

Antiviral Drug Sensitivity of Influenza Virus Isolates Collected from Migratory Birds

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INTRODUCTION

Aquatic birds are the natural reservoirs of a wide variety of influenza A viruses. Novel influenza strains occasionally emerge from wild bird populations and spread to other species. Historically, pandemic influenza strains crossing over to humans have led to serious outbreaks. The rapid evolution of influenza strains in birds presents a major public health concern. Globalization and intensified agriculture have increased human contact with avian influenzas in the past century. Antiviral drugs effective against influenza are an important part of preparing for outbreaks, as well as common seasonal flu's. Two major classes of antiviral drugs widely used against influenza are M2 inhibitors and neuraminidase inhibitors. The M2 inhibitor amantadine was approved by the FDA in 1966. More than 30 years later the neuraminidase inhibitor oseltamivir was approved for use against the flu in 1999, marketed as Tamiflu®. Unfortunately, flu viruses are highly mutable and can quickly develop resistance to treatment. Resistance has been observed to both amantadine and oseltamivir, with resistance to amantadine especially widespread since its introduction 50 years ago. Monitoring levels of drug sensitivity in natural flu strains collected from the wild is one way to understand how different strains are affected by antiviral drugs and how resistance spreads.

METHODS

Twenty-one influenza A viruses were obtained from the American Type Culture Collection (ATCC), from subgroups H1-H9, collected from wild birds. Stocks of each influenza virus isolate were prepared and enumerated (titered). Dose of virus needed to kill 50% of individual cell cultures (CCID50) was determined by endpoint dilution assay.

Endpoint dilution antiviral assay

Drug efficacy of both oseltamivir and amantadine was compared against 11 isolates by cell culture assay in 96-well plates. Madin-Darby canine kidney (MDCK) cells were grown in MEM supplemented with 5% FBS. Virus was prepared in MEM containing Trypsin (10 Units/ml) and EDTA (1 µg/ml) final concentration. Drug diluted in half-log increments was added, each dilution was assayed in three replicates. Cells were incubated with virus and drug dilution and observed for cytopathic effect on days 3 and 6. Cytopathic effect was then measured by neutral red assay.

Viral neuraminidase inhibition assay

The effect of oseltamivir on viral neuraminidase activity was performed using a commercially available kit (NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit, Applied Biosystems, Foster City, CA) in 96-well solid white microplates following the manufacturer's instructions. Oseltamivir in half-log dilution increments was incubated with virus (as the source of neuraminidase). Plates were pre-incubated for 10 minutes prior to addition of chemiluminescent substrate. Following addition of substrate plates were incubated for 10 min at 37°C. The neuraminidase activity was evaluated using a Centro LB 960 luminometer (Berthold Technologies) for 0.5 sec immediately after addition of NA-Star® accelerator solution.

RESULTS

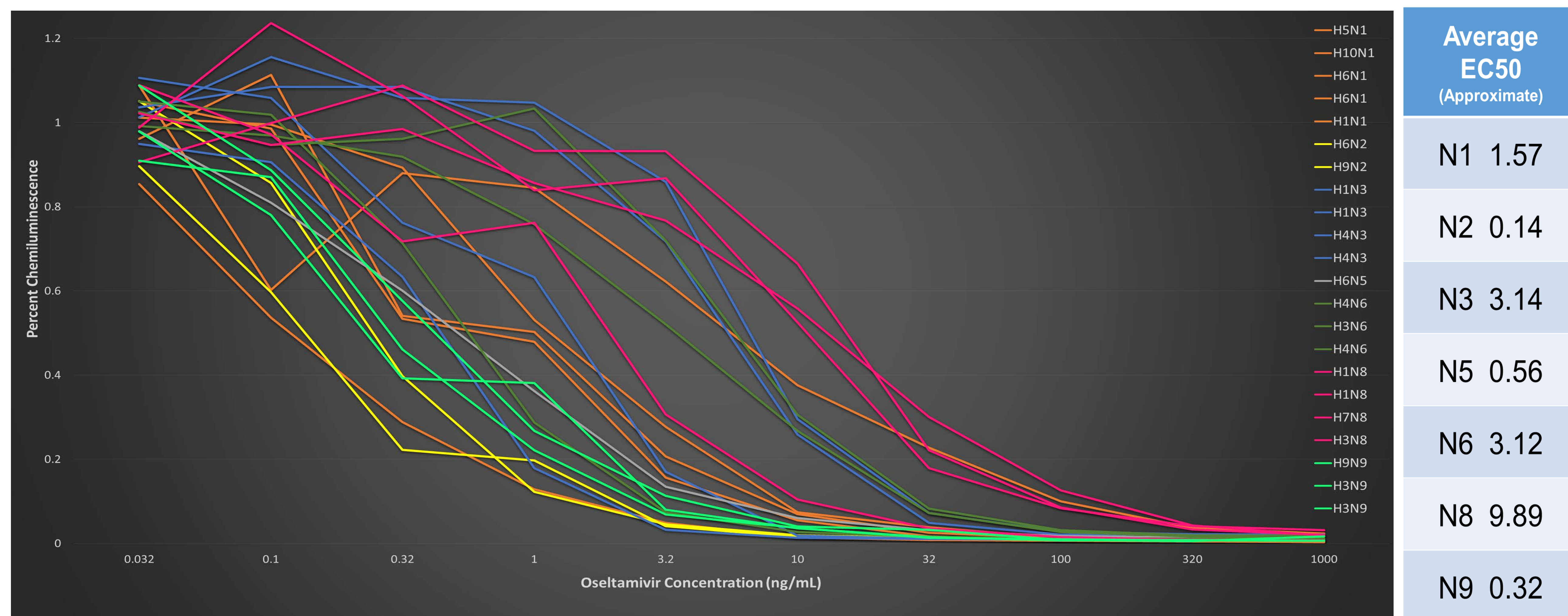


Figure 1- Effect of oseltamivir on neuraminidase activity by subtype

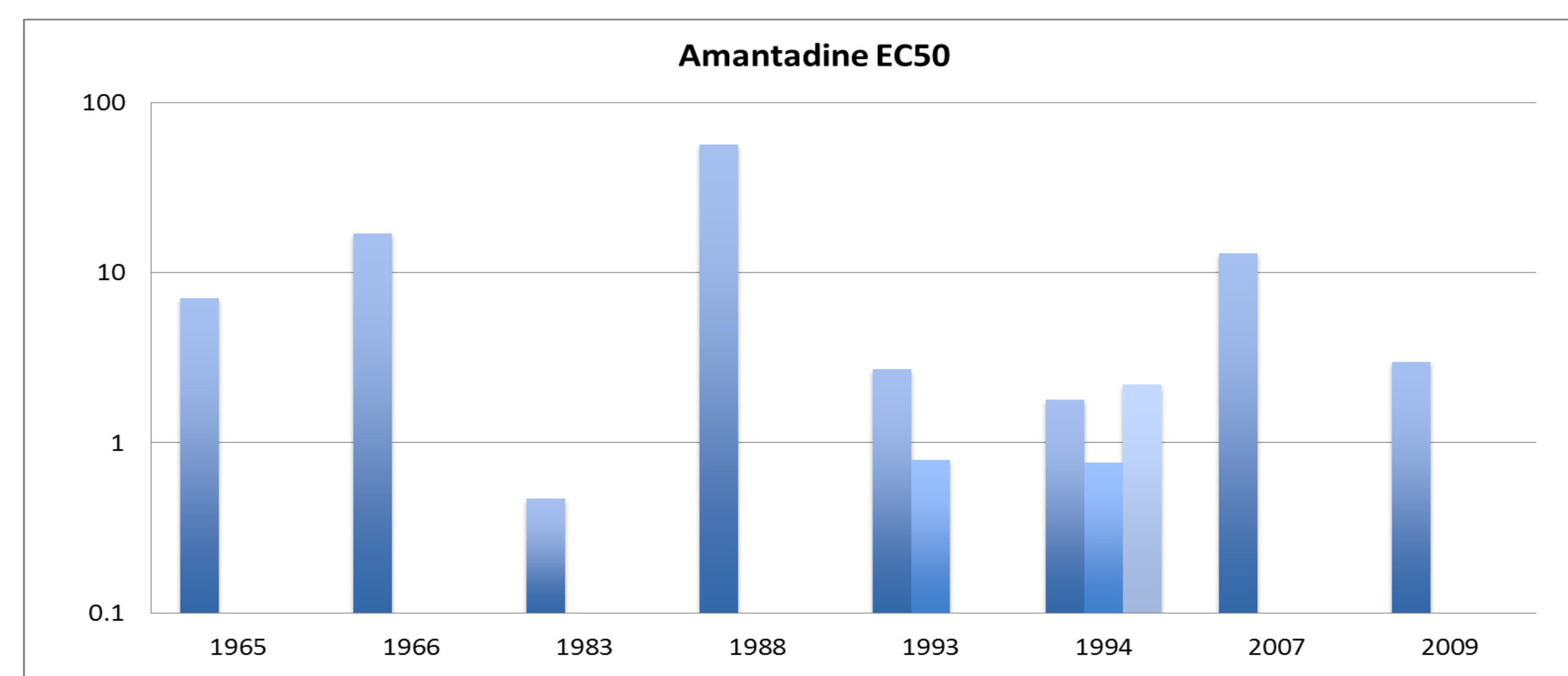


Figure 2- Cell culture EC50 of amantadine by year isolate was obtained

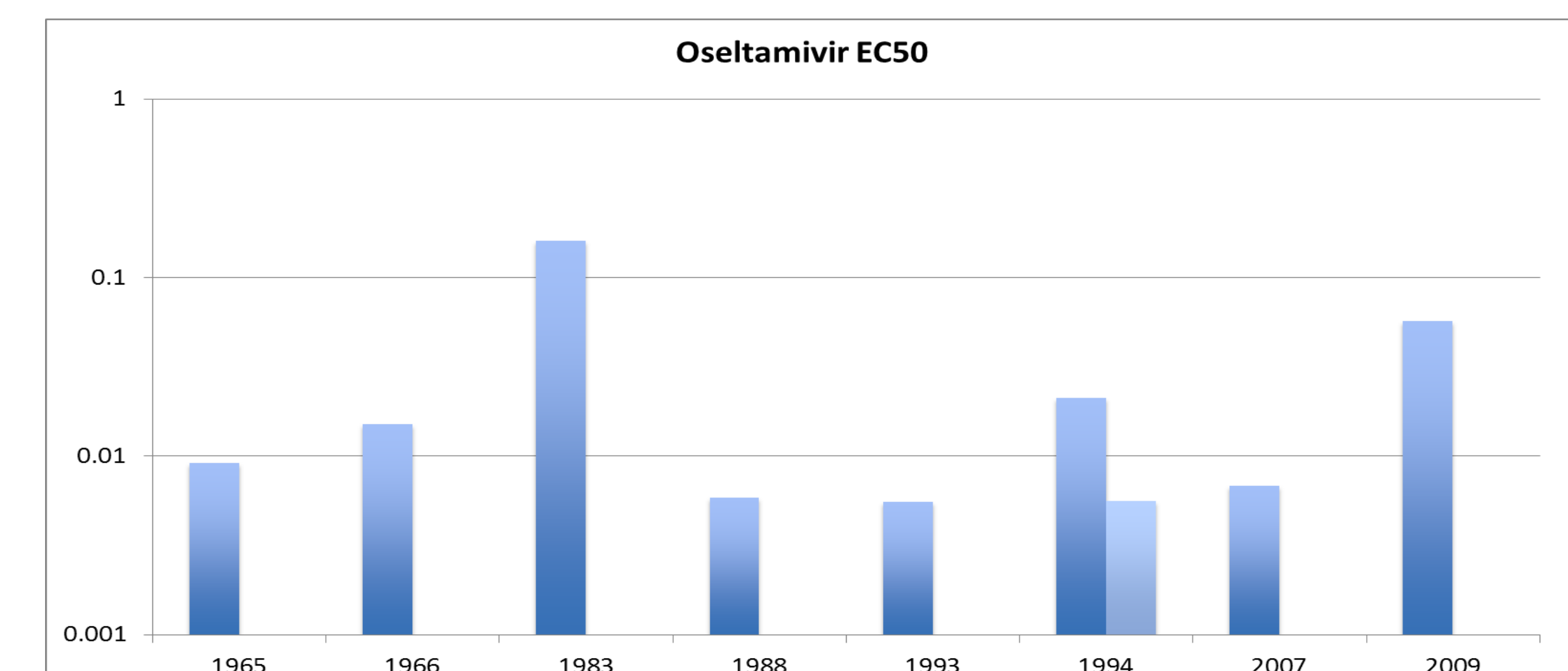


Figure 3- Cell culture EC50 of oseltamivir by year isolate was obtained

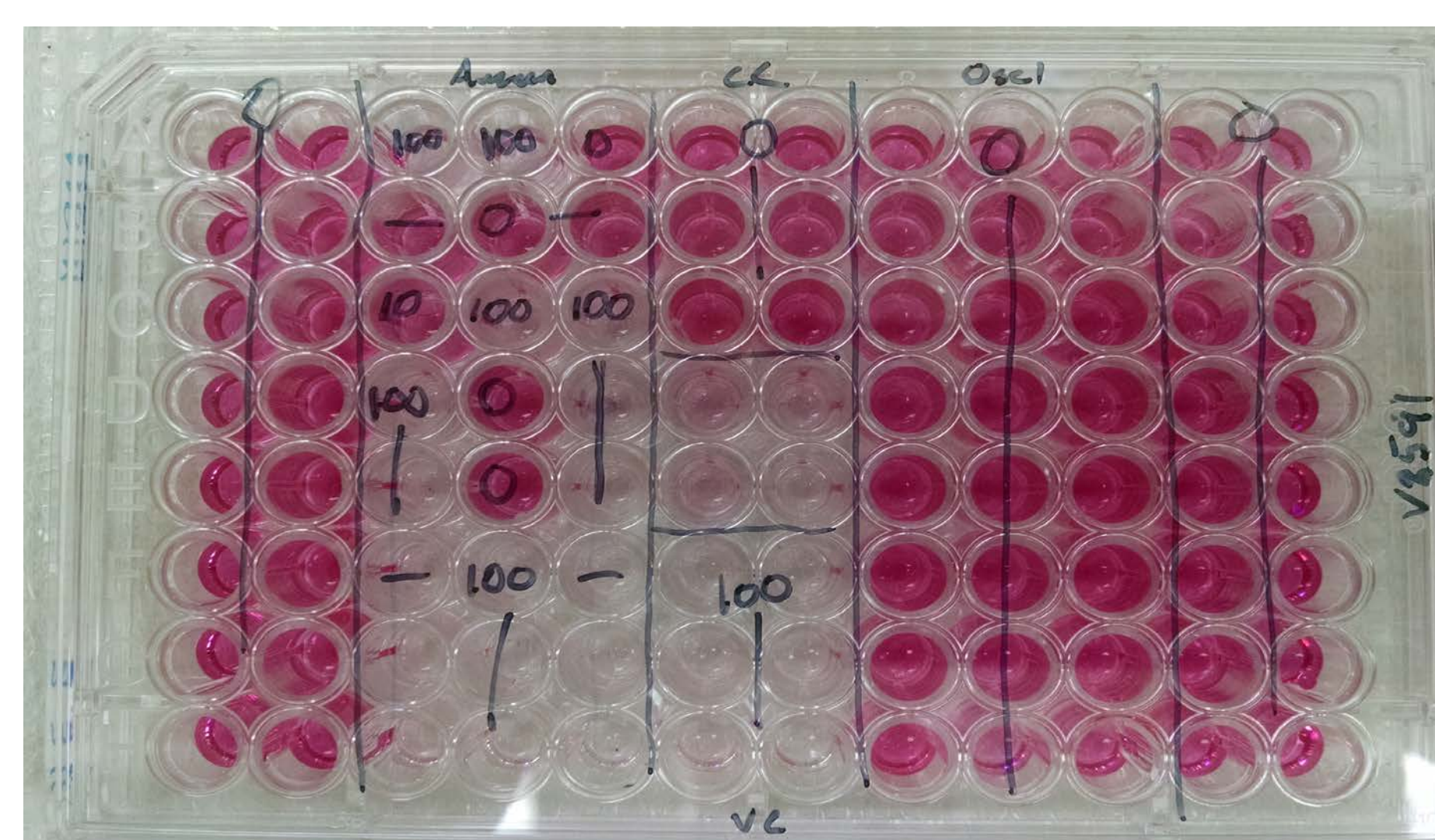


Figure 4- Cytopathic effect visible on 96-well cell culture assay after addition of neutral red stain (day 6)

CONCLUSIONS

These studies showed significant differences in oseltamivir sensitivity between the different N-subtypes in the sample set. The results did not show significant correlation between the year the isolate was obtained and the drug sensitivity profile of the strain. Further studies will verify the results on a larger sample size and examine other factors (region, species, etc.) for correlation with drug sensitivity.

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