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Short communication

Neuraminidase characterisation reveals very low levels of antiviral resistance and the presence of mutations associated with reduced antibody effectiveness in the Irish influenza 2018/2019 season



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ARTICLE INFO	A B S T R A C T
Keywords: Influenza Laboratory surveillance Ireland Antiviral resistance Molecular epidemiology Vaccine effectiveness	Neuraminidase inhibitor (NAI) resistance levels globally are currently low. However, as antivirals are increas- ingly being used, and even in the absence of selective pressure, resistance may increase or emerge. The neur- aminidase (NA) genes from influenza viruses from the Irish 2018/2019 season were sequenced: 1/144 (0.7 %) A (H1N1)pdm09 sequences harboured a substitution associated with highly-reduced susceptibility to NAIs. The very low NAI resistance we describe supports current Irish NAI use recommendations. However, continued monitoring is essential. NA characterisation also identified substitutions associated with reduced antibody effectiveness, thereby highlighting the potential of NA sequence surveillance as an additional tool for investi- gating influenza vaccine effectiveness (VE).

1. Background

In the most recent global analysis of human influenza viruses, the level of neuraminidase inhibitor (NAI) resistance was found to be 0.2 % [1]. The main amino acid substitutions associated with highly reduced or reduced susceptibility are H275Y and Y155H in influenza A(H1N1) pdm09, and E119V and R292K in influenza A(H3N2) viruses [2]. In spite of current low resistance levels, with antivirals increasingly being recommended for use, there is the prospect of emerging resistance. In Ireland, a national network of sentinel general practitioners, covering 6 % of the population, monitors and swabs patients presenting with influenza-like illness. Influenza surveillance systems in Ireland also include the surveillance of: notified influenza cases; hospitalised and intensive care unit cases and deaths; general practitioner out-of-hours syndromic surveillance; sentinel hospital admissions; excess mortality; outbreaks; and influenza vaccine effectiveness (VE). Laboratory antigenic and genetic characterisation of both sentinel influenza like illness and non-sentinel respiratory clinical samples are carried out by the National Virus Reference Laboratory. During the 2018/2019 season

there were 7943 notified influenza cases and 97 deaths reported in Ireland [3]. Influenza A(H1N1)pdm09 predominated, accounting for 73 % of subtyped samples, followed by A(H3N2) at 27 % [3]. Routine molecular characterisation of neuraminidase (NA) antiviral resistance markers is carried out in Ireland but to a lesser extent than for haemagglutinin (HA) characterisation.

2. Objectives

The primary aim of this work was to investigate levels of NAI resistance in influenza isolates for the 2018/2019 season in Ireland. A secondary aim was to investigate the presence of substitutions that may be associated with reduced antibody effectiveness [4,5].

3. Study design

As a WHO National Influenza Centre, the National Virus Reference Laboratory is designated to carry out virus characterisation and antiviral susceptibility monitoring in Ireland. In this study, influenza A samples

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Received 13 July 2020; Received in revised form 29 August 2020; Accepted 27 September 2020 Available online 1 October 2020 1386-6532/© 2020 Published by Elsevier B.V. from the 2018/2019 season, previously subtyped for the influenza HA gene A(H1N1)pdm09 (n = 171) and A(H3N2) (n = 55) were subject to whole genome PCR followed by Sanger sequencing with specific primers for A(H1N1)pdm09 and A(H3N2) NA genes [6]. Resulting sequences were assembled using Gap4 [7] and were aligned, translated, and analysed for the presence of amino acid substitutions associated with antiviral resistance as detailed by the WHO [8] using MEGA 7.0 [9]. Full length sequences were deposited in the GISAID database. Substitutions were verified using flusurver.bii.a-star.edu.sg. Only sequences with complete sequence coverage for all amino acid substitutions were analysed. Maximum likelihood phylogenies of full-length A(H1N1)pdm09 and A(H3N2) NA sequences were constructed using 1000 bootstrap replicates in MEGA 7.0.

4. Results

Of the A(H1N1)pdm09 (n = 144) samples, one sample (0.7 %) harboured an amino acid substitution (Y155H) shown to be associated with highly reduced susceptibility to oseltamivir and zanamivir in A(H1N1) [10]. In the A(H3N2) (n = 32) sequences no antiviral resistance related substitutions were detected. There was 98.2%-99.1% nucleotide sequence identity between A(H1N1)pdm09 gene sequences and the A/Michigan/45/2015 vaccine strain gene (Fig. 1). All A(H1N1)pdm09 NA full length sequences (n = 58) (which included n = 22 from the Irish influenza VE study) clustered within the A(H1N1)pdm09 6B.1 clade, which included the 2018/2019 vaccine strain A/Michigan/45/2015. There was 98.4 %-99.2 % nucleotide sequence identity between A (H3N2) gene sequences and the A/Singapore/INFIMH-16-0019/2016 vaccine strain gene (Fig. 2). All A(H3N2) NA full length sequences (n = 22) (which included n = 18 from the Irish Influenza VE study) clustered with either A(H3N2) 3C.3a (n = 5) or 3C.2a1b (n = 17) representative strains, rather than the 3C.2a1 vaccine strain A/Singapore/INFIMH-16-0019/2016. All A(H3N2) NA sequences harboured the relatively recently emerged S245N/S247T and P468H substitutions shown experimentally to have reduced antibody binding and to induce lower antiserum titres [4]. The earliest Irish A(H3N2) NA sequence identified with this genotype was from December 2015 (data not shown, GISAID database). Similarly, all full length A(H1N1)pdm09 sequences contained substitutions N248D and N369K and 57/58 contained N449D, which has been associated with reduced reactivity to some human monoclonal antibodies specific to the A/California/7/2009 (H1N1pdm09) vaccine strain [5].

5. Conclusions

Vaccination is the primary public health measure used to prevent, reduce, and interrupt the transmission of influenza infection. However, antivirals are also used for prophylaxis as well as treatment of influenza, and are of particular importance during influenza A(H3N2) predominant seasons when lower influenza VE in older people may occur [11]. Antivirals reduce the risk of severe complications such as hospitalisation and death, as well as the duration of illness, and are recommended to be used as early as possible in the infection [12]. Current Irish guidelines for the use of antivirals recommend antivirals for the treatment of uncomplicated influenza in at-risk groups *i.e.* those ≥ 65 years of age, pregnant women, residential care facilities residents, immunosuppressed individuals, individuals with chronic medical conditions and for treatment of clinically complicated influenza [14]. Targeted use of antivirals for post-exposure prophylaxis is also recommended for those in these at-risk groups.

The risk of widespread antiviral use is that resistance will increase. However, resistant strains can still emerge even in the absence of selective pressure from widespread antiviral use. This occurred during the 2007/2008 season when oseltamivir resistant A(H1N1) harbouring the H275Y substitution emerged with an average prevalence in Europe of 20 % [13]. Mirroring the bacterial antimicrobial resistance 'One Health'

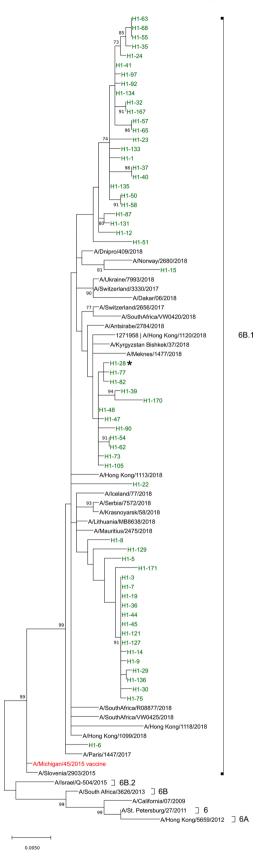


Fig. 1. Maximum likelihood phylogeny of full length A(H1N1)pdm09 neuraminidase nucleotide sequences (n = 58). Irish 2018/2019 samples with H1 prefix and vaccine strain indciated with 'vaccine'. *sequence with Y155H substitution. HA group indicated.

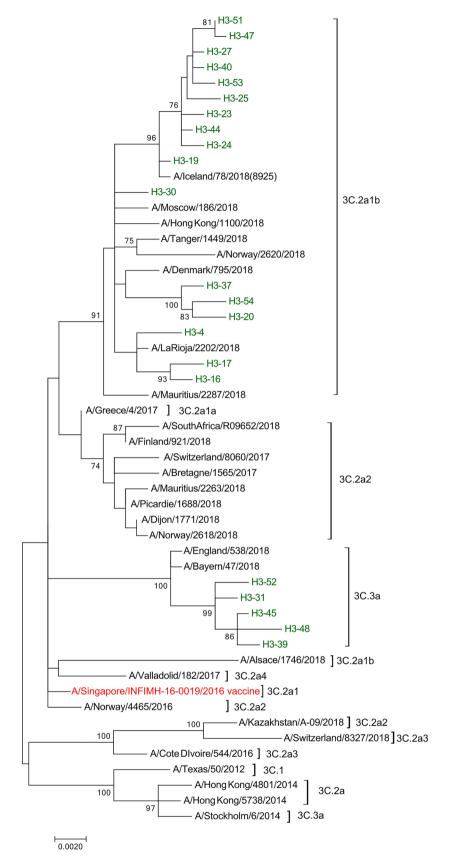


Fig. 2. Maximum likelihood phylogeny of full length A(H3N2) neuraminidase nucleotide sequences (n = 22). Irish 2018/2019 samples with 'H3' prefix and vaccine strain indicated with 'vaccine'. HA group indicated.

framework that has been adopted to tackle it, a multidisciplinary approach has been put forward as a means to detect, contain and prevent the spread of influenza antiviral resistance [14]. Studies have shown the potential for the amplification of antiviral resistance in influenza in natural bird reservoirs upon their ingestion of incompletely degraded antivirals from the environment [15,16].

Seasonal influenza vaccines provide immunity primarily by inducing antibodies that target the protective epitopes on HA. Thus, VE studies primarily concentrate on HA genetic variation: however many factors influence VE, including genetic and antigenic variability, egg adaptation during manufacturing, host related factors (such as age and underlying medical conditions), previous vaccination, and an individual's first influenza infection and subsequent immune response [17]. In Ireland, for the 2018/2019 season, moderate VE against all influenza viruses, and high VE against the predominant A(H1N1)pdm09 subtype [18] was observed, similar to other parts of Europe [19]. However, there was very low VE across Europe against the A(H3N2) subtype 3C.3a clade and amongst the 1964–1983 birth cohort of adults in the 2018/2019 season leading to the suggestion of immune imprinting having a negative impact on VE [20,21].

As a major protein on the influenza membrane surface inducing an independent immune response to that of HA, NA may also play a role in VE. Compared to HA there is a relative lack of characterisation of NA immunogenic epitopes and NA characteristics are not standardised during vaccine production [22]. A recent study demonstrated that a reduction or removal of antibody binding and reduced protection against A(H3N2) strains was due to mutations in the NA gene [4]. These mutations appear to have spread globally and were present in the 2018/2019 A(H3N2) Irish sequences and also in the vaccine strain A/Singapore/INFIMH-16-0019/2016. In addition to potential birth cohort effects, NA mutations may also partly explain the reduced VE observed against influenza A(H3N2) for the 2018/2019 season in Europe. Inclusion of NA genetic characterisation as part of the influenza VE monitoring programme in Ireland therefore will also facilitate identifying potential factors contributing to sub-optimal VE.

The very low levels of NAI resistance identified in our study support the current Irish recommendations for the use of NAIs. However, continued monitoring is essential and must be flexible and timely with the release of new NAIs and other influenza antivirals on to global markets. Influenza surveillance systems, vaccination programmes and control measures need to be strengthened in order to reduce the burden of seasonal influenza on the population, healthcare system and economy and avoid overwhelming health systems with the possible co-circulation of COVID-19 and influenza.

Authors' contributions

Carina Brehony: laboratory work, data analysis, preparation of figures, manuscript writing review and edit.

Linda Dunford: laboratory work, manuscript review and edit.

Charlene Bennett: laboratory work, manuscript review and edit.

Lisa Domegan: conceptualisation of influenza VE objective, selection of VE specimens, manuscript review and edit.

Joan O'Donnell: epidemiological surveillance work, manuscript review and edit.

Eleanor McNamara: supervision, manuscript review and edit.

Cillian De Gascun: project conceptualisation, supervision, manuscript review and edit.

Declaration of Competing Interest

None declared.

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