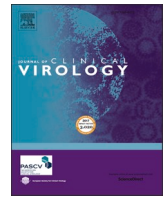




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Short communication

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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ABSTRACT

Neuraminidase inhibitor (NAI) resistance levels globally are currently low. However, as antivirals are increasingly being used, and even in the absence of selective pressure, resistance may increase or emerge. The neuraminidase (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 investigating 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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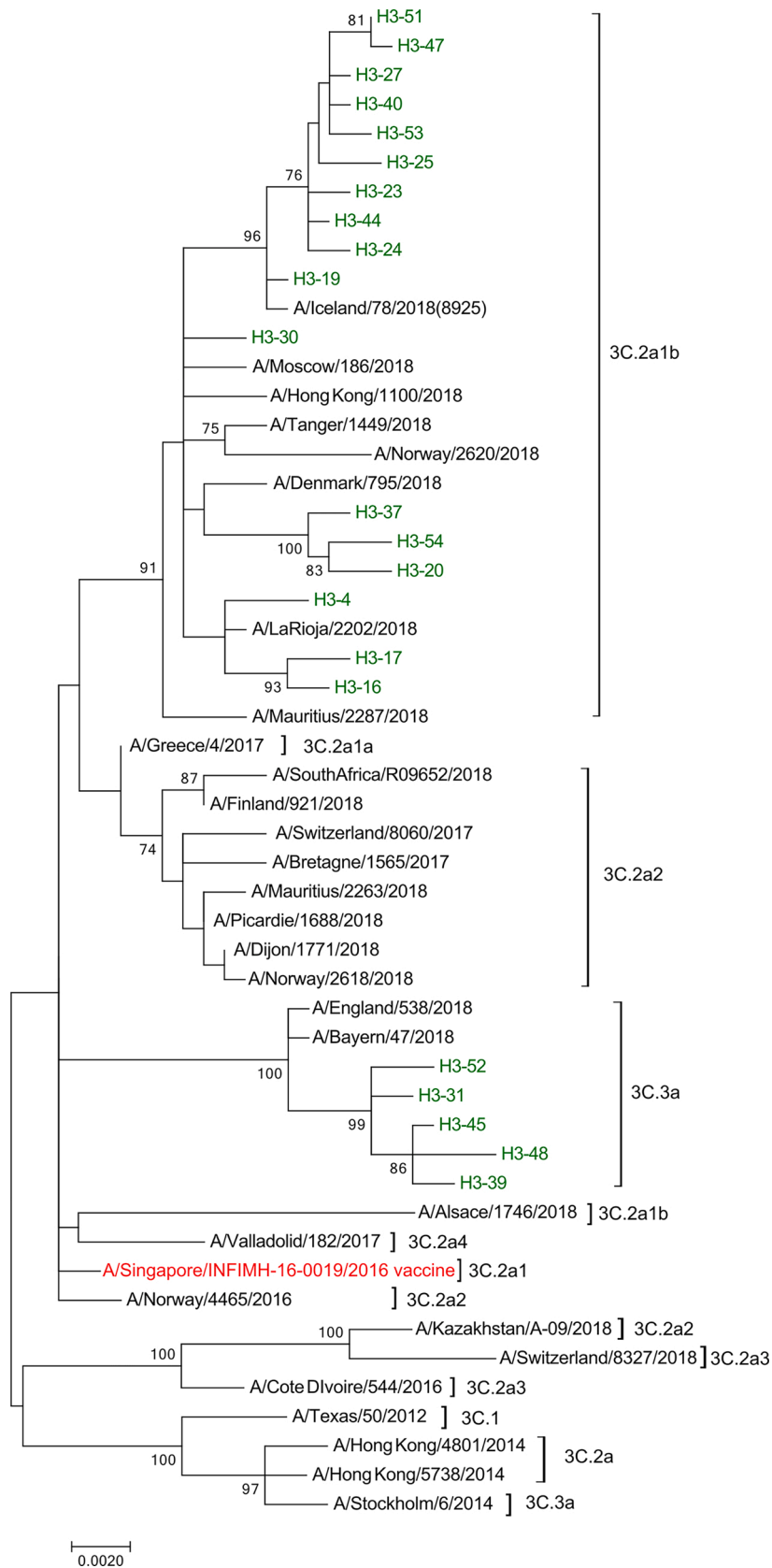


Fig. 2. Maximum likelihood phylogeny of full length A(H3N2) neuraminidase nucleotide sequences (n = 22). Irish 2018/2019 samples with 'H3' prefix and vaccine strain indicated with 'vaccine'. HA group indicated.

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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