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LETTER

Detection of SARS-CoV-2 RNA in Medical Wastewater in Wuhan During the COVID-19 Outbreak

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Dear Editor,

The outbreak of 2019 novel coronavirus disease (COVID-19), caused by the infection of SARS-CoV-2, was first reported in Wuhan, China (Kong *et al.* 2020a, 2020b) and has become the most serious public health emergency in the century (Matsuzaki *et al.* 2010; World Health Organization 2020). The fecal shedding of SARS-CoV-2 has been proven by the viral strains isolated from COVID-19 patient's stool specimens (Wang *et al.* 2020). It proposed the possibility that contaminated waste water and fomites might be involved in disease transmission (Tang *et al.* 2020), especially at the healthcare facilities with large number of patients. Several studies have demonstrated the possible transmission of SARS-CoV-2 by wastewater (Kitajima *et al.* 2020; La Rosa *et al.* 2020; Orive *et al.* 2020). Here we report the results of a small scale experimental investigation, showing that low level of SARS-CoV-2 RNA was present in the wastewater from COVID-19 related facilities in Wuhan, China during the outbreak.

As the first epicenter of the pandemic, Wuhan has experienced a catastrophic medical need that once collapsed the healthcare system of the city. In order to handle the situation and brake the transmission chain of SARS-CoV-2, a three-layer COVID-19 healthcare facility system was built, including 48 designated hospitals for COVID-19 patients in severe or critical conditions, 14 Fangcang shelter hospitals treating patients with mild symptoms and over 100 community quarantine spots for the isolation and health monitoring of recovered patients, suspected patients and close contacts (Chen *et al.* 2020) (Supplementary Table S1).

The study involved four types of facilities, including two designated hospitals, two Fangcang shelter hospitals, two community quarantine spots and two urban wastewater treatment plants (WWTPs) (Table 1). All the six hospitals/quarantine spots were equipped with permanent or temporary onsite liquid waste treatment system (LWTS). On March 4th, 2020, water samples were collected from 10 sampling sites, including the water outlet of onsite LWTS at each healthcare facility, as well as the water inlet and outlet of WWTPs. Two liters of specimen were collected with sterile containers at each site. Specimens were tested for total residual chlorine immediately using 3, 3′, 5, 5′-tetramethylbenzidine colorimetry. The nucleic acid was extracted from 200 μL of specimen using a GeneRotex automated nucleic acid extraction system (Tianlong, Xi'an, China) and a commercial qPCR assay (Daan Gene, Guangzhou, China) was employed to detect the presence of SARS-CoV-2 RNA. The assay's limit of detection (LoD) for SARS-CoV-2 *ORF1ab* and *N* gene was 500 copies/mL with cut-off cycle of threshold (Ct) value of 40. As shown in Table 1, samples from healthcare facilities had higher concentrations of residual chlorine (1 mg/L to > 10 mg/L) than those from WWTPs (< 0.5 mg/L), which was related to the chlorine-containing disinfectant uses at the onsite liquid waste treatment sites. Although most samples were negative in the SARS-CoV-2 RNA qPCR test, sample #6 from a quarantine spot presented a weak positive result for the *N* gene (Ct value = 38.96). The detection rates of viral RNA in 8 facilities were zero for *ORF1ab* fragment and 12.5% for *N* gene.

In order to identify the potential low-level viral RNA contamination, one water sample from each type of facility was chosen to be concentrated and further tested for SARS-CoV-2 RNA (Table 1). For each sample, a total of 500 mL homogeneous specimen was collected on 47 mm diameter

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Table1 Presence of SARS-CoV-2 RNA in the wastewater from different locations in Wuhan, China, during the COVID-19 outbreak

Facility (Patients capacity)		Sampling site	Concentration of chlorine residual (mg/L)	Before concentration		After concentration (250:1)				
				qPCR result (Ct value)		qPCR result (Ct value)		ddPCR result (copies/reaction)		
				ORF1ab	N	ORF1ab	N	ORF1ab	N	E
Designated hospital										
1	Wuchang Hospital (504)	Outlet of the West Zone onsite LWTS	>10	Negative	Negative	NT	NT	NT	NT	NT
2	The Central Hospital of Wuhan (543)	Outlet of the onsite LWTS	6	Negative	Negative	35.90	33.63	134	42	10.2
Fangcang shelter hospital										
3	Jiangxia Cabin Hospital (564)	Outlet of the onsite LWTS	1	Negative	Negative	NT	NT	NT	NT	NT
4	Jiangan Cabin Hospital (1000)	Outlet of the onsite LWTS	3	Negative	Negative	33.64	32.31	402	26	36
Community quarantine spot										
5	Guanggu New Beacon Hotel (60)	Outlet of the onsite LWTS	5	Negative	Negative	NT	NT	NT	NT	NT
6	Jinyinhu New Beacon Hotel (167)	Outlet of the onsite LWTS	NT (high turbidity)	Negative	38.96	Negative	Negative	44	6.6	11
WWTP										
7	Qingshan WWTP	South 3# wastewater inlet	<0.5	Negative	Negative	NT	NT	NT	NT	NT
8	Qingshan WWTP	South 3# water outlet	<0.5	Negative	Negative	NT	NT	NT	NT	NT
9	Hanxi WWTP	Main wastewater inlet	<0.5	Negative	Negative	Negative	36.15	110	0	0
10	Hanxi WWTP	Main water outlet	<0.5	Negative	Negative	NT	NT	NT	NT	NT

WWTP wastewater treatment plant, *qPCR* quantitative PCR, *ddPCR* droplet digital PCR, *LWTS* liquid waste treatment system, *Ct* cycle of threshold, *NT* not tested.

EZ-PAK filter with 0.45 μm pore (Millipore, US) and the retentate was eluted in 2 mL of phosphate buffer saline (pH 9.5) (Zhou *et al.* 2010). The concentrated samples then underwent nucleic acid extraction and qPCR test as above. In addition, droplet digital PCR (ddPCR) assay stargeting *ORF1ab*, *N* gene and *E* gene were exploited. Target 1 (*ORF1ab* gene) comprised forward primer CCCTGTGGGT TTTACACTTAA, reverse primer ACGATTGTGCATCA GCTGA, and the probe 5′-FAM-CCGTCTGCGGTATGTG-GAAAGGTTATGG-BHQ1-3′. Target 2 (*N* gene) comprised forward primer GGGGAACTTCTCCTGCTAGAAT, reverse primer CAGACATTTTGCTCTCAAGCTG, and the probe 5′-FAM- TTGCTGCTGCTTGACAGATT-TAMRA-3′. Target 3 (*E* gene) comprised forward primer ACAGGTACGT-TAATAGTTAATAGCGT, reverse primer ATATTGCAG CAGTACGCACACA, and the probe 5′- FAM-ACACTAG CCATCCTTACTGCGCTTCG-BBQ-3′. The ddPCR tests were performed on a QX200 droplet digital PCR system (Bio-Rad, USA) as previously described (Chan *et al.* 2020; Corman *et al.* 2020; Dong *et al.* 2020). The LoDs of ddPCR were 2 copies/reaction for all three targets. SARS-CoV-2 RNA was detected in all four concentrated samples by either qPCR or ddPCR. The detection rates of qPCR rose to 50% for ORF1ab and 75% for *N* gene, and those of ddPCR were 100% for *ORF1ab*, 75% for *N* gene and 75% for *E* gene. Both were much higher than the detection rates before concentration. Samples from the designated hospital and Fangcang shelter hospital presented higher viral RNA levels than those from quarantine spot and WWTP. Notably, the qPCR result of concentrated sample #6 was negative for *N* gene, which could be related to the high level of the interfering substance in the concentrated wastewater. The ddPCR assay, on the other hand, detected SARS-CoV-2 RNA in the same sample, showing high sensitivity for the complex sample including multifarious wastewater (Singh *et al.* 2017). Besides, as samples were concentrated with 0.45 μm filter, instead of filter with 0.22 μm pore that easily clogged by the wastewater sample in the prior test, the concentration efficiency might be compromised (Ahmed *et al.* 2020; Hennechart-Collette *et al.* 2020).

This study was conducted in the early of March 2020, the later stage of COVID-19 outbreak in Wuhan. Although our observation had a very limited sample number, the SARS-CoV-2 RNA presence in wastewater appeared to be a pervasive phenomenon in Wuhan, when there were still over 20 thousand COVID-19 patients in the city. Viral RNA was not only found in the liquid waste of medical facilities, but also in

the urban sewerage network, which was in accordance with the recent report that viral RNA was detected in the wastewater surveillance in the Netherlands, the United States and Sweden (La Rosa *et al.* 2020; Mallapaty 2020; Orive *et al.* 2020). However, the trace of SARS-CoV-2 RNA did not indicate the presence of infectious viral particles. The viral RNA level detected in our study was very low (under or close to the LoD of qPCR assay), indicating wastewater unlikely to be a spread source in this scenario. Adequate disinfection of wastewater is essential to control the source of infection. In order to eliminate the wastewater contamination caused by centralized COVID-19 healthcare facilities, additional disinfection of drainage system such as continuous disinfectant drip (Supplementary Figure S1) was conducted in Wuhan, as well as the standard onsite wastewater disinfection.

The detection of SARS-CoV-2 RNA from wastewater not only provides a warning sign for the virus's arrival in community, but also implies the possible transmission of SARS-CoV-2, especially in the outbreak city with centralized isolation hospitals. Considering COVID-19 pandemic has caused lack of testing resources in many countries and regions, we call for particular attention to the surveillance and efficient disinfection of wastewater from COVID-19 related facilities, as well as the systematic study on the role of polluted wastewater in SARS-CoV-2 transmission.

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Compliance with Ethics Guidelines

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement This article does not contain any studies with human or animal subjects performed by any of the authors.

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