Potential emergence of antiviral-resistant pandemic viruses via environmental drug exposure of animal reservoirs

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A continuous leak of antiviral drugs into the environment leads to antiviral resistance which compromises the treatment of human viral diseases.[1,2] As the intense search for effective drugs against the novel coronavirus (SARS-CoV-2) is progressing world-wide, several antiviral and anti-parasitic drugs, including those for Ebola (remdesivir), influenza (favipiravir, oseltamivir), HIV (lopinavir/ritonavir) and malaria (chloroquine), have undergone clinical trials on COVID-19 patients.[3,4] Since these drugs and their metabolites are mostly excreted in urine, there is the potential for discharge to the environment depending on removal efficiency at wastewater treatment plants (WWTPs).[1,2,5,6] For example, our preliminary worst-case (treatment by activated sludge process only) estimation shows that rivers and lakes receive 430–2120 ng/L favipiravir hydroxide, the major metabolite of influenza drug favipiravir (Avigan®), or 54–270 ng/L GS-441524, the active form of ebola drug remdesivir, from WWTP effluents if 100 new patients per 1 milllion capita are added every day to existing patients who are treated with the drugs (estimated based on Singer et al. 2007[2] and Azuma et al. 2012[5]). Animals that are a natural reservoir of viruses, including bats, camels, cats, pangolins, and pigs, may then be exposed to the river water containing antiviral drugs (Fig. 1), inducing antiviral selective pressures and mutations in the virus leading to anti-viral drug resistance.

Viruses are known to rapidly undergo genome mutations with successive replications, increasing the chances of resistance to existing antiviral treatments.[7] To date, antiviral drug resistance has been reported for human viral diseases including AIDS, hepatitis B and C, herpes,

and influenza.[7] Likewise, antiviral drug resistance could be accelerated by exposure of animal reservoirs to environmental waters containing antiviral drugs. For example, for influenza viruses, multiple studies[1,2,5] have alarmed the risk of anti-influenza drug resistance in the body of water fowls, which are known as natural reservoirs of influenza virus[8]. During influenza pandemic, water fowls, such as ducks, may ingest anti-influenza drugs and metabolites in environmental waters. In Japan, oseltamivir carboxylate, the active metabolite of oseltamivir (Tamiflu®), was detected in river water at concentrations up to 864.8 ng/L[6] during the past pandemic of novel influenza, exceeding the concentration that inhibits 50% of *in vitro* growth (IC_{50}) of influenza-A virus (97–210 ng/L)[9]. This suggests contaminated natural waters could initiate antiviral selective pressure in animal reservoirs during a pandemic with high rates of anti-viral drug use. Similarly, SARS-CoV-2 is potentially capable of acquiring antiviral drug resistance in its animal reservoirs (e.g., bats and pangolins)[10] in the event of exposure to surface waters contaminated with antiviral drugs during the COVID-19 pandemic. As of May 14, 2020, the average mutation rate of SARS-CoV-2 is 25.3 substitutions per year, which equals approximately one in 14 days.[11] Considering this mutation rate and the numerous populations of wild animal reservoirs, the emergence of antiviral drug-resistance to SARS-CoV-2 during the current waves of COVID-19 could generate challenges for human treatment in the post COVID-19-pandemic Anthropocene.

The unprecedented mass use of antiviral drugs is looming as global researchers race to develop them for COVID-19 applications, alongside vaccine development. In March 2020, U.S. health officials estimated that it may take 12 to 18 months for production and delivery of effective vaccines for COVID-19,[12] with many hurdles to overcome, placing greater pressure on anti-viral drug development. The urgent question to understand is to what extent wild animal reservoirs could be exposed to anti-viral drug residues in environmental waters, and how that may induce antiviral drug-resistant viruses in the future that then challenge the existing treatments for COVID-19-like pandemic diseases.

Presumptive actions are required to study the occurrence, behavior and fate of various antiviral drugs and their metabolites during wastewater treatment and into receiving waters during pandemic events. In addition, the susceptibility of SARS-CoV-2 to antiviral drugs needs an urgent evaluation of potential antiviral drug resistance development in wild animal

reservoirs. Perhaps this current COVID-19 crisis may become an opportunity to invest in upgrading WWTPs with advanced treatment capabilities such as ozonation for the enhanced removal of pharmaceuticals, including antiviral drugs.[1,5] Lack of proper sanitation infrastructure in less developed countries is also a challenge in terms of antiviral spread control in the environment, and thus additional investments in this area is needed as a global responsibility to maintain the efficacy of ani-viral drugs. We believe that significant research is required to safeguard the efficacy and longevity of anti-viral treatments. By responsibly understanding their environmental fate in WWTP and environmental settings, accelerated anti-viral resistance may be avoided, and treatment tools for future viral pandemics preserved.

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615 words; 12 references

Notes:

The authors declare no competing financial interest.

References:

(1) Nannou C.; Ofrydopoulou A.; Evgenidou E.; Heath D.; Heath E.; Lambropoulou D. Antiviral drugs in aquatic environment and wastewater treatment plants: A review on occurrence, fate, removal and ecotoxicity. *Sci. Total Environ*. 2020, 699, 134322.

(2) Singer A.C.; Howard B.M.; Johnson A.C.; Knowles C.J.; Jackman S.; Accinelli C.; Caracciolo A.B.; Bernard I.; Bird S.; Boucard T.; Boxall A. Meeting report: risk assessment of Tamiflu use under pandemic conditions. *Environ. Health Perspect*. 2008, 116(11), 1563-7.

(3) The race against COVID-19. *Nat. Nanotechnol.* 2020, 15, 239–240.

(4) Liu C.; Zhou Q.; Li Y.; Garner L.V.; Watkins S.P.; Carter LJ. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *Acs Cent Sci*, 2020, 6(3), 315-331.

(5) Azuma T.; Nakada N.; Yamashita N.; Tanaka H. Synchronous dynamics of observed and predicted values of anti-influenza drugs in environmental eaters during a seasonal influenza outbreak. *Environ. Sci. Technol.* 2012, 46, 12873-81.

(6) Takanami, R.; Ozaki, H, Giri, R.R.; Taniguchi, S.; Hayashi, S. Detection of antiviral drugs oseltamivir phosphate and oseltamivir carboxylate in Neya River, Osaka, Japan. *J Water and Environ. Technol*. 2011, 8(4), 363-72.

(7) Richman, D.D. Antiviral drug resistance. Antivir. Res. 2006, 71, 117–121.

(8) Olsen B.; Munster V.J.; Wallensten A.; Waldenström J.; Osterhaus A.D.M.E.; Fouchier R.A.M. Global Patterns of Influenza A Virus in Wild Birds. *Science* 2006, 312(5772), 384–388.

(9) Monto A.S.; McKimm-Breschkin J.; Macken C.; Hampson A.W.; Hay A.; Klimov A. Detection of influenza viruses resistant to neuraminidase inhibitors in global

surveillance during the first 3 years of their use. Antimicrob Agents Chemother. 2006, 50 (7), 2395-24021.
(10) Zhang T.; Wu Q.; Zhang Z. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. Curr Biol. 2020, 30 (8) 1346-1351.e2.
(11) Nextstrain web: https://nextstrain.org/ncov/global. Accessed May 14, 2020.
(12) Lovelace, B. First human trial for coronavirus vaccine begins Monday in the US. CNBC https://www.cnbc.com/2020/03/16/first-human-trial-for-coronavirus-vaccine-begins-monday-inthe-us.html (2020). Accessed May 14, 2020.