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Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2012–2013

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ABSTRACT

Emergence of influenza viruses with reduced susceptibility to neuraminidase inhibitors (NAIs) is sporadic, often follows exposure to NAIs, but occasionally occurs in the absence of NAI pressure. The emergence and global spread in 2007/2008 of A(H1N1) influenza viruses showing clinical resistance to oseltamivir due to neuraminidase (NA) H275Y substitution, in the absence of drug pressure, warrants continued vigilance and monitoring for similar viruses. 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 11,387 viruses collected by WHO-recognized National Influenza Centres (NIC) between May 2012 and May 2013 to determine 50% inhibitory concentration (IC_{50}) data for oseltamivir, zanamivir, peramivir and laninamivir. The data were evaluated using normalized IC_{50} fold-changes rather than raw IC_{50} data. Nearly 90% of the 11,387 viruses were from three WHO regions: Western Pacific, the Americas and Europe. Only 0.2% ($n = 27$) showed highly reduced inhibition (HRI) against at least one of the four NAIs, usually oseltamivir, while 0.3% ($n = 39$) showed reduced inhibition (RI). NA sequence data, available from the WHO CCs and from sequence databases ($n = 3661$), were screened for amino acid substitutions associated with reduced

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NAI susceptibility. Those showing HRI were A(H1N1)pdm09 with NA H275Y ($n = 18$), A(H3N2) with NA E119V ($n = 3$) or NA R292K ($n = 1$) and B/Victoria-lineage with NA H273Y ($n = 2$); amino acid position numbering is A subtype and B type specific. Overall, approximately 99% of circulating viruses tested during the 2012–2013 period were sensitive to all four NAIs. Consequently, these drugs remain an appropriate choice for the treatment and prophylaxis of influenza virus infections.

1. Introduction

Neuraminidase inhibitors (NAIs) are the most widely used antiviral drugs for the treatment or prophylaxis of influenza. The older class of influenza antivirals, the adamantanes, are currently not recommended for use due to the high frequency (>99%) of resistance in circulating influenza A viruses [A(H1N1)pdm09 and A(H3N2)] and their ineffectiveness against influenza B viruses (Simonsen et al., 2007; Nelson et al., 2009; Tisdale, 2009; Gubareva et al., 2010). The NAIs oseltamivir and zanamivir have been approved for use in many countries since 1999/2000, while two other NAIs, peramivir and laninamivir, have to date been approved in Japan, and in the case of peramivir also in the Republic of Korea and China.

The NAIs bind to the neuraminidase (NA) glycoprotein on the surface of influenza A and B viruses, restricting the capacity of these viruses to release from host cells, a critical stage of virus replication. Substitutions of amino acids located in or close to the NA active site can lead to reductions in NAI binding and effectiveness of drug treatment. Such viruses are typically described as being 'resistant' to or showing 'reduced inhibition' or 'highly reduced inhibition' by particular NAIs; these terms might be confusing, as 'resistant' relates to clinical effectiveness and 'reduced inhibition/highly reduced inhibition' to the biological characteristics of the NA. Therefore clear definitions were formulated by the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) expert working group on surveillance of influenza antiviral susceptibility (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 mean or median for viruses from the same type/subtype/lineage showing 'normal inhibition' (NI), for reporting and classifying the NAI susceptibility of viruses to individual NAIs (WHO, 2012). The rationale for the selection of specific fold-change values is described in detail in a previous report (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 'highly reduced inhibition' (HRI) are influenza A viruses with a >100-fold increase in IC_{50} or influenza B viruses with a >50-fold increase in IC_{50} (WHO, 2012). Infections with viruses showing HRI are considered 'clinically resistant' if treatment with the antiviral drug for which the virus shows HRI has reduced clinical effectiveness.

In most years the frequency of circulating influenza viruses showing RI or HRI is less than 1%, but occasionally influenza viruses with RI or HRI spread widely within a community (Hurt et al., 2011; Garg et al., 2013). The most extreme example was in 2007/2008, when the former seasonal A(H1N1) virus acquired the NA H275Y substitution and spread globally in approximately 12 months (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., 2009). This NA substitution conferred HRI by both oseltamivir and peramivir, and was shown in clinical situations to render oseltamivir, the most widely used NAI, significantly less effective for the treatment of this virus infection (Kawai et al., 2009; Dharan et al., 2010). Amino acid substitutions D344N and R222Q in the NA of former seasonal A(H1N1) compensated for the detrimental effect of the HRI H275Y substitution on virus fitness, allowing the virus to spread efficiently (Abed et al., 2011; Rameix-Welti et al., 2011). As regional differences in NAI use exist, high use in Japan and the United States of America (USA) and low use in other parts of the world, this could potentially bias the results in a given region if post treatment reduced susceptibility is prevalent and a substantial proportion of such viruses are submitted to the WHO CC. However, to date such regional differences have not been observed.

On an annual basis, the WHO convenes a technical consultation, the WHO-AVWG, comprised of representatives from the WHO Collaborating Centres for Reference and Research on Influenza (WHO CCs), a selection of WHO-recognized National Influenza Centres (NICs) and public health institutes and research laboratories with expertise in influenza antiviral susceptibility surveillance (WHO, 2012, 2013). The meeting of the WHO-AVWG provides an opportunity to analyse the NAI susceptibility data for influenza viruses collected across 126 countries by GISRS laboratories over the previous 12 months. In this paper, which is the first of a series of annual reports, we present the results for viruses collected via GISRS and analysed by five WHO CCs between May 2012 and May 2013 (subsequently referred to as 2012–2013).

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

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/). provided IC_{50} and NA amino acid substitution data for virus isolates, notably for those showing RI or HRI by NAIs, with specimen collection dates between week 21/2012 (19/5/2012) through week 20/2013 (19/5/2013) inclusive. Epidemiologic data for patient gender, age, geographic location, setting (community, hospitalised and sentinel/non-sentinel specimen collection), antiviral treatment history and immune status were also included in the analyses when available. 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.

The WHO CCs tested 11,387 viruses, collected during the 2012–2013 period, for NAI susceptibility using local implementations of the fluorescence-based neuraminidase inhibition assay described by Potier et al., 1979. The viruses rescued are mostly derived from community surveillance specimens collected at first encounter of the patient, so they are unlikely to have been treated with NAI. A small proportion of viruses are likely to be derived from patients during or after treatment with NAI, but the actual number is unknown as antiviral treatment information is not available for many of the specimens submitted to the WHO CCs. The number tested was well distributed across the time period but with increased numbers over the periods of epidemics in the Southern and Northern Hemispheres (Fig. 1A). Across the 12 months there were 5109 (45%) A(H3N2), 2343 (21%) A(H1N1)pdm09, 2172