Molecular epidemiology of influenza B virus among hospitalized pediatric patients in Northern Italy during the 2015-16 season.

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Background

Currently, the influenza B viruses belong to two lineages distinct by their genetic and antigenic characteristics, which are referred to as the Yamagata and Victoria lineages, designated after their original isolates, B/Yamagata/16/88 and B/Victoria/2/87. The assessment of the lineage and the group prevalent in circulation is of importance, in order to select the virus to be included in influenza vaccines and to evaluate the efficacy of vaccination. The relatively common emergence of influenza B reassortants suggests strict monitoring of the genetic characteristics of the circulating strains in order to assure the best selection of B viruses to include in influenza vaccines.

Main aim of this study was to evaluate molecular characteristics of influenza B viruses circulating in a Region of Northern Italy, Lombardia, during the influenza season 2015-2016

Material and Methods

The study was carried out in the Department of Maternal and Pediatric Sciences of the University of Milan and in Section of Microbiology, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy between December 1 and March 31, 2016.

Entire influenza B HA gene was amplified directly from clinical specimens using the SuperScript III One-Step RT-PCR amplification kit (Invitrogen, Carlsbad, USA) and primers B/HA-f-6 5'-cacaaaatgaaggcaataattgtacta-3' and B/HA-r+1730 5'-gacagatggagcawgaaacrttgtctctgg-3'with following cycling condition: 50°C for 30 min, 95°C for 10 min; 50 cycles of 95°C for 30s, 58°C for 30s, and 72°C for 2 min, with a final extension cycle at 72°C for 5 min.

Phylogenetic trees were generated by means of the Maximum likelihood method with the Kimura 3-parameter as an evolutionary model using MEGA version 7 software. Tests for positive selection were conducted using the Datamonkey server.

Results

A total of 763 nasopharyngeal samples were collected, 523 from Milano and 240 from Pavia. Among them, 137 (17.9%) resulted positive for influenza viruses, 66 (48.2%) for influenza A and 71 (51.8%) influenza B.

Phylogenetic analysis showed that the great majority of influenza B strains (47/55, 85.5%) fall in a clade defined by amino acid substitutions I117V, N129D and V146I (based on B/Brisbane/60/2008 numbering; Figure 1).

Of note, three strains were close related with "old" influenza B strains circulated in the previous seasons. Two of them, B/Milano/40NIC/2016 and B/Milano/28CA/2016 strains were characterized by I97M, K209N, and T258A mutations as also observed in another Italian influenza B strain (B/Firenze/2/2016) circulating in the same period. The B/Milano/35CA/2016 strain was characterized by the K54N, V124A and D526E mutations (Figure 1).

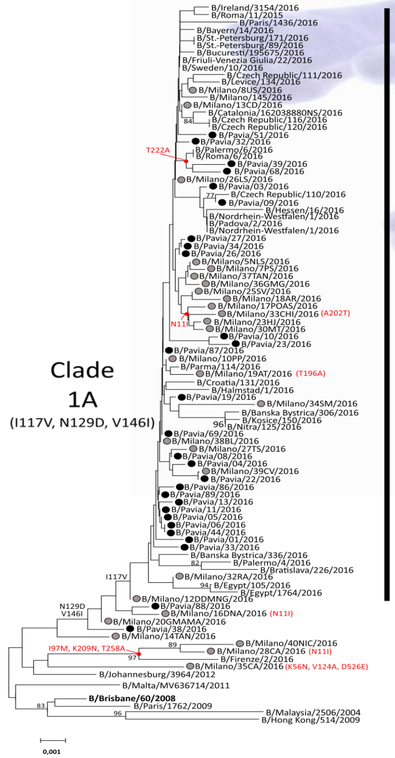

Figure 1. Maximum Likelihood Phylogenetic tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

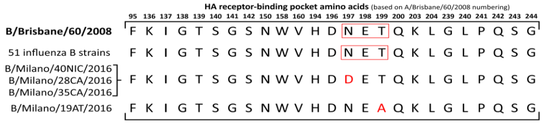

Figure 2. Sequence alignment of the influenza B HA receptor-binding pocket amino acids. The glycosylation site in the sequence of B/Brisbane/60/2008 as well as in other influenza B strains is boxed in red.

Results

In the studied influenza B strains, a series of amino acid changes were observed: I117V, V124A, N129D, V146I, D197N, T199A, and A202T (Table 1). 2 amino acid changes were observed in HA regions involved in the receptor binding or in the antibody recognition [the 120 loop (position 116-137), the 150 loop (141-151), the 160 loop (162-167) and the helix 190 (194-202) (15)] (Figure 2).

Table 1. Amino acid mutations in the antigenic sites of influenza B HA gene.

Subunit	Epitope (residue location)	Mutation (number of strains)
HA1	120-loop (116-137)	I117V(42), V124A (1), D129N (44)
	150-loop (141-150)	I146V (3)
	160-loop (162-167)	-
	190-loop (194-202)	D197N (46), T199A (1), A202T (1)

A global analysis of selective pressure made using the SLAC model indicated an estimated overall dN/dS ratio of 0.175. Overall, no sites were identified as being under positive selection by site-specific analyses in the influenza B alignment by at least three of the methods used (SLAC, FEL, REL, FUBAR and MEME) (Table 2). The IFEL model used to determine the selection pressure acting on the codons along the internal branches of the tree identified a positively selected codon in the influenza B sequences (Table 2).

Table 2. Positive and negative selected sites for influenza B strains identified in this study.

Methods	Flu B condons	
	Positive selection	Negative selection
SLAC	none	80, 210, 244, 256, 461, 519, 532, 549, 550, 556, 559, 562, 563
FEL	none	73, 78, 80, 122, 244, 256, 265, 297, 351, 358, 380, 462, 531, 563, 565
REL	11, 58, 117, 197, 526	73, 80, 244, 256, 265, 351, 358, 380, 531, 563
FUBAR	11	73, 78, 80, 122, 244, 256,265, 297, 351, 358, 380, 462, 531, 563, 565.
MEME	none	none
IFEL	11	80, 244, 256, 265, 351, 563

FEL: fixed-effects likelihood; FUBAR: fast unconstrained Bayesian approximation; IFEL: random effects likelihood; MEME: mixed effects model of evolution; REL: internal branch fixed-effects likelihood; SLAC: single-likelihood ancestor. Positions selected by at least 3 methods are reported in bold.

Conclusions

Flu B viruses that have circulated in Lombardy Region of Italy during the influenza season 2015-2016 belonged to the Flu B Victoria-lineage not included in the trivalent vaccine commonly administered during the 2015-16 influenza season in Italy.

The introduction of quadrivalent vaccine in the Italian vaccination campaign could prevent or reduce the circulation of both lineages of influenza B and improve protection of population against a potential severe disease.