The main option in reducing the impact of influenza is immunoprophylaxis with an inactivated vaccine, a preventive measure particularly recommended for groups with a high risk of complications. Antiviral drugs represent another therapeutic chance to reduce deaths, complications, duration of hospitalization and could play a role in prevention of the infection by chemio-prophylactic action and by reducing the viral shedding of the infected patient. Two classes of antiviral agents have been used to treat influenza, the M2 ion channel inhibitors (MI), that include Amantadine derivates and have been used for more than 30 years [1, 2], and the Neuraminidase inhibitors (NI), Zanamivir and Oseltamivir, available since the half of ninety [3, 4]. These two classes of drugs target different viral proteins and have different mechanisms of action on the replication cycle of the virus. Amantadine inhibit virus replication during the early stage of infection by blocking the ion channel formed by the transmembrane domain of M2 protein [5, 6], and NI interrupt replication cycle by preventing virus release [7]. MI drug resistant mutants are present in about 30% of treated patients although Japanese authors have reported that the frequency of resistant viruses in children treated with Amantadine could be high as 80% [8, 9]. Recent reports on the global prevalence of adamatine-resistant viruses indicate a significant increase of drug resistance, from 1.8% during the 2001/02 influenza season to 12.3% during the 2003/04 season [10]. In USA, > 90% of the virus isolated during the first months of 2005/06 showed amino acid mutations known to be correlated with Amantadine resistance. In contrast to Amantadine resistance, NI resistance remains rare worldwide [11].

In Table I were reported the amino acid substitutions observed in the collected viruses in the hydrophobic sequences in the trans-membrane region of the M2 protein. No strain isolated before 2004 showed amino acid substitution related to Amantadine or Rimantadine resistance, while 4 out of 24 (16.7%) viruses collected during the 2004/05 season showed S31N substitution. Interestingly, in the 4 S31N escape mutants, other mutations at codon 21, 27 and 28 (D21G, V27T and V28I) in the trans-membrane region were observed; in particular, substitution in position 27 was observed in Amantadine or Rimantadine resistant viruses isolated in Asia and America, but Valine was usually substituted with Alanine (V27A) [10, 15].

The 4 escape mutants were A/H3N2 viruses collected in the first weeks of the 2004/05 epidemic and the above mentioned mutations were not present in the A/H1N1 and A/H3N2 viruses isolated during the peak and when the incidence decreased. The patients from whom drug-resistant viruses were isolated were < 8 year children who lived in different areas of Genoa and they did not appear epidemiologically correlated. The hemagglutination inhibition (HI) test and HA-NA sequence analysis allowed to characterize the 4 viruses as belonging to a distinct cluster very close to A/H3N2/Panama/2007/99.
No mutation in NA gene related to resistance to Zanamivir and Oseltamivir were observed in the 40 strains. The alarming increase in incidence of Amantadine-resistant and Rimantadine-resistant influenza A viruses over the past decade described in Asia and America was not entirely observed in this Italian surveillance: the drug-resistant virus proportion of 16.7% observed during the 2004/05 season was similar than that registered in USA (15%) during the same season and very much lower than that reported in China and Hong Kong during the 2003/04 season (70 and 74%, respectively) and in the U.S.A. during the 2005/06 season (92%). The 4 drug resistant viruses present very peculiar epidemiological and virological figures: the early circulation during the 2004/05 epidemic, the young age of the infected patients and the belongings to a antigenic cluster that circulated very rarely during the 2004/05 season. A/H3N2/California/7/04-like viruses, that predominated during the epidemic did not showed mutation related to MI-resistance [16].

The effect of Adamantane use on epidemiological data is very difficult to assess as data on Adamantine treatment or exposure are available only in the U.S.A., where of the 92 patients from whom drug-resistant viruses were isolated since 2004, only two (2%) were known to have receive MI treatment before virus collection [10]. Furthermore, MI are available in over-the-counter formulations and do not need prescription in China, Russia and some other countries [17, 18]. In Italy the use of MI is very limited and no patients from whom viruses were isolated were treated. MI-resistant mutations could be occurred without the drug pressure as reported by Bright [10]: he described A/H1N1 viruses, isolated in 1930’s (A/PR/8/34 and A/WSN/33), before these drugs were developed, presenting amino acid substitution associated with resistance. More studies are needed to define the circulation of MI-resistant viruses, the frequency with which they are transmitted and their impact on effort to control influenza. This data are of fundamental importance in relation to strategies for MI or combined MI-NI use in case of epidemic or pandemic. On the other hand, no virus showed amino acid mutations related to NI-resistance: the lack of emergent resistance make them the drug of choice for prophylactic or therapeutic purpose.

References


[14] Ilyushina NA, Bovin NV, Webster RG, Govorkova EA. Combina-

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Tab. 1. Amino acid substitutions in the hydrophobic sequences in the trans-membrane region of the M2 protein.

<table>
<thead>
<tr>
<th>Period</th>
<th>N. Isolates</th>
<th>M2 amino acid</th>
<th>M2-Inhibitor Resistance Pattern</th>
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</thead>
<tbody>
<tr>
<td>1999-2005</td>
<td>7</td>
<td>21 26 27 28 30 31 32 34</td>
<td>Susceptible</td>
</tr>
<tr>
<td>2003-04</td>
<td>6</td>
<td>21 26 27 28 30 31 32 34</td>
<td>Susceptible</td>
</tr>
<tr>
<td>2004-05</td>
<td>4</td>
<td>21 26 27 28 30 31 32 34</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

20 21 26 27 28 30 31 32 34 Susceptible
nation therapy, a potential strategy for reducing emergence of drug-resistant influenza A variants. Antiviral Res 2006 in press


