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# Genetic changes in influenza a virus genes responsible for formation of drug resistance phenotype

## Abstract

Here we present the results of adaptation and analysis of genetic changes of influenza strains A/FPV/Waybrige/78 (H7N7) and A/Swine/Iowa/30 (H1N1) to blockers of ion channels (Remantadin®) and neuraminidase inhibitor (Tamiflu®). From 6 to 10 times increase in the $IC_{50}$ value of Tamiflu for FPV_RTam and Sw_RTam compared with wild-type variants was shown. The $IC_{50}$ value was increased by 10 to 33 times for Remantadin-resistant mutants. The substitutions S31N and A30T were shown in the M2 protein structure of mutants FPV_RRim and Sw_RRim respectively. The mutations like H274Y in the structure of neuraminidase which are responsible for resistance to Tamiflu, in the mutants FPV_RTam and Sw_RTam was not revealed. But, in the structure of the M1 protein amino acid sequence of these mutants, an unexpected substitution at position 207 was recorded. To study the problems of formation of drug-resistant viruses and find the ways to overcome the resistance, the experiments were carried out on the adaptation of influenza virus strains to high concentrations of antiviral drugs.

**Keywords:** Influenza virus, Drug resistance, Tamiflu, Remantadin, Sequencing, Mutation, Antiviral drugs

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## Introduction

Despite the progress attained in the fight against influenza, it remains one of the most pressing medical and social problems due to the ability to spread rapidly around the world and affect different strata of the population.[1]

Attempts to control the spread of influenza virus through vaccination are complicated by the speed of mutation.[2] Vaccination prevents the development of influenza in 70-90 % cases; nevertheless, vaccines only protect against the limited range of strains and are not effective against the new, potentially pandemic variants.[3]

An alternative to the vaccine treatment is the use of antiviral drugs for treating influenza virus. To date, 2 groups of anti-influenza drugs are widely used: adamantanes (amantadine and rimantadine), being the blockers of ion channels formed by the viral M2 protein.[4] and the neuraminidase inhibitors (NAIs) (oseltamivir and zanamivir) [5]. Since 2010, the neuraminidase inhibitors are the only class of antivirals recommended by the WHO as the first-line treatment for people requiring antiviral therapy as the currently circulating influenza viruses are resistant to the adamantanes.[1]

The emergence and worldwide spread of oseltamivir-resistant seasonal A (H1N1) viruses during the 2007-2009 seasons emphasize the need for continuous monitoring of antiviral drug susceptibilities. Further research priorities should include a better understanding of the mechanisms of resistance to existing antivirals, the development of novel compounds.[5]

To study the problems of formation of drug-resistant viruses and find the ways to overcome the resistance, the experiments were carried out on the adaptation of influenza virus strains to high concentrations of antiviral drugs. The studies have been conducted on evaluating the structural characteristics of NA and M genes in tamiflu- and rimantadine-resistant strains of influenza A virus.

## Materials and methods

Influenza virus strains A/FPV/ Weybridge /78 (H7N7) and A/Swine/Iowa/30 (H1N1) used in the study were passaged in MDCK cell culture. Because H7N7 viruses have caused disease in mammals, including horses, seals, and humans, they may form a pandemic threat to humans.[6] Swine are an important reservoir for influenza A virus. As well as having an impact on animal health, influenza virus in swine is a source for zoonotic and pandemic infections in humans. Classical H1N1 swine influenza viruses are genetically and antigenically similar to the type A influenza viruses implicated in the human pandemic.[7] The mutants of influenza virus were obtained from the wild-type (wt) by serial passage in cell culture, with a gradual increase in the concentration of antiviral drugs. Oseltamivir and rimantadini hydrochloridum were purchased locally as Tamiflu® (F. Hoffmann-La Roche Ltd, Switzerland) and Remantadin® (JSC Olainfarm, Latvia) respectively and dissolved in double distilled water. The mutants which had the resistance to Tamiflu were named FPV_Tam and Sw_Tam. The mutants which had the resistance to Remantadin - were named FPV_Rim and Sw_Rim respectively. To examine the $IC_{50}$ value of the drugs was used hemagglutination (HA) assay. The $IC_{50}$ values of Tamiflu® for mutants FPV_RTam and Sw_RTam increased to > 0.300 mg/mL, while for the wild-type influenza virus A/FPV/Waybrige/78 (H7N7) and A/Swine/Iowa/30 (H1N1), this parameter was of 0.010 and 0.055 mg/mL, respectively. A similar pattern was observed against the mutants FPV_RRim and Sw_RRim, for which the $IC_{50}$ value for the Remantadin® was > 0.100 mg/mL while $IC_{50}$ value against the wild type strains A/FPV/Waybrige/78 (H7N7) and A/Swine/Iowa/30 (H1N1) was of 0.011 and <0.003 mg/mL, respectively.

Sequencing of the full-length gene sequences was performed with use of the Ion Torrent PGM sequencer (Life Technologies, USA). Assembling the reads into contigs was carried out on the combined reference genome of influenza virus type A using the Bowtie2 software. Derived genome annotation was performed using the Lasergene v12 software (DNASTAR, Inc., Madison, Wisconsin, USA).

## Results and discussion

Comparative analysis on genetic changes of resistant mutants and

wild-type influenza A virus strains have revealed specific substitution in the structure of nucleic acids, responsible for formation of the drug resistance phenotype to Tamiflu® and Remantadin®.

Amino acid sequence analysis of the mutants showed the presence of specific substitutions responsible for development of resistance to the drug Remantadin® in the M2 protein structure. The presence in the structure of the M2 protein mutant FPV_RRim the substitution S31N, and mutant Sw_RRim replacement A30T, responsible for the development of resistance to the drug Remantadin® was shown.

Despite a 6 to 10 times increase in the $IC_{50}$ value of Tamiflu® for mutants FPV_RTam and Sw_RTam compared with wild-type variants, the mutation like H274Y in the NA gene, which is responsible for resistance to oseltamivir, was not detected. It is interesting to note that NA mutations in oseltamivir-resistant isolates, such as I222R which has been reported to confer reduced susceptibility to multiple NAIs by itself, in the absence of the H274Y mutation and N294S mutation which demonstrated decreased sensitivity to oseltamivir.[5] was not found in the structure of NA gene also.

However, our analysis the structure of the M1 protein amino acid sequence of these mutants, revealed a specific substitution at position 207 which is not specified in the literature. We assume that a specific substitution of asparagine to serine at position 207 in the M1 protein structure of mutants FPV_RTam and Sw_RTam has a selective value in the formation of resistance to the Tamiflu®. To explain this substitution the work in this field will be continued.

## Conclusion

It has been shown that a specific mutation at position 207 in the M1 protein structure of resistant mutants has a selective value in formation of resistance to the drug Tamiflu®. We suppose that the emergence of drug resistance may have multigenic nature and could be coded by different genes.

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## Conflicts of interest

None.

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