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Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014

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ABSTRACT

Four World Health Organization (WHO) Collaborating Centres for Reference and Research on Influenza and one WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza (WHO CCs) tested 10,641 viruses collected by WHO-recognized National Influenza Centres between May 2013 and May 2014 to determine 50% inhibitory concentration (IC_{50}) data for neuraminidase inhibitors (NAIs) oseltamivir, zanamivir, peramivir and laninamivir. In addition, neuraminidase (NA) sequence data, available from the WHO CCs and from sequence databases ($n = 3206$), were screened for amino acid substitutions associated with reduced NAI susceptibility. Ninety-five per cent of the viruses tested by the WHO CCs were from three WHO regions: Western Pacific, the Americas and Europe. Approximately 2% ($n = 172$) showed highly reduced inhibition (HRI) against at least one of the four NAIs, commonly oseltamivir, while 0.3% ($n = 32$) showed reduced inhibition (RI). Those showing HRI were A(H1N1)pdm09 with NA H275Y ($n = 169$), A(H3N2) with NA E119V ($n = 1$), B/Victoria-lineage with NA E117G ($n = 1$) and B/Yamagata-lineage with NA H273Y ($n = 1$); amino acid position numbering is A subtype and B type specific. Although approximately 98% of circulating viruses tested during the 2013–2014 period were sensitive to all four NAIs, a large community cluster of A(H1N1)pdm09 viruses with the NA H275Y substitution from patients with no previous exposure to antivirals was detected in Hokkaido, Japan. Significant

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numbers of A(H1N1)pdm09 NA H275Y viruses were also detected in China and the United States: phylogenetic analyses showed that the Chinese viruses were similar to those from Japan, while the United States viruses clustered separately from those of the Hokkaido outbreak, indicative of multiple resistance-emergence events. Consequently, global surveillance of influenza antiviral susceptibility should be continued from a public health perspective.

1. Introduction

Neuraminidase inhibitors (NAIs) are currently the only licenced antiviral drugs which are effective for the treatment or prophylaxis of seasonal influenza. National and regional antiviral stockpile policies, where they exist, rely primarily on oseltamivir and to a far lesser extent zanamivir, both of which have been approved for use in many countries since 1999–2000. In Japan, two other NAIs, peramivir and laninamivir, have been approved for seasonal use, and favipiravir (T705; Toyama Chemicals), a viral RNA dependent RNA polymerase inhibitor, has recently been approved for pandemic preparedness stockpiling only (http://www.toyama-chemical.co.jp/eng/news/news140324e.html). Peramivir is also approved for use in the Republic of Korea, China, and the United States.

Experience from 2007–2008, when the former seasonal A(H1N1) virus acquired oseltamivir resistance due to an H275Y neuraminidase (NA) amino acid substitution and spread globally within 12 months, has demonstrated that surveillance for NAI-resistant viruses is essential both to guide seasonal clinical management and inform pandemic preparedness strategies (Lackenby et al., 2008; Collins et al., 2009; Dharan et al., 2009; García et al., 2009; Hauge et al., 2009; Hurt et al., 2009; Meijer et al., 2007; Ujike et al., 2010).

The former seasonal A(H1N1) H275Y virus exhibited highly reduced inhibition (HRI) by oseltamivir and peramivir *in vitro* and was shown to be clinically resistant to oseltamivir (Kawai et al., 2009; Dharan et al., 2010; Matsuzaki et al., 2010; Saito et al., 2010). Additional NA substitutions (R222Q, V234M, D344N and D354G) compensated for the detrimental effect of the H275Y substitution on virus fitness, allowing the virus to spread efficiently (Bloom et al., 2010; Rameix-Welti et al., 2011; Abed et al., 2011; Bouvier et al., 2012).

The World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) expert working group on surveillance of influenza antiviral susceptibility (WHO-AVWG) was established in 2011 to provide advice on GISRS surveillance strategies for influenza antiviral susceptibility and to provide practical guidance to WHO-recognized National Influenza Centres (NICs) (WHO, 2012, 2013).

To standardise interpretation and reporting of NAI susceptibility of influenza viruses to individual NAIs, clear definitions were formulated by the WHO-AVWG using 50% inhibitory concentration (IC_{50}; the concentration of drug required to inhibit a standardised amount of NA activity by 50%) fold-change thresholds, compared to the median for viruses from the same type/subtype/lineage showing 'normal inhibition' (NI) (WHO, 2012). Those showing 'reduced inhibition' (RI) are influenza A viruses that have a 10- to 100-fold increase in IC_{50}, or influenza B viruses with a 5- to 50-fold increase in IC_{50}. Viruses showing HRI are influenza A viruses with > 100-fold increase in IC_{50} or influenza B viruses with >50-fold increase in IC_{50} (WHO, 2012).

Recently, we published a global update on the antiviral susceptibility of human influenza viruses collected by NICs between May 2012 and May 2013 as the first of a series of annual reports (Meijer et al., 2014). Only 0.2% ($n = 27$) of 11,387 viruses tested showed HRI against at least one of the four NAIs, usually oseltamivir, and mainly in A(H1N1)pdm09 viruses (21/27). Despite >99% of circulating viruses being sensitive to all four NAIs during the 2012–2013 period, localised community circulation of influenza viruses with RI or HRI has occurred in recent years, most notably with A(H1N1)pdm09 viruses containing the H275Y NA substitution (Hurt et al., 2012; Garg et al., 2013). Animal models have shown that A(H1N1)pdm09 H275Y viruses with additional NA amino acid substitutions, V241I and N369K, have increased replication and transmission fitness (Butler et al., 2014; Abed et al., 2014). Importantly, >97% of the N1 sequences from circulating A(H1N1)pdm09 viruses in 2012–2013 contained the two NA substitutions V241I and N369 K that improve viral fitness of the variant virus (Meijer et al., 2014). These observations, together with those from the former seasonal A(H1N1) 2007–2008 event, illustrate the potential for the global emergence of fit A(H1N1)pdm09 viruses with HRI by oseltamivir and peramivir.

Here, we analysed the NAI susceptibility data for influenza viruses collected across 113 countries by GISRS laboratories between May 2013 and May 2014 (subsequently referred to as 2013–2014).

2. Overall analysis of phenotypic antiviral susceptibility data from WHO CCs

NIC within each country receives or collects clinical specimens from the national laboratory network in order to conduct preliminary analyses. NICs also send representative virus isolates to at least one of the five WHO CCs (Atlanta, United States; Beijing, China; London, United Kingdom; Melbourne, Australia, and Tokyo, Japan; http://www.who.int/influenza/gisrs_laboratory/collaborating_centres/list/en/) for more advanced analyses. At the WHO CCs, viruses are in general passaged one or two times in MDCK cells before being subjected to phenotypic antiviral susceptibility testing.

Five WHO CCs provided IC_{50} and NA amino acid substitution data for virus isolates, notably for those showing RI or HRI by NAIs, recovered from clinical specimens collected between week 21/2013 (20/5/2013) through week 20/2014 (18/5/2014). When available, patient-specific epidemiologic data such as gender, age, geographic location, healthcare setting (community, hospitalised and sentinel/non-sentinel specimen collection), antiviral treatment history and immune status, were included in the analyses. All five WHO CCs tested for oseltamivir and zanamivir susceptibility, and additionally the Atlanta, Melbourne and Tokyo WHO CCs tested for peramivir and laninamivir susceptibility (Supplementary Table 1).

The WHO CCs tested 10,641 viruses from the 2013–2014 period for NAI susceptibility using local adaptations of the fluorescence-based NA enzyme inhibition assay described by Potier et al. (1979) (Supplementary Table 1). The majority of viruses tested were derived from community surveillance specimens, typically collected for influenza diagnosis, and therefore prior to any NAI treatment. However, while antiviral treatment information was not available for many of the specimens, a proportion of viruses were probably derived from patients during or after treatment with NAI, in hospital or community settings. The number of viruses tested was well distributed across the time period but with a small peak during the Southern Hemisphere winter and a prominent peak during the Northern Hemisphere winter (Fig. 1A).

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