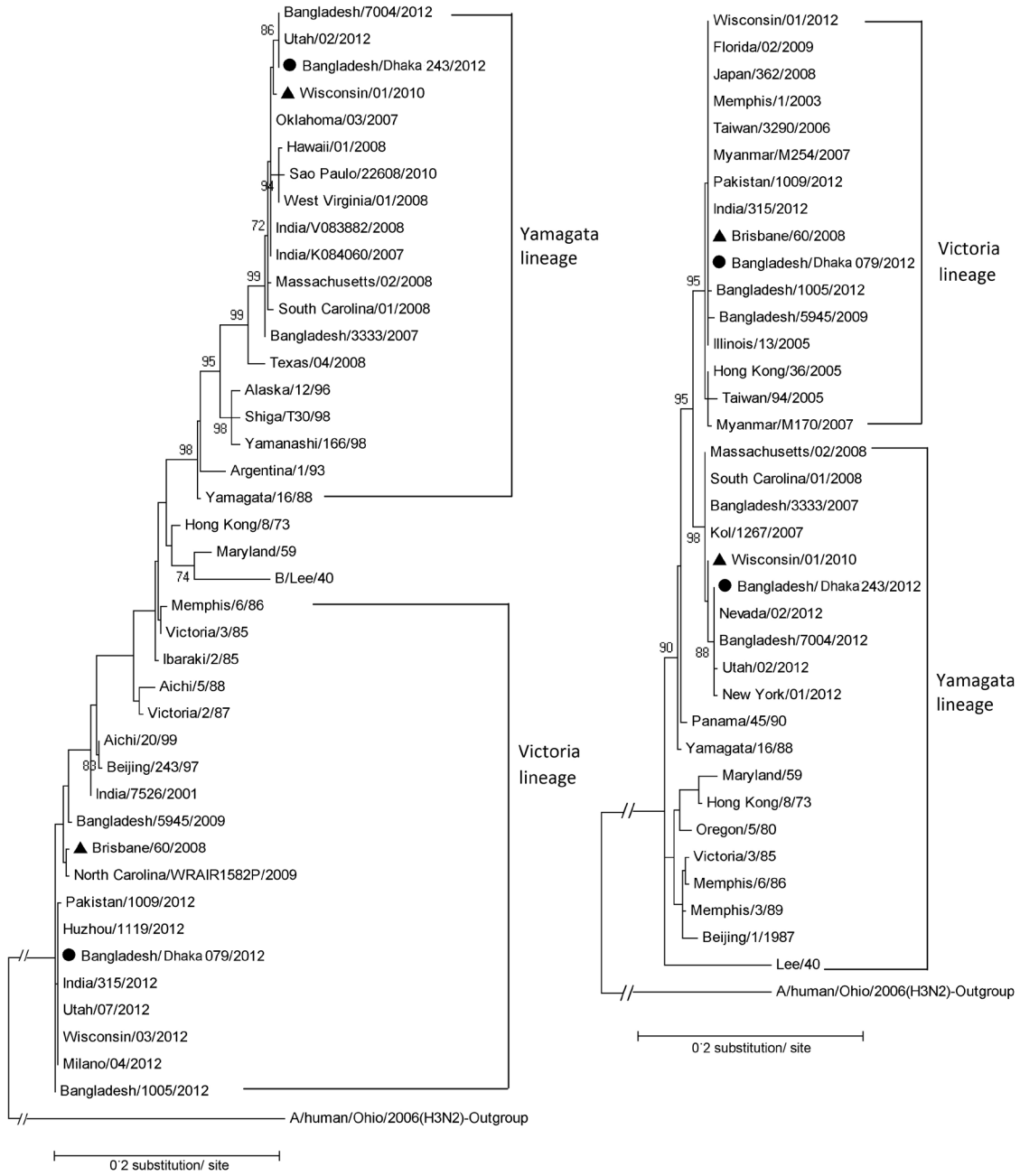

Fig 1–Phylogenetic tree of influenza B virus strains. The tree was constructed based on (a) partial gene sequence of hemagglutinin (HA) and (b) neuraminidase (NA) from Bangladesh strains (accession nos. KP759590-KP759593) and from other countries available in GenBank and GISAID. Black triangles represent vaccine strains and black circles represent strains from this study.

of WHO recommended vaccine strains for Bangladesh.

The limitations of this study include its relatively small sample size obtained from a single geographical origin over a short period of time and the data based on partial sequences of HA and NA genes. Active slum-based influenza virus surveillance is required to elucidate the true burden of influenza virus infection among this populace who constitute one third of the urban population of Bangladesh.

ACKNOWLEDGEMENTS

The research was supported through a Higher Education Quality Enhancement Project funded by Government of Bangladesh and the Ministry of Education (Grant # CP-2212). The authors acknowledge icddr,b, Mohakhali, Dhaka for their cooperation and constructive support.

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