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Short communication

Predominance of influenza A(H3N2) virus genetic subclade 3C.2a1 during an early 2016/17 influenza season in Europe – Contribution of surveillance data from World Health Organization (WHO) European Region to the WHO vaccine composition consultation for northern hemisphere 2017/18

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ABSTRACT

During the European 2016/17 influenza season, A(H3N2) viruses have predominated and the majority clustered in genetic subclade 3C.2a1. Genetic analyses showed that circulating viruses have undergone considerable genetic diversification of the haemagglutinin gene from the current vaccine virus A/Hong Kong/4801/2014 (clade 3C.2a), but the antigenic data that is limited by the challenges with the antigenic characterisation of currently circulating A(H3N2) viruses, showed no clear evidence of antigenic change. The recommended A(H3N2) vaccine component for the northern hemisphere 2017/18 influenza season remained unchanged. However, early and mid-season vaccine effectiveness (VE) estimates were suggestive of reduced VE against A(H3N2) viruses.

1. Introduction

The influenza season 2016/17 started early in week 46/2016 in the northern and western parts of the World Health Organization (WHO) European Region, which has been the earliest start of an influenza season since 2009/10. The season was dominated by A (H3N2) viruses by week 5/2017 [1]. As exemplified during the A (H3N2)-predominated 2014/15 influenza season when 217,000 excess deaths were estimated in the elderly population in Europe [2], the circulation of A(H3N2) viruses is in general associated with more severe outcomes [3]. Several influenza outbreaks particularly affecting long-term care facilities and nosocomial outbreaks [4] have been reported this season by the media and national public health institutes [5]. A(H3N2) viruses drifted in 2015 [6] and recent 2016/17 season reports have indicated genetic diversification of the haemagglutinin (HA) protein of circulating viruses [4,7–9]. Simultaneous circulation of genetically diverse strains in Europe may partly explain suboptimal (<50%) vaccine effectiveness (VE) estimates reported this season in different geographic regions. Early and mid-season VE estimates for 2016/17 showed that VE against A(H3N2) illness has been suboptimal for all age groups in Europe (38%) [10], the US (43%) [11], and Canada (42%) [8], as well as for the elderly in Stockholm county (28%) and Finland (32%) [9]. WHO convened the vaccine composition consultation at the end of February 2017 and recommended to retain the A(H3N2) component for the 2017/18 influenza season for the northern hemisphere [12]. We analysed virological surveillance data reported by the National Influenza Centre's (NICs) from the WHO European Region to describe the genetic and antigenic characteristics of the currently circulating viruses and compare them with the vaccine and WHO reference viruses representing various genetic clades. In addition, we analysed the genetic subclusters of A(H3N2) viruses in relation to the patient's vaccination status.

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2. Virological influenza surveillance in Europe, influenza season 2016/17

Virological influenza surveillance data in the WHO European Region including the European Influenza Surveillance Network are collected on a weekly basis and reported to The European Surveillance System (TESSy) [6]. From week 40/2016 to week 5/2017, 24 countries, including 21 European Union/European Economic Area, reported influenza virus detections together with antigenic and/or genetic characterisation data, according to predefined, by the Collaborating Centre for Reference and Research on Influenza at the Francis Crick Institute London, United Kingdom (WHO CC London), categories as previously described [6,14]. Depending on their resources, NICs perform characterisation of influenza viruses and antiviral susceptibility testing of a sample of virus isolates of all (sub)types, from different age groups, surveillance systems, geographical locations and phases of the epidemic [13]. WHO CC London provides NICs with post-infection ferret antisera raised against vaccine viruses and reference viruses for the preliminary antigenic characterization. Genetic characterisation is commonly performed using the Sanger sequencing method; three laboratories are using Next Generation Sequencing techniques, but do not report minority variants.

Twenty-four countries reported 69,454 influenza detections from sentinel and non-sentinel sources. Influenza type A viruses (66,295, 95%) prevailed over type B (3159, 5%). The great majority (20,702, >99%) of subtyped influenza A viruses were A(H3N2), outnumbering A(H1N1)pdm09 viruses (74, <1%). The lineage of 422 B viruses was determined of which 125 (30%) fell in B/Victoria and 297 (70%) in B/Yamagata lineages.

2.1. Genetic and antigenic analysis of circulating A(H3N2) influenza viruses, 2016/17

Genetic and/or antigenic characterisation (n = 1531 and n = 601, respectively) results were reported for a total of 1878 viruses from 24 countries (Table 1). Three hundred and eighteen of 483 (66%) A (H3N2) viruses were attributed to a predefined category and were reported as antigenically similar to the vaccine strain A/Hong Kong/4801/2014, supporting the WHO recommendation to retain the current virus in the 2017/18 vaccine. Approximately one third (154/483) of A(H3N2) viruses were not assigned to an antigenic reporting category, indicating reduced antigenic titres compared to the vaccine strain, lack of the appropriate reference virus antisera against the different subclusters of A(H3N2) viruses or other difficulties with the haemagglutination assay. Due to the challenges in the antigenic characterisation of A(H3N2) viruses, because of the accumulation of amino acid substitutions resulting to a partial loss of their ability to agglutinate erythrocytes and neuraminidase-driven haemagglutination, antigenic data were not available for all viruses and from all countries. Countries have reported the use of oseltamivir to enhance the performance of the HI assays. However, two countries provided 39% of antigenic reports: Germany (20%) and Slovakia (19%), while genetic data were provided by a larger number of countries (Table 1).

For 959 of the 1531 genetically characterised viruses, reference to a public-access database entry was provided. Of 924 reported A (H3N2) viral HA sequences, 905 (99%) belonged to the 3C.2a clade, represented by A/Hong Kong/4801/2014, while 13 (1%) belonged to the 3C.3a clade represented by A/Switzerland/9715293/2013. The majority (667/918, 74%) of 3C.2a viruses belonged to a newly emerged subclade 3C.2a1 represented by A/Bolzano/7/2016, often with additional amino acid substitutions (Table 2, Fig. 1).

The A(H3N2) 3C.2a1 genetic subclade emerged at the end of the 2015/16 season [14] and has become predominant in the 2016/17 season. In addition to the predominance of the 3C.2a1 subclade, the surveillance data revealed genetic evolution of A(H3N2) viruses (Fig. 1). HA1 sequences of the A(H3N2) strains showed that subclusters within clade 3C.2a and subclade 3C.2a1 have emerged and expanded in recent months. The various subclusters contain viruses from 24 reporting countries across Europe, which supports the conclusion of widespread circulation of 3C.2a1 viruses. According to the WHO CC London interim report, 10% of viruses could be successfully propagated and antigenically assessed [15]. Data from the WHO CCs show that the viruses within the 3C.2a1 subclade remain antigenically similar to the vaccine component, A/Hong Kong/4801/2014 [12]. However, in vaccinated individuals, HI titres of antibodies against several representative circulating cell culture-propagated A(H3N2) viruses belonging to the different 3C.2a1 subclusters were reduced significantly compared to the egg-propagated vaccine virus [12].

All analysed 2016/17 viruses carried substitutions at viral HA antigenic epitopes, which are the main drivers of immunogenicity. Further enhancement of the changes in antigenicity and virulence of influenza viruses has been attributed to shielding of the major antigenic epitopes by alteration of N-linked glycosylation sites, which also applied to some of the emergent subclusters [16,17]. All of the identified subclusters, along with the information on changes on antigenic and/or glycosylation sites and the vaccination status of the patients, are mentioned in Table 2.

2.2. Virus characteristics and patient vaccination status in A(H3N2) influenza viruses, 2016/17

A total of 153 HA sequences originated from vaccinated and 520 from unvaccinated patients. A notable observation was a 3C.2a1 subcluster of viruses that carried the amino acid substitution T135K. This large subcluster included 105 viruses, 13% of the 829 A(H3N2) viruses analysed that were widely circulating in Austria, Finland, Germany, Greece and Sweden, and to a lower extend in the Netherlands, Norway, Slovenia and Spain. Of the viruses within this subcluster that were isolated from cases with known vaccination status, 40% (33/82) originated from vaccinated patients. Substitution T135K is located in a conserved element of the receptor-binding site in the antigenic epitope A and causes a loss of the glycosylation site, while amino acid T135 is known to be conserved in 62% of human H1, H2 and H3 viruses [18]. Antigenic analysis has been performed on 25 of these viruses, of which 7 were not attributed to a predefined category and 18 were characterised as A/Hong Kong/4801/2014-like. According to the WHO CC London interim report, the antiserum raised against the egg-propagated vaccine virus A/Hong Kong/4801/2014 (3C.2a) recognised none of the four analysed viruses with substitutions N121K, T135K and N171K. However, the antiserum raised against the cell culture-propagated A/Hong Kong/7295/2014 (3C.2a) recognised all four viruses with those HA1 substitutions. The egg-propagated vaccine virus is similar but not identical to the cell culture-propagated virus. Some cell-based vaccine candidate viruses have been listed as options for inclusion in the vaccine [15].

Another less populated subcluster within 3C.2a (57/829, 7% of the analysed viruses) with variations on antigenic sites, T131K combined with R142G and R261Q, also accumulated a high proportion of viruses that originated from vaccinated individuals (13/42, 30%); T131 is also known to be conserved in 45% of human H1, H2 and H3 viruses [18] (Table 2).

Early-season results from the I-MOVE project for VE estimates in Europe have also included the aforementioned emerging genetic subclusters [10]. In addition to the common substitutions in amino acid positions 171 and 121 of 3C.2a1 viruses, 22% carried the I140M substitution, 22% carried the R142G mutation, 18% carried the K92R and H311Q substitutions and 7% carried the T135K muta-

Table 1
Antigenic and genetic characterisations per reporting category and country, WHO European Region, weeks 40/2016–5/2017.

	ANTIGENIC										GENETIC										
Reporting category/ Country	AH1, A/ California/ 7/2009-like	AH1, A/ Michigan/ 45/2015-like	AH3, A/ Switzerland/ 9715293/ 2013-like	AH3, A/Hong Kong/4801/ 2014-like	AH3, not attributed to category[a]	BVic, B/ Brisbane/ 60/2008-like	BVic, not attributed to category[a]	BYam, B/ Massachusetts/ 02/2012	BYam, B/ Phuket/ 3073/ 2013	TOTAL ANTIGENIC	AH1, A/ Michigan/ 45/2015 (subgroup 6B.1)[b]	AH1, A/ South Africa/ 3626/2013 (subgroup 6B)	AH3, A/ Bolzano/7/ 2016 (subgroup 3C.2a1)	AH3, A/Hong Kong/4801/2014 (subgroup3C.2a)[c]	AH3, A/ Switzerland/ 9715293/ 2013 subgroup (3C.3a)	AH3, subgroup not listed	BVic, B/ Brisbane/ 60/2008 (clade 1 A)[b,c]	BVic, not attributed to clade	BYam, B/Phuket/ 3073/2013 (clade 3)[d]	BYam, not attributed to clade	TOTAL GENETIC
Austria	1	1	0	0	0	0	0	2	0	4	0	3	30	9	0	0	0	0	2	0	44
Belgium											0	0	15	3	0	0	0	0	0	0	18
Czech Republic											0	0	24	0	0	0	0	0	0	0	24
Denmark	1	0	3	0	0	0	0	0	1	5	0	0	47	47	0	0	0	0	0	0	94
Finland											0	0	56	31	1	0	0	0	2	0	90
France	0	0	4	8	0	0	0	0	0	12	0	0	78	5	0	26	0	0	0	0	109
Germany	0	3	0	0	108	2	0	0	6	119	0	0	88	26	0	0	0	0	0	0	114
Greece	0	0	0	77	0	10	0	0	0	87	0	0	91	13	0	0	3	0	0	0	107
Ireland											0	0	24	1	9	0	0	0	0	0	34
Italy											0	0	22	2	0	0	0	0	0	0	24
Kyrgyzstan	0	0	0	33	0	19	7	0	3	62											
Latvia	0	0	0	14	0	0	0	0	6	20	0	0	0	0	0	0	0	11	0	56	67
Luxembourg	0	0	0	8	0	0	0	0	0	8	0	0	28	9	0	0	0	0	0	0	37
Netherlands											1	0	26	9	0	0	0	0	2	0	38
Norway											4	0	50	91	0	0	13	0	13	0	171
Portugal											0	1	37	13	0	0	1	0	0	0	52
Romania	0	0	0	0	45	2	0	0	0	47	0	0	29	8	0	0	1	0	0	0	38
Russian Federation	1	0	0	45	0	31	0	0	0	77	0	0	1	7	0	0	0	0	0	0	8
Slovakia	0	0	0	112	0	1	0	0	1	114											
Slovenia	0	0	0	2	0	0	0	0	13	15	0	0	15	14	0	0	0	0	5	0	34
Spain											3	0	185	53	4	0	6	0	0	0	251
Sweden											0	0	51	34	0	0	4	0	3	0	92
Switzerland	0	0	4	19	1	1	0	0	1	26											
United Kingdom	0	0	0	0	0	1	0	4	0	5	0	0	62	11	0	0	1	0	11	0	85
Total	3	4	11	318	154	67	7	6	31	601	8	4	959	386	14	26	29	11	38	56	1531

[a] Vaccine component for northern hemisphere 2016/17 season.
[b] Vaccine component for southern hemisphere 2017 and northern hemisphere 2017/18 seasons.
[c] An antigenic drift from vaccine virus is not necessarily indicated.
[d] Vaccine component of quadrivalent vaccines for both northern and southern hemisphere.

Table 2
Influenza A(H3N2) viruses characterised by clade and subclade, amino acid substitutions and patient vaccination status, WHO European Region, weeks 40/2016–5/2017. Coloured circles indicate branch in the phylogenetic tree (see Fig. 1). A total of 153 haemagglutinin sequences originated from vaccinated and 520 from unvaccinated patients.

Reference virus (clade/ subclade) Clade/subclade-specific amino acid substitutions[a] +Additional frequent substitutions	Number of viruses	Number of vaccinated / total number with known vaccination status (% vaccinated)[b]
A/Hong Kong/4801/2014 (3C.2a)	228	33/168 (20)
N121K(D)+S144K(A,-CHO)+/−other substitutions[c]	*152*	*19/111 (17)*
+S219Y(D), I58V	16	2/14 (14)
+122D(A,-CHO),S262N(E)	48	7/31 (23)
+R33Q(C)+/− I230L(D)	7	–
+I140M(A)	4	–
+S199L(B)	4	–
+I214T(D)	4	–
+D53N(C)	3	–
+S144K(A,-CHO)	2	–
T131K(A)+R142G(A)+R261Q(E)	*57*	*13/42 (30)*
Q197K(B)+R261Q(E)	*4*	–
A/Bolzano/7/2016 (3C.2a1)	604	98/468 (21)
N171K(D)+/−other substitutions (w/o N121K)	*193*	*26/156 (17)*
+K259R(E)	14	4/13 (30)
+V213I(D)	4	–
+S47T(C)+G78S	45	5/29 (17)
+R142G(A)+/−other substitutions	13	2/11 (18)
+D53N(C)	2	–
N171K(D)+N121K(D)+/−other substitutions	*409*	*75/310 (24)*
+ I140M(A)+/−other substitutions	161	23/128 (18)
+K2Q	22	2/15 (13)
+N122D(A,-CHO)	5	–
+ T135K(A,-CHO)+/−other substitutions	105	33/82 (40)
+N122D(A,-CHO)	19	4/16 (25)
+Q197K(B)	3	–
+N96S	3	–
+T160K(B,-CHO)	1	–
+ N31S+/−R33Q(C)	15	1/14 (7)
+ K92R(E)+/−other substitutions	92	13/58 (22)
+H311Q(C)	30	4/16 (25)
+Q197K(B)	1	–
+ R142G(A)	45	6/36 (17)
A/Switzerland/9715293/2013 (3C.3a)	3	–
T128A(B,-CHO)+R142G(A)+A138S(A)+S144K(A,-CHO)+N145S(A)+F159S(B)+N225D(D)	3	–

[a] Indicated in parenthesis: the antigenic sites A-E on, or adjacent to which the amino acid substitutions are located; -CHO refers to loss of an N-linked glycosylation site.
[b] Number of vaccinated not shown for subclades with <s10 viruses.
[c] Major subgroups in italics.

tion, compared to the vaccine virus. There were a few discrepancies from the results of the present study, probably due to the variable circulation of specific subclusters of viruses in different counties in Europe that might have not be participated in I-MOVE.

Several studies indicated that simultaneous circulation of the different A(H3N2) subgroups in Europe may partly explain the suboptimal VE estimates reported this season [8–10]. Based on the Canadian report on the circulation of the various subclusters in the region, the higher VE estimates compared to a Scandinavian study could be partly attributed to the relatively infrequent (15%) circulation of the T135K variant in Canada [8], compared to Sweden and Finland [9]; however, relevant antigenic data were not presented. The suboptimal VE results are similar to previous A (H3N2)-dominated seasons [19,20]. Moreover, VE estimates may vary within a given season in the same region, depending on whether the analysis is performed early/mid or end of season, in most cases resulting in lower end-of-season estimates [21,22]. Decreasing influenza vaccine protection with increasing time since vaccination across influenza types and subtypes is associated with intra-season waning of host immunity [23]. These intra-seasonal differences could also be partly explained by the shift in the circulation of emergent variant strains in the community. Real-time VE estimates from Finland and Sweden have shown that after an initial moderate VE of about 50% in the elderly, VE sharply declined, consistent with the observed rise in the circulation of 3C.2a1 emergent subgroups [9].

If the emerging subgroups of A(H3N2) viruses continue to diversify from the vaccine component, in terms of their antigenic properties or a wider circulation of variant strains, the VE might be further reduced by the end of the 2016/17 season.

2.3. Antiviral susceptibility of A(H3N2) influenza viruses, 2016/17

None of the 11 A(H1N1)pdm09, 789 A(H3N2) and 37 influenza type B viruses, for which antiviral susceptibility data were reported, showed molecular or phenotypic evidence of reduced inhibition by the neuraminidase (NA) inhibitors oseltamivir and zanamivir.

3. Limitations

The data are still preliminary for this season as they were collected up to week 5/2017, covering 24 countries of the Region and may therefore not represent the overall situation at the end of the season and in all countries. The characterised viruses were not selected randomly and selection biases may have affected the choice of viruses for sequence analysis and therefore the genetic changes associated with vaccination status have to be interpreted

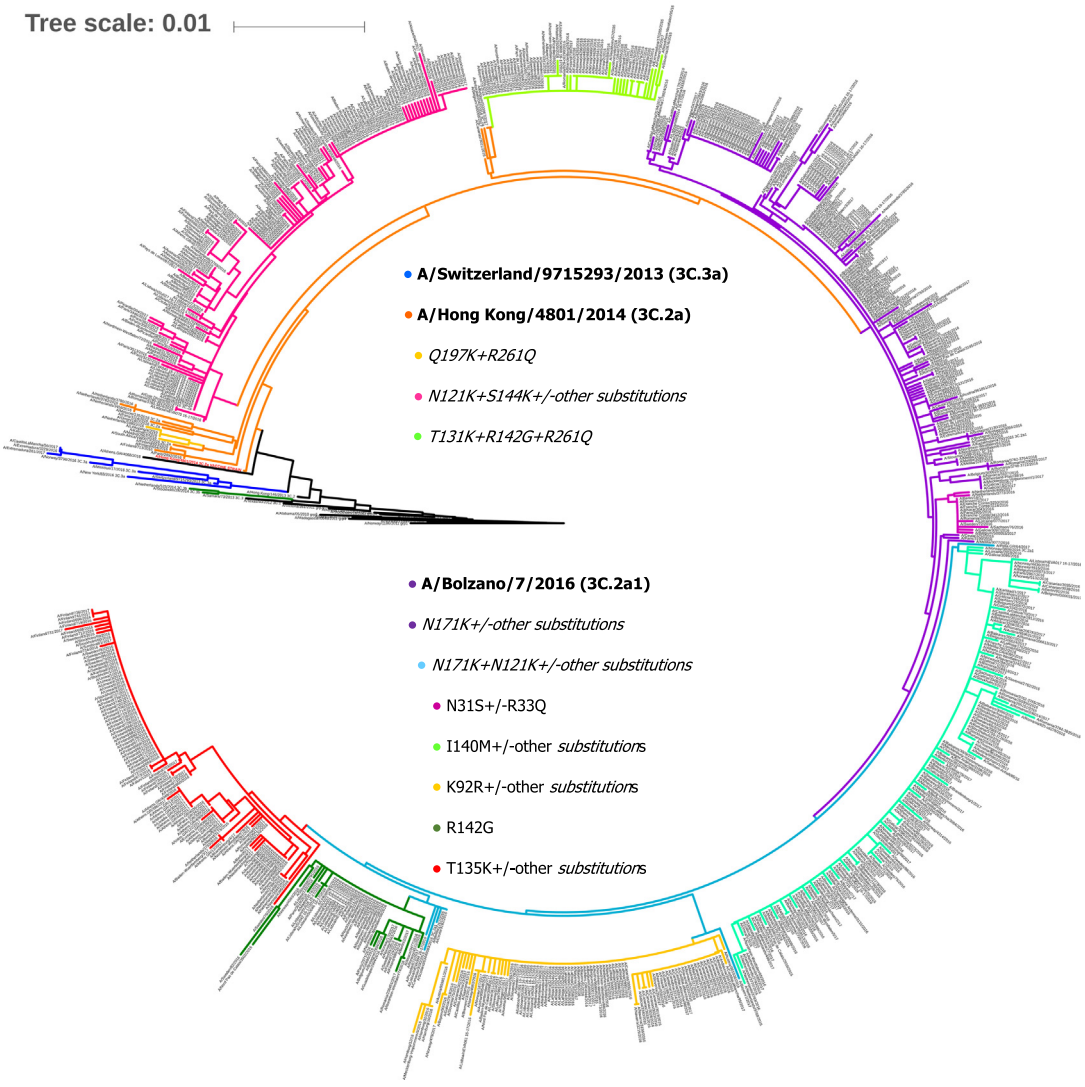

Fig. 1. Phylogenetic comparison of influenza A(H3N2) viruses based on the haemagglutinin (HA) 1 coding gene region. TESSy-reported HA sequences were retrieved from Global Initiative for Sharing All Influenza Data (GISAID) EpiFlu influenza sequence database and used for the genetic and phylogenetic analysis and all the sequence data providers and submitters are acknowledged for their contributions. The phylogenetic trees were constructed with the Neighbour-Joining method, using Kimura-2 parameter-corrected distances and are in the units of the number of base substitutions per site and bootstrapped with 1000 replicates using MEGA software v7.0. The circular tree was prepared with the Tree Of Life v3.4.3. Comparison of aligned sequences and amino acid substitution analysis was done using BioEdit Sequence Alignment Editor v7.2.5. Amino acid substitutions were determined compared to the vaccine virus A/Hong Kong/4801/2014 HA sequence, indicated in red colour. Colour coding of the branches indicates the various phylogenetic clades, subclades and subclusters (see also Table 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with caution. The interpretation of genetic and antigenic data sources is complex due to a number of factors, which can lead to a biased view of the properties of circulating viruses. The viruses that can be recovered and analysed antigenically, may not be fully representative of the majority variants, and on the other hand genetic characterisation data does not always predict the antigenic characterisation result. Vaccination status was known for 54% of the virus strain-based reports and VE results originate from a subset of reporting countries; it was therefore not possible to extend the genetic analysis in relation to the vaccination status to all detected viruses.

4. Conclusion

The available antigenic data from WHO European Region influenza surveillance supporting the WHO influenza vaccine composition recommendation for the 2017/18 northern hemisphere influenza season, were all of the A(H3N2) viruses attributed to a category were reported as antigenically similar to the vaccine virus. The reports on viruses that could not be allocated to a category cause concern, as those may reflect technical challenges in the antigenic analysis of recent A(H3N2) viruses but may also indicate antigenic changes. The genetic data showed that circulating A (H3N2) viruses have undergone considerable genetic diversification. The variable circulation of the emerging subgroups may partly explain the suboptimal early and mid-season VE observed and affect the end-of season A(H3N2) VE estimates. Circulating A (H3N2) viruses continue to be susceptible to NA inhibitors, and antiviral treatment may be administered in accordance to national recommendations. End-season VE estimates against specific 3C.2a1 subclusters, in which included genetic data would originate from randomly selected strains, could provide important information on the relationship of specific amino acid substitutions and

influenza vaccine performance. Influenza surveillance in the WHO European Region and thereby the vaccine virus selection process would be strengthened by linking the genetic with the antigenic data, as well as increasing the number of reporting countries and especially the number of antigenic reports. Due to the varying effectiveness of influenza vaccines from season to season, efforts are needed to improve influenza vaccine effectiveness.

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Conflict of interest

None.

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AM and EB: study initiative, data cleaning and analysis, first draft and revisions of the report. Both authors contributed equally to the study and manuscript.

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