Global Epidemic Trend Analysis of Influenza Type B Drug Resistance Sites From 2006 To 2018

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Short report

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Title page

Global epidemic trend analysis of influenza type B drug resistance sites from 2006 to 2018

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Running title: Drug resistance sites analysis of Flu B virus

Abstract:

Background:
Influenza is a severe respiratory viral infection that causes significant morbidity and mortality, due to annual epidemics and unpredictable pandemics. At present, drugs used in influenza virus B infection treatment are mainly neuraminidase inhibitors (NAIs). With the extensive use of NAI drugs, the influenza virus B has carried different drug-resistant mutations. This study aims to analyze the drug resistance mutations of the NA sequence in Flu B and provide guidance for clinical medication.

Methods:
Near full-length sequences of the NA region of all influenza B viruses from January 1, 2006 to December 31, 2018 were downloaded from public databases GISAID and NCBI. Multiple sequence alignments were performed using Clustal Omega 1.2.4 software. Subsequently, phylogenetic trees were constructed by FastTree 2.1.11 and clustered by ClusterPickergui_1.2.3.JAR. Then, the major drug resistance sites and surrounding auxiliary sites were analyzed by Mega-X and Weblogo (http://weblogo.threeplusone.com/) tools.

Results:
Among the amino acid sequences of neuraminidase (NA) from 2006 to 2018, only Cluster 4 in 2018 carried D197N mutation of NA active site, while other drug resistance sites were conserved without mutation. According to the Weblogo analysis, a large number of N198, S295, K373, and K375 mutations were found in the amino acid residues at the auxiliary sites surround D197, N294, and R374 in the influenza B virus.

Conclusion:
We found the D197N mutation in Cluster 4 of the 2018 influenza B virus, with a large number of N198, S295, K373, and K375 mutations in the helper sites around N197, N294, R374 from 2006 to 2018. NA inhibitors are currently the only kind of specific antiviral agent for the influenza B virus, although these mutations cause mild NAIs resistance.

Keywords: influenza B virus; drug resistance mutation; phylogenetic tree

From person to person, influenza is an acute viral infection of the respiratory tract which can spread globally, infect any age group, and cause a serious public health problem. Its typical symptoms are high fever, runny nose, sore throat, muscle aches, headaches, cough, and feeling of tiredness[1]. According to the latest estimates by the US Centers for Disease Control and Prevention, World Health Organization (WHO), and global health partners, as many as 650,000 people die of seasonal influenza caused by respiratory diseases each year [2]. The human influenza virus belongs to the orthomyxoviride virus family in virological classification[3]. It is divided into A, B, and C types according to the antigenicity of their nuclear proteins, while influenza B viruses are the primary pathogens causing human influenza in recent years, with minor outbreaks in some areas.

Since 1983, circulating influenza B viruses split into two distinct lineages, B/Victoria (B/Vic) and B/Yamagata (B/Yam), represented by B/Victoria/2/87 and B/Yamagata/16/88 strains, respectively [4]. The HA proteins of B/Vic and B/Yam viruses were significantly different, with about a 5% amino acid difference. Most of the differences locate at the antigenic sites of influenza B virus HA protein would lead to antigenicity differences between strains. In addition, the NA amino acid difference between B/Vic and B/Yam viruses is about 2%. Studies found NA amino acid differences in various flu B strains mainly located in the open reading frame of the NB gene[5], coding the head and stem of NA proteins. The recombination from those differential fragments forms new strains, which is more likely to lead to the popularity of influenza B.

Nowadays, neuraminidase inhibitors, such as Oseltamivir, Zanamivir, Peramivir, etc, are the main clinical drugs for type B influenza treatment [6]. With the worldwide prevalence of influenza caused by type B influenza virus and the wide use of NAI drugs, the drug resistance mutations of influenza type B viruses have been accumulating. It has been reported that influenza B viruses have evolved varying degrees of resistance to clinically used NAI drugs above-mentioned [7]. However, there is no systematic study on drug resistance of NAI all over the world. In this study, we analyzed the near full-length neuraminidase (NA) gene sequences of influenza Type B virus that had been submitted to the public database around the world to characterize the crucial drug resistance mutations in NA proteins to provide a theoretical basis for better scientific prevention and control of influenza B.

Methods

Nucleotide sequence download and processing

This study downloaded the NA region sequences of all influenza type B viruses from January 1. 2006 to December 31, 2018, as the internationally approved anti-influenza B virus drugs are mainly developed for neuraminidase. These sequences were from Global Initiative on Sharing All Influenza Data (GISAID, http://platform.gisaid. org/epi3/frontend) and National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?Go=database). The Python script was used to get rid of the repeated sequences and edit the unified sequence name with the format{accession}{continent}{country}{year}. The Clustal Omega 1.2.4 software was used to carry out multi-sequence alignment, retain the qualified NA sequences and delete the sequences missing more bases at both ends to obtain the near full-length NA sequences.

Clustering analysis based on phylogenetic trees

In order to understand the molecular evolution of the influenza B virus, we used FastTree 2.1.11 software to perform a phylogenetic analysis of the near full-length NA sequences and constructed approximately-Maximum-likelihood phylogenetic trees. The command to run is fasttree-gtr-nt< alignment. File >tree_file. Then, clustering analysis was performed using ClusterPickerGUI_1.2.3.jar. Parameters were set as initial Threshold = 0.9, Main Support Threshold = 0.9, Genetic Distance Threshold =4.5, and Large Cluster Threshold = 20 to extract in-cluster sequences and non-cluster sequences.

Analysis of amino acid mutation site

Using online Consensus Maker tool (https://www.hiv.lanl.gov/content/sequence/CONSENSUS/SimpCon.html), clusters are consistent with non - cluster in sequence.

The consistent sequences of in-clusters and non-cluster were translated into amino acid sequences using MEGA-X[8], the differences in amino acids were compared, and the changes in in-clusters' drug-resistant mutations of amino acid sequences in each year were counted.

Ten amino acids surrounding the resistance loci were selected and analyzed by WebLogo (http://weblogo.threeplusone.com/) to reflect the mutation of auxiliary amino acid residues around the drug-resistant sites.

Results

Number of influenza B virus NA sequences every year from 2006 to 2018

A total of 23,357 NA sequences of influenza B virus from 2006 to 2018 were downloaded from NCBI and GISAID (The database was retrieved on November 01, 2019.). The repeated sequences were removed, and sequences with dozens of nucleotide bases missing at both ends were deleted. A total of 19,136 nucleic acid sequences in the near full-length NA region were obtained after preprocessing. The above NA nucleic acid sequences were classified by year to analyze the epidemic distribution of NA sequences each year. From 2006 to 2018, the related research on the NA sequences of the influenza B virus showed an upward trend year by year (Fig. 1). These data provide the basis for epidemiological and drug resistance analysis of influenza B virus.

Cluster number analysis of influenza B virus every year from 2006-2018

The nearly full-length NA sequences of the influenza B virus were aligned, and the ML phylogenetic tree was generated by FastTree 2.1.11 each year. We clustered the NA sequences through ClusterPicker, and the sequence number of in-Cluster from 2006 to 2018 was obtained and statistically plotted each year. The number of epidemic clusters of influenza B virus' NA sequences also increased year by year (Fig. 2), indicating that the NA sequence of influenza B virus never stopped evolving, and it was constantly mutated with diversified variation. With the increased number of Clusters, the variation of influenza B is becoming more and more complex, which is likely to contribute NAIs resistant mutants.

Mutations in NA protease resistance sites

The resistance of influenza viruses to NAIs drugs is mainly due to the mutation of NA protease catalytic activity center sites. At present, the reported mutation sites of influenza B virus resistance to NAIs drugs are mainly G407S, R374K, N294S, H273Y, I221 (I221V, I221T), D197 (D197E, D197Y, D197N), R150K and E117A (N1 numbering)[9]. We counted mutations in NA resistance sites each year from 2006 to 2018. It was found that only Cluster 4 in 2018

had the mutation of neuraminidase active site D197N, and other drug resistance sites were conserved without mutation. However, D197N can destroy the interaction between the D197 site and the R150 site, and reduce the stability of critical inhibitory binding sites, which will lead to potential drug resistance. Some studies have pointed out that the three-dimensional structure of the D197N site is not directly related to the substrate or inhibitor, suggesting that the influenza B virus is still sensitive to NAIs drugs.

Weblogo analysis of nearby auxiliary amino acid mutations

Weblogo (http://weblogo.threeplusone.com) was used to describe the frequency of changes in 10 amino acids surrounding the drug resistance sites of influenza B virus from 2006 to 2018 (Fig. 3). According to the statistical results, many mutations of N198, S295, K373, and K375 (N1 numbering) occurred in the amino acid residues at the auxiliary sites around D197, N294 and R374 (Supplementary Table 1).

Discussion

NAIs can mimic the natural substrate, sialic acid, to bind to NA and block its active sites so that it cannot catalyze the hydrolysis of sialic acid and prevent the release of virus particles. The RNA polymerase of the influenza virus is prone to cause mismatches. With the increase of clinical use of NAIs, the corresponding NAIs drug-resistant strains gradually appeared. At present, it is believed that the molecular mechanism of influenza virus resistance to NAIs is mainly the mutation of the viral RNA sequence encoding NA, which changes one or more amino acid residues constituting NA. The most common changes include amino acid residue substitution [10] and deletion[11]. Drug-resistant strains with the substitution or deletion of NA protease active sites or nearby amino acid residues can directly or indirectly cause the spatial conformation change of NA protease active sites and the damage of enzyme function, the failure of NA binding to NAIs with high affinity.

The NAIs resistant strains of influenza B virus are not as common as influenza A virus, and the detection rate is very meager at 0-1%[12]. Drug resistance mutations of the influenza B virus mainly occurred at D197 (D197E, D197Y, D198N) and R150K (N1 numbering)[13]. It is very consistent with our analytical results. In addition, mutations in I221T, G407S, R374K, and E117 (E119A, E119D, E119G) can also lead to resistance to oseltamivir (I221T, G407S, R374K, and E119A/D/G), paramivir (E119A/D/G), and zanamivir (G407S, R374K, and E119A/D/G)[14]. In general, the influenza B virus is still highly sensitive to NA inhibitors.

Although the mechanism of NAI resistance caused by some sites of influenza virus has been studied, the specific mechanism of NAI resistance of influenza virus has not been generally accepted and fully explained. HA mainly recognizes and binds receptors, which recognize and act on the same receptor. NA is mainly involved in the release of virus particles from cells. Therefore, the balance of HA and NA plays a vital role in viral replication. Mutations in some HA sites cause the decrease of affinity between HA and receptors and reduce the virus's dependence on NA enzyme activity. Therefore, the resulting NAIs resistance is a kind of multiple cross-resistance. In addition, HA mutation may also have a particular compensation effect on the decline of virus viability caused by NA resistance mutation[15].

Conclusions

According to this study, we know that neuraminidase inhibitors are still the only specific

antiviral drugs for the treatment of the influenza B virus, and their early use will significantly improve the prognosis of patients. Although some viruses have drug resistance mutations, the currently prevalent influenza B virus is generally sensitive to NAIs.

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Figures

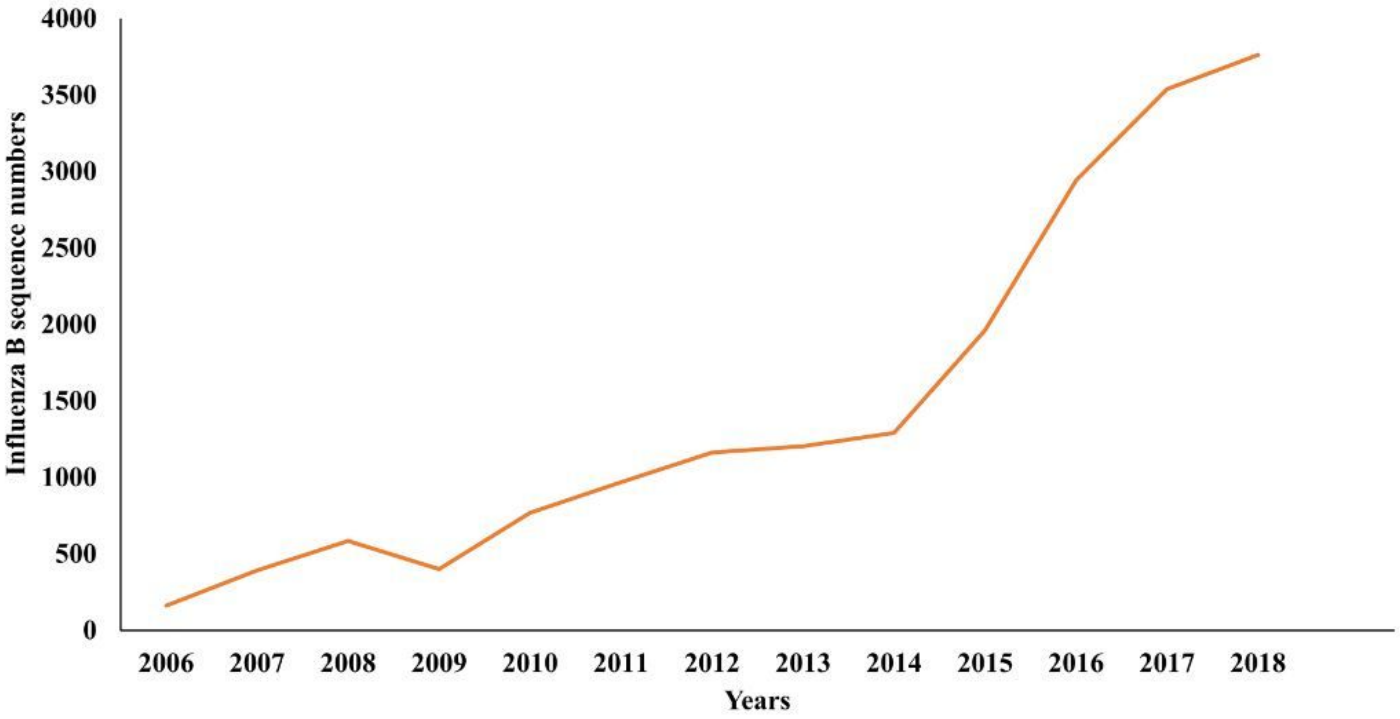

Figure 1

From 2006 to 2018, the related research on the NA sequences of the influenza B virus showed an upward trend year by year (Fig. 1).

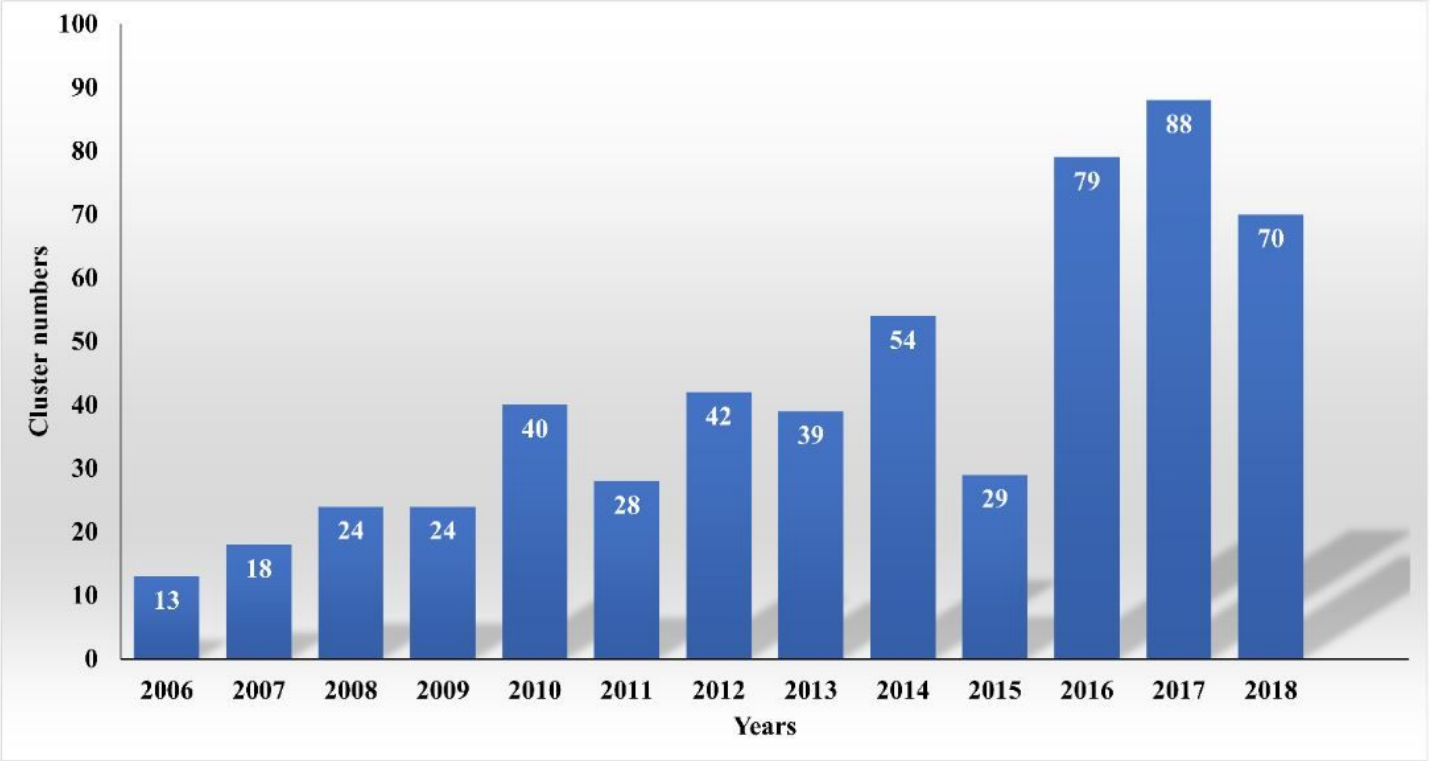

Figure 2

The number of epidemic clusters of influenza B virus' NA sequences also increased year by year (Fig. 2), indicating that the NA sequence of influenza B virus never stopped evolving, and it was constantly mutated with diversified variation.

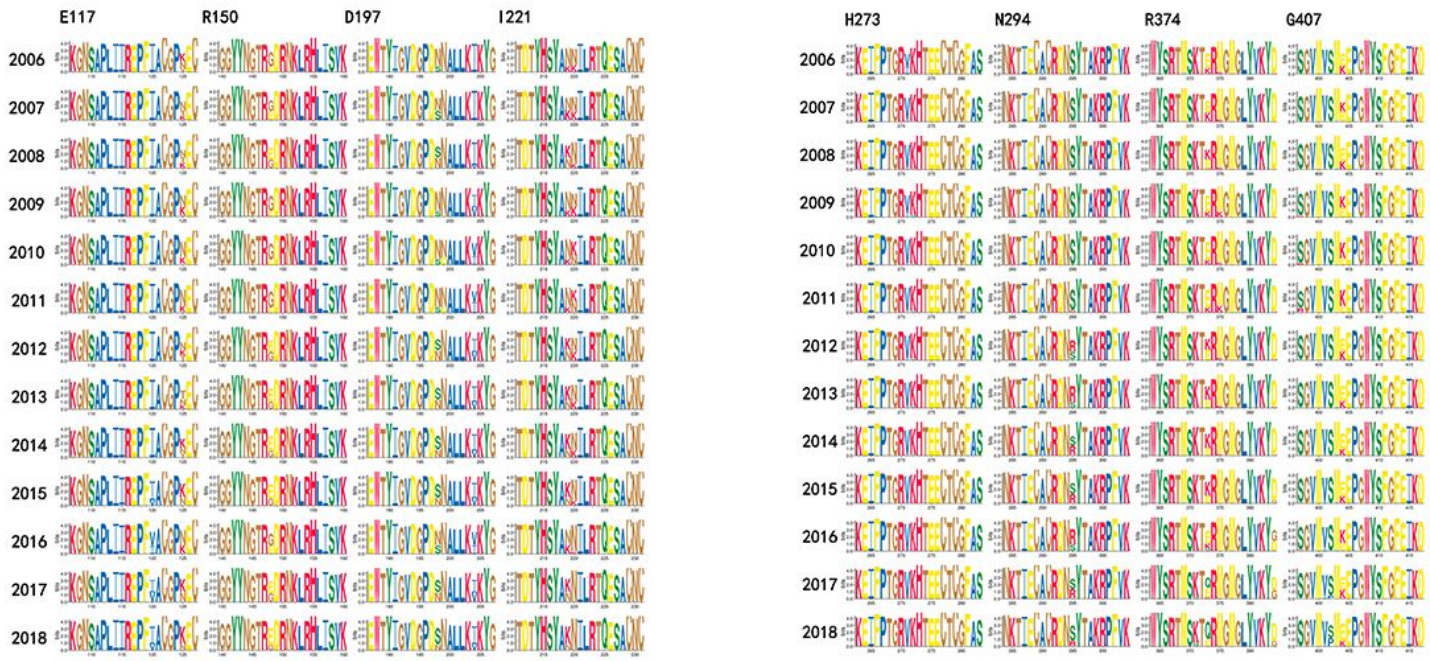

Figure 3

Weblogo (http://weblogo.threeplusone.com) was used to describe the frequency of changes in 10 amino acids surrounding the drug resistance sites of influenza B virus from 2006 to 2018 (Fig. 3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx