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Guidance for clinical and public health laboratories testing for influenza virus antiviral drug susceptibility in Europe

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ABSTRACT

Two classes of antiviral drugs are licensed in Europe for treatment and prophylaxis of influenza; the M2 ion-channel blockers amantadine and rimantadine acting against type A influenza viruses only and the neuraminidase enzyme inhibitors zanamivir and oseltamivir acting against type A and type B influenza viruses.

This guidance document was developed for but not limited to the European Union (EU) and other European Economic Area (EEA) countries on how and when to test for influenza virus antiviral drug susceptibility. It is aimed at clinical and influenza surveillance laboratories carrying out antiviral drug susceptibility testing on influenza viruses from patients suspected of harbouring viruses with reduced susceptibility or for the monitoring of the emergence of such among circulating viruses, respectively. Therefore, the guidance should not be read as a directive or an algorithm for treatment.

Monitoring for emergence of influenza viruses with reduced drug susceptibility in hospitalized cases is crucial for decision making on possible changes to antiviral treatment. Therefore, it is important to test for antiviral susceptibility in certain patient groups, such as patients treated with influenza antiviral drugs. It is also important to determine the frequency of viruses with natural (not related to drug use) reduced susceptibility among community and hospitalized cases, as this knowledge is essential for making empirical antiviral treatment decisions. Furthermore, testing of specimens from community influenza patients is needed to determine the frequency of viruses with reduced susceptibility and good viral fitness that are readily transmissible, as they may become dominant among circulating viruses.

Phenotypic neuraminidase enzyme inhibition assays are recommended to determine the level of inhibition of the neuraminidase enzyme by antiviral drugs as a measure of drug susceptibility of the virus. Genotypic assays are recommended to identify amino acid substitutions in the neuraminidase and M2 ion-channel proteins that have been associated with reduced antiviral susceptibility previously. By 2012 all circulating seasonal influenza A(H1N1)pdm09 and A(H3N2) viruses were naturally resistant to the M2 ion-channel blockers, so priority should be given to testing for neuraminidase inhibitor susceptibility.

Abbreviations: CNRL, Community Network of Reference Laboratories for Human Influenza in Europe; ECDC, European Centre for Disease Prevention and Control; EEA, European Economic Area; EISN, European Influenza Surveillance Network; EQA, External Quality Assessment; EU, European Union; GISRS, Global Influenza Surveillance and Response System; HRI, highly reduced inhibition; (H)RI, reduced or highly reduced inhibition; IC_{50}, 50% inhibitory concentration; IQR, interquartile range; NA, neuraminidase; NAI, neuraminidase inhibitor; NI, normal inhibition; NIC, National Influenza Centre; QCMD, Quality Control for Molecular Diagnostics; RI, reduced inhibition; RT-PCR, reverse transcription polymerase chain reaction; SMAD, Standard Deviation of the Median Absolute Deviation of the Median; SNP, single nucleotide polymorphism; TESSy, The European Surveillance System; WHO, World Health Organization.

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1. Introduction

At the time of writing of this guidance M2 ion-channel blockers amantadine and rimantadine, of the adamantane class of drugs, and oseltamivir and zanamivir, of the neuraminidase (NA) inhibitor (NAI) class of drugs, are registered for influenza therapeutic and prophylactic use in the European Union (EU) and other European Economic Area (EEA) countries. As resistance against these influenza antiviral drugs may emerge within a treated patient or spontaneously without drug pressure, monitoring for antiviral drug susceptibility is critical to inform clinicians and public health officials about the appropriate antiviral drugs that can be used for patient treatment and in outbreak situations.[1]

This document aims to provide guidance for, but not limited to, EU and EEA countries for:

(a) Clinical laboratories carrying out antiviral drug susceptibility testing on influenza viruses from patients suspected of harbouring viruses with reduced antiviral susceptibility.
(b) Laboratories involved in influenza surveillance activities that monitor the emergence of reduced susceptibility among circulating viruses.
(c) How and when to perform tests for influenza antiviral drug susceptibility.

This document should not be read as either a directive or an algorithm for patient treatment schedules (which drug to use, which dose, single or multi-drug therapy, continuous or intermittent therapy, etc.), as such decisions will depend on many pieces of information among which antiviral drug susceptibility of the disease-causing virus is only one component. Rather:

(a) Antiviral drug susceptibility data, generated in accordance with this guidance on who and when to test and with which assays, should allow clinical virologists or microbiologists to give appropriate advice to clinicians on the potential effectiveness of particular drugs to support treatment decisions and management of patients and contacts.[1]
(b) More generally, public health authorities could provide general advice about the use of the appropriate antiviral drugs in outbreaks or pandemics, especially in relation to stockpiled antiviral drugs.[1,2]
(c) Based on the provided recommendations regarding the most suitable assays to determine influenza antiviral drug susceptibility, laboratories should be able to select a testing method that fits with available resources.
(d) These recommendations should be referred to for possible courses of action rather than viewed as requirements for compliance.

The present guidance focuses mainly on the NAIs oseltamivir and zanamivir since circulating A(H1N1)pdm09 and A(H3N2) viruses are naturally resistant to the adamantane class of influenza antiviral drugs.[3]

2. Methods

This guidance has been written by the Antiviral Susceptibility Task Group of the European Influenza Surveillance Network (EISN), Community Network of Reference Laboratories for Human Influenza in Europe (CNRL), coordinated by the European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden. The majority of the authors have extensive knowledge of influenza antiviral research, surveillance and clinical practice, working at World Health Organization (WHO) designated National Influenza Centres (NIC) and a Collaborating Centre for Reference and Research on Influenza, Public Health Laboratories, and laboratories linked to university clinics where influenza patients are treated, providing the expertise needed for writing this guidance. Literature searches were used to underpin the relevance of the guidance. Concepts of the guidance were presented and discussed at the annual ECDC influenza surveillance meetings of 2010, 2011 and 2012, of which the latter two were jointly organised with the WHO Europe regional office. At these meetings, virologists, epidemiologists and clinicians gathered to discuss achievements and outlooks for future development of EISN and the WHO EUROFLU influenza surveillance networks. In addition, drafts of the guidance were discussed at the separate ECDC EISN-CNRL virology task group meetings in 2010 and 2011. The ECDC Advisory Forum reviewed a final draft. Comments and suggestions from all these reviews were taken into account for this final version that is endorsed by the ECDC.

3. Terminology

Amino acid substitutions in the M2 ion-channel protein of type A influenza viruses that cause a highly reduced inhibition of virus growth by adamantanes *in vitro* (Table 1) are known to cause resistance to antiviral therapy in clinical cases.[4] Therefore, it is considered appropriate to designate viruses carrying such amino acid substitutions in the M2 ion-channel protein, resistant. For NAIs this is different. The only amino acid substitution in the NA for which resistance to antiviral therapy in clinical cases has been demonstrated in large population studies is the H275Y substitution in the NA of former seasonal A(H1N1) influenza virus.[5] Currently, the globally accepted tests to detect reductions in susceptibility of influenza viruses to NAIs are phenotypic neuraminidase enzyme inhibition tests that generate IC_{50} values, the concentration of antiviral drug needed to inhibit enzyme activity by 50%.[3] However, such assays allow neither prediction of the level of inhibition of virus propagation *in vitro*, as virus replication might be less dependent on neuraminidase activity in cell culture, nor about the effect on treatment of a patient. Therefore, to ensure consistency in reporting of NAI susceptibility data, the Antiviral Working Group of the WHO Global Influenza Surveillance and Response System (GISRS) has defined categories of susceptibility based on the fold-change of IC_{50} values compared to IC_{50} values for reference susceptible viruses.[6] For type A influenza viruses these are: normal (<10-fold increase), reduced (10–100-fold increase) and highly reduced (>100-fold increase) inhibition. The same categories are used for type B viruses but with <5-fold, 5–50-fold and >50-fold increases. For consistency throughout the document, for M2 ion-channel blockers and NAIs the terms normal inhibition (NI), reduced inhibition (RI) and highly reduced inhibition (HRI) are used; also in relation to amino acid substitutions. To indicate reduced or highly reduced inhibition the abbreviation (H)RI is used.

Amino acid substitutions in NA known to cause (H)RI based on either detection in the indicated virus type and subtype during surveillance, and/or in hospitalised treated patients, and/or clinical trials, as presented in the literature[4,7,8] and from analyses conducted by the authors are shown (Table 1). (H)RI conferring amino acid substitutions in NA identified *in vitro* by passage of virus in the presence of antiviral drug or by incorporating certain residues by reverse genetics, have been excluded from Table 1. Fold-difference IC_{50} values associated with individual amino acid substitutions (not shown) have been used to categorise specific amino acid substitutions in the NA. All substitutions in the M2 ion-channel listed in Table 1, but for G34E, have been documented many times during surveillance or clinical treatment.[4] G34E has only occasionally been documented *in vivo*, but is considered important for monitoring.[4,9]