

Risks associated with the presence of SARS-CoV-2 in sewage and possible approaches to limit its spread through aquatic matrices

Riscos associados à presença do SARS-CoV-2 em esgotos e possíveis abordagens para limitar sua propagação através de matrizes aquáticas

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ABSTRACT

Introduction: SARS-CoV-2 is a new type of coronavirus capable of infecting humans and cause the Coronavirus Disease (COVID-19), an illness that has causing enormous impacts in Brazil and worldwide. The disease, due to its high-level dissemination and lethality rates, was declared pandemic by the World Health Organization in the first half of 2020. Several studies have frequently indicated the detection of SARS-CoV-2 RNA fragments in samples from sewage networks, treatment plants and natural waters. The presence of SARS-CoV-2 in those environments has raised the possibility of transmission through the contact with contaminated waters and aerosols generated during their flow or treatment. **Objective:** Describe detection reports of the new coronavirus in samples obtained from sewage networks, from waste sludges of treatment plants and from natural water bodies, and present the viability of this virus when artificially inoculated in those environments. **Method:** Integrative literature review based on scientific articles written in English or Portuguese, indexed in the *Web of Science*, *Scopus*, *PubMed*, *ScienceDirect*, *Google Scholar* and *MedRxiv* databases. **Results:** It was possible to highlight the risks that the SARS-CoV-2 poses to human and wildlife populations when present in wastewater, appropriate strategies to be used to limit the spread of this pathogen in aquatic matrices, and the importance of implementing epidemiological monitoring systems in those places. **Conclusions:** In order to reduce the risks of emerging and re-emerging outbreaks of COVID-19 through aqueous matrices, precautionary approaches regarding the presence of SARS-CoV-2 in those environments have been strongly recommended.

KEYWORDS: Epidemiological Monitoring; Disease Outbreaks; Residual Waters, Water Resources; Preventive Medicine

RESUMO

Introdução: O SARS-CoV-2 é um novo tipo de coronavírus capaz de infectar humanos e causar a *Coronavirus Disease* (COVID-19), enfermidade que tem causado enormes impactos no Brasil e no mundo. A doença, devido às suas altas taxas de disseminação e letalidade, foi declarada pandêmica pela Organização Mundial da Saúde no primeiro semestre de 2020. Vários estudos têm frequentemente indicado a detecção de fragmentos de RNA do SARS-CoV-2 em amostras de redes de esgoto, estações de tratamento e águas naturais. A presença do SARS-CoV-2 nesses ambientes tem levantado a possibilidade de transmissão pelo contato com águas contaminadas e aerossóis gerados durante seus fluxos ou tratamentos. **Objetivo:** Descrever relatos de detecção do novo coronavírus em amostras obtidas em redes de esgotos, em lodos residuais de plantas de tratamento e em corpos d'água naturais, e apresentar a viabilidade desse vírus quando inoculado artificialmente nesses ambientes. **Método:** Revisão integrativa de literatura fundamentada em artigos científicos escritos em inglês ou português, indexados nas bases

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de dados do *Web of Science*, *Scopus*, *PubMed*, *ScienceDirect*, *Google Scholar* e *MedRxiv*. **Resultados:** Foi possível destacar os riscos que o SARS-CoV-2 proporciona às populações de humanos e de animais selvagens quando presente nas águas residuais, estratégias cabíveis de serem utilizadas para limitar a propagação desse patógeno nas matrizes aquáticas, e a importância da implementação de sistemas de monitoramento epidemiológico nesses locais. **Conclusões:** A fim de reduzir os riscos de surtos emergentes e reemergentes da COVID-19 por meio de matrizes aquosas, abordagens preventivas em relação à presença do SARS-CoV-2 nesses ambientes têm sido fortemente recomendadas.

PALAVRAS-CHAVE: Monitoramento Epidemiológico; Surtos de Doenças; Águas Residuais; Recursos Hídricos; Medicina Preventiva

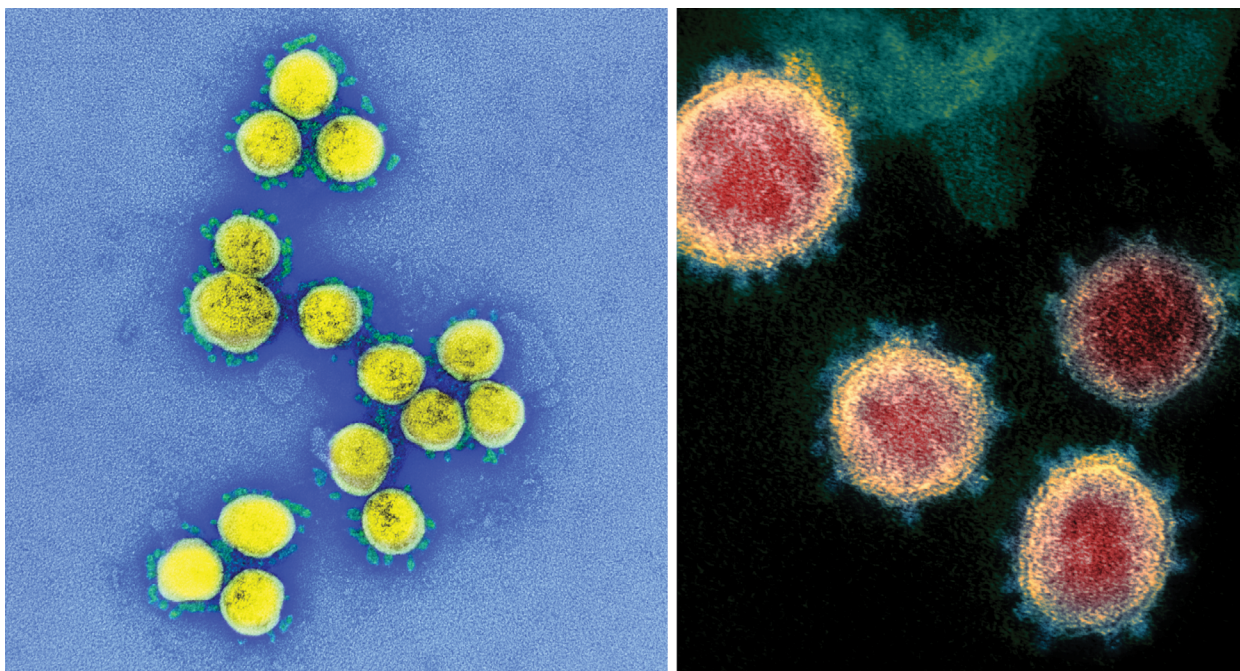
INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new type of coronavirus that is infectious to humans and causes the *coronavirus disease* (COVID-19), a fatal disease that is highly transmissible and has been responsible for enormous social and economic impacts throughout planet Earth¹. According to clinical studies, COVID-19 has manifestations in various human systems, including cardiovascular, renal, musculoskeletal, neurological, immune, visual, gastrointestinal, and especially the respiratory system^{2,3,4,5,6,7,8}. Symptomatic patients have presented fever, fatigue, taste and smell dysfunctions, rhinorrhea, lethargy, dry cough, dyspnea, headache, muscle pain, vomiting, diarrhea, and severe pneumonia^{9,10}. According to the World Health Organization, in the year 2020, there were approximately 81.5 million cases and 1.8 million deaths caused by COVID-19 in about 222 countries, areas and/or territories across the world¹¹.

SARS-CoV-2 belongs to the family Coronaviridae and the genus *Betacoronavirus*. The virus has a genome of approximately

30,000 single-stranded nucleotides of positive-sense RNA and is surrounded by a fragile lipid envelope whose surface contains glycoproteins called *spike*, crown-shaped structures from which it got its name (*corona* is the Latin word for crown)^{12,13} (Figure 1). SARS-CoV-2 has a sequence rate of change of approximately 1.1×10^{-3} sites per year, which represents two mutations per month when the world population is considered^{14,15}. Although most of the mutations caused in the genome of this virus were probably deleterious or neutral, a small portion caused significant changes in its infectivity and interaction with hosts¹⁵. The most important variants, the so-called variants of interest, were classified as Alpha (B. 1.1.7), Beta (B. 1.351, B. 1.351.2, B. 1.351.3), Gamma (P. 1, P. 1.1, P. 1.2) and Delta (B. 1.617.2, AY.1, AY.2, AY.3)¹⁶.

The Alpha variant, first described in the UK in December 2020, was observed to have 23 mutations, 17 amino acid changes, and a 46% increase in transmissibility^{16,17}. The Beta variant, initially reported in South Africa in October 2020, was observed to have



Source: National Institute of Allergy and Infectious Diseases - Rocky Mountain Laboratories (NIAID-RML).

Figure 1. Transmission electron micrograph of SARS-CoV-2 particles isolated from a patient.



23 mutations, 17 amino acid changes, and a 32% increase in transmissibility^{16,17}. The Gamma variant, first reported in Brazil in January 2021, was observed to have 35 mutations, 17 amino acid changes, and a 43% increase in transmissibility^{16,17}. The Delta variant, on the other hand, initially described in February 2021 in India, was observed to have approximately 15 mutations, six amino acid changes and a remarkable 60% increase in transmissibility when compared to the Alpha variant^{16,18}. All variants of interest, Alpha, Beta, Gamma, and Delta, have shown a higher tendency to require hospitalization^{19,20}. Moreover, the Delta variant presented a significant increase in viral load and higher risks of disease progression^{21,22}.

SARS-CoV-2 has been spread by direct contact with contaminated secretions that are carried and dispersed through the air, such as respiratory droplets, saliva, and airborne aerosol particles^{23,24}. In addition, reports have also indicated possible viral transmission through indirect contact with contaminated surfaces²⁵. According to viral persistence studies, aerosolized particles were able to provide SARS-CoV-2 infectivity for up to 16 hours²⁶, whereas surfaces like plastic, stainless steel and surgical masks could do it for up to 7 days^{27,28}. The best practices to contain the transmission of the disease have been protective measures like personal hygiene, face masks, eye protectors, physical distancing, adequate ventilation of enclosed spaces, disinfection of surfaces, and immunization by vaccines^{29,30,31,32,33,34}.

Elimination of SARS-CoV-2 viral particles through urine and feces has been commonly observed in COVID-19 patients, including mild, pre-symptomatic, and asymptomatic cases^{35,36,37}. The presence of viral particles of the new coronavirus in these samples, which were infectious in some cases, has evidenced the possibility of viral transmission through direct contact or aerosols generated by the excreta of contaminated patients^{38,39,40,41}. In addition, the excretion of SARS-CoV-2 through the feces and urine of patients with COVID-19 confirms the importance of viral monitoring in sewers and natural bodies of water, which collect and concentrate human excrement, and store water for public supply, irrigation or recreational activities^{42,43}.

In this context, this review aimed to describe reports of detection of SARS-CoV-2 in samples obtained in sewage networks, sludge from treatment plants and natural bodies of water, and the infectivity that this virus presents when artificially inoculated in these environments. The review highlighted the risks that SARS-CoV-2 poses to humans and wildlife when present in sewers, and approaches that can be adopted to limit the spread of this pathogen through aquatic media, including the implementation of epidemiological monitoring strategies in these environments.

METHOD

Database and search criteria

Our integrative literature review was based on scientific articles written in English or Portuguese and found in virtual repositories

of public access or accessed through academic institutions. The methodological procedure involved electronic searches between July 22, 2020, and September 21, 2021, in the databases of the *Web of Science*, *Scopus*, *PubMed*, *ScienceDirect*, *Google Scholar* and *MedRxiv*. At this stage, when conducted in Portuguese, the research used the combination of the following terms: “SARS-CoV-2”, “presença”, “detecção”, “água residual”, “esgoto”, “tratado”, “água natural”, “água de rio”, “infectividade”, “viabilidade”, “variantes”, “risco ambiental”, “vigilância”, and “monitoramento”. When conducted in English, the searches were done by combining the terms: “SARS-CoV-2”, “presence”, “detection”, “wastewater”, “sewage”, “treated”, “natural water”, “river water”, “infectivity”, “viability”, “variants”, “environmental risk”, “surveillance”, and “monitoring”.

Classification and refinement of scientific works

After the documents were obtained, they were screened for their titles and abstracts so we could verify whether they met the proposed topic and to eliminate duplicates. Then, the works were classified according to the following topics: (i) presence and detection of SARS-CoV-2 in wastewater or natural waters, (ii) infectivity and viability of SARS-CoV-2 in wastewater or natural waters, (iii) risks associated with the presence of SARS-CoV-2 in wastewater or natural waters, (iv) approaches to limit the spread of SARS-CoV-2 through aquatic media, and (v) epidemiological monitoring of SARS-CoV-2 and its variants in wastewater and natural waters. After classification, the articles were read in full and refined according to the criteria mentioned below in order to determine the possibility of including them as theoretical references in this study:

- The samples used in the studies were collected from aquatic media or in stages of sewage treatment processes;
- The studies presented detailed descriptions of the place of origin of the samples and the date of sample collection;
- The studies presented detailed descriptions of the procedures used to determine the viability of SARS-CoV-2 and to detect and/or quantify fragments of the genetic material of the virus;
- Quantification studies of the genetic material of SARS-CoV-2 had values expressed in the form of “copies per sample volume”;
- Systematic, narrative or integrative reviews addressed original research and relevant discussions on the topic of this article.

RESULTS AND DISCUSSION

After searching the databases by combining the keywords mentioned in the previous topic, the integrative review retrieved a total of 445 scientific articles. After the initial screening, it was found that 221 articles fit the topic proposed in this review and that 224 did not fit it or were duplicates. Of the 221 articles with relevant content: 110 were classified as belonging to the topic (i); nine as belonging to the topic (ii); 24, to the topic



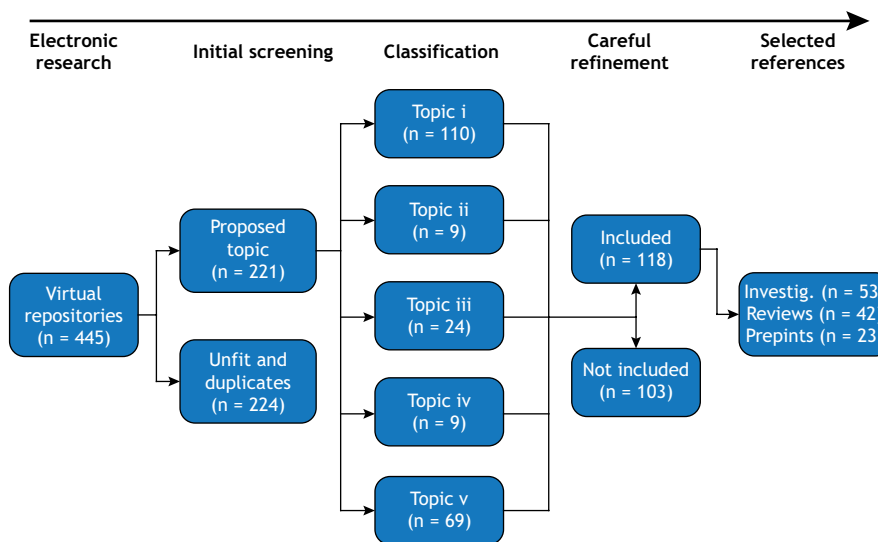
(iii); nine, to the topic (iv); and 69, to the topic (v). After the refinement based on the inclusion criteria, 118 scientific articles were chosen to be used as the theoretical foundation of this work, and 103 were excluded. Of the 118 articles selected and included in the references of this review, 53 were novel investigations, 42 were literature reviews, and 23 were preprints. The methodological path used to prepare this review is illustrated in Figure 2.

Presence of SARS-CoV-2 in sewers and in natural waters

SARS-CoV-2 is often found in expectorated body fluids, vomit, feces and urine of individuals with COVID-19. It has entered sewage systems through discharge wastewater from hospitals, isolation centers and homes inhabited or visited by infected people^{44,45,46}. Through leaks caused by infrastructure failures

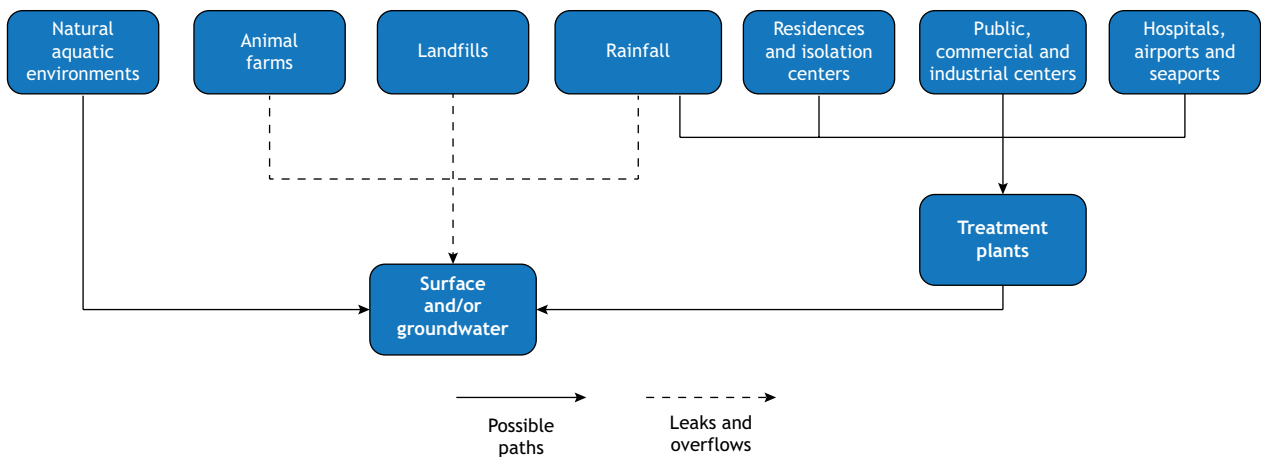
and/or untreated wastewater discharges, SARS-CoV-2 has been able to contaminate receiving water bodies like streams, rivers, ponds, estuaries, lakes and groundwater^{47,48,49,50}. In addition, combined sewage overflows, usually due to heavy rainfall, have increased the possibility of SARS-CoV-2 entering natural water systems^{12,46}. Figure 3 illustrates potential paths that the new coronavirus can take until it reaches sewage systems and natural waters.

Detection of SARS-CoV-2 in samples from these environments has been done through molecular biology procedures, based on the technique of Reverse Transcription followed by Real-Time Polymerase Chain Reaction (RT-qPCR). The methods, also called quantitative PCR, have provided the copy and quantification of the presence of fragments of genetic material of the virus through *in vitro* replication⁵¹. RT-qPCR methods are considered



Source: prepared by the authors, 2021.

Figure 2. Methodological approach used in the preparation of this review.



Source: prepared by the authors, 2021.

Figure 3. Main routes that SARS-CoV-2 can take to reach sewage systems and natural waters.



the gold standard for the detection of low amounts of genetic material in a sensitive and specific way in several types of media⁵². Reports indicate that the technique could successfully determine the diversity and abundance of various viral pathogens in wastewater and natural water samples, including those of Hepatitis A and E, Polyomavirus, Papillomavirus, Enterovirus, Rotavirus, Adenovirus, Parechovirus, Salivirus, Astrovirus, and Zika^{53,54,55,56,57,58}.

The analyses, although highly influenced by several physical-chemical-biological and methodological parameters, were able to detect concentrations ranging from 5.6×10 to 4.6×10^8 particles of the SARS-CoV-2 viral genome per liter of untreated wastewater^{59,60}. When carried out on samples of treated wastewater, that is, from the effluent generated by sewage treatment plants (STP), approximate amounts of up to 1.09×10^6 of viral gene particles of the new coronavirus were detected per liter⁶¹. In natural waters, like river and stream waters, analytical methods were able to detect concentrations of up to 3.28×10^6 SARS-CoV-2 genome particles per liter of sample⁶². Table 1 summarizes studies that detected particles of the SARS-CoV-2 viral genome in different samples from aquatic environments.

Viability of SARS-CoV-2 in sewage systems and natural waters

Methods applied in virology and are based on *in vitro* cell culture techniques and have been used to provide estimates of the infectious potential or viability that SARS-CoV-2 has when present in wastewater or natural waters⁹⁶. Protocols have been created using techniques and reagents that do not interfere with the integrity of the lipid bilayer surrounding SARS-CoV-2⁹⁷. These studies have been used to assess the potential risks that contaminated wastewater and natural waters pose to humans, especially regarding possible transmission by aerosols and/or fecal-oral route⁴⁶.

The assessment of the risks that a sample poses to human hosts and also to animals, as well as the detailed understanding of how an animal virus has crossed species boundaries to infect humans, as highlighted by Andersen et al.⁹⁸, has supported studies aimed at preventing future zoonotic events. In addition, to promote more accurate epidemiological models, research has considered the influence of several physical-chemical-biological factors on the survival of SARS-CoV-2 in aquatic environments, such as temperature, pH, retention time, amount of organic matter, chemical reagents, and the presence of antagonist microorganisms^{52,99,100}.

Although to date no studies have proven the existence of infectious particles of SARS-CoV-2 in samples from sewers, studies reported in the literature indicated that the new coronavirus, when artificially inoculated, was able to remain viable for up to 4 days in this environment at a temperature of 24 °C and 17.5 days at a temperature of 4 °C¹⁰¹. In natural waters, it was seen that SARS-CoV-2 was able to remain active and infectious for up to 6.4 days in river water at 24 °C and 18.7 days at 4 °C¹⁰¹. Additionally, the transmission of the new coronavirus through contaminated

sewage systems was evidenced in a survey conducted in a low-income community in China¹⁰². Table 2 summarizes the viability that infectious particles of SARS-CoV-2 can have when artificially inoculated in different aqueous media.

Presence of SARS-CoV-2 in sewage systems and natural waters

Fragments of the SARS-CoV-2 viral genome in sewage systems and natural waters have made researchers concerned about possible risks of indirect transmission of COVID-19 via fecal-oral route^{102,106}. Researchers have emphasized the possibility of SARS-CoV-2 infection through direct contact with sewage or contaminated water, aerosols generated in drainage and treatment systems, flushing toilets, and also through faulty connections of drains and sewage pipes in homes and buildings^{34,39,46}. Transmission by this route was evidenced during the 2003 SARS-CoV-1 outbreak, when studies indicated that aerosolized droplets of water contaminated with feces were responsible for the spread of the virus in a residential building in Hong Kong^{45,107}.

The presence of SARS-CoV-2 genome particles in natural waters, like lakes, rivers, springs, and streams, has also indicated the possibility of the spread of the new coronavirus in humans through recreation and fishing activities, as often happens with other diseases transmitted by enteric viral pathogens^{42,49,108}. The occurrence of the virus in these environments has also raised the possibility of the spread of SARS-CoV-2 in domestic and wild animals, new hosts that would tend to propitiate the spread, resurgence, and evolutionary adaptation of the pathogen in future outbreaks through cross-infection^{109,110,111,112,113}. Furthermore, the spread of SARS-CoV-2 through untreated wastewater has shown immense potential to have devastating consequences in populations of susceptible species, like terrestrial and marine mammals^{114,115}.

The reuse of treated water and activated sludge from treatment plants for processes like irrigation and fertilization of crops and urban parks, groundwater recharge and industrial activities has also been considered of concern^{12,116,117,118,119}. To date, although studies have not provided sufficient data to determine the risks related to the spread of SARS-CoV-2 by these routes, they have evidenced the existence of particles of the viral genome of the new coronavirus in treated wastewater and in the activated sludge generated in treatment plants, highlighting possible risks associated with its reuse^{83,88}. Similar viruses, like human Coronavirus 229E (CoV229E) and bovine Coronavirus (BCoV), have even been able to remain infective for up to four and 14 days in lettuce (*Lactuca sativa*) leaves, respectively^{120,121}.

Limiting the spread of SARS-CoV-2 through aquatic media

Inadequate sanitation and discharge of untreated sewage directly into surface waters have been considered possible sources of water and soil contamination by SARS-CoV-2^{12,106,122}. Consequently, the possible spread of COVID-19 among humans, domestic and wild animals that have had contact with contaminated waters has been hypothesized^{45,123}. The possibility of



Table 1. Reports of detection of fragments of the SARS-CoV-2 viral genome in samples of untreated sewage, waste sludge from treatment plants, treated sewage and natural waters.

Country	Location	Sample source	Viral load (copies per liter)	Reference
Australia	Southeast Queensland	Untreated sewage	$0.19 \times 10^2 - 1.2 \times 10^2$	63
Brazil	Florianópolis, Santa Catarina	Untreated sewage	$5.49 \pm 0.02 \log_{10} - 6.68 \pm 0.02 \log_{10}$	64
Brazil	Niterói, Rio de Janeiro	Untreated sewage	30.7 - 71.2	65
China	Dongxihu district	Untreated sewage	$0 - 1.4 \times 10^4$	66
Spain	Barcelona	Untreated sewage	$0 - 8.3 \times 10^2$	67
Spain	Valencia	Untreated sewage	$0 - 5.99 \log_{10}$	68
United States of America	Southeastern Virginia	Untreated sewage	$10^2 - 10^5$	69
United States of America	Massachusetts	Untreated sewage	$\sim 10^3$	70
United States of America	Bozeman, Montana	Untreated sewage	0 - 1710.8	71
United States of America	Southern Louisiana	Untreated sewage	$0 - 3.2 \pm 0.4 \log_{10}$	72
United States of America	Detroit, Michigan	Untreated sewage	$1.24 \times 10^4 - 2.85 \times 10^5$	73
Finland	Helsinki	Untreated sewage	$26 \pm 0.2 \log_{10} - 44 \pm 0.2 \log_{10}$	74
Netherlands	Tilburg	Untreated sewage	$0 - 2.2 \times 10^6$	75
India	Amedabad, Gujarat	Untreated sewage	$5.6 \times 10 - 3.5 \times 10^2$	59
Italy	Bologna	Untreated sewage	$0 - 3.3 \times 10^4$	76
Japan	Ishikawa and Toyama municipal administrations	Untreated sewage	$0 - 4.4 \times 10^4$	77
Japan	Yamanashi municipal administration	Untreated sewage	$0 - 2.4 \times 10^3$	78
Pakistan	Lahore, Punjab	Untreated sewage	$0 - 4.00 \times 10^3 \log_{10}$	79
United Kingdom	Southeast Region	Untreated sewage	$0 - 5.78 \pm 0.07 \log_{10}$	80
Spain	Orense	Primary sludge	$0 - 24.5 \times 10^3$	81
United States of America	New Haven, Connecticut	Primary sludge	$1.7 \times 10^6 - 4.6 \times 10^8$	60
Mexico	Santiago de Queretaro	Activated sludge	$0 - 10.753 \times 10^3 \log_{10}$	82
Turkey	Istanbul	Activated sludge	$1.17 \times 10^4 - 4.02 \times 10^4$	83
Germany	North Rhine-Westphalia	Treated sewage	$2.7 \times 10^3 \times 37 \times 10^3$	84
Chile	Santiago	Treated sewage	$0 - 167 \times 10^3$	85
France	Paris	Treated sewage	$\sim 10^5$	86
Iran	South of Tehran	Treated sewage	Qualitative	87
Iran	Tehran	Treated sewage	$7.18 \times 10^4 - 1.09 \times 10^6$	61
Israel	Jerusalem	Treated sewage	$>100 \times 10^3$	88
Italy	Padua, Veneto region	Treated sewage	Qualitative	89
Sweden	Gothenburg	Treated sewage	$0.14 \log_{10} - 6.27 \log_{10}$	90
China	Wuhan	Effluent from a hospital septic tank	$0 - 14.7 \times 10^3$	91
Brazil	State of Minas Gerais	River water	$0 - 1.1 \times 10^5$	92
Brazil	São Paulo City	Creek water	$1.40 \times 10^4 - 3.28 \times 10^5$	62
Ecuador	Quito	River water	$2.07 \times 10^5 - 3.19 \times 10^6$	47
Italy	Milan metropolitan area	River water	Qualitative	48
Mexico	Mexico City	River water	$0 - 79 \times 10^3$	93
Mexico	Monterrey metropolitan area	Groundwater	$0 - 38.3 \times 10^3$	94
Serbia	Belgrade	River water	$0 - 1.32 \times 10^4$	95

Source: prepared by the authors, 2021.



Table 2. Persistence of the viability of SARS-CoV-2 in different samples of sewage and natural waters (artificial inoculation).

Country	Location	Medium	Temperature	T ₉₀ (days)*	T ₉₉ (days)**	Virion survival time (days)	Reference
Brazil	Nova Lima, Minas Gerais	River water	24 °C	1.9	6.4	-	101
			4 °C	7.7	18.7	-	
		Sewage	24 °C	1.2	4.0	-	
			4 °C	5.5	17.5	-	
Ireland	Dublin	River water	4 °C	3.8	-	-	103
			20 °C	2.3	-	-	
		Sea water	4 °C	2.2	-	-	
			20 °C	1.1	-	-	
United States	Northern Indiana	Sewage	20 °C	1.6 - 2.1	3.2 - 4.3	-	104
		Tap water	20 °C	2.0	3.9	-	
Korea	Inje-gun and Sokcho	Tap water	23 °C	-	-	6	105
		Fresh water	23 °C	-	-	2	
		Sea water	23 °C	-	-	1	

* 90% reduction time of viable virions¹⁰⁴ * Reduction time of 99% of viable virions¹⁰⁴.
Source: prepared by the authors, 2021.

secondary transmission of SARS-CoV-2 via sewage systems or contaminated natural waters has been a major concern in countries with few financial resources, which often have unsatisfactory sanitation and health facilities^{124,125}. In these places, where there is a greater chance of transmission of waterborne pathogens¹¹⁵, improving the existing water and sewage treatment infrastructure is highly recommended.

To reduce the viral load and limit secondary transmission, researchers have recommended that the inactivation treatment of SARS-CoV-2 in wastewater be done in a decentralized manner, especially in critical points that tend to have a higher probability of receiving the new coronavirus, such as hospitals, community clinics and nursing homes^{45,50}. Wastewater treatment plants, which conventionally do not guarantee the inactivation of SARS-CoV-2, have been advised to implement effective disinfection systems to ensure that the virus does not spread through wastewater disposal or reuse schemes^{12,117,123}.

Given the substantial risk of SARS-CoV-2 infection through exposure to contaminated aerosols, it has also been widely recommended that STP workers follow precautionary and safety procedures against viral exposure, such as the use of appropriate personal protective equipment (PPE), frequent personal and facility hygiene, and regular operational training^{118,126,127,128,129}. There is also the need to investigate the impact of SARS-CoV-2 on the microbial community that performs the biological degradation of contaminants in wastewater, including possible horizontal gene transfers to microbial hosts^{45,123,130}.

Epidemiological monitoring of SARS-CoV-2 in sewage systems and natural waters

Considering studies conducted to date, researchers have been advocating a precautionary and surveillance approach to the

spread of SARS-CoV-2 and its variants through contaminated sewage systems and natural water. Environmental monitoring is considered an economical and effective measure to assess the circulation of pathogens in a community and it could be used as a diagnostic tool for the spread of SARS-CoV-2 viral particles in samples collected in sewage systems and natural waters^{122,131}. The strategy, called Wastewater-Based Epidemiology (WBE), could be used as a non-invasive tool to alert communities of new COVID-19 infections and thus promote better measures to contain viral spread^{132,133,134}.

The approach would make it possible to enumerate mild, pre-symptomatic and asymptomatic cases of people who do not have access to healthcare, who are often not detected by clinical diagnoses, but who can still spread COVID-19^{135,136,137,138}. Thus, strategies focused on epidemiological surveillance in sewage systems could be used to more accurately infer the actual number of people infected with SARS-CoV-2 in communities and foster better practices for coordinating efforts, allocating health resources, and administering vaccination^{139,140,141,142}.

Sewage monitoring could also be used to quickly and simply detect the SARS-CoV-2 variants of interest that are circulating in communities and assess the dynamics of the spread of these variants in populations^{71,80,86,127,143,144,145,146,147}. These analyses, for example, were able to monitor the mutational spectrum and evolutionary trends of the Alpha, Beta, Gamma, and Delta variants of SARS-CoV-2 in different areas of cities in France and the United States^{148,149}. Research of this type tends to be conducted on previously frozen and archived wastewater samples and, thus, promote future studies aimed at understanding the ancestry of SARS-CoV-2¹⁵⁰.

Monitoring SARS-CoV-2 in sewage could also be used to assess the environmental impact and public health risks associated



with viral transmissibility through water bodies, slurry, biosolids, aerosolized particles, and animal hosts^{116,151,152,153}. Studies could be conducted to determine the efficiency of disinfection systems and promote strategies regarding the suitability of water and sewage treatment plants, including waste transport and discharge procedures^{91,119}. Research would also enable the assessment of risks associated with natural and reused waters, for example, in recreational activities, fishing, irrigation of crops and urban areas, recharge of groundwater and industrial processes^{12,42,88,118}.

Regular monitoring of sewage systems and natural waters, as illustrated in Figure 4, would enable better assessment of the spread of SARS-CoV-2 in the hydrological cycle and its impact on the environment and human health^{45,96,127}. Monitoring programs could support international collaborative repositories of novel coronavirus surveillance in wastewater (www.covid-19wbec.org)¹⁵⁴ to provide comparison of detection results on global scales and improve epidemiological surveillance methods in these environments^{52,123,155}. Detection efforts, along with other epidemiological models like serological data, rhino pharyngeal diagnoses, clinical records, and hospital admissions, could be used to increase the effectiveness of future public health interventions^{156,157}. This approach has proven to be a valuable tool for authorities to assess and take quick action in the face of epidemic outbreaks, either by SARS-CoV-2 or by any other pathogen^{158,159,160}.

CONCLUSIONS

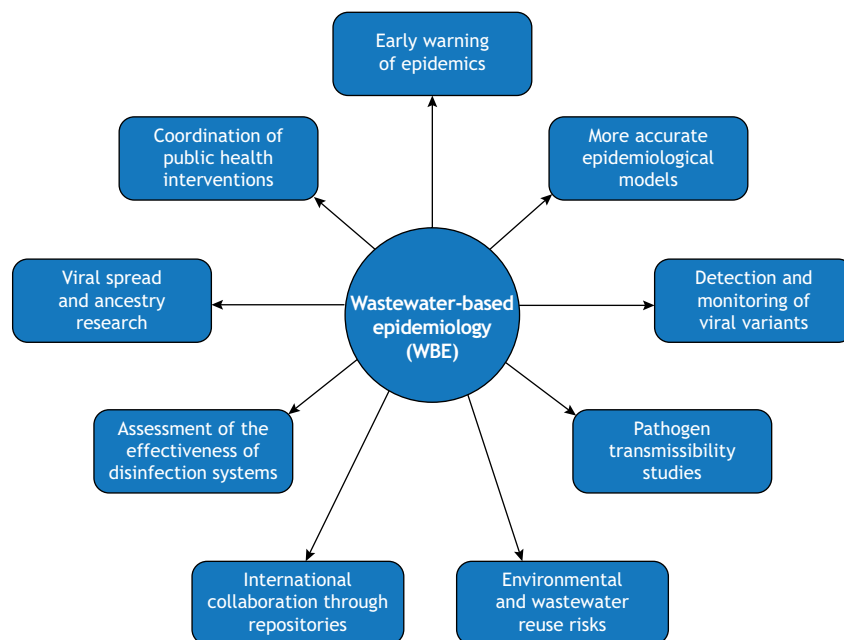
Although there is no scientific proof yet, the possible transmission of SARS-CoV-2 through direct contact or by aerosols

generated during the flow and treatment of sewage, handling of waste sludge, and also by natural waters contaminated with the new coronavirus, has had great repercussion among researchers. The possibility of cross-contamination of the new coronavirus through intermediate hosts, wild or domestic, has further increased attention to the presence of this viral pathogen and its variants in these environments. The situation tends to be worse in countries with fewer resources and notoriously poor sanitation infrastructure.

Therefore, in order to reduce the risks of emerging and re-emerging outbreaks of SARS-CoV-2 through aquatic media, precautionary approaches have been strongly recommended regarding the presence of the new coronavirus in these sites. In this context, we emphasize the importance of new public policies in the area of sanitation, especially in countries with high rates of waterborne diseases.

The recommendations are particularly aimed at:

1. Establishing efficient methods to detect and quantify the viral particles of SARS-CoV-2, as well as the pathogenicity and survival conditions of this virus and its variants in sewage, waste sludge, reuse waters, and in natural waters.
2. Identifying the best strategies for effluent treatment, waste management, and readjustment of water and sewage treatment plants, aiming at reducing the possibility of transmission of SARS-CoV-2 through the water system. In this context, we also recommended considering the risks that treatment plants can pose to employees and nearby communities, as well as to study and implement prophylactic measures.



Source: prepared by the authors, 2021.

Figure 4. Possible applications of epidemiological monitoring of SARS-CoV-2 in sewage systems by wastewater-based epidemiology.



3. Considering the possibility of implementing methods that inhibit the spread of SARS-CoV-2 and its variants through the use of reuse water or solid waste generated in STPs in manufacture and/or agriculture, including in industrial processes, groundwater recharge, irrigation of crops and urban parks.
4. Planning and implementing a surveillance system based on the analysis of sewage and natural waters (WBE) in order to monitor the spatial and temporal dynamics of SARS-CoV-2 and provide an early warning system for future emerging and/or re-emerging outbreaks of this and other pathogenic viruses.
5. Encouraging research aimed at understanding the survival and spread of SARS-CoV-2 and its variants through aquatic

ecosystems, including the elucidation of the interactions that the new coronavirus has with aquatic biota, its infection mechanisms, and the aspects that have led to its transmission to humans.

Finally, considering the enormous harmful potential that pandemic diseases such as COVID-19 can have, and that three coronavirus outbreaks have already occurred and that possibly others will occur in the near future, greater investment in the areas of sanitation, water resources and environmental monitoring are sorely needed. Investment in water, sanitation and similar processes, as emphasized by Melo et al.¹⁶¹, can boost the economy, help reestablish globally recommended production standards, and directly improve public health and the quality of life of human beings.

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Authors' contribution

Mainardi PH - conception, planning (study design), acquisition, analysis, interpretation of data and writing of the manuscript. Bidoia ED - conception, planning (study design) and writing of the manuscript. All authors approved the final draft of the manuscript.

Disclosures

The authors report that there is no potential conflict of interest with peers and institutions, nor political or financial conflicts in this study.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.



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