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Wastewater-Based Epidemiological Surveillance for SARS-CoV-2

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Abstract

Background:

Similar to enteric or diarrheal coronaviruses (CoV), SARS-CoV-2 is also known for its gastrointestinal and fecal shedding even in asymptomatic Covid-19 individuals.

Methods:

An online literature search was conducted using Google scholar. PubMed, Europe PMC, Medline etc. to collect published articles on the countrywide detection of SARS-CoV-2 in various water and wastewater sources.

Result:

Ample of recent studies showed detection SARS-CoV-2 RNA in municipal and hospital wastewater as well as river and pond samples, suggesting its potential waterborne spread. Most of the reports used the molecular method (RT-qPCR) to detect the viral RNA, whereas some also quantified the RNA. Notably, the analyzed studies used different methods of water sampling and virus concentration, chose different gene-targets for RT-qPCR and presented RNA quantity as genome copy/mL or log10. Several of studies also described different physical and chemical methods of decontamination or inactivation of SARS-CoV-2 in treated water. Overall, while considerable amount of viral RNA was reported widely, couple of study tested the viability and infectivity of the retrieved particles in cultured cells.

Conclusion:

The available data on occurrences of SARS-CoV-2 in various water sources suggest an urgent need of wastewater-based epidemiological surveillance as an early-warning tool for COVID-19. This would help prevent unexpected contamination and safeguard drinking water.

Keywords: SARS-CoV-2; Covid-19; Fecal shedding, Waterborne infection, Wastewater surveillance.

1. Introduction

Enteric viruses are primarily manifested and shed in the gastrointestinal tract of infected individuals. Waterborne enteric viruses are thus transmitted through ingestion of feces contaminated water or food. The most significant feature of enteric viruses is their transmission potential at a low infectious amount and viability under environmental stresses [1]. In humans, most of the enteric virus infections occur asymptomatically or cause self-limiting gastroenteritis, diarrhea or respiratory infections. Of these, rotavirus, norovirus, astrovirus, enterovirus, cytomegalovirus, adenovirus, hepatitis A virus and hepatitis E virus cause vomiting, diarrhea, jaundice or liver disease [2]. Coronaviruses (CoVs) are enveloped RNA viruses that primarily cause respiratory tract infections in humans, bronchitis in chickens, hepatitis in mice, and severe gastroenteritis in calves, piglets and dogs [3]. In addition, enteric manifestation and waterborne transmission of human CVS are also known. Previously, ample of studies have reported occurrence of CoV-like particles in fecal samples of individuals with or without gastroenteritis [4-6]. Of the several species of mammalian CoVs, six (HCoV-OC43, HCoV-229E, HCoV-NL63 HCoV-HKU1, SARS-CoV-1, and MERS-CoV) are known to primarily cause respiratory infections in humans [7]. The seventh and newly identified SARS-CoV-2 that has caused the SARS-CoV-2 disease (COVID-19) pandemic, is the third most pathogenic CoV after SARS-CoV-1 and MERS-CoV [8, 9]. Much has been learnt from the previous experience with the SARS-CoV-1 and MERS-CoV outbreaks, which could provide significant insights into the current COVID-19 scenario. Similar to SARS-CoV-1 and MERS-CoV, a proportion of COVID-19 patients have shown a relatively ‘asymptomatic’ state where the incubation period may include a time when the first diagnostic specimen is tested positive before the onset of typical symptoms. In view of this, the potential transmission of SARS-CoV-2 during the incubation period in a ‘pre-symptomatic’ state has been underlined [10, 11]. This review article presents an update on the growing evidence on detection of SARS-CoV-2 in different water sources from different geographical regions, and the need of wastewater-based epidemiological (WBE) surveillance for COVID-19. In view of this, an online literature search was conducted using Google scholar, PubMed, Europe PMC, Medline etc. to collect published articles, using phrases like water contamination of SARS-CoV-2, waterborne COVID-19, SARS-CoV2 RNA in wastewater etc.

2. Enteric Manifestations of Human CoVs

In general, all human CoVs have been variably associated with enteric manifestations, including diarrhea and stool shedding. In previous studies, while an equal proportion of OC43 or NL63 infected patients showed digestive symptoms and fecal shedding [12], a higher frequency was observed among NL63 patients with positive respiratory specimens than those with negative tests [13]. Moreover, in NL63 infected individuals with respiratory disease, nearly one-third had stomach ache or diarrhea [14-16]. Though HKU1 is commonly associated with acute respiratory infection, it has been also linked to the intestinal issues [17, 18]. Taken together, the OC43, NL63, and HKU1 associated gastrointestinal symptoms with almost similar frequency have been suggested. In line with other human CoVs, diarrhea has been reported as a common manifestation of SARS-CoV-1 [19]. SARS-CoV-1 associated enteric disease has been further supported by histopathology from patients' biopsy or autopsy specimen [19-22]. In addition, both intestinal mucosal epithelium and lymphoid tissues were shown SARS-CoV-1 RNA [19, 21, 22]. In cases of MERS-CoV, digestive symptoms, mainly diarrhea have been reported in about one-third of patients [23-25]. Of these, up to 50% of patients showed shedding of viral RNA in stool samples [25, 26]. Similar to SARS-CoV-1 and MERS-CoV, a proportion of SARS-CoV-2 infected patients have shown gastrointestinal and hepatobiliary manifestations, such as nausea, vomiting, abdominal pain, diarrhea, liver dysfunction and hepatitis [27-30]. In addition, SARS-CoV-2 RNA has been detected in patients' biopsy-specimen from esophagus, duodenum, stomach and rectum, as well as in stool and urine samples [31-36]. Notably, higher titer of viral RNA has been observed in the rectal and stool samples than nasopharyngeal specimen [34-36]. Most importantly, the SARS-CoV-2 RNA remains detectable in stool samples for several days even after the patients' respiratory specimen are tested negative. The duration of virus shedding in stools with means of 2-3 weeks, as well as the amount of detectable viral RNA has been observed to vary among patients [32, 34, 37, 38]. Notably, a recent study has demonstrated the infectivity of stool-derived SARS-CoV-2 to cultured cells [39].

3. Occurrences of SARS-CoV-2 in Sewage and Wastewater

In general, transmissions of enteric or diarrheal viruses have been well associated with various water sources, such as drinking water pipelines, wells, lakes, and wastewater. Therefore, the enteric and fecal shedding of infectious SARS-CoV-2 further increases the risk of its waterborne or fecal-oral transmission [40]. Most low-income countries have poor sanitation and inadequate wastewater treatment facilities, which potentially aggravate the risks of COVID 19 spread. The SARS-CoV-2 contamination of water sources may occur through various pathways. The surface water, such as ponds, lakes and rivers where wastewater is often discharged directly without proper treatment, can further transport the virus through the water channels into the communities. In view of the highlighted plausible fecal contamination and waterborne transmission of SARS-CoV-2, several wastewater surveillance studies have reported detection of viral RNA in raw and treated wastewater samples collected from wastewater treatment plants, river and hospital septic tanks [41-48] (**Table 1).**

Notably, assessment of wastewater samples in a Spanish low prevalence area has strongly supported enteric and stool shedding of SARS-CoV-2 even before the first cases of COVID-19 were reported [49]. In a recent report, higher titers of SARS-CoV-2 have been found in wastewater samples than clinically confirmed cases [50]. Wastewater surveillance has been thus suggested as an early-warning preventive instrument to monitor COVID-19 spread [51, 52].

4. Wastewater Treatment and Inactivation of SARS-CoV-2

The half-life of SARS-CoV-2 in wastewater has been reported to be significantly affected by temperature [53], UV ozone [54], and chlorine. For effective centralized disinfection,

No.	Country	Sample source	Positive samples	Quantitative RT-PCR (gc/l)
1	Australia	Raw wastewater	4 (44%)	19–120
2	France	Raw wastewater	23 (100%)	3×10^{6}
	France	Raw wastewater	6 (75%)	5×10^{4}
3	Spain	Raw wastewater	4 (66.7)	7.5×10^{3}–15×10^{3}
	Spain	Primary sludge	9 (100%)	0.1×10^{5}–4×10^{4}
	Spain	Biological sludge	9 (90%)	7.5×10^{3}–10×10^{3}
	Spain	Raw wastewater	35 (83.3%)	2.5×10^{5}
	Spain	Secondary effluent	2 (11%)	2.5×10^{5}
	Spain	Raw Wastewater	13 (86.7%)	5.2–5.9 log10
4	Italy	Raw wastewater	6 (50%)	NA
	Italy	Raw wastewater	7 (50%)	NA
	Italy	River water	3 (75%)	NA
5	Germany	Raw wastewater	9 (100%)	3.0×10^{3} – 20×10^{3}
	Germany	Secondary effluent	4 (100%)	2.7–37×10^{3}
	Germany	Effluent	44 (86%)	2.0 x10^{3} – 3.0×10^{6}
6	China	Hospital septic tank	2 (33%)	0.5 – 18.7×10^{3}
7	Nertherland	Airport wastewater	1 (100%)	NA
	Nertherland	City wastewater	1 (100%)	NA
	Nertherland	Sewage water	14 (58.3%)	2.6×10^{3}-30×10^{3}
8	USA	Raw wastewater	7 (100%)	$>3\times10^{4}$
	USA	Raw wastewater	10 (71.4%)	0.1×10^{5} – 2×10^{5}
	USA	Raw wastewater	18 (82%)	42.7×10^{3}
	USA	Primary sludge	36 (10%)	1.7x10^{6}– 4.6×10^{8}
	USA	Raw wastewater	2 (13.2%)	3.2 log10
	USA	Raw wastewater	120 (60.6%)	10^{2}–10^{5}
	USA	Raw wastewater	126 (61%)	66–390
9	UK	Sewage water	4 (100%)	3.5 – 4.2 log10
10	Japan	Raw wastewater	7 (26%)	2.1×10^{4} – 4.4×10^{4}
	Japan	Treated wastewater	1 (20%)	2.4×10^{3}

11	India	Sewage water	2 (100%)	$0.78x10^2 – 8.05x10^2$
	India	Raw wastewater	30 (100%)	$3.08\ x10^4 – 2.19\ x10^5$
	India	Raw wastewater	6 (35.3%)	NA
	India	Raw wastewater	6 (100%)	NA
12	Iran	Sewage water	8 (80%)	$0.1×10^4$
13	Pakistan	Raw wastewater	21 (26.9%)	NA
14	UAE	Wastewater	33 (85%)	$2.8×10^2 – 2.9×10^4$
15	Israel	Sewage water	3 (17.6%)	NA
16	Turkey	Raw sewage	5 (71.3%)	$2.9×10^3– 1.8×10^4$
	Turkey	Raw sewage	9 (100%)	$1.1×10^4 – 4.\ ×10^4$
17	Ecuador	River water	3 (100%)	$2.9×10^5– 3.2×10^6$

Table 1: Worldwide wastewater-based epidemiological surveillance for SARS-CoV-2.

the World Health Organization (WHO) has suggested free chlorine (0.5 mg/L) pH 8.0) and at least 30 min of contact time [55]. The half-life of SARSCoV-2 in hospital wastewater was estimated to range between 4.8 and 7.2 h at 20°C [53]. Recently, a disinfection guideline, requiring free chlorine (6.5 mg/L) and contact time of 1.5 h for medical sewage has been initiated in China [54]. In view of this, there is a proposed measure that includes decentralization of wastewater treatment facilities, community-wide monitoring and testing of SARS-CoV-2 in wastewater samples, improved sanitation, developing point-of-use decontamination devices, and implementation of more focused policy [56]. Nonetheless, in some cases, even when the cause of water contamination is resolved, the drinking water still gets contaminated by the sewage through blockage of the drainage system, pipe leakage or pump failure. In other cases, inadequate or failing treatment processes also lead to partial removal of enteric viruses from water sources.

5. Wastewater-based Epidemiological Surveillance for SARS-CoV-2

In a pandemic situation, diagnostic tests have never been intended for mass surveillance because these are costly and time-consuming. Wastewater-based epidemiology (WBE) serves as an important instrument to trace and monitor enteric viruses excreted in feces in a community [57]. The provision of safe water, sanitation, waste management and hygienic conditions is therefore, essential for protecting public health during infectious disease outbreaks. In the recent decade therefore, WBE has been applied to a wide range of waterborne enteric viruses, including CoVs which are ultimately discharged into urban sewage [58-60]. WBE is an integrated technique that implies extraction of infectious agents its biomarkers (RNA or DNA) from water samples, genetic identification, data analysis and processing, and epidemiological interpretation. Wastewater samples are collected from different community sources, which are regarded as collective stool and analyzed where positive tests reflect the health status of the community in near real-time. In view of this, WBE could be a valuable surveillance tool to monitor SARS-CoV-2, providing opportunities to estimate its prevalence, genetic diversity and geographic distribution [61-62]. The WHO guidelines recommend a preventive management framework for sanitation and water surveillance for authorities who set the health-based targets for the protection of drinking water from waterborne infections [55]. This includes assessing the adequacy of systems, defining and monitoring control measures, and establishing management strategies for water safety. Such framework for safe water can be therefore, adapted according to environmental, socio-economic and cultural circumstances on the national, regional and local levels [63-66]. In addition, sewages of hostels, housing complexes, hotels, hospitals, quarantine centers, prisons, factories and warehouses, railway stations, seaports, airports, malls, stadiums, military cantonments and other confined areas should be monitored for SARS-CoV-2.

6. Sensitive Methods of Detection of SARS-CoV-2

Development of nucleic acid-based sensitive surveillance techniques has allowed detection of enteric viruses, including human CoVs. In terms of the COVID-19 pandemic, WBE is currently being applied to detect SARS-CoV-2 in wastewater to screen potential carriers and provide early warning of COVID-19 outbreaks in the community [67-75]. The molecular diagnostic tool that implies rear-time quantitative reverse-transcription polymerase chain reaction (RT-qPCR) targeting SARS-CoV-2-specific genes is the most sensitive method of detection in wastewater samples [76-84]. In brief, samples are collected either using automated sampling techniques (refrigerated or submersible autosampler) or the grab sampling techniques, and transported in cold conditions to the laboratory and stored at 4°C. Virus particle are concentrated using water ultracentrifugation and ultrafiltration, following RNA extraction and RT-qPCR using SARS-CoV-2 gene-specific primers [85-100]. Based on the positive-test results, the prevalence of COVID-19 in an area is estimated using a mass balance on the total number of viral RNA copies present in the wastewater, and those shed in feces [41]. Owing to the samples' origin of different water sources and geographic regions of variable endemicity or socioeconomic status, variable occurrences of SARS-CoV-2 have been reported. Nonetheless, the overall detection rate of SARS-CoV-2 RNA in raw sewage or wastewater samples ranged between 13.0 and 100% with optimal concentrations over 106 gc/L (Table 1). Notably, the first published report by Medema et al. [46] on detection of SARS-CoV-2 in Dutch untreated sewage used the ultrafiltration method of virus concentration and RT-qPCR for RNA quantification that ranged from 2.6×103 to 2.2×106 gc/L. Comparatively, while Wu et al. [50] data based on polyethylene glycol precipitation and ultracentrifugation of raw sewage, had demonstrated viral RNA detection ranging between 103 and 105 gc/L in the USA, Wurtzer et al. [42] detected SARS-CoV-2 RNA in French wastewater samples in the range of 5×104–3×106 gc/L. Randazzo et a [43]. using aluminum flocculation-based concentration methods, reported 2.5 ×105 gc/L in Spanish wastewater, which corroborated that of the German data based on ultracentrifugation and ultrafiltration of SARS-CoV-2 RNA [65]. Interestingly, Haramoto et al. [66] showed a comparatively lower level (2.5×103 copies/L) of SARS-CoV-2 RNA in secondary-treated wastewater samples in Japan, indicating the beneficial effect of water treatment on reducing the viral load.

7. Conclusions and Future Prospects

Since the first reporting on the detection of SARS-CoV-2 in the fecal samples, its plausible waterborne transmission through contaminated water has become an important epidemiological issue. Recently, ample of data on wastewater or sewage surveillance for SARS-CoV-2 has emerged from different countries. Though most of the WBE studies focused on SARS-CoV-2 detection and quantification in different water samples, couple of study has tested its viability and infectivity in cultured cells. Notably, different studies have described different methods of water sampling and virus concentration, selected different gene-targets for RT-qPCR and presented RNA quantity as genome copy/mL or log10. Therefore, it would not be possible to analyze and conclude the outcomes of these data, and propose a uniform protocol and benchmark limit.

Moreover, in such a pandemic situation, costly and time-consuming diagnostic tests have never been intended for mass surveillance. Implementation of WBE as an early-warning tool is thus very much required for community-wide monitoring of COVID-19. In view of this, sewage samples of hostels, housing complexes, hotels, hospitals, quarantine centers, prisons, public toilets, roadway and railway stations, airports, malls, stadiums, and military cantonments should be tested for SARS-CoV. Nonetheless, tracking the source, spread, and changing trends of this novel virus and its rapidly emerging variants in near real-time would be one of the most challenging aspects.

8. Conflicts of Interest

The authors declare no conflict of interests.

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