



## **Review** Transmission Pathways and Genomic Epidemiology of Emerging Variants of SARS-CoV-2 in the Environment

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can spread to the environment through several routes and persist for a more extended period. Therefore, we reviewed pertinent literature to understand the transmission dynamics of SARS-CoV-2 and genomic epidemiology of emerging variants of concern (VOCs) in the environment, their inactivation strategies, and the impact of COVID-19 on the ecosystem. The fallouts of the reviewed studies indicate that SARS-CoV-2 transmits through air and fomite, contaminated surfaces, biomedical wastes, and stool, which contaminates the environment through wastewater. As a result, multiple VOCs of SARS-CoV-2 were circulating in the environment. Genomic epidemiology revealed that the most prevalent VOC was Delta (B.1.617.2; 44.24%), followed by Omicron (B.1.1.529; 43.33%), in the environment. Phylogenetic analysis showed that environmental strains are clustered with a likeness of the human strains of the same or nearby countries, emphasizing the significance of continued environmental surveillance to track the emergence of the new variant. Thus, we should reduce viral dispersion in the environment through rapid and appropriate disinfection strategies. Moreover, the increased production and use of macro and microfiber plastic products should be brought under strict legislation with integrated waste management to control the unrelenting propagation of viral RNA. Finally, a comprehensive understanding of the environmental transmission pathways of SARS-CoV-2 is crucial for forecasting outbreak severity in the community, allowing us to prepare with the correct tools to control any impending pandemic. We recommend wastewater-based SARS-CoV-2 surveillance and air particulates to track the emerging VOCs of SARS-CoV-2 spread in the environment.

Keywords: variant of concern; wastewater; airborne; pollution; alpha variant

## 1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected human health regardless of geographical boundaries, with the first cases reported at the end of December 2019 in Wuhan, Hubei Province, China. Even with vaccination doses of approximately 3.4 billion worldwide, approximately 186.8 million confirmed cases and 4.0 million



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). deaths were reported by the second week of July 2021 [1,2]. Infection is characterized by the presence of high fever with coughing, breathing difficulty, and fatigue with evidence of acute respiratory distress, which may cause the death of an individual. Initial evidence on airborne transmission of the virus through air fomites of  $\leq 5 \mu$ m-sized particles suggests maintaining a 1 m social distance policy [3]. Furthermore, a list of factors related to the environment and human behaviors are considered responsible for transmitting SARS-CoV-2 between individuals. Although there is rare evidence of the potent virus in feces and contact with the coronavirus disease 2019 (COVID-19) via aerosolization from feces, transmission via contact with a contaminated surface to the mucous membrane of the mouth, nose, and eyes was confirmed [3]. Consequently, environmental factors such as temperature, humidity, sustainability of fomites, aeration, and filtering systems in households, hospitals, and other mass gathering places may influence the viral spread that we need to dig out.

Transmission of the virus at the community level is mainly responsible for converting the outbreak into a pandemic. Though it has been proven that the virus can be transmitted directly through an infected person's cough, oral and nasal secretion, and interceded contaminated droplets [4], the indirect route of virus transmission is still unreported or poorly understood [5]. Therefore, the existence of the virus in environmental samples indicates that the virus is present in the community. In spite of having a higher reproduction number [6] and low incubation period [7], one study has reported the non-infectiousness of SARS-CoV-2 RNA recovered from wastewater to humans [8]. However, further studies are required to target the survivability of the virus in water and wastewater under different environmental conditions to detect whether the contaminated wastewater is an emerging concern for transmission of SARS-CoV-2 to humans [9].

Earlier, SARS-CoV-1 was transmitted from feces to air and the environment [10]. The "virus-laden droplets" that occur through bathroom ventilation in the room can be a source of airborne spread [11]. Similarly, the SARS-CoV-2 droplets may spread through the wastewater sanitation arrangement of a building's different floors and air by cross-contamination [12]. Further, one study has already reported that the environment is a potential medium of transmission of SARS-CoV-2 after detecting positive samples from the toilet bowl, sink, and swab samples of air exhaust outlets of COVID-19 patient rooms [13].

Overall, the COVID-19 pandemic has a detrimental effect on public health caused by environmental risk factors [1,14,15]. So, the safe management of domestic and household waste could be critical during the ongoing COVID-19 pandemic. Further, there is a knowledge gap about other possible environmental transmission routes, such as air fomites and surface-level contamination, which have come across due to the steady increase in infection rates. Since SARS-CoV-2 is considered highly infectious among the coronavirus family, it is essential to unveil the pattern and possible environmental transmission pathways and inactivation.

Therefore, we conducted this review to understand the transmission pathways, persistence, and inactivation of SARS-CoV-2 in environmental contact surfaces and the impact of plastic waste pollution globally. We have highlighted sewage tracking to surveil COVID-19 in this pandemic situation and identify the credibility of the existing wastewaterbased epidemiology (WBE) for SARS-CoV-2 surveillance and monitoring in different geographical regions.

#### 2. Methodology

We screened published literature containing the following information: 1. SARS-CoV-2 transmission pathways from humans to the environment; 2. persistence of SARS-CoV-2 in different environments and surfaces; 3. the inactivation strategies of SARS-CoV-2; and 4. global plastic waste pollution due to SARS-CoV-2. We used the Google scholar, PubMed, Scopus, and the Web of Science databases accessed through Hinary (https://www.who.int/hinari/en/; accessed on 25 September 2021). We developed Boolean words under descriptive, outcome, and population terms (Table 1) for searching the literature.

Term Keywords				
Descriptive terms	Occurrence OR Identification OR Detection OR Investigation OR Diagnosis OR Frequency OR Prevalence OR Survey			
Outcome term	Coronavirus OR SARS-CoV OR SARS-CoV-2 OR COVID-19			
Population Terms	Environment OR Water OR Mask OR PPE OR Wastebin OR Grocery shop OR Currency OR Floor OR Wastebin OR Disposal area OR Infected surfaces OR Inanimate surfaces OR Inert surfaces OR Sewer OR Fecal OR Feces OR Stool OR Droplet OR Airborne			

For the emerging variant epidemiology, we retrieved all the complete genome sequences of environmental strains of different variants of SARS-CoV-2 from the GISAID on 22 September 2021. We calculated the percentage of each emerging variant as the number of each variant's sequences over the total number of sequences. We illustrated the possible cyclic pathway of risk of transmission of the viral particles from infected human to the environment and genomic surveillance in Figure 1. We graphically showed the temporal distribution of emerging variants from environmental samples using MS-Excel 2015. We created the spatial distribution map for emerging variants of concerns (VOCs) using ArcGIS software [16].



Figure 1. Possible cyclic risk pathway and source of genomic surveillance.

Table 1. Boolean operator to search databases.

We selected genomes (Supplementary File S1) for phylogenetic analysis because we verified their grouping quality for additional investigation in the GISAID dataset. In contrast, genome arrangements have >5% Network Neurobehavioral Scale (NNNS), and <29,000 nt were avoided considering poor-quality successions. The reference SARS-CoV-2 Wuhan genome (NC\_045512) was utilized. Succession Dataset developer (SEDA; https:// www.sing-group.org/seda/, accessed on 25 March 2022) was used to eliminate all interior stop codon containing arrangements. Moreover, we utilized numerous grouping arrangement program (MAFFT) order lines (https://mafft.cbrc.jp/arrangement/programming/, accessed on 25 March 2022) [14] to adjust all recovered genome successions over the reference succession. We used the MEGA 7 apparatus for the phylogenetic examination as portrayed by [6,15]. We constructed the phylogenetic trees using the neighbor-joining technique [17] and the Kimura–Nei method [18] for all the detailed developmental relationship examinations where the bootstrap test (1000 reproduces) is displayed close to the branches. During the essential decision making, we pondered declaring time, the geographical region close by human-environmental interface reports, unpredictable model combination dates, close by, and the significance between every plan and their uncovered pathogenic power. This phylogenetic tree addressed the developmental relationship of and delegates emerging variants of SARS-CoV-2 from both humans and the environment. Its fundamental design was to clarify the human-environment interfacial transformative relationship alongside the transmission dynamic of SARS-CoV-2.

# **3.** Risk of Transmission Dynamics and Persistence of SARS-CoV-2 in the Environment 3.1. Risk of Transmission of SARS-CoV-2 through Stool

Human pathogenic viruses pass into the environment through fecal, urogenital, and oropharyngeal secretions, blood, and sweat (Figure 1) [19]. The overall SARS-CoV-2 shedding period varies from 2 to 10 days for symptomatic cases, extending to 20 days for immunocompromised patients [20,21]. Another study detected the RNA in COVID-19 patients' stool after 10–30 days of onset of illness [22]. Liu et al. [23] detected SARS-CoV-2 RNA for up to 5 days in the urine of COVID-19 patients. The median lifespan of SARS-CoV-2 in stool specimens of infected patients was 22 days (17–31 days) [24], longer than that of SARS-CoV-1 ( $\leq$ 4 days) [21,25], higher than that of respiratory droplets (18 days, 13–29 days) and serum samples (16 days, 11–21 days) [24], which is alarming because in experimental setup, viral RNA isolated from a COVID-19-affected patient's stool sample has demonstrated the infection ability of the African green monkey kidney cell (Vero) [26].

Evidence of fecal contamination was first identified in Macau, China [27], and simultaneously, several other studies in China detected viral RNA in feces (Table 2). Almost 66.67% (6/9) of SARS-CoV-2-positive fecal samples has been observed in Munich, Germany [28]. In Canada and England, the alpha variant was identified from sewage samples, which implies the possible transmission of the virus through fecal shedding and water contamination [29,30].

Although the current knowledge is aggregated based on a few studies, the findings are significant because of environmental contamination by releasing contaminated feces into affluent and onsite hygiene systems and open defecation [31]. The recent trend of the multifaceted SARS-CoV-2 transmission favors the probable fecal shedding and spread of the SARS-CoV-2 virus through water [32–34]. In addition, most of the studies identified a high concentration of SARS-CoV-2 RNA in fecal samples in infected patients, regardless of the infectivity of the virus. It is essential to conduct further research in environmental and laboratory setups to confirm the hypothesis of viral spread through fecal shedding [13].

Country	Location	<b>Detection Time</b>	Detection Methods	PCR Target Regions	Positive Rate n/N (%)	Reference
China	Hubei, Shandong, and Beijing	1 January to 17 February 2020	rRT-PCR	Open reading frame 1ab gene	44/153 (29%)	[35]
China	Jinhua	27 January to 10 February 2020	RT-PCR	Not found	5/14 (35.7%)	[36]
China	Shanghai and Qingdao	Early February	RT-PCR	1ab gene and nucleocapsid protein gene	5/10 (50%)	[22]
China	Zhuhai	1 to 14 February 2020	rRT-PCR	Not found	39/73 (53.4%)	[37]
China	Zhoushan		RT-PCR	N gene	1/1 (100%)	[32]
Singapore	Singapore City	January–February, 2020	RT-PCR	Not found	5/18 (27.8%)	[38]
China	Shanghai	20 January to 10 February 2020	RT-PCR	Not found	11/66 (16.7%)	[39]
China	Guangdong	February 2020	RT-PCR	N Gene	5/6 (83.3%)	[40]
Singapore	Kallang	13 February 2020	rRT-PCR	N gene	1/1 (100%)	[41]
China	Sichuan	January	RT-PCR	Not found	8/9 (88.9%)	[42]
China	Macau	21 January to 16 February 2020	qRT-PCR	Not found	10/10 (100%)	[27]
China	Zhuhai	16 January to 15 March 2020	RT-PCR	RdRp gene, N gene, E gene	41/74 (55%)	[43]
China	Shandong	17 January to 6 March 2020	RT-PCR	Not found	3/3 (100%)	[44]
China	Tianjin	3 to 17 February 2020	RT-PCR	N gene	3/3 (100%	[45]
Korea	Seoul	April 2020	RT-PCR	RdRp gene	2/46 (4.34%)	[46]
China	Wuhan	9 to 20 February 2020	RT-PCR	Not found	28/42 (66.67%)	[47]
USA			RT-PCR	S gene, N gene	2/7 (28.57%)	[48]
USA	Illinois	Not found	RT-PCR	S gene	2/2 (100%)	[49]
Germany	Munich	23 January 2020	RT–PCR	E gene	6/9 (66.67)	[28]
France	Paris	Not found	RT–PCR	E gene	2/5 (40%)	[50]
South Korea	Chungbuk	25 February– 5 March 2020	qRT-PCR	SARS-CoV-2 RNA	100%	[46]
China	Wuhan	27 January– 7 February 2020	qRT-PCR	SARS-CoV-2 RNA	12/28	[47]
USA	Massachusetts	Not found	qRT-PCR	N1, N2, E, RdRp gene	35/60	[51]
Brazil		Jan to Jul 2020	qRT-PCR	NSP3 segment and ORF1/2 junction region	10/121 (8.3%)	[52]

#### Table 2. Detection of SARS-CoV-2 RNA in stool.

rRT-PCR: real-time reverse transcriptase-polymerase chain reaction; RT-PCR: reverse transcriptase-polymerase chain reaction.

## 3.2. Risk of Transmission Dynamics and Persistence of SARS-CoV-2 in Sewage

Long before the emergence of SARS-CoV-2, other coronaviruses were detected in the effluent of sewage treatment plants. Evidence suggests that the survival of different coronavirus strains depends on the nature and type of wastewater and temperature variation. Human coronaviruses (HCoVs) are inactivated rapidly in water, i.e., HCoV-229E survived only for seven days at 23 °C in water [53]. Temperature is a crucial factor in the persistence of the virus. HCoV-229E survives with a wide fluctuation of temperature variations as low as 4 °C to as high as 25 °C for 21 days. However, viral persistence also varied among different strains, such as transmissible gastroenteritis virus (TGEV) for 35 days on pasteurized sewage at 40 °C. Its persistence decreased to 21 days while the temperature increased to 25 °C [54]. Similarly, for SARS-CoV-1, the persistence depends on the temperature of domestic sewage [55]. After experiments on the primary and secondary effluent, one study reported that the persistence of HCoV-229E was similar for two days at 23 °C [53].

The usual phenomenon is that feces and urine of infected patients are discharged into sewer systems (Figure 1), ultimately finding their way into wastewater and sewage treatment systems/plants [34]. This is considered the primary route of SARS-CoV-2 transmission to water and wastewater [56]. Thus, there is a chance of SARS-CoV-2 spread via gasp of open toilet setup and filthy oropharyngeal drops from effluent, particularly in crowded domestic areas [12,57]. COVID-19 patients can shed the virus for a more extended period than asymptomatic humans. This may increase the transmission of the virus particles in the sewage for an extended period. These, in turn, will end up in water streams if no treatment facility is available in place.

SARS-CoV-2 droplets may spread through the wastewater sanitation arrangement of a building's different floors and air by cross-contamination [12]. Further, one study has already reported that the environment is a potential medium of transmission of SARS-CoV-2 after detecting positive samples from the toilet bowl, sink, and swab samples of air exhaust outlets of COVID-19 patients' rooms [13]. On the contrary, a recent study found that following culture, extracted RNA from the exterior surface of continuous positive airway pressure helmets has no cytopathic effect [58]. Again, SARS-CoV-2 has been identified in wastewater in almost all regions of Europe, America, Asia, and the Middle East, regardless of the country's economic classification (Table 3). Eleven studies detected SARS-CoV-2 in the effluent Asia-Pacific region, namely Bangladesh, India, China, Australia, Pakistan, United Arab Emirates, and Japan [59]. Among the European countries, Italy, Spain, France, Germany, The Netherlands, Turkey, the Czech Republic, and Slovenia detected SARS-CoV-2 RNA in the wastewater [60–69] (Table 3).

Country	Location	Sample Type	Detection Date	Detection Methods	PCR Target Regions	Positive Rate/Output	Reference
Bangladesh	Noakhali	Untreated wastewater	29 August 2020	qRT-PCR	ORF-lab	12/16	[59]
India	Ahmedabad	Untreated wastewater	27 May 2020	qRT-PCR	ORF-lab	100%	[70]
India	Jaipur	Wastewater	04 May 2020 to 12 June 2020	RT-PCR	S gene, E gene, ORF1ab gene, RdRp gene, and N gene	6/17 (35%)	[71]
Israel	Multiple locations	Wastewater	April 2020	qRT-PCR	Е	9/11 (82%)	[72]
China	Zhejiang University	(Sewage) Inlets of preprocessing disinfection pool (Sewage) The outlet for preprocessing disinfection pool The final outlet for the sewage disinfection pool	19 February 2020 to 24 February 2020	qRT-PCR	Not found	3/3 (100%) 1/1 (100%) 0/1	[73]
China	Wuchang Cabin Hospital, Wuhan	Hospital septic tank Influent Hospital septic tank effluent	26 February 2020, 01 March 2020, 10 March 2020	qRT-PCR	CCDC-ORF1 CCDC-N	0/4 (0%) 7/9 (78%)	[74]
Australia	Brisbane, Queensland	Untreated wastewater	N/M	qRT-PCR	Not found	2/22(22%)	[34]
United Arab Emirates	Dubai	Wastewater	7 May to 7 July 2020	RT-PCR	N gene and S gene	829/2900 (28.6%)	[75]

Table 3. Detection of SARS-CoV-2 RNA in wastewater.

Country	Location	Sample Type	Detection Date	Detection Methods	PCR Target Regions	Positive Rate/Output	Reference
Pakistan	Lahore	Sewage water sample	13-25 July 2020	qRT-PCR	ORF1ab, N gene	16/28 (54.1%)	[76]
Iran	Tehran	Influent and effluent	June to July 2020	qRT-PCR	ORF1ab, N	80-100%	[77]
Czech Republic	Multiple locations	Untreated wastewater	April to June 2020	qRT-PCR	E-Gene	13/112 (11.6%)	[62]
Germany	Multiple cities in North Rhine- Westphalia	Untreated wastewater Treated effluent	08 April 2020	qRT-PCR	S Gene	9/9 (100%) 4/4 (100%)	[78]
France	Paris	Wastewater	05 March 2020 to 23 April 2020	qRT-PCR	RdRp	3/3 (100%)	[67]
Italy	Milan and Rome	Untreated wastewater	N/M	qRT-PCR	ORF-lab, S gene	12/12 (100%)	[61]
The Netherlands	Multiple Cities and an airport	Wastewater	26 March 2020	qRT-PCR	E gene	9/9	[63]
Italy	Milan	Wastewater Effluent	14 April 2020 to 22 April 2020	qRT-PCR	ORF1ab, N, E	3/4 (75%) 0/2	[64]
Italy	Padua	Untreated wastewater		qRT-PCR	N gene	4/9 (44.4%)	[69]
Spain	Multiple locations	Wastewater	06 April 2020 to 21 April 2020	qRT-PCR	Not found	7/7 (100%)	[60]
Turkey	Istanbul	Wastewater	07 May 2020	RT-PCR	RdRp	9/9 (100%)	[65]
Spain	Valencia	Influent Secondary treated Tertiary effluent	12 March 2020 to 14 April 2020	qRT-PCR	N1, N2, N3	35/42 (83%) 2/18 (11%) 0/12 (0%)	[66]
Slovenia	Not found	Wastewater	1 to 15 June 2020	qRT-PCR	RdRP and E genes	10/15 (66.7%)	[68]
USA	Louisiana	Wastewater	January to April 2020	qRT-PCR	N1, N2	2/15 (13%)	[79]
Ecuador	Quito	Wastewater	05 May 2020	qRT-PCR	N1, N2	3/3 (100%)	[80]
USA	Southeastern Virginia	Wastewater	09 March 2020	RT-ddPCR	N, N2, N3	98/198 (49.5)	[81]
USA	Michigan	Wastewater	08 April to 26 May 2020	qRT-PCR	N1	18/18 (100%)	[82]
USA	Massachusetts	Wastewater	25 March 2020	qRT-PCR	N1, N2, N3	10/10 (100%)	[83]
USA	Bozeman, Montana	Wastewater	March to April 2020	qRT-PCR	N1, N2	7/7 (100)	[84]
USA	New York	Wastewater	06 to 13 May 2020	RT-PCR	Not found	18/22 (82)	[85]
Brazil	Niterói, Rio de Janeiro	Raw sewage	15 April 2020	qRT-PCR	Ultracentrifugation	5/12 (41.6%)	[86]
Mexico	Queretaro State	Influent from the wastewater treatment plant	April to July 2020	RT-PCR	RdRp, S, N	36%	[87]
Switzerland	STEP de Vidy, Lausanne and alpine ski resort	Three wastewaters treatment plant	21 December 2020	Next- Generation Sequencing (NGS)	Ultracentrifugation	Detection of Alpha and Beta Variants	[88]
England	London	Sewage plant	14 to 26 January 2021	RT-PCR and NGS	Ultracentrifugation	B.1.1.7, B1.351 and P.1 lineages	[29]
Canada	Canadian municipality	Composite influent wastewater	26 January 2020	qRT-PCR	Ultracentrifugation	Alpha Variant	[30]

Table 3. Cont.

qRT-PCR: quantitative reverse transcriptase-polymerase chain reaction; ORF: open reading frame; RdRp-RNAdependent RNA polymerase.

### 3.3. Risk of Transmission of SARS-CoV-2 through Biomedical Wastage

Moreover, in Switzerland, wastewater-based surveillance reported the existence of both the alpha (B.1.1.7) and beta variant (501.V2) with a variant-specific signature mutation in sewer water [88]. The same study identified three co-occurring signature mutations of alpha variants from wastewater in Switzerland, suggesting a new strain of the SARS-CoV-2 virus in the community [88]. In the North and South American regions, eight studies have identified the existence of the virus in wastewater and raw sewage in the U.S., Brazil, and Ecuador [80–86]. Therefore, it is suggested to treat wastewater, raw sewage, and river water as potential environmental media for the dispersal of SARS-CoV-2 [9]. Nevertheless, no study determined the infectiousness of SARS-CoV-2 from different types of raw and treated wastewater. Thus, we recommend conducting further studies to determine the infectiousness of SARS-CoV-2 from wastewater, different sewage, and sludge at various stages of treatment plants. The absence of different enteric and respiratory viruses, such as other coronaviruses, noroviruses, hepatitis A virus, hepatitis E virus, adenovirus, and astrovirus, in treated wastewater or sewer indicates the better efficacy of the treatment plant [89–92]. Although enveloped and non-enveloped viruses act differently in the environment, the enveloped SARS-CoV-2 is also an indicator virus, detection of which in the treated wastewater or sewer determines that the treatment plant or system is not safe for public health [89–92]. However, no study determined the infectiousness of SARS-CoV-2 ribonucleic acid (RNA) detected from sewage samples. Further, SARS-CoV-2 is sensitive to free chlorine [10]. So, the infectiousness of SARS-CoV-2 in wastewater is still in question considering their vulnerability to disinfection processes.

Due to the pandemic, the demand and use of different personal protective equipment (PPE) have increased dramatically. A study estimated that approximately 2.3 billion face masks were used as of 31 July 2020 in 49 Asian countries [93]. Individual Asian countries produced more than 16 thousand tons of medical waste during this pandemic [93]—the amount of medical waste increased along with the rise of COVID-19 cases. From the beginning of the pandemic to May 2020, South Korea produced 2000 tons of COVID-19 waste [94]. In Malaysia, the generation of clinical waste has increased by up to 27% during the pandemic compared to pre-pandemic time [95]. Romania produced more than 4 thousand tons of medical waste during lockdown from 26 February to 15 June 2020 [96]. Indonesia was approximately 13 thousand tons in 60 days from the hospital and household settings [97].

Waste generated by COVID-19 patients treated in households or private hospitals and medical centers or individuals in quarantine increases the likelihood of infection transmission to the environment [98]. Biomedical waste produced from hospitals and clinics engaged in COVID-19 patients treatment is potentially a bearer of SARS-CoV-2 [99,100]. Other biomedical waste generated from households and hospitals, such as disposable gowns, face masks, hand gloves, goggles, and face shields, can easily be mixed with domestic and hospital waste (Figure 1) [101]. Studies on the persistence of SARS-CoV-2 on biomedical waste also support the risk of infection through both hospital and household waste. However, the SARS-CoV-2 virus can survive from hours to days of COVID-19 waste, including disposable gowns, masks (inner and outer layer), tissue paper, testing kits, and gloves, depending on the temperature [102]. COVID-19 medical waste dumped without being appropriately treated has the possibility of mixing with the environment through water, food, soil, air, and livestock. This puts the environment and human lives at risk [103–105].

## 3.4. Risk of Transmission of SARS-CoV-2 through Diverse Inanimate Environmental Surface Contact

The role of environmental factors has long been studied for different coronaviruses. Several studies have identified potential environmental pathways through inanimate surface contact before this pandemic (Figure 1). The persistence of other coronaviruses on different porous and non-porous surfaces, including steel, aluminum, paper, wood, metal, glassware, plastics, polyvinyl chloride (PVC), rubber and surgical gloves, onetime gowns, ceramic, Teflon, cloth, surgical masks, tissue paper, cardboard, polymer notes, paper, cotton, and vinyl has been presented in Table 4. Different coronaviruses can remain infectious on steel surfaces for 4 h to more than 28 days, mainly depending on temperature, humidity, and viral load [102,106]. Further, the virus can survive on the contaminated surface for 6 to 9 days post-contamination [107]. SARS-CoV-2 can persist for up to 9 days at room temperature [108].

Moreover, lower temperatures increase the duration of the tenacity of the virus. For instance, at 4 °C, the transmissible gastroenteritis virus (TGEV) remains active on steel for more than 28 days, but it is reduced to 4–96 h when the temperature increases to 40 °C. The earlier study corroborated that at 22 °C, SARS-CoV-2 may survive for four days on steel,

one day on wood, 30 min on paper and tissue, two days on glass, one day on cloth, and 4–7 days on single used face masks [102].

Types of Liquid Media and Inert Surface	Virus Name	Temperature	Relative Humidity	Persistence (Hrs or Days)	References	
Wastewater and tap water	SARS-CoV-2	20 °C	NM	7 d	[109]	
¥47 .	SARS-CoV	20 °C	NM	2 d	[]	
Water		4 °C	NM	14 d	[55]	
		20 °C	40%	48 h	[110]	
	MERS-CoV	30 °C	30%	8–24 h	[110]	
Steel	HCoV	21 °C	30-40%	5 d	[111]	
-		22 °C	65%	4 d	[102]	
	SARS-Cov-2	20 °C	50%	≥43 h	[106]	
Aluminum	HCoV	21 °C	55-70%	2–8 h	[112]	
Metal	CARC C.V	20–22 °C	NM	5 d	[110]	
<b>X47</b> 1	SARS-Cov	20–22 °C	NM	4 d	[113]	
Wood	SARS-CoV-2	22 °C	65%	1d	[102]	
		20–22 °C	NM	4–5 d	[113]	
Paper	SARS-CoV	20–22 °C	NM	24 h	[25]	
-	SARS-CoV-2	22 °C	65%	30 min	[102]	
	SARS-CoV	20–22 °C	NM	4 d	[113]	
-	HCoV	21 °C	30-40%	5 d	[111]	
Glass	SARS-CoV-2	22 °C	65%	2 d	[102]	
		22 °C	50%	$\leq 2 d$	[106]	
	SARS-CoV	22–25 °C	40-50%	≤5 d	[114]	
-	SARS-CoV	20–22 °C	NM	4 d	[113]	
Plastic		20–22 °C	NM	6–9 d	[115]	
-	MERS-CoV	21 °C	40%	48 h	[110]	
-	HCoV	20–22 °C	NM	2–6 d	[115]	
PVC, ceramic, Teflon			30-40%	5 d	[111]	
Silicon rubber	HCoV	21 °C	30-40%	4 d	[111]	
Surgical gloves		-	55-70%	$\leq 5 h$	[112]	
Disposable gown	SARS-CoV	20–22 °C	NM	2 d	[25]	
	SARS-CoV	21–25 °C	NM	5 d	[113]	
Cloth	SARS-CoV-2	22 °C	65%	1 d		
Surgical mask—outer layer		22 °C	65%	7 d		
Surgical mask—inner layer	SARS-CoV-2	22 °C	65%	4 d	[102]	
Tissue paper	SARS-CoV-2	22 °C	65%	30 min		
Cardboard	SARS-CoV-2	21–23 °C	40%	1 d		
	SARS-CoV	21–23 °C	40%	8 h	[116]	
Polymer note		20 °C	50%	≥49 h	[106]	
Paper note		20 °C	50%	≤3 d		
Cotton	SAKS-CoV-2	20 °C	50%	$\leq 2 d$		
Vinyl		20 °C	50%	$\leq 2 d$		

**Table 4.** Presence and persistence of different coronaviruses in the diverse environmental media.

MERS: Middle East Respiratory Syndrome; NM: not mentioned.

The empirical evidence suggests that the persistence of HCoV, SARS-CoV, and MERS-CoV varied from 2 to 7 days at 21-25 °C on different surfaces (Table 4). Again, SARS-CoV-2

has been detected from various environmental samples collected from a light switch, bathroom doorknob, inner wall of the toilet, towel, sewer inlet, inner surface of washbowl, floor, bedside table surface, pillow, and duvet cover of a quarantine room by Hu et al. [117]. SARS-CoV-2 is more contagious compared to other coronaviruses [117]. The current data on SARS-CoV-2 persistence suggest that although there is still limited evidence to establish the idea of the virus spreading through surface contact, it is arguable to say that surface contamination may also increase the chance of infection [5,117].

#### 3.5. Presence and Risk of Transmission of SARS-CoV-2 Virus through Air

A vital transmission route of SARS-CoV-2 is via respiratory droplets and close contact with aerosol particles [118]. The virus can be attached to any medium, i.e., respiratory droplets from humans, which will carry it to another human [119]. These droplets can spread or settle down on surfaces and subsequently infect humans. Humans can be infected after inhaling hundreds of virus particles, whereas a single droplet can become tens of thousands of virus particles [120]. Aerosols are usually less than 5  $\mu$ m, and droplets are more significant than 5  $\mu$ m [119]. The large particles settle down easily due to their weight; thus, there is less chance of the virus spreading to a wider area [121].

On the other hand, aerosols can remain suspended in the air from 1.0 to 15.0 s depending on the velocity and direction of wind [122]. The study reported that SARS-CoV-2 could travel via air up to 4 m [123].

Aerosol transmission depends on various criteria such as virus concentration in aerosols, virus' survival time, and infective dose [124]. Nevertheless, all these parameters are still not known. Additionally, air circulation is less indoors than outdoors, and indoor air samples were more contaminated [125]. SARS-CoV-2 can remain viable for 3 h in aerosols in laboratory conditions detected in the half-life of SARS-CoV-2 at 1.1 to 1.2 h. However, the authors of [126] reported that in aerosols, SARS-CoV-2 could remain infective for 16 h although the viability of viruses decreases when the temperature [127] and humidity [128] increase.

Many researchers argue that airborne transmission can cause more infection and possibly spread in three ways—(i) through air circulation in confined compartments with infected patients; (ii) recirculating air in building ventilation systems; (iii) through ventilation, air conditioning, and heating systems' connection with outside air of the health facilities [129]. Respiratory droplet transmission (>5  $\mu$  m) is the primary mode of spread for SARS-CoV-2. The persistence of the virus in the aerosols lasts for more than 3 h (<5  $\mu$ m), which is infectious in humans [111,130,131]. Believing in aerosol-driven infections, several researchers showed that aerosol transmission of the disease in closed environments may cause community transmission [22,130–135]. Another study detected SARS-CoV-2 in the air within approximately 4 m of COVID-19 patients [136].

In addition, the identification and perseverance of SARS-CoV-2 on different porous and non-porous surfaces, water environment, and stool have long been documented, which may increase plausible air-fomite transmission. Moreover, researchers argued that air pollution and microfiber contamination (2.5 m-sized particles) are risk factors for the transmission and severity of SARS-CoV-2 infection [137] and regardless of allergy status, co-exposure to airborne pollen increases susceptibility to SARS-CoV-2 virus infections [138]. Although SARS-CoV-2 transmission through fomites is relatively low compared to sneezing or coughing droplets, microfiber or pollen may act as a vehicle for virus transfer at a high concentration, or the particles may injure the lungs when inhaled. As a result, the severity of SARS-CoV-2 increased dramatically. Moreover, the Centre for Disease Control (CDC) recommends practicing handwashing and sanitizing after contact with possible contaminated surfaces such as door handles, tables, gas pumps, shopping carts, or electronic cashier registers/screens, which are frequently touched by other people [139]. However, most of the stated studies were performed in experimental conditions. Therefore, the researcher should test the persistence of SARS-CoV-2 in a real-life environment to show airborne transmission effectiveness.

## 4. Genomic Epidemiology of Emerging Variants of SARS-CoV-2 in the Environment

By 15 April 2022, in GISAID, 5860 complete sequences of SARS-CoV-2 RNA collected from different environmental sources including wastewater, clinic material, and surfaces worldwide were deposited and are used in this paper (Supplementary File S1). Among them, 5013 sequences (Alpha n = 605, 12.07%; Beta n = 8, 0.16%; Gamma n = 9, 0.18; Delta n = 2218, 44.24%; MU n = 1, 0.02%; Omicron n = 2172, 43.33%) were of emerging variants of concern. The delta variant was reported from environmental samples in November 2020. However, from January 2021, the alpha variant was slowly increasing in environmental samples [140]. This trend persisted until June 2021. Nevertheless, delta replaced the alpha variant among the environmental samples (Figure 2). Emerging variants detected in several countries from environmental samples have been shown in Figure 2.



**Figure 2.** Distribution of emerging variants of SARS-CoV-2 in environmental samples globally. **(A)** Spatial distribution. **(B)** Temporal distribution.

The phylogeny of environmental strains of SARS-CoV-2 is shown in Figure 3. Different VOCs formed a separate cluster in the tree, having close relations with human strains isolated from the same country (Figure 3). Another phylogeny for the omicron variant from environmental samples has been shown in Figure 4, whereas the phylogeny for the delta variant has been shown in Figure 5. This variant of the environment and humans in the same regions shows genetic resemblance [140]. For Omicron, the environmental strains from the USA are grouped with human strains from Italy, the USA, and Mexico; environmental strains from Austria are grouped with human strains from Belgium and Austria; environmental strains from Liechtenstein are grouped with human strains from Belgium, USA, and Austria. However, interestingly, strains from different countries were also grouped: environmental strains from Austria and Liechtenstein; human strains from the Netherlands and environmental strains from Austria; human strains from Belgium, USA, Germany, England, and environmental strains from Austria (Figure 5).



**Figure 3.** Phylogenetic analysis of emerging variants from environmental samples. Green, red, violet, purple- and indigo-colored blocks represent alpha, beta, gamma, mu, and delta variants from the respective environment. The Fuchsia pink color indicates the reference sequence from Wuhan, China.



**Figure 4.** Phylogenetic analysis of Omicron variants from environmental samples. Deep Indigocolored blocks represent omicron variants from the environment, whereas the fuchsia pink color indicates the reference sequence from Wuhan, China.



**Figure 5.** Phylogenetic analysis of Delta variants from environmental samples. Indigo-colored blocks represent delta variants from the environment, whereas the fuchsia pink color indicates the reference sequence from Wuhan, China.

#### 5. Inactivation Strategies of SARS-CoV-2 in Different Environmental Conditions

The virus may be inactivated using different methods such as ultraviolet (U.V.) rays, heat, and alcohol treatment [141], in water treatment plants, health care settings, and agricultural fields [142,143]. However, biocidal efficacy depends on various factors, including virus strain, titer, nature of the surface, and ambient conditions [144]. Below, described methods are followed to inactivate viruses in the environment.

#### 5.1. Inactivation of SARS-CoV-2 Using Biocidal Agents

Alcohol-based disinfectant solutions such as isopropyl alcohol at different concentrations are widely used to inactivate different viruses in household and hospital settings. A comprehensive study on inactivation of different coronaviruses showed a wide variety of disinfectant such as 78–95% ethanol, 70–100% 2-propanol, 45% 2-propanol in combination with 30% 1-propanol, 0.5–2.5% glutardialdehyde, 0.7–1% formaldehyde and 0.23–7.5% povidone-iodine can be useful for readily inactivation of coronavirus infectivity at 4 log10 fold [145]. However, p-chloro-m-xylenol (PCMX) can inactivate the SARS-CoV-2 virus on glass surfaces within 0.5 to 10 min at ambient temperature [146]. In addition, 0.21% sodium hypochlorite and 0.5% hydrogen peroxide are also effective against SARS-CoV-2. Hospitals are using ethanol as a hand sanitizer. These alcohol-based disinfectants can be used on inanimate contact surfaces such as doorknobs, telephones, and lift buttons, reducing the chance of infection. Other products for solid surface decontamination include–quaternary ammonium compounds, peroxy compounds, sodium hypochlorite (NaClO), alcohol, and organic acids [147].

It is critical to inactivate the virus before they pollute the water bodies. In this regard, Hypochlorite (HClO) is the most efficient way to inactivate the pathogen of wastewater [148]. NaClO disinfection combined with U.V. rays for tertiary treatment can remove SARS-CoV-2 from wastewater [66]. Compared to other means such as U.V./Ozone, disinfections work more effectively for SARS-CoV-2 than chlorine-based solutions and offer several benefits, including lesser power consumption, lower toxicity, simple equipment, and setup [144]. Enveloped viruses such as SARS-CoV-2 can be easily removed from wastewater as they frequently adhere to organic biomass [149]. It was reported that moving bed biofilm reactors and sequencing batch reactors are efficient secondary treatment strategies to abolish the virus from wastewater [71]. Other efficient inactivation processes include activated sludge, biological nutrient removal, and algae bioreactors [150]. However, the aerosolization of viable viruses from water and wastewater may risk people being involved in treatment activities [151]. Open aerobic wastewater treatment plants, activities such as pumping wastewater, discharge, and flow are highly likely to participate in virus aerosolization.

#### 5.2. Inactivation of SARS-CoV-2 Using Non-Biocidal Agents

Heat and U.V. irradiation-based inactivation techniques have been widely used in the hospital and biomedical sectors to sterilize medical equipment and apparatus. Both methods effectively kill SARS-CoV-2 from the surfaces [152]. Several types of research have been carried out to estimate the efficiency of UV-C irradiation on inanimate surfaces [153]. While other U.V. methods showed no significant inactivation for up to 15 min, UV-C increased the virus deactivation rate by 400 fold within 6 minutes [154]. In addition, this method was effective while stabilizing the virus from biomedical waste [145].

Furthermore, for decades, heat and thermal deactivation methods have been used for virus inactivation. The temperature's effect on virus deactivation was an approximately 102-fold reduction within 12 days at 24 °C and a log4 unit reduction at 70 °C within 2.5 min for human/murine norovirus and a log3 fold reduction within two days at 71 °C for feline calicivirus [155]. In another experimental study, the virus was inactivated at 90% within 7 to 14 min in different culture media [156]. The dosages and methods of irradiation are crucial factors to ponder in their application as an essential means to battle the SARS-CoV-2 pandemic.

## 6. Environmental Pollution Is Due to the COVID-19 Pandemic

The main compounds of disposable masks are polypropylene, polyethylene, and other polymers such as polyesters, polyurethane, and polystyrene [157]. The surge in manufacturing and use of face masks and other PPE items raised a challenge for proper disposal in the environment [158]. Plastic molecules ultimately their way to the freshwater and marine environment [158]. Studies have reported that more than 200 masks enter Indonesia's aquatic ecosystem per day [159]. Sea turtles and seabirds consume plastic litter [160] and this may obstruct their gastrointestinal tracts, resulting in debilitation and death. An adult Magellanic penguin (*Spheniscus magellanicus*) was found dead in Brazil due to ingesting a face mask [161].

Further, SARS-CoV-2 can survive in a surgical mask, gloves, and other plastic material for several days, and in developing countries, sewage waste goes to the ocean directly without any treatment, which may increase the chance of the virus migrating long distance [162,163]. Although SARS-CoV-2 has not yet been detected in aquatic mammals [163], previous research linked contaminated wastewater to SARS-CoV-2 reverse zoonotic transmission to wildlife [164]. Moreover, the scientific community expects that by highlighting the vulnerability and transmission of SARS-CoV-2 among wildlife [165], policy decisions about wastewater management worldwide will be shaped to help safeguard at-risk wild species and marine mammals that may be exposed to this coronavirus. Furthermore, other subfamily, gamma ( $\gamma$ ) coronaviruses, are infective to aquatic mammals, mainly beluga whales and bottlenose dolphins and mammals of the Cetacea family [166].

Dedicated waste management legislation has been found in approximately 24% of world countries and approximately 33% of countries have general legislation, whereas 43% of countries are deprived of health care waste management legislation (Figure 6). Although North American countries have the highest dedicated legislation in contrast to all the countries globally, they face the challenge of gradually increasing contamination of SARS-CoV-2 in wastage. The global use of approximately 89 million face masks each month is due to COVID-19, which is recognized as plastic or plastic derivatives pollutants [167]. Face masks of polyethylene polymers [168,169] ultimately get in the way of dumpsites and water streams and pollute the aquatic and terrestrial environment [168]. Discarded face masks, gloves, and sanitizer bottles in the open environment such as parks, walkways, and even on main roads increase the challenges of environmental pollution, with adverse effects on the human and wildlife ecosystem. Waste and sewer treatment plants release their effluents into the water bodies and should be sensitive to the efficacy of their treatment [77]. Improperly treated effluents may increase environmental contamination and increase the possibility of exposure of the community population to SARS-CoV-2. This emphasizes the need for all of us to make better decisions and respond more quickly to infection spillover and the challenges posed by environmental contamination [170].



Figure 6. Region-wise health care waste management legislative status.

Furthermore, the spread of 'VOCs' in the environment warns of the risk of SARS-CoV-2 establishing in the environment, and this could spill back to other animal species with significant population densities [171]. Indeed, Omicron's genetic variants are sufficiently numerous that they could have been acquired by circulation in an animal reservoir, which is a plausible alternative explanation for its formation [170,171]. As a result, we urge strengthening the OneHealth approach of surveillance practice at the human–animal interface and in the environment to prevent future epidemics and pandemics.

#### 7. Conclusions and Recommendations

SARS-CoV-2 has different transmission pathways, leading to environmental persistence and further spreading to remote areas. The spread of SARS-CoV-2 via wastewater and sewage has recently been a concern for the scientific community. Airborne transmission and transmission from contaminated surfaces are also seriously considered due to the considerable length of virus survival in air particles and inanimate surfaces. Moreover, the prevalent emerging VOCs, Delta and Omicron, are mostly circulating in the diverse environmental media which are genetically related to human strains of SARS-CoV-2. Thus, different methods must be adopted to inactivate the virus using potential agents. Human health, wildlife, and aquatic mammals are in danger due to environmental contamination of SARS-CoV-2 through household and medical wastage. The virus may spread to more expansive areas through environmental contaminants and have a much more expansive impact than we could imagine. Thus, wastewater surveillance may be an efficient tool to detect emerging variants circulating in the community and may act as an early warning system for public health mitigation. Wastewater surveillance and sequencing of SARS-CoV-2 variants are needed to integrate with public health initiatives. This review will help in preventing and controlling the environmental contamination of SARS-CoV-2 and help in understanding integrated medical waste management.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/covid2070067/s1, File S1: Environmental detection of SARS-CoV-2 metadata.

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