Antiviral Resistance in Influenza Viruses

Laboratory Testing

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KEYWORDS

• Pyrosequencing • Matrix gene • Neuraminidase • Inhibition assays

KEY POINTS

- Influenza continues to be a significant health care issue. Although vaccination is the major line of defense, antiviral drugs play an important role in prophylaxis and disease management. Approved drug treatments for influenza are currently limited to those that target the viral matrix protein or neuraminidase enzyme.
- Resistance-associated sequence changes in the genes encoding these proteins have been extensively studied. Available methods for genotypic and phenotypic antiviral susceptibility testing have been expanded and are being further developed and improved.
- Rapid molecular techniques including real-time polymerase chain reaction and pyrosequencing assays can be used to screen large numbers of samples but extensive sequencing and phenotypic assays are required for a more comprehensive assessment of antiviral susceptibility.
- The sporadic emergence of drug-resistant variants and the global spread of resistant strains several times in recent years have demonstrated the ongoing need for vigilant patient testing and surveillance programs.

INTRODUCTION

Influenza continues to be a health care challenge, causing annual epidemics that typically affect 10% to 20% of the population from winter to early spring in temperate climates. In addition, the viruses are rapidly evolving, with the continual emergence of new strains and constant threat of another pandemic. In the last century these have occurred in 1918 with the H1N1 "Spanish influenza," in 1957 with the H2N2 "Asian

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influenza," in 1968 with H3N2 "Hong Kong influenza," and most recently in 2009 with the new H1N1 strain that emerged from swine in Mexico. Vaccination is typically the first line of defense against influenza. However, evolutionary changes in the virus during a season can result in suboptimal correlations between vaccine strain antigens and those of the circulating strains, and manufacturing problems may cause delays in vaccine availability, either of which results in poor community protection. Furthermore, even in ideal circumstances, as with all vaccines, efficacy is less than 100%; there are always members of the community who are unable to be vaccinated for medical reasons. Therefore, antiviral drugs play an important role in the prevention of influenza infection and in disease management.

Currently there are only 4 antiviral drugs approved by the Food and Drug Administration (FDA) in the United States for the treatment or prophylaxis of influenza. These drugs include 2 adamantanes, amantadine and rimantadine, which block the M2 protein channel in the virus envelope, and 2 neuraminidase (NA) inhibitors, oseltamivir and zanamivir. Additional drugs that target the NA enzyme as well as those directed at other viral and cell targets are in various stages of development and approval. Although influenza viruses do not readily generate resistance to antiviral drugs, resistant strains can spread rapidly, replacing the susceptible virus population globally within 1 to 2 seasons. Therefore, testing for the presence of resistance is an important component of influenza surveillance as well as an important diagnostic service for patients who are not responding to treatment.

MICROBIOLOGY

Members of the family *Orthomyxoviridae*, influenza viruses, types A, B, and C, infect humans, although type C viruses usually cause insignificant and asymptomatic infections and are not included in this review. Influenza A viruses are further subtyped based on the 2 major glycoproteins in the viral envelope, the hemagglutinin (HA) and the NA. A total of 18 different HA and 9 different NA have been described, and although other influenza A subtypes occasionally infect humans and cause disease, only subtypes H1N1, H1N2, H2N2, and H3N2 circulate in humans, together with influenza B viruses.

Both influenza A and B viruses contain a segmented genome comprising 8 negative-sense RNA fragments. Three of the largest segments encode the proteins of the replication machinery PB1, PB2, and PA and a fourth contains the information for the nucleoprotein, NP. Two other segments encode the HA and NA genes. Finally, 2 smaller segments encode the matrix (M) gene proteins M1 and M2 (or BM2 in influenza B viruses) on one segment and an interferon antagonist and nuclear export protein, NS1 and NEP, on the other segment. A few additional genes have been identified in some segments, not all of which have had their function elucidated. At both ends of all segments are conserved sequences that function as replication and transcription promoters.

Receptor-mediated binding is facilitated by the HA envelope proteins of the virus, which bind to sialic acid residues on glycoprotein or glycolipids on the external cell membrane surface. Following endocytosis, a decrease in pH inside the endosome causes a conformational change in the HA protein. This decrease initiates fusion of the viral and endosomal membranes and results in the release of genomic segments and internal structural proteins into the cell cytoplasm. These viral components are imported into the cell nucleus where replication and transcription occur. As is seen in most RNA viruses, the polymerase has a relatively high error rate, resulting in the frequent generation of sequence changes. In addition, because of the segmented