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# Genetic characterization of influenza A(H3N2) viruses from 2014 to 2017 in Yantai, east of China

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**Abstract**

**Background:** The genetic variations of influenza viruses pose a real challenge to the vaccine strategies and medical treatment of patients.
**Methods:** In this study, the molecular epidemiology and evolution of influenza A(H3N2) strains were analyzed from April 2014 to March 2017 in Yantai area of eastern China.
**Results:** The phylogenetic analysis of the hemagglutinin (HA) sequences of influenza A(H3N2) showed that all of the influenza A(H3N2) strains during the study period belonged to the genetic clade 3c with the mutations N145S (epitopeA), V186G (epitopeB), P198S (epitopeB) and F219S (epitopeD). Most strains (12/14) of the 2014/2015 season fell into the subgroup 3C.3a characterized by A138S (epitopeA), R142G (epitopeA), F159S (epitopeB) and T128A (epitopeB), while strains isolated from the 2015/2016 and 2016/2017 seasons clustered in 3C.2a shared mutations N144S (epitopeA), F159Y (epitopeB), K160T (epitopeB) and Q311H (epitopeC). The strains isolated from the 2014/2015 and 2015/2016 seasons was were genetically and antigenically distinct from the given vaccine strains. The evaluation of vaccine efficacy (VE) against circulating strains estimated using the pepitope model suggested that little or no protection against circulating strains from 2014/2015 and 2015/2016 seasons was afforded by the given vaccine strains. The sequence analysis of the neuraminidase (NA) showed that all of the analyzed strains had no substitution in the catalytic sites or the framework sites or the supporting the catalytic residues or the oseltamivir resistance substitutions.
**Conclusions:** The results of the study suggested that the vaccine strains provided suboptimal protection against influenza A(H3N2) strains, especially in the 2014/2015 and 2015/2016 seasons and the A(H3N2) strains circulating in the Yantai area were still susceptible to NA inhibitors. Continued systematic antigenic and molecular surveillance of the influenza virus is essential to developing strategies for the prevention and control of influenza.

**Keywords:** antigenic drift; hemagglutinin; H3N2; influenza A virus; neuraminidase.

## Introduction

Influenza viruses are important causative pathogens of respiratory infections, causing occasional pandemics and seasonal epidemics in humans and animals globally [1]. Influenza viruses are estimated to result in about 3–5 million cases of severe illness, and about 250–500 thousand cases of death annually [2]. The A(H3N2) strain evolves significantly faster than the other subtypes, and thus has rapidly spread in multiple countries since its emergence in 1968 [3]. The surface proteins hemagglutinin (HA) and neuraminidase (NA) of influenza A(H3N2) viruses are two major viral targets for the host immune system [4]. The HA protein plays a major role in binding to the host receptor and represents major antigenic sites (defined as epitopes A, B, C, D, E) [4]. Meanwhile, the neuraminidase (NA) protein with sialidase activity is responsible for releasing the newly produced virions from the host cell [5, 6]. The genetic variations of HA and NA lead to circulating viruses to evade host immune responses and reduce vaccine effectiveness and sensitivity to antivirals. Combined genetic and antigenic as well as phenotypic analyses of circulating strains could provide improvements in the process of vaccine virus selection and treatment regimens of the patient [7]. In this study, we analyzed the genetic and antigenic characteristics of the influenza A(H3N2) strains circulating in the Yantai area of eastern China from 2014 to 2017 and evaluated vaccine efficacy (VE) and susceptibility to NA inhibitors, which was necessary to develop strategies for the prevention and control of influenza.

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# Materials and methods

## Specimen collection

Respiratory samples were collected from April 2014 to March 2017 among patients with an influenza-like illness (ILI) (sudden onset of fever ≥38 °C combined with respiratory symptoms such as cough, sore throat and absence of other diagnoses) who sought medical attention at two sentinel surveillance hospitals in Yantai. The mean number of outpatients in the two sentinel surveillance hospitals is about 300 per day. The Yantai area is located in eastern China, covering an area of more than 14,000 $km^2$, with a population of about 7.06 million population. A total of 5183 swabs were collected with the informed consent of all patients or guardians of children. These swabs were placed in virus transport media tubes immediately and sent to the Yantai Center for Disease Control and Prevention within 24 h.

## Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

## Virus isolation and subtype identification

These clinical specimens were isolated in Madin-Darby canine kidney (MDCK) cells, and the presence of virus in the culture was confirmed by hemagglutination assay. The subtype of influenza, which was isolated with a minimum of eight hemagglutination units, was determined by hemagglutination inhibition (HAI) assay with the standard antigen and antiserum from the Chinese Nation Influenza Center, according to the surveillance protocol published by the Ministry of Health (MoH) of China [8]. HAI assays were performed in V-bottom 96-well microtiter plates with the standard antigen controls, 1% human type O erythrocytes controls and negative controls using 1% suspension of human type O erythrocytes. The standard serum was tested with two-fold serial dilutions from 1:10 to 1:1280. The HAI titer was the reciprocal of the highest dilution of the antiserum that cause complete inhibition of hemagglutination. A virus isolate was recognized as a particular subtype when it reacted with one standard antiserum at a four-fold or greater HAI titer than when it reacted with other standard antisera. A total of 45 cultures confirmed as positive for influenza A/H3N2 were selected based on their distribution over time (14 in the 2014/2015 season, 10 in the 2015/2016 season, 21 in the 2016/2017 season) for the *HA* and *NA* gene analysis.

## Nucleic acid extraction and sequencing

Viral RNA was extracted using the RNeasy Mini Kit (Qiagen 52904, Germany) according to the manufacturer's instructions, and the HA and NA sequences were subsequently amplified by reverse transcription polymerase chain reaction (RT-PCR) using a PrimeScript One Step RT-PCR Kit (Qiagen210212, Germany). The primers and cycling conditions were used for amplification as described before [9]. RT-PCR products were sequenced in both directions using the Sanger method. Sequences were submitted to the GenBank database with the accession numbers MF593700-MF593789.

## Phylogenetic analysis

Sequences from the vaccine and reference strains (the accession numbers in Table 1) were retrieved from the Global Initiative on Sharing All Influenza Data (GISAID) databases for phylogenetic analyses. The phylogenetic trees were generated with the Neighbor-Joining method, using the MEGA 7.0.26 software package (Philadelphia, PA, USA). The Kimura 2-parameter model was used and the reliability of the trees was provided by bootstrap analysis of 1000 replications. The potential N-linked glycosylation sites were predicted using the NetNGlyc 1.0 server, with a threshold value of $> 0.5$.

## Estimation of vaccine efficacy using the pepitope model

The potential VE of influenza A/H3N2 was evaluated using the pepitope model, which measures the antigenic distance between the vaccine strains and circulating strains and has a better correlation with vaccine VE than antisera HAI assay or phylogenetic analyses [10]. Pepitope is calculated by the fraction of substituted amino acid in the dominant epitope of HA [11]. The association between VE efficacy for influenza A(H3N2) and pepitope is given by $VE = -2.47 \times \text{pepitope} + 0.47$ [12–14]. If pepitope = 0, VE will be 47% as a perfect match between vaccine and the circulating strains [10].

**Table 1:** Sequence information of reference A(H3N2) strains included in the phylogenetic analyses.

| Isolate name | Accession number | | Group |
|---|---|---|---|
| | HA | NA | |
| A/Fujian/411/2002 | EPI358784 | | |
| A/California/7/2004 | EPI367105 | | |
| A/Wisconsin/67/2005 | EPI160218 | | |
| A/Brisbane/10/2007 | EPI165489 | | |
| A/Stockholm/18/2011 | EPI326139 | | 3A |
| A/England/259/2011 | EPI346607 | | 3B |
| A/Maryland/02/2012 | EPI358041 | | 3B |
| A/Victoria/361/2011 | EPI349106 | | 3C (vaccine for 2013/2014) |
| A/Texas/50/2012 | EPI391247 | EPI391246 | 3C.1 (vaccine for 2014/2015) |
| A/Quebec/44/2014 | EPI573951 | | 3C.3X |
| A/Finland/385/2013 | EPI502957 | | 3C.3 |
| A/Switzerland/9715293/2013 | EPI530687 | EPI530688 | 3C.3a (vaccine for 2015/2016) |
| A/Norway/1903/2014 | EPI539623 | | 3C.2 |
| A/Hong_Kong/5738/2014 | EPI539806 | | 3C.2a |
| A/Hong Kong/4801/2014 | EPI614437 | EPI614436 | 3C.2a (vaccine for 2016/2017) |

# Results

## The activity of influenza viruses

From April 2014 to March 2017, 5183 samples from ILI patients were tested for influenza by virus isolation. Based on the HAI assay results, 8.95% (464/5183) were positive for influenza viruses: 36% (n = 167) were typed as influenza B, while 64% (n = 297) were influenza A. The confirmed influenza A viruses comprised 114 (38%) A(H1N1) pdm09 and 183 (62%) A(H3N2). In each surveillance year, there were at least two subtypes co-circulating in the Yantai area, and a seasonal pattern of influenza activity was observed. Influenza A(H3N2) appeared in August 2014, lasted about 5 months and peaked in November 2014. From December 2014, the A(H1N1)pdm09 influenza virus was observed, persisted until April 2015 and replaced the influenza A(H3N2) gradually. The influenza A(H3N2) viruses circulated at low level in 2015/2016 influenza season, and became the dominant subtype again until November 2016. The prevalence of A(H3N2) persisted until March 2017, and was replaced by A(H1N1) pdm09 (Figure 1).

## Hemagglutination inhibition (HAI) assay results

During the study period, a total of 183 isolates were identified as influenza A(H3N2) through HAI assay. One hundred and twenty one isolates, 14 isolates and 48 isolates were identified in the 2014/2015 season, 2015/2016 season and 2016/2017 season, respectively. In the 2014/2015 and 2015/2016 seasons most of the viruses were recognized (115/121, 95.04% in 2014/2015 and 12/14, 85.71% in 2015/2016) by the corresponding standard antisera at titers four-fold and ≥eight-fold lower than the homologous titer.

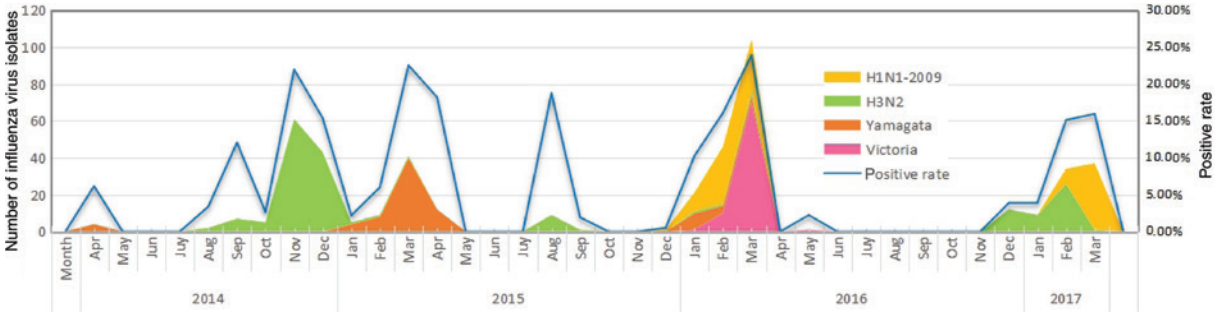


**Figure 1:** Month-wise distribution of influenza subtypes from April 2014 to Mar 2017 seasons.

**Table 2:** HAI reactions of influenza A/H3N2 circulating in Yantai from April 2014 to Mar 2017.

| Influenza season | Reference A(H3N2) viruses | No. of isolates | Reduction in HAI titer compared to homologous titer | | |
|---|---|---|---|---|---|
| | | | ≤2-Fold | 4-Fold | ≥8-Fold |
| 2014/2015 | A/Texas/50/2012 (3C) | 121 | 6 (4.96%) | 42 (34.71%) | 73 (60.33%) |
| 2015/2016 | A/Switzerland/9715293/2013 (3C.3a) | 14 | 2 (14.28%) | 7 (50%) | 5 (35.71%) |
| 2016/2017 | A/Hong Kong/4801/2014 (3C.2a) | 48 | 42 (87.5%) | 5 (10.42%) | 1 (2.08%) |

However, in the 2016/2017 season, almost all of the isolates (42/48, 87.5%) from Yantai area were recognized well by the standard antisera at titers ≤two-fold reduced compared to the homologous titers of the antisera (Table 2).

## Phylogenetic analysis

The HA phylogenetic analysis was conducted on 45 studied isolates along with 15 HA genes of the northern hemisphere vaccine and reference strains, and the NA phylogenetic analysis was performed on the NA gene of the 45 studied isolates and three northern hemisphere vaccine strains.

In comparison with A/Texas/50/2012 (vaccine strain for 2014/2015), the average nucleotide and amino acid similarities on the HA of the 2014/2015 season were estimated to be 98.62% and 97.36%. While the similarities of nucleotide and amino acid between the A(H3N2) strains from the 2015/2016 seasons and A/Switzerland/9715293/2013 (the vaccine for 2015/2016) were 98.3% and 97.4%, respectively. When compared to A/Hong Kong/4801/2014 (the vaccine strain for 2016/2017), the average HA nucleotide and amino acid identities among the 2016/2017 strains were 99.2% and 98.9%, respectively.

For NA of the influenza A(H3N2), the average similarities of nucleotide and amino acid between strains isolated from Yantai city and the given vaccine strains were 99.27% and 99.56% in the 2014/2015 season, 98.77% and 98.72% in the 2015/2016 season, and 98.47% and 97.92% in the 2016/2017 season, respectively.

The HA phylogenetic tree showed that all of the influenza A(H3N2) strains from the April 2014 to March 2017 seasons belonged to the genetic clade 3C and shared mutations N145S (epitopeA), V186G (epitopeB), P198S (epitopeB) and F219S (epitopeD). Most strains (12/14) of the 2014/2015 season fell into the subgroup 3C.3a and possessed the mutations A138S (epitopeA), R142G (epitopeA), F159S (epitopeB) and T128A (epitope), while strains isolated from the 2015/2016 and 2016/2017 seasons clustered in 3C.2a characterized by N144S (epitopeA), F159Y (epitopeB), K160T (epitopeB) and Q311H (epitopeC). Then, the 3C.2a strains underwent further evolution to yield two subclades. The strains form the 2015/2016 season possessed mutations N171k (epitopeD), and the

**Table 3:** Amino acid substitutions in the HA and NA genes of influenza A(H3N2) viruses circulating in Yantai compared to A/Texas/50/2012.

| Gene subclade | No. of isolates | Amino acid substitutions | |
|---|---|---|---|
| | | HA | NA |
| 3C.3a | 2004/2015(12) | N128A(***B***,12), A138S(***A***,12), R142G(***A***,12), N145S(***A***,12), F156S(***B***,12), V186G(***B***,12), P198S(***B***,12), F219S(***D***,12), N225D(12), K326R(12) | E221D(11/12), I392T(10/12) |
| 3C.2a | 2014/2015(2)<br>2015/2016(10)<br>2016/2017(21) | L3I(33), N128T(***B***,33), T131K(***A***,21), R142K(***A***,21), N144S(***A***,33), N145S(***A***,33), F159Y(***B***,33), K160T/I(***B***,29/2), N171K(***D***,8), V186G(***B***,33), P198S(***B***,33), F219S(***D***,33), N225D(33), R261Q(17) Q311S(33), I406V(8), G484E(8), D489N(33) | E221D(31), T267K(28/31), S245N(30/31), S247T(30/31), I380V(30/31), P468H(28/31) |

Antigenic sites are identified in bold italic.

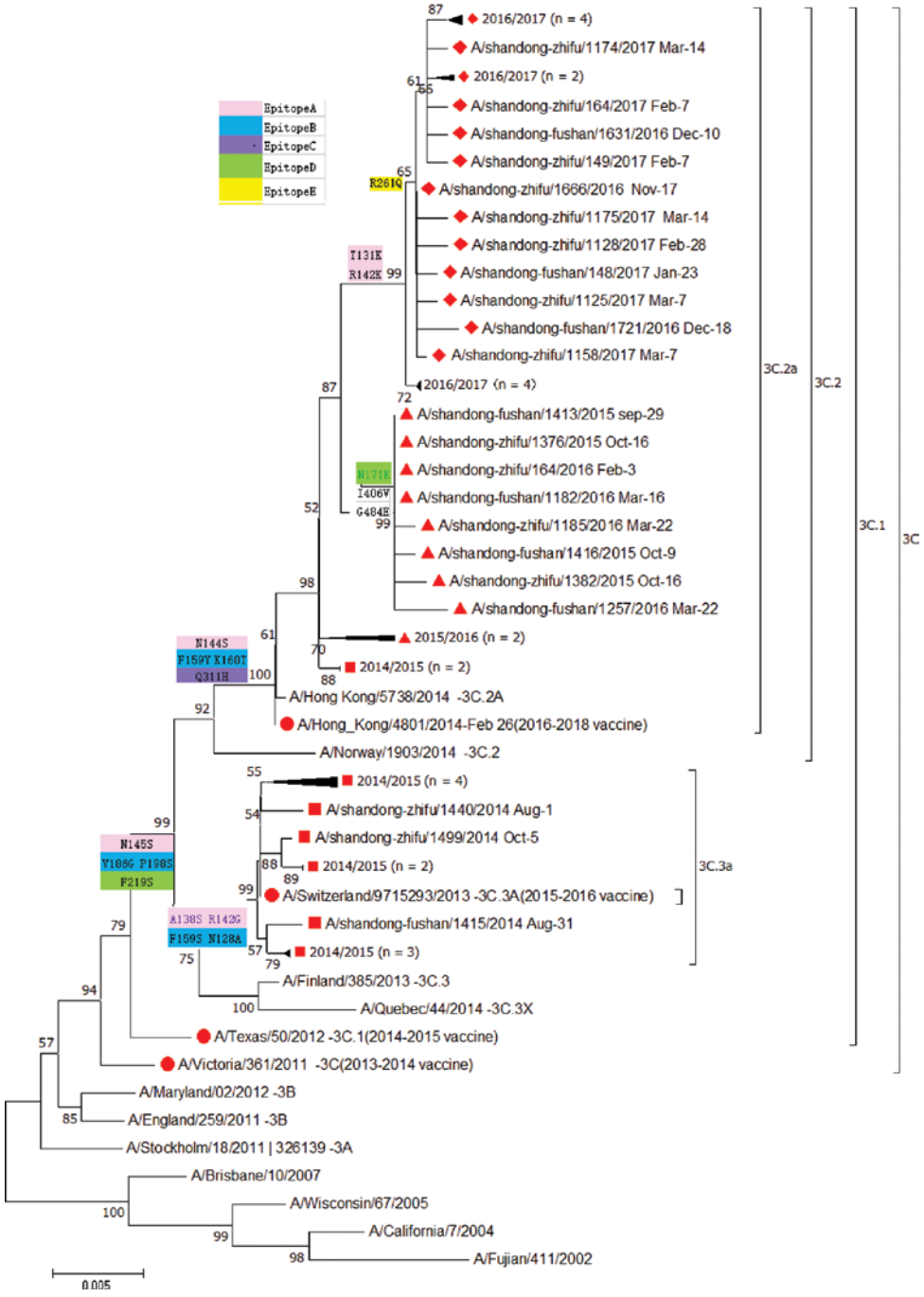


**Figure 2:** Phylogenetic tree comparing the HA gene of A/H3N2 Yantai isolates and vaccine strains.

2016/2017 strains were characterized by T131K (epitopeA) and R142K (epitopeA) (Table 3, Figure 2).

No mutation was observed in the HA receptor binding sites (positions 98Y, 136T, 153W, 183H, 190D, 221P and 227E). The HA proteins of the A(H3N2) clade 3C.3a strains had 12 potential glycosylation sites (8, 22, 38, 45, 63, 122, 133, 144, 165, 246, 285 and 483). Comparing to the 3C.3a strains, the A128T mutation which resulted in the gain of a potential glycosylation site occurred in the clade 3C.2a. The N144S substitution caused the loss of a potential glycosylation site, and the 158 glycosylation site was observed in 3C.2a strains due to the mutations F159Y and K160T.

Compared to the NA sequence of A/Texas/50/2012, most strains in the 2014/2015 season carried the substitutions E221D(13/14) and I392T(10/14), and those strains in

the 2015/2016 and 2016/2017 seasons had substitutions T267K(28/31), S245N(30/31), S247T(30/31), I380V(30/31) and P468H(28/31) (Table 3).

The NA proteins of A(H3N2) circulating in the Yantai area in the 2014/2015 season had eight potential glycosylation sites (61, 70, 86, 146, 200, 234, 329 and 367), while the strains in the 2015/2016 and 2016/2017 seasons had an additional N-linked glycosylation site due to the substitutions S245N.

No substitution was observed in the catalytic sites (118, 151, 152, 224, 276, 292, 371 and 406) or framework sites supporting the catalytic residues (119, 156, 178, 179, 198, 222, 227, 274, 277, 294 and 425) in the strains circulating in the Yantai area [15]. Most importantly, all of the analyzed strains had no the oseltamivir resistance substitutions E119V and R292k [16].

## Evaluations of vaccine efficacy

To estimate the effect of the accumulated amino acid substitutions in the HA1 domain on predicted VE in a given year, the pepitope model was used to evaluate how well the vaccine strains match the circulating strain. Theoretically, the VE becomes negative when the pepitope is higher than 0.19 [17]. For the 2014/2015 season, the pepitope between A/Texas/50/2012 and A/H3N2 strains circulating in Yantai area showed a mean value of 0.20 (dominant epitope = B, substitutions: 128, 159, 186, 198), which indicated a negative VE against those strains present that year of −5.18% (E = −2.44% of 47%, pepitope = 0). For the 2015/2016 season, the mean pepitope value between the vaccine strains and A/H3N2 strains in Yantai area was 0.158 (dominant epitope = A; substitutions: 138, 142, 144), which suggested the VE against those strains was 16.97% (E = 7.97% of 47%, pepitope = 0.198) of that of a perfect match with the A/Switzerland/9715293/2013 vaccine strain, while the mean pepitope value for the 2016/2017 season was 0.11 (dominant epitope = A; substitutions: 131, 142), which indicated the VE against those strains was 42.05% (E = 19.76% of 47%) of that of a perfect match with the A/Hong Kong/4801/2014 vaccine strain (Table 4).

# Discussion

This study analyzed the prevalence of influenza A(H3N2) in Yantai area of eastern China from April 2014 to Mar 2017. The monitoring data analysis showed that in addition to the circulation in winter months, influenza A(H3N2) also circulated during summer in 2014 and 2015.

**Table 4:** Efficacy among the vaccine strains and amino acid residue differences on the dominant epitopes of the influenza A/H3N2 in Yantai, China.

| Influenza season | Vaccine strain | No. of strains | Dominant epitope | No. of mutations | Residue differences | Pepitope | Vaccine efficacy 47% | Vaccine efficacy 100% |
|---|---|---|---|---|---|---|---|---|
| 2014/2015 | A/Texas/50/2012 | 2 | B | 5 | 128 159 160 186 198 | 0.238 | −11.81% | −25.13% |
| | 2014/2015 vaccine strain | | | | | | | |
| | | 10 | B | 4 | 128 159 186 198 | 0.190 | −0.05% | −0.10% |
| | | 2 | A | 4 | 124 138 142 145 | 0.211 | −5.00% | −10.64% |
| | | | | | Mean | 0.200 | −2.44% | −5.18% |
| 2015/2016 | A/Switzerland/9715293/2013 | 10 | A | 3 | 138 142 144 | 0.158 | 8.00% | 17.02% |
| | 2015/2016 vaccine strain | | | | | | | |
| | | | | | Mean | 0.158 | 7.97% | 16.97% |
| 2016/2017 | A/Hong_Kong/4801/2014 | 1 | A | 3 | 131 142 144 | 0.158 | 8.00% | 17.02% |
| | 2016/2017 vaccine strain | | | | | | | |
| | | 1 | A | 3 | 131 135 142 | 0.158 | 8.00% | 17.02% |
| | | 19 | A | 2 | 131 142 | 0.105 | 21.00% | 44.68% |
| | | | | | Mean | 0.110 | 19.76% | 42.05% |

Seasonal influenza is generally prevalent during winter in the temperate zone, but its prevalence is irregular in the tropics. The Yantai area is located in the temperate zone of the northern hemisphere. The prevalence of influenza A(H3N2) during summer in 2014 and 2015 indicated that the antigenicity of circulating strains may be changed greatly, and most people were susceptible to these strains.

The phylogenetic analysis showed that all of the influenza A(H3N2) strains isolated in Yantai area during the study period had diverged into two genetically different groups. One group was 3C.3a, including predominant circulating strains in the 2014/2015 season, and was considered antigenically different from the vaccine reference strain for the 2014/2015 season (A/Texas/50/2012(H3N2) clade 3C.1) [18–20]. And the other group was 3C.2a, which were observed in the 2014/2015 season occasionally and became predominant circulating strains in the 2015/2016 and 2016/2017 seasons. These strains were divergent from the 2015/2016 vaccine strain A/Switzerland/9715293/2013 (clade 3C.3a), and were genetically similar to the 2016/2017 vaccine strain A/Hong Kong/4801/2014 (clade 3C.2a). This was consistent with the previously reported result that the A(H3N2) strains had phylogenetic discordant clustering patterns with the vaccine strains in the 2014/2015 and 2015/2016 seasons [21, 22]. Meanwhile, the 2016/2017 strains were genetically similar to the 2016/2017 vaccine strain A/Hong Kong/4801/2014 (clade 3C.2a). A real challenge to vaccine strategies was posed by this diversity in circulating influenza types and subtypes [23].

Due to the antigenic change of influenza virus strains, influenza virus vaccines must be updated regularly so that the vaccines can match the currently circulating strains well [24]. The surface protein HA is the primary target of the protective immune system, and antigenic changes on the HA can lead to the escape of immune system. The HA consists of five epitopes (A–E) located on the globular head of HA as main targets for specific antibodies [11, 20]. Prior studies suggested that when four or more amino acid mutations were found on at least two epitopes on the HA protein, the influenza variants were considered to drift from the parental strain [25]. However, Koel et al. [26] have found that substitutions responsible for the major antigenic change were exclusively located in the antigenic sites A (position 145) and B (positions 155, 156, 158, 159, 189 and 193) immediately adjacent to the receptor binding site (RBS) and a single amino acid substitution could cause a major antigenic change.

Compared to the recommended vaccine strain, A/Texas/50/2012 during the 2014/2015 season, the HA gene of the circulating A(H3N2) strains possessed N145S and F159S amino acid mutations causing major antigenic change. In the 2015/2016 seasons, compared to the vaccine strain (A/Switzerland/9715293/2013), major antigenic changes also occurred due to F159Y substitution. In the 2016/2017 season, the circulating strains and the corresponding vaccine strain A/Hong Kong/4801/2014 had similar antigenic properties because no substitution was observed on the amino acid responsible for the major antigenic change. This was in accordance with the result of the HAI assay: most of A(H3N2) viruses in 2014/2015 and 2015/2016 showed clear antigenic divergence from the corresponding vaccine virus based on the HAI assay. And almost all Yantai isolates showed better reactivity with antisera raised against vaccine strains in 2016/2017.

The current vaccines recommended every year can elicit effective antibody responses to both HA and NA. However, when the newly emerging viruses arise due to antigenic variations, these vaccines afford little to no protection [27]. The pepitope model was used to quantify antigenic distance between the circulating strains and the corresponding vaccine strains. The results of the the pepitope model showed a vaccine mismatch of A(H3N2) in the 2014/2015 and 2015/2016 seasons, which was consistent with several studies reported previously [21, 22]. However, the vaccine strains for the 2016/2017 season were capable of affording more protection against the circulating influenza A(H3N2), which was in accordance with some studies in Thailand and Cameroon [22, 28]. The pepitope model provides a new tool to quantify the antigenic distance between the dominant circulating strains and candidate vaccine strains more precisely and serves as an additional assessment to the supplement of HAI assay data when vaccine strains are selected [29].

The changes at potential glycosylation sites may be due to the selection imposed by the host immune system [30]. Compared to A/Texas/50/2012, the HA gene of the 3C.2a strains circulating in Yantai area during the study period gained two potential glycosylation sites, and lost one potential glycosylation site, meanwhile the NA gene of the 3C.2a strains had an additional N-linked glycosylation site which not only affects the antigenic and functional properties of surface proteins, but also can provide the chance to escape the surveillance of the host immune system [31].

Vaccine effectiveness reduced due to the antigenic drift of the circulating influenza A(H3N2), so influenza antiviral medications become very important [32]. Evaluating the efficacy of the influenza NA inhibitors is one of the tasks in the systematic monitoring for influenza viruses. In the present study, the catalytic sites and framework sites that support the catalytic residues within NA gene of the analytic strains were conserved. And the

oseltamivir resistance mutation was not detected as the incidence of NA inhibitor resistance was extremely rare in A(H3N2) strains [33]. The result of this study revealed that the A(H3N2) strains circulating in Yantai area from 2014 to 2017 were susceptible to NA inhibitors.

## Conclusions

In conclusion, the predominant A(H3N2) strains circulating in Yantai area belonged to the subclade 3C.3a during the 2014/2015 season, then became subclade 3C.2a during the 2015/2016 and 2016/2017 seasons. The vaccine strains recommended by the World Health Organization provided suboptimal protection against influenza A(H3N2) strains because of the genetic variations in the HA and NA genes during this study period, especially in the 2014/2015 and 2015/2016 seasons. The A(H3N2) strains circulating in Yantai area were still susceptible to NA inhibitors. The finding of this study shows that continual surveillance of the subtypes and genetic changes of the circulating seasonal influenza viruses are necessary to find the new antigenic or drug-resistant variants which is important to develop the strategies for the prevention and control of influenza.

**Conflict of interest and author contributions:** All the authors declare that they have no conflict of interest and have accepted responsibility for the entire content of this submitted manuscript and approved submission.
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**Honorarium:** None declared.
**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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