Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Short Communication

Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters

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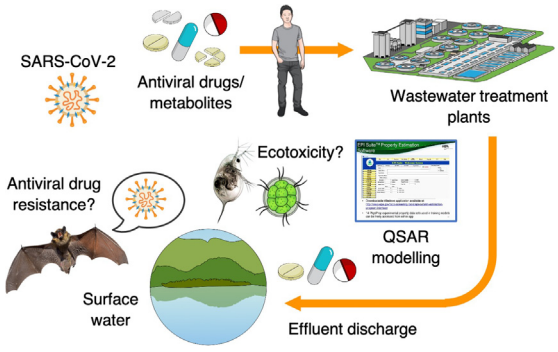
HIGHLIGHTS

- Environmental concentrations of antiviral drugs for COVID-19 were predicted.
- Many drugs and metabolites are not readily removed by wastewater treatment.
- Residues of several drugs may pose high ecotoxicological risk in receiving waters.
- Potential of environmental development of antiviral drug resistance is small.
- Proper usage and waste management of antiviral drugs are urgently needed.

GRAPHICAL ABSTRACT



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ABSTRACT

Antiviral drugs have been used to treat the ever-growing number of coronavirus disease, 2019 (COVID-19) patients. Consequently, unprecedented amounts of such drug residues discharging into ambient waters raise concerns on the potential ecotoxicological effects to aquatic lives, as well as development of antiviral drug-resistance in wildlife. Here, we estimated the occurrence, fate and ecotoxicological risk of 11 therapeutic agents suggested as drugs for COVID-19 treatment and their 13 metabolites in wastewater and environmental waters, based on drug consumption, physical-chemical property, and ecotoxicological and pharmacological data for the drugs, with the aid of quantitative structure-activity relationship (QSAR) modelling. Our results suggest that the removal efficiencies at conventional wastewater treatment plants will remain low (<20%) for half of the substances, and consequently, high drug residues (e.g. 7402 ng/L ribavirin, 4231 ng/L favipiravir, 730 ng/L lopinavir, 319 ng/L remdesivir; each combined for both unchanged forms and metabolites; and when each drug is administered to 100 patients out of 100,000 populations on a day) can be present in secondary effluents and persist in the environmental waters. Ecotoxicological risk in receiving river waters can be high (risk quotient >1) by a use of favipiravir, lopinavir, umifenovir and ritonavir, and medium (risk quotient >0.1) by a use of chloroquine, hydroxychloroquine, remdesivir, and ribavirin, while the risk will remain low (risk quotient <0.1) for dexamethasone and oseltamivir. The potential of wild animals acquiring antiviral drug resistance was estimated to be low. Our prediction suggests a pressing need for proper usage and waste management of antiviral drugs as well as for improving removal efficiencies of drug residues in wastewater.

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1. Introduction

Coronavirus disease, 2019 (COVID-19), a highly infectious disease caused by severe acute respiratory syndrome-related coronavirus (SARS-CoV-2), has been declared a pandemic, with 101 million confirmed cases and 2.2 million deaths worldwide as of February 2021 (WHO, 2020). As there are no specific therapeutic drugs recognised for targeting the cure from the SARS-CoV-2, various existing pharmaceuticals have been tested as therapeutic agents to treat COVID-19 patients ("The Race", 2020; Liu et al., 2020a). Studies suggest that remdesivir, an antiviral drug against Ebola, may be effective in shortening the time to recovery in adults hospitalized with COVID-19 (Beigel et al., 2020), and has been approved for COVID-19 treatment in countries such as US and Japan ("Japan Approves Remdesivir", 2020), Recently, low-dose dexamethaxone, a synthetic corticosteroid, has been suggested effective in reducing deaths in COVID-19 patients with ventilation, which is potentially a major breakthrough in COVID-19 treatment ("Dexamethasone Reduces Death", 2020). Several vaccines have been rapidly developed and their mass-delivery and uses have already been started in some countries, but there are many hurdles and uncertainties to overcome, such as logistics and public hesitancy ("Hope of a COVID-19 Vaccine", 2020). Therefore, the unprecedented mass use of these therapeutic drugs is expected to continue worldwide (Kumar et al., 2020a).

After human consumption, pharmaceuticals are excreted from human body and discharged into wastewater as unchanged drugs or metabolites, which are often only partly removed in conventional wastewater treatment plants (WWTPs) (Joss et al., 2005; Nannou et al., 2020). These residues present in receiving environmental waters have posed ecotoxicological concerns (Al Aukidy et al., 2012; Fick et al., 2010; Godoy and Kummrow, 2017; Santos et al., 2010). In particular, during pandemic events, high amounts of antiviral drugs and their metabolites released into environmental waters are likely to pose a high risk to aquatic ecosystems (Jain et al., 2013; Nannou et al., 2020). In addition, such high amounts of antiviral drugs in environmental waters may lead to the development of antiviral drug-resistant viral strains inside the body of specific wild animals, which are natural reservoirs of viruses (Kumar et al., 2020a). That is, when animal reservoirs continuously ingest environmental waters containing elevated levels of antiviral drugs and their metabolites, the viruses inside their bodies may develop resistance through rapid mutations (Jain et al., 2013; Nannou et al., 2020; Singer et al., 2007). We define this type of antiviral drug resistance as *environmentally acquired antiviral drug resistance* (EDR). EDR has been concerned for influenza A virus during past outbreaks (Fick et al., 2007; Ghosh et al., 2010; Singer et al., 2007). SARS-CoV-2 might similarly develop EDR inside animal hosts such as bats (Zhou et al., 2020), owing to expected mass use of antiviral drugs during the current waves of COVID-19 (Kumar et al., 2020a; Kumar et al., 2020b; Race et al., 2020; Sims and Kasprzyk-Hordern, 2020). To date, however, there has been no quantitative evaluation of EDR of SARS-CoV-2.

The occurrence, fate and ecotoxicity of a diverse range of pharmaceuticals, including antiviral drugs, in WWTPs and in environmental waters during past outbreaks as well as normal times have been summarized (Aymerich et al., 2016; Jain et al., 2013; Kasprzyk-Hordern et al., 2009; Nannou et al., 2020; Ncube et al., 2018; Tran et al., 2018). However, those past studies hardly include most of the therapeutic agents which have been considered for COVID-19 treatment, except for oseltamivir (anti-influenza drug) (Azuma et al., 2012; Fick et al., 2007; Ghosh et al., 2010; Prasse et al., 2010; Singer et al., 2007) and lopinavir/ritonavir (anti-HIV drugs) (Abafe et al., 2018; Wood et al., 2015). The potential occurrence and fate in aquatic environments, the general physical-chemical properties, and ecotoxicological risks of various COVID-19-associated drugs are largely unknown. For screening environmental fate and toxicity of pharmaceuticals, quantitative structure-activity relationship (QSAR) models have been applied to diverse pharmaceuticals (Escher et al., 2011; Kar et al., 2020; Sanderson et al., 2004).

The objective of the study is to provide a model-based evaluation on the occurrence, fate and ecotoxicological effects of a suite of therapeutic agents associated with COVID-19 treatment and their metabolites in wastewater and environmental waters during pandemic events. Predicted environmental concentrations (PECs) were calculated with assumed patient numbers treated with these drugs (100 patients out of 100 k populations are on the course of treatment every day), taking into account drug consumption patterns, excretion from human body, and elimination at WWTPs. QSAR models were used to predict elimination at WWTPs and chronic toxicity to aquatic lives for the substances for which measurement-based data were not available. Furthermore, potential of EDR by animal reservoirs was assessed by *in vitro* pharmacological data of the drugs against SARS-CoV-2. To our knowledge, this is the first study to estimate ecotoxicological impacts of mass use of multi-antiviral drugs associated with COVID-19 on ambient waters and suggest necessary global precautionary measures.

2. Materials and methods

We evaluated 11 representative potential therapeutic drugs for COVID-19 treatment (chloroquine, dexamethaxone, favipiravir, hydroxychloroquine, lopinavir, oseltamivir, remdesivir, ribavirin, ritonavir, teicoplanin and umifenovir) and their 13 major metabolites (Table 1), which were selected from literature (Liu et al., 2020a; Wu et al., 2020; Yousefi et al., 2020). The drugs' original purposes are shown in Table 1, and their CAS number and simplified molecular-input line-entry system (SMILES) in Table S1 in the Supplementary data.

2.1. Predicted environmental concentrations

The concentrations of the target substances in raw wastewater, secondary effluent, and river waters were predicted by the following Eqs. (1)–(3), which were adapted from past modelling studies on antivirals and/or down-the-drain chemicals (Singer et al., 2007; Ghosh et al., 2010; Keller et al., 2014):

$$PEC_{raw} = \frac{N_t}{100,000} \times \frac{D_d \times f \times 10^6}{W_c} \quad (1)$$

$$PEC_{se} = PEC_{raw} \times (1-R) \quad (2)$$

$$PEC_{riv} = \frac{PEC_{se}}{10} \quad (3)$$

where PEC_{raw} is a predicted concentration in raw wastewater; N_t is a number of patients on the course of treatment with a drug per 100,000 population in a day (assumed as 100); D_d is an average daily drug dose expected for COVID-19 treatment; f is a fraction of excreted substances (to urine and feces) to drug dose; W_c is water consumption per person per day of 200 L, which has been used by European Medicine Agency (EMA) for environmental risk assessment of pharmaceuticals (EMA, 2018); 10^6 is a conversion factor from mg of substances to ng; PEC_{se} is a predicted concentration in secondary effluent; R is removal efficiency in conventional WWTPs (mentioned below); and PEC_{riv} is a predicted concentration in rivers.

The average daily drug dose D_d ranged from 6 mg/day for dexamethasone to 2473 mg/day for ribavirin. The details of drug dose can be found in Table S1. The fraction of excretion (f), identified based on literature and database search, varied largely, ranging from 0.8% to 83% for unchanged drugs and from 1.5% to 80% for metabolites (Table 1). We assumed dilution of secondary effluent by ten times in the receiving rivers, which represents a minimum dilution in many countries (Keller et al., 2014) and was also used for environmental risk

Table 1
Predicted physical-chemical properties and ecotoxicological characteristics of potential therapeutic agents for COVID-19.

Drugs and metabolites	Original purpose	Average daily dose, D_d (mg/day)[a]	Substances and excreted fraction, f (%)[b]		Antiviral activity	M.W.[p]	$LogK_{ow}$	PNEC (ng/L)	Removal in WWTP, R	Primary biodegradation
Chloroquine	Malaria	343	Chloroquine (urine and feces)	50%[c]	Active	319.9	4.63[q]	3700[s]	63%	weeks to months
-metabolite			*N*-desethylchloroquine (urine)	10%[c]	Unknown	291.8	3.79	55	22%	days to weeks
Dexamethasone	Corticosteroid	6	Dexamethasone	10%[d]	Active	392.5	1.92[q]	50[t]	2.2%	weeks to months
Favipiravir	Influenza	1,600	Favipiravir (urine)	0.8%[e]	Prodrug	157.1	0.72	91	1.9%	days to weeks
-metabolite			T705M1 (urine)	53.1%[e]	Inactive	173.1	0.99	81	1.9%	days to weeks
Hydroxychloroquine	Malaria	354	Hydroxychloroquine (urine and feces)	47%[f]	Active	335.9	3.03	170	6.0%	weeks to months
Lopinavir	HIV	800	Lopinavir (mostly feces)	22%[g]	Active	628.8	5.94	4.7	92%	days to weeks
-metabolite			M1 (mostly feces)	71% in total[g]	Unknown	642.8	5.54	5.9	89%	days to weeks
-metabolite			M2 (mostly feces)		Unknown	644.8	3.48	30	71%	days to weeks
-metabolites			M3/M4 (mostly feces)		Unknown	644.8	3.46	30	71%	days to weeks
Oseltamivir	Influenza	150	Oseltamivir (urine and feces)	15%[h]	Prodrug	312.4	0.95	4700	1.9%	days to weeks
-metabolite			Oseltamivir carboxylate (mostly urine)	80%[h]	Active	284.4	0.18	120000	1.9%	hours to days
Remdesivir	Ebora	110	Remdesivir (urine)	10%[i]	Active	602.6	1.74	31	2.1%	days to weeks
-metabolite			GS-451524 (urine)	49%[i]	Active	291.3	−1.76	240	1.9%	days to weeks
Ribavirin	HCV, RSV	2473	Ribavirin (urine)	17%[j]	Active	244.2	−1.85[q]	2700	1.9%	hours to days
-metabolite			$TCONH_2$ (urine)	44%[j, k]	Inactive	112.1	−1.37	830	1.9%	days to weeks
Ritonavir	HIV	200	Ritonavir (mostly feces)	37%[l]	Active	720.9	6.27	2.9	93%	days to weeks
-metabolite			M2 (mostly feces)	60%[l]	Active	736.9	5.17	20	82%	days to weeks
Teicoplanin	Antibiotic	400	Teicoplanin (urine and feces)	83%[m]	Active	1879.7	−1.1[r]	n.a.[u]	0%[v]	n.a.
Umifenovir	Influenza, SARS	600	Umifenovir (feces)	40%[n]	Active	477.4	5.4	9.3	87%	weeks to months
-metabolite			M10 (feces)	3%[o]	Unknown	556.5	2.91	160	5.0%	weeks to months
-metabolite			M18 (urine)	1.5%[o]	Unknown	653.5	3.34	25000	87%	weeks to months
-metabolite			M20 (urine)	2.1%[o]	Unknown	669.5	0.76	240000	1.9%	days – weeks

[a] Average daily dose (mg) was calculated as the total amount of a drug for expected use for COVID-19 treatment, divided by expected treatment duration (see Table S1).
[b] Excretion (%) is the amount, expressed as a fraction of dose, of a parent drug (unchanged drug) or its metabolites which are eliminated from human body via urine and feces. The excretion data were obtained from literature and drug database search.
[c] Ducharme and Farinotti (1996).
[d] FDA Approved Drug Products: Hemady Dexamethasone Oral Tablets (2019).
[e] Ministry of Health, Labour and Welfare (2014).
[f] Browning (2014).
[g] Health Canada (2019). The fraction of each of four metabolites of lopinavir (M1 to M4) is not available, thus the sum of total metabolite fractions was evaluated.
[h] He et al. (1999).
[i] FDA: Fact Sheet For Health Care Providers EUA of Remdesivir (2020).
[j] FDA Approved Drug Products: Rebetol (ribavirin) oral capsules (2019).
[k] Lin et al. (2006).
[l] FDA Approved Drug Products: NORVIR (ritonavir) Capsules, Soft Gelatin for Oral use (2012).
[m] Electronic Medicines Compendium: Targocid (teicoplanin) 400 mg powder Monograph (2020).
[n] Liu et al. (2009).
[o] Deng et al. (2013).
[p] Molecular weight.
[q] Experimentally determined (US EPA ECOTOX knowledgebase, 2020, https://cfpub.epa.gov/ecotox/)
[r] Experimentally determined (Rowland, 1990)
[s] Based on Zurita et al., 2005; eEC_{50} for *D. magna* at a 72h exposure.
[t] Based on DellaGreca et al., 2004; chronic toxicity for *C. dubia* at a 7d exposure.
[u] Not available.
[v] The removal efficiency of teicoplanin in WWTP was not predictable by EPISuite, thus a removal efficiency of 0% was assumed.

assessment by EMA (2018). To give a conservative estimation, no in-stream attenuation was assumed.

2.2. Physical-chemical properties

Removal efficiency in conventional WWTPs (employing activated sludge process as secondary treatment) was obtained as 'total removal at STPs' predicted by STPWIN program in EPI Suite™ (EPA, 2021). $LogK_{ow}$ was searched for experimentally derived octanol-water distribution coefficient (K_{ow}), but it was available only for chloroquine, dexamethasone, ribavirin and teicoplanin (Table 1). For the other drugs and metabolites, $LogK_{ow}$ was estimated by Kowwin v.1.68 in EPI Suite™. Considering its importance in determining environmental fate, $LogK_{ow}$ was also calculated by SPARC program (Hilal et al., 2003) for comparison. Kowwin modelling is based on a database of substances with known K_{ow}, whereas SPARC program calculates strictly from molecular structure (Hilal et al., 2003). If there is more than an order of magnitude difference in K_{ow} between the two programs, the ALOGPS 2.1 program by VCC labs (VCC Laboratory, 2009) was tested, and the K_{ow} from either Kowwin or SPARC closer to the value predicted by AGLOPS program was used. The $LogK_{ow}$ values evaluated by Kowwin, SPARC and ALOGPS 2.1 are shown in Table S2.

2.3. Ecotoxicity

Chronic toxicity of the target substances was evaluated, either using experimentally derived ecotoxicity (when available) or otherwise using

predicted ecotoxicity by ECOSAR, a computerized structure activity relationship for aquatic toxicity (EPA, 2020). Experimentally derived ecotoxicity data was searched by US EPA Ecotox knowledgebase (https://cfpub.epa.gov/ecotox/) and Google Scholar, and was obtained for only chloroquine (Zurita et al., 2005) and dexamethasone (DellaGreca et al., 2004). As ecotoxicity of chloroquine was obtained for only acute toxicity, the median effective concentration (EC_{50}; hereafter, denoted as *eEC_{50}* to differentiate from viral inhibitory concentration) was converted to chronic toxicity by acute-to-chronic ratio of 10 (Mayo-Bean et al., 2017). For the remaining substances, chronic ecotoxicity was predicted by ECOSAR, and the smallest values of chronic ecotoxicity for three model organisms (daphnia, algae and fish) were taken for a conservative estimate. For each substance, the predicted no-effect concentration (PNEC) was estimated as the chronic toxicity value divided by UF, a standard uncertainty factor, as shown in Eq. (4); the UF value of 1000 was conventionally adopted to consider the intra- and interspecies variability in the sensitivity (Hernando et al., 2006):

$$\mathrm{PNEC} = \frac{\mathrm{eEC_{50}}}{1000} \tag{4}$$

In addition, the mode of action in aquatic organisms was predicted by VEGA (2019) for each substance.

Risk quotient (RQ) was calculated for each substance as the ratio between PEC_{riv} and PNEC, as shown in Eq. (5):

$$\mathrm{RQ} = \frac{\mathrm{PEC_{riv}}}{\mathrm{PNEC}} \tag{5}$$

The risk is classified into three levels: RQ 0.01–0.1, low risk; RQ 0.1–1, medium risk; and RQ >1, high risk (Hernando et al., 2006).

2.4. Environmentally acquired antiviral drug resistance

The drug concentration which inhibits *in vitro* viral growth by 50% (the half maximal inhibitory concentration; IC_{50}) is a measure of susceptibility of viruses to antiviral agents (Pillay and Zambon, 1998), and it can also be expressed as half maximal effective concentration (EC_{50}); here, we denote IC_{50} and EC_{50} of antiviral agents as *vIC_{50}* and *vEC_{50}* to differentiate from ecotoxicological median effective concentration eEC_{50}.

The likelihood of developing antiviral resistance by a virus is the largest when the drug concentrations are close to vIC_{50} (Pillay and Zambon, 1998). Thus, we evaluated the potential of EDR by animal reservoirs exposed to environmental waters, by defining *EDR potential* (EDRP) as the minimum values between the ratio of PEC_{riv} to vIC_{50} values of an antiviral drug and its reciprocal (Eq. (6)):

$$\mathrm{EDRP} = \mathrm{Min}\left(\frac{\mathrm{PEC_{riv}}}{\mathrm{vIC_{50}}}, \frac{\mathrm{vIC_{50}}}{\mathrm{PEC_{riv}}}\right) \tag{6}$$

By definition, EDRP of 1 is the maximal value. The vIC_{50} and vEC_{50} values of the target pharmaceuticals determined *in vitro* against SARS-CoV-2 were summarized from literature (Table 2). Note that, the determined vIC_{50}/vEC_{50} values varied by an order of magnitude, depending on experimental conditions (e.g., multiplicity of infection: MOI (Liu et al., 2020b), time after infection of test cells (Gonçalves et al., 2020)). To make a conservative estimate, the lowest vIC_{50}/vEC_{50} values were used for EDRP calculation. Metabolites are also evaluated for EDRP, assuming the same vIC_{50} values as those of the parent substances.

3. Results and discussion

3.1. Physical-chemical properties and environmental fate

The predicted physical-chemical properties of the target substances are summarized in Table 1. Approximately half of the parent drugs (6/11, 54%) and the metabolites (6/12, 50%) were found to be hydrophilic ($LogK_{ow}$ <3). These hydrophilic substances mostly have low molecular weight (mw <400), but a few substances had high molecular weight (e.g., remdesivir, mw 602.6; teicoplanin, mw 1709.4; umifenovir M10, mw 556.5; and umifenovir M20, mw 669.5) but low $LogK_{ow}$ values (1.74, −1.10, 2.91 and 0.76, respectively). The predicted removal in conventional WWTPs was low for the half of the

Table 2
Summary of determined vIC_{50} and vEC_{50} of antiviral drugs against SARS-CoV-2.

Antiviral drugs	vIC_{50}/vEC_{50} (μM)	vIC_{50}/vEC_{50} used for EDRP calculation	
		μM	Converted to μg/L
Chloroquine	1.03 (Holwerda et al., 2020) 1.13 (Wang et al., 2020a) 1.31 (Ohashi et al., 2020) 5.47 (Yao et al., 2020) 2.71–7.36 (Liu et al., 2020b) 7.28, 12.0 (Jeon et al., 2020) 9.27 (Xiong et al., 2020)	1.03	329
Favipiravir	62 (Wang et al., 2020a) >500 (Jeon et al., 2020)	62	9740
Hydroxychloroquine	0.72 (Yao et al., 2020) 4.51–12.96 (Liu et al., 2020b) 9.21–11.17 (Weston et al., 2020)	0.72	242
Lopinavir	1.73 (Ohashi et al., 2020) 4.9–5.2 (Gonçalves et al., 2020) 5.73 (Yamamoto et al., 2020) 9.12, 15.27 (Jeon et al., 2020)	1.73	1088
Oseltamivir	>100 (Tan and Jin, 2020) >100 (Wang et al., 2020b)	100	31,200
Remdesivir	0.77 (Wang et al., 2020a) 1.842 (Holwerda et al., 2020) 2.5 (Liu et al., 2020c) 8.24, 11.41 (Jeon et al., 2020)	0.77	464
Ribavirin	109.5 (Wang et al., 2020a)	109.5	26,740
Ritonavir	8.63 (Yamamoto et al., 2020)	8.63	6222
Teicoplanin	1.66 (Zhang et al., 2020)	1.66	3120
Umifenovir	4.11 (Wang et al., 2020b) 30 (Lu, 2020)	4.11	1962

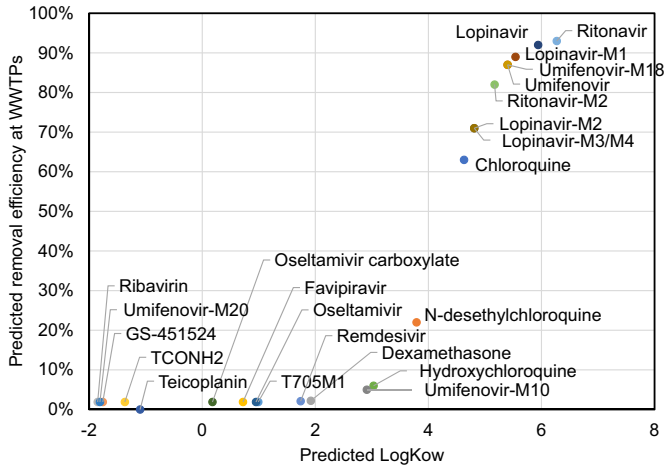

Fig. 1. Comparison between $LogK_{ow}$ values and removal efficiencies at WWTPs, both predicted by EPI Suite™. Note that removal efficiency of teicoplanin was not predictable by EPI Suite™, thus was assumed as 0.

substances (removal efficiency <20% for 12 substances), whereas high removal efficiency (>80%) was predicted for only six substances (chloroquine, lopinavir, ritonavir, umifenovir, and two metabolites). The predicted high removal efficiency would be largely associated with adsorptive behavior of the substances; predicted $LogK_{ow}$ values and predicted removal efficiencies were expressed in a sigmoid-like growth curve (Fig. 1). In addition, biodegradability at WWTPs predicted by STPWIN ('Biodegradation in STP') was only less than 0.77%. 'Primary biodegradation', which indicates the time required for the transformation of a substance to an initial metabolite (EPA, 2021), was 'days to weeks' and 'weeks to months' for most of the target substances.

Measurement-based removal efficiencies of the target substances during activated sludge treatment processes were largely unavailable, except for oseltamivir, oseltamivir carboxylate (the active metabolite of oseltamivir), lopinavir and ritonavir. Regarding oseltamivir and oseltamivir carboxylate, the predicted removal efficiencies at conventional WWTPs (2% for both) were in accordance with their low removal efficiencies determined during activated sludge treatment (none for oseltamivir carboxylate: Fick et al., 2007; 10% for both substances: Azuma et al., 2012; none for oseltamivir and 59% for oseltamivir carboxylate: Prasse et al., 2010). Regarding lopinavir and ritonavir, high removal efficiencies (92% and 93%, respectively) were predicted. In comparison, their measurement-based removal efficiencies in two municipal WWTPs in South Africa (employing activated sludge with nutrient removal, anaerobic digestion, followed by maturation ponds) somewhat differed (43% to 71% and −192% to −58%, respectively (Abafe et al., 2018)); however, this results could be treated with care, because the wastewater samples were taken by grab sampling. Favipiravir was predicted to be persistent during activated sludge process (2% removal); this is in accordance with its persistence against biodegradation in a batch-scale experiment (Azuma et al., 2017). Note that, favipiravir was easily degraded by sunlight in the latter study, indicating a rapid decrease in environmental waters.

3.2. Predicted occurrence in wastewater and environmental waters

The large concentrations in secondary effluents were predicted for $TCONH_2$ (5339 ng/L), the major active metabolite of ribavirin, followed by T705M1 (4168 ng/L; the major inactive metabolite of favipiravir) and ribavirin (2063 ng/L), as shown in Table 3. On the contrary, low PECs in secondary effluents were predicted for dexamethasone (2.9 ng/L), ritonavir (26 ng/L) and remdesivir (54 ng/L), because of low dose (dexamethasone), high removal at activated sludge process (ritonavir) and high rate of transformation to metabolites (for remdesivir, with 265 ng/L of the active metabolite, GS-451524). For all substances, concentrations in the river waters were lower by a factor of 10, because of assumed dilution.

As for oseltamivir, the PEC in secondary effluents in this study (118 ng/L and 589 ng/L) was similar with the maximum concentrations in treated wastewater (293 ng/L to 672 ng/L) determined during pandemic events in Japan (Azuma et al., 2017; Azuma et al., 2012; Ghosh et al., 2010), but lower than the predicted concentrations of oseltamivir carboxylate in UK and US rivers of 31.8 μg/L during the peak of influenza outbreaks (Singer et al., 2007). Favipiravir has been rarely detected in wastewater effluents after activated sludge process and in river waters in Japan during the past influenza season, presumably because of low usage of favipiravir to influenza patients in Japan and low excretion unchanged (0.8%) (Azuma et al., 2017). Lopinavir was abundant in wastewater in South Africa (1200–2500 in influents, 130–3800 ng/L in effluents)(Abafe et al., 2018; Wood et al., 2015). Concentrations of ribavirin were below limit of quantification in raw wastewater and treated wastewater in Germany and China (Peng et al., 2014; Prasse et al., 2010). Ritonavir has been determined in wastewater in South Africa (mean 1600–3200 ng/L) (Abafe et al., 2018), treated hospital effluent in Switzerland (max 108 ng/L)(Kovalova et al., 2012), and surface water in France (max. 12 ± 5 ng/L)(Aminot et al., 2015). The predicted concentrations of lopinavir and ritonavir in raw wastewater were several times lower than the abovementioned high concentrations of lopinavir and ritonavir in wastewater in South Africa, probably owing to the daily usage for HIV treatment in South Africa (Abafe et al., 2018). The occurrence of the other substances has not been determined in environmental waters.

Clearly, the PECs of the target substances greatly depend on N_t, the number of treated patients per 100 k population in a day. Since expected treatment duration is 5–10 days for most therapeutics in this study, the assumed N_t value of 100 would imply that there are additional 10–20 patients treated by a drug per 100 k population each day. As of February 2021, multiple countries have reported more than 10 new daily confirmed cases per 100 k population; for example, Gibraltar (364), Belgium (143), Portugal (126), Switzerland (98), France (78), the US (75), Spain (74), Germany (69), Qatar (64), Italy (64), Chile (46), South Africa (32), Brazil (27), and Russia (20) (7-day moving average; WHO, 2020). These numbers would have been much larger at regional/county level; in the US, 7-day moving average of daily new cases per 100 k population has been up to more than 500 in 88 counties (1.63 million population in total), and up to more than 1000 in 34 counties (0.74 million population in total) as of Feb 1, 2020 (USAFacts US Coronavirus Cases and Deaths, 2021). Considering these numbers and the ratio of severely or critically ill patients of COVID-19 (19%) (Wu and McGoogan, 2020), the predicted number of patients given in this study can be a likely scenario in many parts of the world, and the number can be even larger in areas with high infection rates.

3.3. High predicted ecotoxicological risk for favipiravir, lopinavir and umifenovir

Ritonavir showed the highest chronic toxicity to aquatic organisms (PNEC 2.9 ng/L; Table 3), followed by lopinavir (4.7 ng/L), lopinavir M1 (5.9 ng/L), umifenovir (9.3 ng/L) and ritonavir M2 (20 ng/L). Ritonavir has been widely concerned for its high ecotoxicological risk because of its exceptionally high hydrophobicity (Escher et al., 2011). PNEC of the other substances were predicted at more than 30 ng/L, up to 120,000 ng/L.

Consequently, high ecotoxicological risk was suggested in the receiving river waters for favipiravir-T705M1 (RQ 5.2), metabolites of lopinavir (0.78–3.9), umifenovir (1.7) and lopinavir (1.5) (Table 3). Medium risk was predicted for seven substances: ritonavir (0.89) and its metabolite M2 (0.53), $TCONH_2$, the major metabolite of ribavirin (0.64), hydroxychloroquine (0.47), *N*-desethylchloroquine, the major metabolite of chloroquine (0.25), remdesivir (0.17), and GS-451524,

Table 3
Predicted Environmental Concentration (PEC), Predicted No Effect Concentration (PNEC), ecological Risk Quotients (RQ) and Environmentally acquired antiviral Drug Resistance Potential (EDRP) of potential therapeutic agents for COVID-19 in wastewater and environmental waters.

Substances	PEC (ng/L)			PNEC	RQ			EDRP (x 10^{-3})		
	Raw wastewater	Secondary effluent	River	(ng/L)	Raw wastewater	Secondary effluent	River	Raw wastewater	Secondary effluent	River
Chloroquine	857	320	32	3700	0.23	0.086	0.0086	2.6	0.97	0.097
***N*-desethylchloroquine**	171	135	13	55	3.1	2.5	0.25	0.57	0.45	0.045
Dexamethasone	3.0	2.9	0.29	50	0.060	0.058	0.0060	n.a.[c]	n.a.	n.a.
Favipiravir	64	63	6.3	91	0.71	0.69	0.069	0.0066	0.0064	0.00064
T705M1	4248	4168	417	81	53	52	5.2	0.40	0.39	0.039
Hydroxychloroquine	833	783	78.3	170	5.0	4.7	0.47	3.4	3.2	0.32
Lopinavir	880	71	7.1	4.7	190	15	1.5	0.81	0.066	0.0066
Lopinavir-M1	2840[a]	659[a]	66[a]	5.9	96–480[b]	7.8–39[b]	0.78–3.9[b]	2.6[d]	0.59[d]	0.059[d]
Lopinavir-M2				30						
Lopinavir-M3/M4				30						
Oseltamivir	113	110	11	4700	0.024	0.023	0.0023	0.0035	0.0035	0.00035
Oseltamivir carboxylate	600	589	59	120000	0.0049	0.0048	0.00048	0.021	0.021	0.0021
Remdesivir	55	54	5.4	31	1.8	1.7	0.17	0.12	0.12	0.012
GS-451524	270	265	26	240	1.1	1.1	0.11	1.2	1.2	0.12
Ribavirin	2102	2063	206	2700	0.77	0.75	0.075	0.079	0.077	0.0077
$TCONH_2$	5440	5339	534	830	6.5	6.4	0.6	0.44	0.44	0.044
Ritonavir	373	26	2.6	2.9	128	8.9	0.89	0.060	0.0042	0.00042
Ritonavir-M2	604	106	11	20	30	5.3	0.53	0.095	0.017	0.0017
Teicoplanin	1654	1654	165	n.a.	n.a.	n.a.	n.a.	0.53	0.53	0.053
Umifenovir	1200	157	16	9.3	130	17	1.7	0.61	0.080	0.0080
Umifenovir-M10	90	86	8.6	160	0.58	0.55	0.055	0.039	0.037	0.0037
Umifenovir-M18	45	6	0.6	25000	0.0018	0.00023	0.000023	0.017	0.0022	0.00022
Umifenovir-M20	63	62	6.2	240000	0.00027	0.00026	0.000026	0.023	0.022	0.0022

[a] The fraction of each of the four metabolites of lopinavir (M1 to M4) was not available, thus the sum of the four metabolites is shown.
[b] The fraction of each of the four metabolites of lopinavir (M1 to M4) was not available, thus the range of RQ is shown, using the maximum and the minimum PNEC to the sum of four metabolites.
[c] n.a.; not available. EDRP of dexamethasone was not available because it is a corticosteroid, not an antiviral agent.
[d] The fraction of each of the four metabolites of lopinavir (M1 to M4) was not available, thus the EDRP was evaluated with the sum of the four metabolites.

the major metabolite of remdesivir (0.11). Low risk was predicted for the remaining nine substances: chloroquine, dexamethasone, favipiravir, oseltamivir, oseltamivir carboxylate, ribavirin and metabolites of umifenovir. The predicted high ecological risk posed by lopinavir and ritonavir in this study is in accordance with a previous study, in which the two antiretroviral drugs dominated RQ in hospital effluents (Escher et al., 2011). Our predictions, however, suggests an appreciably high ecotoxicological risk posed by the metabolites of remdesivir and favipiravir, which have been recommended for the treatment of COVID-19 in several countries. Prediction by VEGA shows that narcosis is the major mode of action in aquatic organisms for all the substances, except for remdesivir, ritonavir and ritonavir-M2 (acetylcholinesterase inhibition), and ribavirin (reactivity). Apart from these antiviral drugs, high ecotoxicity by narcosis has also been suggested for some pharmaceuticals derived from domestic and hospital effluents (e.g. diclofenac) in receiving waters (Al Aukidy et al., 2012; Escher et al., 2011); thus, the ecotoxicity of these pharmaceuticals can be added up in the receiving waters and lead to even higher ecotoxicity. Overall, significant ecological adverse effects can be posed in river waters receiving the discharge of antiviral drugs and metabolites.

3.4. Environmentally acquired antiviral drug resistance potential (EDRP) of SARS-CoV-2 in the natural reservoirs

For all the antiviral drugs in this study, the risk of EDRP against SARS-CoV-2 appears to be insignificant, because there are at least three orders of magnitude difference between PEC and vLC_{50}/vEC_{50} for all substances (Tables 3). In river waters, largest EDRP was found for hydroxychloroquine (0.00032), followed by GS-451524 (the major active metabolite of remdesivir; 0.00012), and chloroquine (0.000097). The small EDRP in the present study was primarily due to the large vLC_{50}/vEC_{50} values of the therapeutic drugs in this study against SARS-CoV-2 (0.72 to >100 μM; 242 to >31,200 μg/L). While in the case of influenza, vLC_{50} of oseltamivir carboxylate, the active form of oseltamivir, is much smaller (e.g., 0.28–0.81 nM; 80–230 ng/L) (Gubareva et al., 2001; Monto et al., 2006). Hence, environmental concentrations of oseltamivir carboxylate can be comparable to vLC_{50} during influenza outbreak, suggesting a significant risk of EDR in the body of water fowls (the natural reservoir of influenza virus) in wastewater-impacted water bodies (Azuma et al., 2012; Fick et al., 2007; Ghosh et al., 2010; Jain et al., 2013; Nannou et al., 2020; Singer et al., 2007).

Regardless of the small EDRP as above, we must note that numerous populations of wild or domestic animals potentially possess SARS-CoV-2; coronaviruses are known to circulate in mammals, and various animals can be direct or intermediate host for SARS-CoV-2. In the case of SARS-CoV-2, bats have been suggested as animal reservoirs as they carry a coronavirus named RaTG13, which is genetically 96.2% identical to SARS-CoV-2 (Zhou et al., 2020). Pangolins also have coronaviruses similar to SARS-CoV-2 (Lam et al., 2020), but they are unlikely the reservoir and they likely acquired these coronaviruses after spillover from the natural hosts (Hu et al., 2021). Besides bats and pangolins, some of domestic or cultured animals such as cats, ferrets and minks are susceptible to SARS-CoV-2, and infections between individuals have been observed for cats and minks (Oreshkova et al., 2020; Shi et al., 2020). In the past coronavirus-outbreaks, palm civets were found to be the intermediate host animals for SARS-CoV, and dromedary camels for MERS-CoV (Hu et al., 2021).

Therefore, regardless of the small EDRP as above, it is recommended that residues of antiviral drugs in wastewater must be reduced. In domestic/cultured settings, wastewater-impacted waters should not be given to animals which are susceptible to SARS-CoV-2. Similarly to antiviral drugs, excessive usage of therapeutic or non-therapeutic antimicrobials has been a matter of concern over disruption of natural biological systems as well as development of antimicrobial resistance in the aquatic systems (Usman et al., 2020).

3.5. Uncertainties

We acknowledge following uncertainties in our predictions. First, usage of each drug would differ depending on regulatory status, drug characteristics (e.g., dosage form, utility, adverse reactions) and patients' health conditions. There are also cases where practitioners and general public are using more than one drug for their own precautions from COVID-19, perhaps owing to a lack of reliable guidelines on specific drug usage for COVID-19. Such practice would result in even higher amount of drugs and their metabolites releasing into the environment, further exacerbating the ecotoxicological impacts. Second, the QSAR models used in this study are supposed to be only for screening analysis of chemicals (EPA, 2021). Therefore, further studies are required for precise evaluations of chemical properties, environmental behavior and ecotoxicity of the substances. Third, the prediction we provided is for a given snapshot concentrations of drug residues, which needs to be substantiated through time-course analysis of drug concentrations in ambient waters during pandemic events, as was done for oseltamivir during an influenza outbreak (Singer et al., 2007). In terms of spatial distribution of drug residues in environmental waters, specific facilities (i.e. hospitals or quarantined hotels and residences) can be important point sources, to which a large proportion of symptomatic patients are often transferred. The impact of such specific medical facilities on environmental pharmaceutical discharge can be particularly significant in small catchments (Kuroda et al., 2016).

4. Conclusions

In the fight against COVID-19, medication is obviously essential in saving human lives and speeding up the recovery. Meanwhile, the potential negative environmental impacts of increased drug usage should not be overlooked. Our study suggests the following:

1. Conventional WWTPs are not capable of efficiently eliminating (removal efficiency <20%) dexamethasone, favipiravir, hydroxychloroquine, oseltamivir, remdesivir, ribavirin and their metabolites from raw wastewater. Therefore, effluents from conventional WWTPs may contain high concentrations of these drugs and their metabolites (up to 7402 ng/L, combined for ribavirin and its metabolite $TCONH_2$), potentially posing high risk to aquatic lives.
2. High risk quotients in effluent-receiving rivers are predicted for T705M1, a metabolite of favipiravir (RQ 5.2), metabolites of lopinavir (0.78–3.9), umifenovir (1.7) and lopinavir (1.5). Use of chloroquine, hydroxychloroquine, remdesivir, ribavirin and ritonavir also implies medium ecotoxicological risk (RQ >0.1) by the parent compounds or their metabolites in rivers.
3. EDR is less concerning for SARS-CoV-2, because PECs of the antiviral drugs in rivers are more than a thousand times smaller than reported vEC_{50}/vLC_{50} values of antiviral drugs against SARS-CoV-2. Nevertheless, efforts to reduce environmental discharge of antiviral drugs and their metabolites are important in terms of EDR prevention, as there are numerous populations of SARS-CoV-2-susceptible animals.

In order to address these issues, proper usage and management of antiviral drugs, and proper management of unused pharmaceuticals must be shared and implemented. Direct disposal of drugs into wastewater must be avoided, and using wastewater-impacted waters for animals must be refrained. In the long term, upgrading WWTPs with advanced treatments, such as ozonation, must be facilitated to efficiently remove diverse pharmaceuticals. On-site treatment of hospital effluents can also be effective in reducing environmental discharge of pharmaceuticals. Proper collection and treatment of wastewater in developing communities are a challenge, and thus additional investment is necessary. In order to facilitate such discussions, measurement-based evaluation of occurrence, fate, and ecotoxicological risk of various therapeutics associated with COVID-19 in WWTPs and environmental waters is urgently warranted in many parts of the world.

CRediT authorship contribution statement

Keisuke Kuroda: Conceptualization, Visualization, Investigation, Data curation, Writing – original draft. **Cong Li:** Formal analysis. **Kiran Dhangar:** Investigation. **Manish Kumar:** Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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