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## **SENSITIVITY OF 2015 KAZAKHSTAN INFLUENZA VIRUSES TO CHEMOTHERAPY DRUGS**

**Abstract.** One of the most important characteristics of influenza viruses is resistance to specific medicines. Practice shows that it is impossible to select an etiotropic antiviral drug effective against the whole variety of circulating viruses.

The purpose of this work was to study the resistance of the Kazakhstan strains of influenza virus to commercial chemotherapy drugs with different mechanisms of action. Studies were conducted on new isolates of the influenza A/H1N1 viruses isolated in 2015. Sensitivity to influenza drugs was assessed by the level of inhibition of reproduction of 100 EID<sub>50</sub> (50% embryo infectious dose) of the virus by different drug concentrations in chick embryos.

It was established that the 2015 Kazakhstan strains of the influenza A/H1N1 viruses are sensitive to tamiflu and resistant to arbidol and ingavirin. With respect to remantadine, both sensitive and resistant variants have been detected among the viruses studied which indicates the heterogeneity of the influenza virus strains circulating in Kazakhstan. The results obtained indicate the need to monitor the epidemiological surveillance and study drug resistance in viruses – infectious agents.

**Key words:** influenza virus, chemotherapy drugs, anti-influenza agents, sensitivity, resistance.

**Introduction.** Acute respiratory viral infections (ARVI) and influenza are the most massive infections of mankind and represent a serious problem for public health. Influenza is in the first place among all human diseases in terms of social importance, huge damage to the health of the population and economy [1]. The share of influenza and ARVI accounts for 10-30% of temporary disability among the population. Influenza infection causes up to 40% of all adult diseases, more than 80% of all infectious pathology, more than 60% of diseases among children. Each year, seasonal epidemics result in about 3-5 million cases of serious illness and about 250-500 thousand cases of death [2].

The ability of the influenza causative agent to change constantly in the process of replication presents a serious problem for practical medicine and virology. Influenza viruses can acquire new properties even due to point mutations in the genome, which leads to an ineffective treatment. One of the most important characteristics of the virus is its resistance to specific drugs [3, 4].

Chemotherapy of viral infections, as a method of treatment, originates from the accidental detection of antiviral properties of the adamantane derivatives in the late 1960s. An extensive experience in the development and use of new agents for the treatment and prevention of viral infections has been accumulated for this time. In the case of influenza, it is recommended to use drugs that have a direct inhibitory effect on the virus reproduction, with different mechanisms of action. The most widely used etiotropic drugs are represented by four groups [5]:

- ion channel blockers (adamantanes, including remantadine);
- hemagglutinin(HA)-specific chaperone (arbidol);
- neuraminidase (NA) inhibitors (tamiflu (oseltamivir), relenza, peramivir);
- NP-directed inhibitors (ingavirin).

The purpose of this work was to study the resistance of the Kazakhstan new epidemically important influenza viruses to commercial chemotherapy drugs recommended for the treatment and prevention of influenza infection.

**Materials and methods.** The following Kazakhstan influenza A/H1N1 viruses, isolated in 2015, were used in the study: A/Aktobe/02/15, A/Atyrau/60/15, A/Atyrau/64/15, as well as reference strains stored in the laboratory collection: A/Kostanay/353/15, and A/California /04/09 pdm, A/Solomon Islands/03/06, A/New Jersey /8/76. The viruses were cultured in the allantoic cavity of developing 8-10-day chick embryos for 48 hours at 36 °C. Hemagglutination activity was determined according to a conventional technique on 96-well plates using a 0.75% suspension of chicken red blood cells [6]. The infectivity was calculated by the L. Reed and H. Mench method [7].

To determine the drug resistance of viruses, the effect of four commercial drugs (remantadine, tamiflu, arbidol, and indavirin) from various manufacturers in an active form was examined. Remantadine (Olainfarm, Latvia) was used in the form of rimantadine hydrochloride (alpha-methyltricyclo [3.3.1.1<sup>7</sup>] decane-1-methanamine); tamiflu (F. Hoffmann-La Roche, Switzerland) as oseltamivir phosphate (ethyl(3R,4R, 5S)-5-amino-4-acetamido-3- (pentan-3-yloxy) -cyclohex-1-en-1- carboxylate); arbidol (Pharmstandard-Leksredstva, Russia) as umifenovir hydrochloride monohydrate (6-bromo-5-hydroxy-1-methyl-4-dimethylaminomethyl-2-phenylthiomethylindole-3-carboxylic acid ethyl ester); ingavirin (Valenta Pharmaceuticals, Russia) as imidazolethyanamide pentanedioic acid.

Sensitivity of viruses to anti-influenza drugs was assessed by the level of inhibition of reproduction of 100 EID<sub>50</sub> of the virus by different drug concentrations [8] in chick embryos. A drug dose suppressing the virus titer in the hemagglutination reaction twice compared to the control was considered inhibitory concentration (IC<sub>50</sub>). Three independent experiments were carried out for every combination of the drug concentration and viral material in three chick embryos for each of them.

**Results.** To determine the drug resistance of the Kazakhstan influenza 2015 viruses, chemotherapy drugs with different mechanisms of action were used in non-toxic concentrations for chick embryos. The table presents the results of a study on the sensitivity of influenza viruses A/Aktobe/02/15, A/Atyrau/60/15, A/Atyrau /64/15 and A/ Kostanay /353/15 to antiviral drugs in comparison with the reference strains of the influenza A/H1N1 virus.

Study on the sensitivity of the Kazakhstan and reference strains of influenza 2015 A/H1N1 viruses to antiviral drugs

Strain	Inhibitory concentration*, mg/mL			
	Remantadine	Tamiflu	Arbidol	Ingavirin
A/Aktobe/02/15	6.3±0.4	3.1±0.3	does not inhibit	does not inhibit
A/Atyrau/60/15	3.5±0.3	13.0±0.1	does not inhibit	does not inhibit
A/Atyrau/64/15	3.9±0.7	7.2±0.2	does not inhibit	does not inhibit
A/Kostanay/353/15	does not inhibit	3.4±0.3	does not inhibit	does not inhibit
A/California/04/09 pdm	does not inhibit	3.5±0.02	does not inhibit	does not inhibit
A/Solomon Islands/03/06	6.4±0.02	3.4±0.02	does not inhibit	does not inhibit
A/New Jersey/8/76	12.65±0.2	6.25±0.1	does not inhibit	does not inhibit
*A drug concentration which causes a decrease in the reproduction of the virus in developing chick embryos by 2 times is indicated.				

As can be seen from the table, the IC<sub>50</sub> values against the West Kazakhstan viruses (A/Aktobe /02/15, A/Atyrau /60/15, A/Atyrau /64/15), as well as the reference variant A/Solomon Islands/03/06, were 3.50 to 6.4 mg/mL for remantadine. The A/Kostanay/353/15 strain, like the reference virus A/California/04/09 pdm, showed resistance to remantadine.

Reproduction of the A/Atyrau /60/15 strain was inhibited by the drug Tamiflu at a concentration of 13.0 mg/mL. Three other Kazakhstan influenza 2015 viruses, like the reference strains taken in the experiment, showed a high degree of sensitivity, since their reproduction was suppressed by the drug at low concentrations of 3.1 to 7.2 mg/mL.

All the viruses studied showed absolute resistance to the drugs Arbidol and Ingavirin.

**Discussion.** Practice shows that it is impossible to select an etiotropic antiviral drug effective against the whole variety of circulating viruses. As is known, remantadine is the most widely used drug among the adamantane series, which blocks  $M_2$  protein and thus stops the regulation of pH level and disrupts the process of virus uncoating. Remantadine was the main drug for the treatment of influenza for more than 35 years. In the early 1980's, the first data on viruses resistant to this drug were published [9]. By 2006, the number of resistant strains had increased to 70-100% in various regions of the world, and later began to decline [10]. Data from numerous studies described in the literature confirm the resistance of the variants of the pandemic A/H1N1/2009 strain to the adamantane drugs [11].

The results of studying the sensitivity of the Kazakhstan strains to remantadine showed that the A/Kostanay/353/15 virus exhibited resistance like the reference variant A/California /04/09 pdm; in contrast, the strains A/Aktobe/02/15, A/Atyrau /60/15, and A/Atyrau/64/15 were found to be sensitive to this drug. This may be one of the major signs of the heterogeneity among the population of influenza viruses circulating in the Republic.

Inhibitors of influenza virus neuraminidase (tamiflu, oseltamivir) are used in clinical practice since the late 1990s, when more than 80% effectiveness of the drug has been shown [12, 13]. They interact with the active center of the enzyme and are competitive inhibitors, disrupting the penetration of viruses into the cell and budding of mature virions from the membranes of infected cells. Use of oseltamivir shortens the average duration of disease by 37%, diminishes the symptom manifestation in 30-38%, and reduces the incidence of influenza-related complications by 67% and complication-associated mortality in high-risk patients by 71% [10]. At the same time, the influenza virus demonstrates a high potential for the development of oseltamivir-resistant strains. For example, resistance to this drug has reached 95-100% by 2007-2009 [14].

At the present time tamiflu is effectively used in the treatment of influenza, because the currently circulating strains related to the pandemic 2009 virus, resistant to remantadine, retain sensitivity to tamiflu. At the same time there are reports on the detection of oseltamivir-resistant pandemic variants of the influenza A virus [15]. In the conducted studies, tamiflu was effective against all viruses taken in the experiment, both reference and Kazakhstan.

In the literature there are a number of references to the effectiveness of arbidol against influenza viruses and absence of resistant strains [16]. The mechanism of action of this drug lies in the violation of conformational changes in the second HA subunit, necessary for penetration through the endosomal membrane, which leads to disturbances in the reproduction of the virus at a stage of the virion assembly [17]. During the work with the Kazakhstan isolates of the influenza virus, those sensitive to arbidol and ingavirin were not detected among them.

Drug resistance in viruses is the result of changes in hereditary properties [18] and develops with repeated administration of drugs [19, 20]. There are described cases of isolation of resistant strains from the samples obtained from patients who had not previously taken specific antiviral agents, which can be explained by the transmission of such strains from person to person [16]. The stability of influenza viruses is caused by mutations in that viral protein, which is the target of the drug action [21, 22].

Drug resistance poses a threat to the effective prevention and treatment of influenza infection, since resistant pathogens are not amenable to standard therapy, which leads to a prolonged course of the disease, increased health care costs, and the risk of death. Patients remain infectious for a longer time, which increases the risk of spreading the viruses among other people.

**Conclusions.** The study on the resistance of Kazakhstan strains of 2015 influenza viruses to commercial chemotherapy drugs showed their sensitivity to tamiflu, and resistance to arbidol and ingavirin. With respect to remantadine, both sensitive and resistant variants have been detected among the viruses studied, which indicated the heterogeneity of the influenza virus strains circulating in Kazakhstan. The research findings indicate the need to monitor epidemiological surveillance and study drug resistance in viruses.

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### **2015 Ж. ҚАЗАҚСТАНДЫҚ ТҰМАУ ВИРУСТАРЫНЫҢ ХИМИЯЛЫҚ ПРЕПАРАТТАРҒА СЕЗІМТАЛДЫҒЫ**

**Аннотация.** Тұмау вирусының маңызды сипаттамаларының бірі – препараттарға қарсы төзімділігі. Тәжірибеде көрсеткендей, айналымдағы барлық вирус түрлеріне этиотропты вирусқа қарсы дәрілік препараттарды тиімді таңдау мүмкін емес.

Жұмыстың мақсаты коммерциялық химиялық препараттарға қатысты Қазақстандағы тұмау вирус штамдарының тұрақтылығын зерттеу. Зерттеулер 2015 жылы оқшауланған А/Н1N1 тұмауының вирусының жаңа изоляттарына жүргізілді. Вирусқа қарсы дәрілерге сезімталдығын тауық эмбриондарында препараттардың әртүрлі концентрацияларымен вирустардың 100 ЭИД<sub>50</sub> репродукциясын төмендету деңгейі арқылы бағаланды.

2015 ж. Қазақстандық А/Н1N1 тұмау вирус штаммдары тамифлюге сезімталдығы және арбидолмен ингавиринге тұрақтылығы анықталды. Ремантадинге қатысты зерттелген вирустардың арасында сезімталды және төзімді нұсқалары анықталды, бұл дегеніміз Қазақстан айналымдағы тұмау вирус штаммдарының біркелкі емес екендігін көрсетеді. Нәтижелер эпидемиологиялық қадағалауды бақылау және вирустық жұқпалы агенттердің дәрілік төзімділігін зерттеудің қажеттілігін көрсетеді.

**Түйін сөздер:** тұмау вирусы, химиопрепараттар, тұмауға қарсы дәрілер, сезімталдық, тұрақтылық.

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### ЧУВСТВИТЕЛЬНОСТЬ КАЗАХСТАНСКИХ ШТАММОВ ВИРУСОВ ГРИППА 2015 г. К ХИМИОПРЕПАРАТАМ

**Аннотация.** Одной из важнейших характеристик вирусов гриппа является резистентность к специфическим лекарственным средствам. Практика показывает, что невозможно подобрать этиотропный противовирусный препарат, эффективный против всего многообразия циркулирующих вирусов.

Цель настоящей работы состояла в изучении резистентности казахстанских штаммов вируса гриппа по отношению к коммерческим химиопрепаратам различного механизма действия. Исследования проводили на новых изолятах вируса гриппа А/Н1N1, выделенных в 2015 г. Чувствительность к противовирусным средствам оценивали по уровню подавления репродукции 100 ЭИД<sub>50</sub> вируса различными концентрациями препаратов в куриных эмбрионах.

Установлено, что казахстанские штаммы вируса гриппа 2015 г. А/Н1N1 чувствительны к тамифлю и устойчивы к арбидолу и ингавирину. По отношению к ремантадину среди исследованных вирусов обнаружены как чувствительные, так и резистентные варианты, что свидетельствует о неоднородности циркулирующих в Казахстане штаммов вирусов гриппа. Полученные результаты указывают на необходимость проведения мониторинга по эпидемическому надзору и изучения лекарственной устойчивости вирусов – возбудителей инфекционных болезней.

**Ключевые слова:** вирус гриппа, химиопрепараты, противовирусные средства, чувствительность, резистентность.

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