REVIEW

Intestinal viral infections of novel SARS-CoV-2 in the Indian community: Risk of virus spread in India

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Abstract

In December 2019, novel severe acute respiratory syndrome coronavirus 2 (nSARS-CoV-2) virus outbreaks emerged from Wuhan, China, and spread all over the world, including India. Molecular diagnosis of Coronavirus Disease 2019 (COVID) 19 for densely and highly populated countries like India is time-consuming. A few reports have described the successful diagnosis of nSARS-CoV-2 virus from sewage and wastewater samples contaminated with fecal matter, suggesting the diagnosis of COVID 19 from the same to raise an alarm about the community transmission of virus for implementation of evacuation and lockdown strategies. So far, the association between the detection of virus and its concentration in stool samples with severity of the disease and the presence or absence of gastrointestinal symptoms have been rarely reported. We led the search utilizing multiple databases, specifically PubMed (Medline), EMBASE, and Google Scholar. We conducted a literature survey on gastrointestinal infection and the spread of this virus through fecal–oral transmission. Reports suggested that the existence and persistence of nSARS-CoV-2 in anal/rectal swabs and stool specimens for a longer period of time than in naso-pharyngeal swabs provides a strong tenable outcome of gastrointestinal contamination and dissemination of this infection via potential fecal–oral transmission. This review may be helpful to conduct further studies to address the enteric involvement and excretion of nSARS-CoV-2 RNA in feces and control the community spread in both COVID-19 patients ahead of the onset of symptoms and in asymptomatic individuals through wastewater and sewage surveillance as an early indication of infection. The existence of the viral genome and active viral particle actively participate in genomic variations. Hence, we comprehended the enteric spread of different viruses amongst communities with special reference to nSARS-CoV-2.

KEYWORDS
COVID19, Faecal material, gastrointestinal tract, Microbiota, Novel SARS-CoV2, Sewage and sewage treatment., viral transmission

1 | INTRODUCTION

In December 2019, Wuhan city, China, reported a massive outbreak of viral pneumonia caused by novel SARS-CoV2. The nSARS-CoV-2 emerged in China and rapidly spread across the globe, and produced the pandemic COVID-19 to more than 216 countries worldwide in approximately eight months.[1,2] The SARS-CoV, MERS-CoV, and the ongoing nSARS-CoV-2 are virulent viral pathogens that have crossed the species barrier from animals to humans and caused substantial mortality and morbidity in humans. Other strains of the coronaviridae family infect birds and mammals.[3] Indeed, just after the outset of the outbreak by this virus, many studies addressing the environmental transmission of viruses had also earlier highlighted the potential new epidemic risk postured by a coronavirus.[4–6] Predominantly, the nSARS-CoV-2 transmits among humans in close contact via respiratory droplets (aerosols) through the respiratory route.[7] Although the spread of the virus among humans is through respiratory droplets, the queries related to other different potential modes of transmission remain that may contribute to the initial cross-species infection, the large size of asymptomatic cases, and fast and weird pattern of the dissemination across the world. The rise in COVID19 cases across the globe even after preventive measures such as mask, social distancing, and travel restrictions clearly suggest a new mode of viral transmission and fecal–oral route could be one (Figure 1).

From the outbreak of nSARS-CoV-2 to date, several world waves have witnessed multiple waves of coronavirus with multi-fold level growth in the transmission. The basic reproduction number (R0) value represents the average number of people infected from one source/ one infected individual. Based on published data on nSARS-CoV2, R0 was found as 2.5 which is significantly higher than other coronavirus outbreaks, that is, SARS-CoV and MERS-CoV.[8] The R0 values during each wave across the globe were reported differently, that is, 1.4–2.5. It is possibly due to increasing genomic variations and selective mutations in nSARS-CoV2 strains.[9–11] Recently, cases of nSARS-CoV2 reported an upsurge in many countries despite vaccination and preventive measures like a mask, social distancing, and lockdown. Apart from aerosol-based transmission (airborne), it is evident that physical contact is initially considered the prime mode of virus transmission.[12] New transmission modes potentially contribute to higher cases of COVID19. It is important to emphasize the mode of transmission of nSARS-CoV2 as crucial in managing COVID19 over finding the origin of the virus. COVID19 patients remain associated with a live infectious virus/viral RNA in stool and fecal material.[13] Fecal material is associated with a potential source of virus transmission, and contamination of water resources further enhances the risk of viral infection. Several findings demonstrated that hospital toilets, including toilet seat, wash basin sink, and floor, contain live infectious nSARS-CoV2 confirmed via RT-PCR.[14] Naicker et al.[15] revealed urine samples of COVID19 patients discharge active and live nSARS-CoV2. The survival time of nSARS-CoV-2 reported up to 90 h in fecal samples was significantly higher than any other carriers.[10]

2 | nSARS-CoV-2 AND TRANSMISSION MODES

Since the outbreak of nSARS-CoV2 virus, the aerosol-based spread of the virus is considered a major mode of the route of transmission. Infected COVID-19 patients are a potential source of the virus and spread via respiratory droplets and aerosols generated during
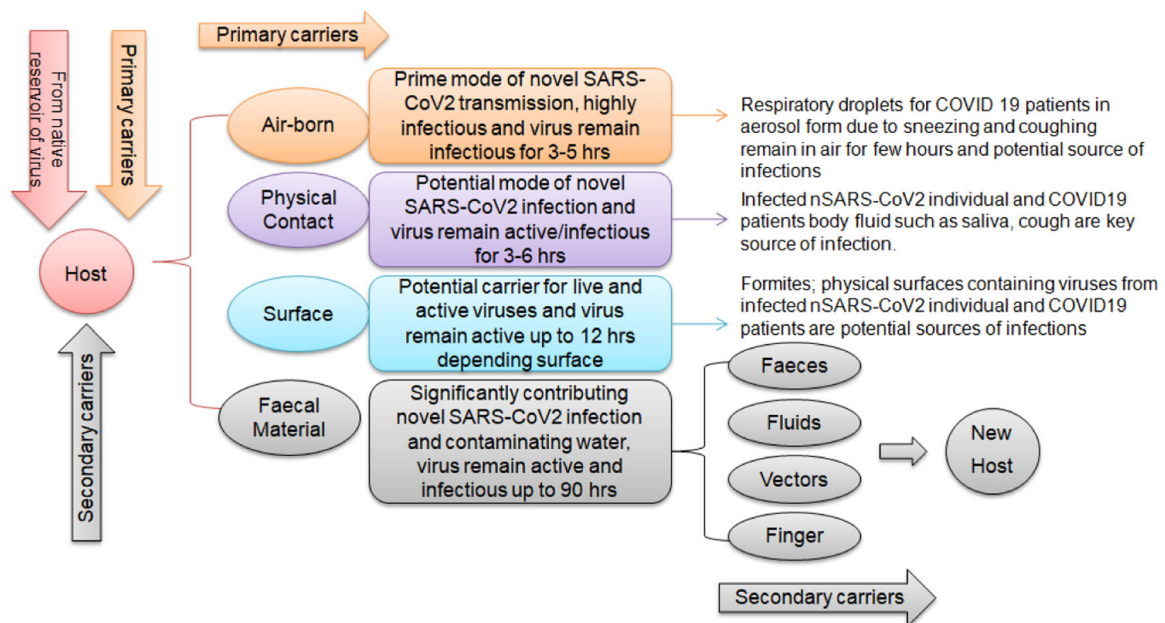
FIGURE 1 The figure demonstrates the mode of transmission of novel SARS-CoV2. The figure also depicts the contribution of fecal material in viral transmission via secondary carriers

coughing or sneezing.[16] Additionally, at the beginning of the pandemic, emphasis was given on fomite-based transmission/physical contact. It is evident the virus spread via fomites, however, the nature of physical contact is an essential factor here. Studies have demonstrated fomites may serve as a potential source of the virus genome, however, infection/infectivity remains of concern over time. These transmission modes and viral transmission also depend on several environmental factors including temperature, humidity, and precipitation.[17] These environmental factors affect virus viability in the different materials/fomites. Sobral et al.[18] demonstrated the global spread of nSARS-CoV2 and its role in environmental factors. A study had shown that temperature and humidity provide a substantial role in virus survival and infection.[18] Clinical studies have demonstrated gastrointestinal symptoms in a large number of patients. The intestinal enterocytes in the ileum show higher expression of ACE2. Additionally, ACE2 expression is also higher in the vascular endothelium and vascular smooth muscle cells in the submucosa of the ileum.[19] These finding clearly demonstrates the fecal–oral transmission of nSARS-CoV2.

3 | FECAL–ORAL TRANSMISSION

Recently supporting evidence has accumulated for the spread of this virus via the fecal–oral route that may have contributed to developing and disseminating this pandemic COVID-19.[20–24] The cause of virus shedding in feces of humans is that the nSARS-CoV-2 virus makes its entry inside the host cells by attaching its spike protein to the Angiotensin-Converting enzyme 2 (ACE 2), recognized as a host receptor and manifested in different human tissues that interact with the spike protein of the virus. ACE 2 is commonly expressed in the gut, kidneys, lungs, cardiovascular system, central nervous system, and adipose tissue.[25] ACE 2 is highly expressed in the respiratory tracts, including the oral mucosa.[26,27] The ACE 2 receptor is also highly expressed in the gastro epithelial cells of the small intestine. A recent immunostaining study done by Xiao et al.[23] has validated the high expression of ACE 2 protein, the prerequisite for nSARS-CoV-2 infection, in the glandular cells of gastric, duodenal, and rectal epithelia. Furthermore, viral Nucleocapsid protein staining was also seen in the cytoplasm of glandular epithelial cells of the gastric, duodenal, and rectum. Such evidence suggested that the presence of nSARS-CoV-2 in anal/rectal swabs and stool specimens for a longer duration than in nasopharyngeal swabs provides strong possibilities of gastrointestinal infection (GI) and the spread of this virus through fecal–oral transmission.[17,21,27–33] Until now, none of the studies have investigated the associations between detection of virus and its concentration in the stool with the severity of disease and presence or absence of gastrointestinal symptoms, and warrants addressal to elucidate the enteric involvement and excretion of nSARS-CoV-2 RNA in feces. However, research studies on fecal–oral transmission should be undertaken in conjunction with environmental studies to determine the virus's viability in conditions that would support such transmission.

4 | ASSOCIATION OF nSARS-CoV-2 VIRUS WITH A GASTROINTESTINAL INFECTION

COVID-19 patients' symptoms typically present fever, respiratory illness, and some reports GI symptoms such as abdominal pain, vomiting, and diarrhea. GI symptoms may precede or trail the respiratory signs and symptoms in COVID-19 patients.[17,29,32–36] The patient even after recovery from nSARS-CoV-2 infection, shed viruses in the stool. Cheung et al.,[37] in a meta-analysis, demonstrated that 48.1% of patients were shedding viruses, and SARS-CoV-2 was detected in their stool when their respiratory samples were tested negative; it was also found that 17.6% of patients with nSARS- CoV-2 infection were facing gastrointestinal problems. Two laboratories from China have successfully isolated viable SARS-CoV-2 virus from feces of infected patients.[17] Macau's study reported the detection of SARS-CoV-2 RNA in feces in 90% of patients, and one patient showed positive results after 14 days of showing symptoms.[38] On the other hand, the study conducted by Xiao et al.[23] declares that the nSARS-CoV-2 virus can last in the gastrointestinal tract for months in patients even after they test negative in respiratory samples, which indicates that the patient can be a potential carrier and fecal–oral transmitter for months. Apart from these studies, there is also evidence of patients who were tested positive from fecal samples without showing the symptoms of diarrhea and gastrointestinal problems.[17,39] One child tested positive from stool whose parents were tested negative in saliva, respiratory samples, and fecal samples.[29] However, studies have also detected the nSARS-CoV-2 RNA in the fecal samples of the COVID-19 patients with and without GI symptoms suggesting the GI tract may be the potential route of transmission and target for nSARS-CoV-2 infection.[38] Thus, the presence of nSARS-CoV-2 in the stool samples of COVID-19 patients and a few patients exhibiting diarrhea suggests a possible link between the lung and the intestine.

4.1 | Association of gut microbiota and viral infection

Elderly, immune-compromised patients, and patients with other comorbidities like diabetes, cardiovascular disorders, asthma, and so on, are more likely to fall sick when exposed to this virus. In such patients, dysbiosis has been implicated by the nSARS-CoV-2 infection of the GI tract. Gut dysbiosis has also been shown to be associated with Irritable Bowel Syndrome (IBD), Type 2 diabetes, depression, cardiovascular diseases.[40–42] Cheung et al.[43] had shown in a meta-analysis high viral particle shedding in fecal matter from clinical isolates in Hong Kong. According to the findings, nearly 17.6% of COVID 19 patients had shown gastrointestinal symptoms where 48.1% were COVID 19 negative.[43] A diverse and dynamic microbes population colonizes the human gastrointestinal (GI) tract, the gut microbiota, and exerts a marked influence during homeostasis and illness on the host. Alterations or decreases in the microbes in the microbial composition can lead to dysbiosis and may contribute to several diseases such as

gastroenterological disorders, metabolic, hepatic, cardiovascular, respiratory, and neurologic illnesses.[44] In murine models, it has also been shown that removal of gut microbiota by antiseptic treatment in mice increases the susceptibility of influenza virus infection. It has also been previously demonstrated by Trompette et al.[45] that bacterial metabolites not only influence the intestinal microbiota but also modulate lung immunity. Like the intestinal microbiota, there is accumulating evidence for lung microbiota, which through a cross-talk between the gut microbiota and the lungs is referred to as the "gut-lung axis" (GLA) have been shown to influence pulmonary health. The GLA cross talk is a two-way dialogue process that involves endotoxins and microbial metabolites through blood, influences the intestinal microbiota, and controls the lung microbiota.[46] Furthermore, lung immunity and inflammation in the lungs can transform the lung microbiota composition and affect the gut microbiota. Gut microbiota regulates type I interferon receptors in the respiratory epithelia in response to virus infection affects the secretion of IFN-α and IFN-β. It is evident IFN-α and IFN-β secretion is mediated via gut microbiota and IFN-1 signaling.[47] Furthermore, natural killer cells become ineffective and affect the expression of IFN-α, IFN-β, IL-6, TNF, IL-12, and IL-18 cytokines from macrophages and DCs.[48]

Studies have demonstrated the role of gut microbiota influencing pulmonary immunity in respiratory diseases through GLA.[49,50] Viral pneumonia caused by nSARS-CoV-2 and acute respiratory distress syndrome (ARDS) is one of the pivotal clinical outcomes of the severe form of the coronavirus disease (COVID-19), particularly in people with older age and poor immune response; however, it has quickly become apparent that severe disease can likewise occur in younger patients with no prior ailments.[51,52] Many experimental and clinical observations have demonstrated gut microbiota's role in the pathogenesis of ARDS. It has been shown by Dickson[53] that in critically ill patients, lung microbiome enrichment with the gut-associated microbe correlates with the development of ARDS. ACE2, the nSARS-CoV-2 receptor, apart from lung, expression in gut epithelial cells is needed for antimicrobial peptide expression, up-take of dietary amino acids, and modulation of gut microbiota. Given the ability of ACE II to modulate microbiota, reports are available that suggest dysbiosis of the gut microbiota contributes to the risk of respiratory illnesses that links to changes in immune response and inflammation.[24] Additionally, ACE2 expression prevents intestinal inflammation, as witnessed in murine models where ACE2 alterations/ deficiency in mice with reshaped gut microbiota resulted in severe colitis and increased the susceptibility to intestinal inflammation diarrhoea.[54] Taken collectively together, all this evidence speculates on the communication between the lung and the gut microbiota influences the respiratory symptoms, the gastrointestinal tropism of nSARS-CoV-2, and gut microbiota in severe cases of COVID-19. It is also quite possible that even the gut-lung axis may also influence the viral load, host immune response, and severity of disease associated with nSARS-CoV-2 infections, however further studies are required for these co-relations to be established. Thus, it should be worth considering the gastrointestinal tract as a potential target in the management and transmission of COVID-19.

5 | CLINICAL SURVEILLANCE OF COVID-19

In the clinical surveillance of COVID-19, only the nSARS-CoV-2 is detected among symptomatic patients, but the virus in most infections in an asymptomatic individual remains undetected.[55] However, it is evident asymptomatic patients are carriers for nSARS-CoV2 and are actively associated with virus transmission. Asymptomatic infection refers to the detection of nSARS-CoV-2 in clinical samples by reverse transcriptase-polymerase chain reaction (RT-PCR), without having any typical clinical signs or symptoms, and abnormalities in images, including lung computed tomography (CT).[56] Most asymptomatic infected persons do not seek medical attention as they do not manifest any apparent clinical signs/symptoms and even do not have the awareness to follow good hygiene practices that enable the COVID-19 to spread rapidly. Thus, a significant challenge is ahead to keep track of these patients to stop and control the transmission of SARS-CoV-2 viruses by such kind of infected person, as they are potentially contagious and need more attention globally. In the early stages of this COVID-19 outbreak, in China, 1.6% incidences were reported due to underestimation. With evidence accumulating across the globe, the rise in the incidence of asymptomatic infection was reported.[57–59] Even nSARS-CoV-2 virus nucleic acid detection in stool samples of asymptomatic patients also reported in different studies indicates the possibility of involvement of the gastrointestinal tract in transmitting nSARS-CoV-2 through the fecal–oral route.[60–64] Therefore, for effective prevention and early control of this global epidemic, screening of high-risk populations such as close contacts, individual households, and follow-up of the source of infection in confined space, especially in regions with poor sanitation, is recommended to identify asymptomatic patients likely to shed virus in feces and possibly spread the disease through the fecal–oral route. The nSARS-CoV-2, after shedding in feces from the human body, can survive for up to several days in the environment.[65] The stability of the virus in external environmental conditions depends on temperature, humidity, and UV radiation. The environment could get enough time to reach other organisms, mutate and change characteristics leading to proliferation and transmission. Thus, tracking wastewater for the coronavirus strain could serve as an essential data source and potential early warning system to public health authorities for COVID-19 infections to trace nSARS-CoV-2 carriers in some local regions.

6 | GENETIC EPIDEMIOLOGY OF nSARS-CoV-2

Since the first unraveling of the whole genome of nSARS-CoV-2, the virus has evolved with genomic diversities resulting in several genetic variants that may affect virulence, pathogenicity, and transmissibility.[66–68] Initial analysis of 103 SARS-COV-2 genome sequences identified two sub-types designated as L (Leucine) and S (Serine) types that are defined by two different single nucleotide

polymorphisms (SNPs).[29] The L-type was more prevalent (70%), aggressive, and contagious than S-type (30%). The virus has further mutated and spread in the form of clades/clusters over different geographical spaces. The geographical diversity exhibited by the different coronavirus strains may correlate with the virus's antigenic properties, virulence, severity, rate of morbidity, and mortality associated with the COVID-19. Foster et al.[69] on phylogenetic analysis of 160 complete genomes of nSARS-Cov-2, identified Clusters as A, B, and C in early March 2020.[69] Cluster A is the ancestral type closest to the bat genome & mainly found in the Americas and Europe and the C type, while Cluster B is the most common type in East Asia as they seem to be immunologically and environmentally adapted to the East-Asian population.[70] Majumder and Biswas retrieved 3636 viral sequences of nSARS-CoV-2 collected from 55 different countries and deposited to the Global Initiative on Sharing All Influenza Data (GISAID) until March 2020 on analysis revealed different 11 types of clades for nSARS-CoV-2 based on amino acid mutations that lead to clade separations such as A1a, A2, A2a, A3, A6, A7, B, B1, B2, and B4.[68] As on September 12, 2020, more than 100 000 viral genomic sequences data have been contributed by several laboratories around the world in the GISAID database (https://www.gisaid.org/).[71]

Furthermore, due to the expanding genetic diversity, the phylogenetic diversity of nSARS-CoV-2 at present revealed the evolution and existence of seven clades of SARS-CoV-2 in the GISAID database, S, L, V, G, GR, GH, and O. Rambaut et al. has defined two lineages A and B which correspond to clade S and L described in Tang et al.[29,72] The recent and most accepted nomenclatures introduced for SARS-CoV-2 by the Next strain provide a grouping of SARS-CoV-2 viruses into five different clades such as 19A, 19B, 20A, 20B, and 20C, and providing broad categorization of globally circulating diversity of nSARS-CoV-2.[73] In the Indian context, during the month of April to May 2020, 19A clade was prevalent in India's northern part (Delhi), while the western part of India (Maharashtra) was dominated by two clades 20A and 20B. At the same time, 19A was dominant in the southern part of India (Telangana) in April month but shifted to 20A and 20B in May 2020, whereas in the eastern part of India (Odisha) 20A dominated in April, while 19A prevailed in May month.[74] Altogether, these findings suggest that the nSARS-CoV-2 virus is clustering, still evolving and widely disseminating across different geographic locations but has a common ancestor. Thus, it would be reasonable to consider the possibility that mutational variants may modulate the clinical onset and disease spread. Therefore, this virus's mutational variants' conceivable outcomes to modulate the clinical manifestation and spread of the disease should be not be ignored but warrant addressal. The water-based epidemiological approach provides an effective way to predict the potential spread of the nSARS-CoV-2 in the community so that effective intervention measures should be counteracted for mitigation of the threat of this virus spread.[53] Thus, concurrent analysis of a genomic sequence from environmental isolates for comparison with sequence from the clinical sample will provide molecular epidemiological data for prevention and control activities of environmental dissemination of this virus in the current and potential future outbreaks. Genome investigation and

gene/s profiling provide a scientific basis for antigenicity and vaccine effectiveness. The virus copies/virus load is directly associated with the infection rate. Virological assessment of clinical samples demonstrated higher virus titer is critical in the profiling not only infection but also severity of disease.[54]

7 | PERSISTENCE OF VIRUSES AMONG VARIOUS SAMPLES AND ITS TRANSMISSION AMONG THE HUMAN POPULATION

Given the current COVID-19 disease situation, between 2% and 35% of patients with nSARS-CoV-2 manifested GI, had diarrhea and viable virus confirmation in stool and sewage samples raises the possibility of fecal–oral transmission.[32,75–78] The initial reports of nSARS-CoV-2 RNA detection have been documented in several studies in the wastewater from the Netherlands, the United States, France, and Australia.[79–84] A recent study in Japan and India successfully demonstrated the presence of nSARS-CoV-2 RNA in secondary treated wastewater samples in Japan and influent samples from the wastewater treatment plant in India.[85,86] The nSARS-CoV-2 RNA detection in these countries' wastewater was accomplished by employing different concentration methods, such as ultrafiltration, PEG precipitation, and electronegative membrane adsorption followed by direct RNA extraction.[69,71,72] One of the reports claims that the patient tested positive with the nSARS-CoV-2 virus without coming in contact with an infected person, raising the possibility of acquisition of infection through the fecal–oral transmission route.[87] The two reports of detection of nSARS-CoV-2 genetic material-viral RNA in wastewater came from the Netherlands from the collection of the samples of human wastewater from the airport, and six sewage samples of seven cities indicated that the sewage surveillance could be a sensitive tool to monitor the circulation of the SARS-CoV-2 virus in the population.[81] Although several studies within a few months after the emergence of COVID-19 began to appear for tracking of nSARS-CoV-2 using water-based epidemiology had claimed the molecular detection of RNA of nSARS-CoV-2 from untreated and treated wastewater, there is one study that shows that there is little risk of infection from wastewater because despite getting high RNA copy number, they failed to isolate the virus in cell culture.[88] Despite high RNA concentration, few studies demonstrated the isolation of infectious viruses from stools,[32,89] while another study failed to isolate the infectious virus.[90] This disparity may be attributed to the minor variation in virus isolation protocols between laboratories. The use of a recently developed engineered Vero cell line may enable enhanced isolation of infectious cultivable nSARS-CoV-2 from stools of symptomatic and asymptomatic individuals with nSARS-CoV-2 infection.[91]

Toilet waste, including feces, urine, soapy water from bath and showers, many pathogens, especially enteric viruses, kitchen sink/basin contents such as water left over from washing dishes and clothes, organic matter, particulate solids, micropollutants, and so on,

that leaves the houses or hospitals or commercial or industrial sites meet up with the sewer through the drainpipes into the "sub-mains" that are laid just beneath the roads. Sub-mains together from different areas as a whole unload their contents into the "outfall-sewers." These outfall sewers are linked to sewage treatment plants that are functional in different parts of the city. Feces reaching the sewerage system undergo dilutions; thus, it is likely that nSARS-CoV-2 viruses similar to enteric viruses contained in the feces undergo transformation and possibly may undergo a reduction in number and viability due to precipitation, low pH, temperature, light exposure, high content of particulate solids and pollutants, and so on.[92] However, a previous study on coronavirus reported that it remains infectious in water and sewage for days to weeks.[93] Despite the aforementioned challenges in sewage and wastewater, several studies have reported the molecular detection of SARS-CoV-2 RNA in treated and untreated wastewater with concentrations up to 10^3 to 10^6 copies per liter.[79–94] Several studies conducted on wastewater samples collection have revealed that the viral load or abundance of nSARS-CoV-2 genetic material in sewage/sewage sludge/wastewater positively correlates with the number of confirmed COVID-19 cases,[79,84,86,95–97] indicating water-based epidemiology to be a promising tool for tracking the spread of COVID-19 within a population.

However, two studies by Trottier et al. and Wu et al. did not observe a correlation observed between nSARS-CoV-2 in wastewater/sewage sludge with confirmed COVID-19 cases.[98,99] Several studies have been conducted until now from the beginning of the COVID-19 epidemic from the different regions across the globe, such as the Netherlands, Turkey, France (Paris), Spain, Brazil, the United States, Italy, indicated that wastewater based surveillance/epidemiology could provide an alternative and early detection tool for identification of COVID-19 spread, early detection of its emergence, an early sign of identification of high-risk areas.[81,83,84,100–105] Studies from India by Arora et al. and Kumar et al. have evaluated wastewater-based surveillance as an early detection tool for COVID-19 spread.[106,107] The findings of both the studies correlated higher viral load in wastewater with an increased number of COVID-19 patients, however, reduction in viral particles were observed in treated wastewater with no sign of viral RNA spotted in effluent released from wastewater treatment plants (WWTPs). Though none of the above-mentioned studies were able to recover infectious nSARS-CoV-2 from sewage or wastewater that can affirm the fecal–oral route transmission of nSARS-CoV-2. Several studies have reported the occurrence of non-enveloped enteric viruses like adenoviruses, polioviruses, enteroviruses, noroviruses, and rotaviruses in wastewater[86,108] and re-emerging Zoonotic hepatitis E Virus.[108,109] They have been detected from virtually all kinds of water: seawater, fresh waters, groundwater, and drinkable and are related to drinking and recreational water outbreaks.[110] Certain enveloped viruses such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and influenza, HIV, and Ebola viruses are known to have been responsible for devastating pandemics.

There are only a few studies available on the presence and survival of enveloped viruses in wastewater which have developed and optimized the methods to concentrate Coronaviruses or other enveloped viruses from wastewater, biosolids, surface waters, and other types of water (river, lake, and reservoir).[111–116] These studies highlighted the decrease of virus survival with increased temperature, thus demonstrating temperature, an essential factor in determining the virus persistence in the aquatic environment. Therefore, when a significant incidence of cases associated with an enveloped virus such as nSARS-CoV-2 in the community and wastewater are cooler, the chances of the high level of infectious viruses in the influent draining into wastewater treatment plants cannot be ignored. The non-enveloped enteric viruses are highly resistant to environmental conditions such as aquatic environment wastewater, wastewater treatments, and disinfection compared to the enveloped viruses.[117–120] However, the study by Casanova et al. and Gundy et al. have demonstrated that the coronavirus, an enveloped virus remains infectious, in water and sewage for days to weeks which could provide enough time for the virus to reach other organisms, mutate and change their characteristics.[121,122] The researchers have reported that the time required to reduce viral infectivity was several days at room temperature in pure water, pasteurized settled sewage, and wastewater. These are possible sources of detection of nSARS-CoV-2 RNA in individuals in communities if the quality of personal hygiene is poor. Viral metagenomics study of sewage sludge in wastewater and surface water has also revealed a large diversity of human viruses, including enveloped viruses.[123–126] The presence of organic matter, suspended solids may have a significant effect on Coronaviruses' survival.

The research on the survival or persistence of nSARS-CoV-2 in the wastewater, depending on the temperature, presence of organic matter, and suspended solids, is an essential requirement to improve the authenticity of the information utilized for water-based epidemiological studies and to comprehend the chances of transmission. The other potential source of transmission through virus-laden aerosol or droplets generated from wastewater flushing or plumbing system in the buildings/apartments cannot be ignored. The nSARS-CoV-2 outbreak of many residents living in an apartment block in Hong Kong in 2003 was traced to droplets containing coronavirus from the wastewater system.[127,128] On the other hand, there are evidence available for the presence of potential pathogenic viral particles (norovirus, adenovirus, and the hepatitis E virus)[129] in the aerosol generated at wastewater treatment plants; thus similarly, a significant risk of spreading of nSARS-CoV-2 through aerosol produced over the uncovered aerobic wastewater treatment facilities such as activated sludge operation, and so on, could not be underestimated.[130] In the nSARS-CoV outbreak of 2002–2003, no evidence of people working in wastewater treatment plants having contracted nSARS-CoV2 was found; however, the viral transmission via aerosol from the treatment unit to the workers handling these units in the present scenario of COVID-19 could not be neglected. Therefore, the role of aerosol generated from sewage or wastewater needs to be further investigated for COVID-19 transmission. Thus, after shedding from feces, the nSARS-CoV-2 virus can transmit by fecal–oral route directly by human-to-human contact and indirectly by wastewater, sewage, drinking water, food, fishes, other aquatic

and soil organisms, vegetable, and other unknown fomites and can infect the new host by adopting several mutations.[131] Recent reports are available that indicate the viral load in sewage/wastewater positively correlates with the number of COVID-19 cases.[132,133] Thus, while estimating the number of infected cases through sewage/wastewater studies, the wastewater temperature and dilution should be accounted for to measure the viral hotspots. Identification of viral hot spots would enable channeling resources to protect vulnerable populations through social distancing initiatives, removing the restrictions in virus-free regions, and mitigating economic and social disturbance.

## 7.1 | ERADICATION OF nSARS-CoV-2 BY CONVENTIONAL SEWAGE TREATMENT METHODS

The conventional wastewater treatment's main aim is to extract suspended solids, organic matter, and pathogen removal to some extent, likely more successful for bacteria than viruses.[134–137] Few studies have reported the nSARS-CoV-2 RNA removal during wastewater treatment.[118,138] However, finding from these studies did not conclude on the viability of the virus RNA and infectivity as well. The challenge in the isolation and study of nSARS-CoV2 from wastewater treatment plants is viral inactivation for other enveloped viruses or Coronaviruses.[139] Coronavirus is generally unstable in the environment and susceptible to chlorine treatment than non-enveloped viruses as natural oxidants such as chlorine disrupt the receptor present on the viral envelope, responsible for infection of sensitive cells.[140–142] A recent study from China showed how a wastewater disinfection pool with a sodium hypochlorite solution could completely eradicate the nSARS-CoV-2 sewage load generated from the isolation ward of Zhejiang University, China.[143,144] However, studies have shown that chlorine provides residual disinfection in the distribution pipelines.[145–147] Therefore, excessive use of disinfectants should be avoided, and effective dosage of residual chlorine should be adjusted to prevent potential adverse environmental effects such as in the ecosystem or in agriculture. Like chlorination, several studies have also reported effective inactivation of Coronaviruses by UV irradiation achieved through the formation of pyrimidine damaging the nucleic acids.[148,149] Altogether, these pieces of evidence indicate that nSARS-CoV-2 should be expected to be eradicated by conventional sewage treatment methods with disinfection and inactivation.

However, for heavily populated countries such as India with inadequate sewage treatment facilities or poor wastewater treatment infrastructures, chances of getting surface water, drinking water, river, or water reservoir contaminated pollution are tremendously high because SARS-CoVs and other coronaviruses can live in untreated sewage for up to several days; even in low-temperature regions for a much longer time. A recent study has monitored the river water samples for the presence of nSARS-CoV-2.[150] Few river water samples tuned out positive by real-time PCR positive for nSARS-CoV-2, possibly due to the discharge of untreated wastewater or through the sewage overflows; the virus from the sewage goes into the river. Although there is no current evidence that Coronaviruses live in wastewater or are transmitted through contaminated drinking water, still there is an increasing concern about the effect of the recent pandemic wave on underprivileged communities with a lack of quality sewer infrastructure or weak wastewater treatment systems, and densely populated cities without proper sanitation where fecal–oral transmission could occur and increase the risk of community transmission (Figure 2).

Many of the undiagnosed clinical cases and viral infections asymptomatic in nature not evident by clinical surveillance can be epidemiologically monitored using the wastewater-based epidemiology approach.[151,152] Thus, with this approach, not only the fecally shed viruses of asymptomatic nature such as adenovirus, norovirus, sapovirus, enterovirus, rotavirus, and hepatitis A virus[153,154] but other viruses such as Saffold virus, cosavirus, and salivirus/klassevirus seldom or rarely reported by epidemiological surveillance program can also be identified in the feces of an entire region.[155,156] Environmental surveillance for sewage monitoring has already been established as a tool for detecting poliovirus in sewage samples to monitor the virus circulation and extent or duration of epidemic poliovirus in specific populations.[157–160] Wastewater epidemiology played a significant part in the eradication of polio in India in 2011. The polio eradication program included the weekly testing of wastewater and sewage for poliovirus in the selected areas, followed by an immunization program if they found traces of viruses.[161] The nSARS-CoV-2 virus and Polio Virus both are dissimilar, and the protocol for diagnosing nSARS-CoV2 from wastewater will have to be standardized for proper monitoring. Pakistan is already using its existing polio environment surveillance network for monitoring nSARS-CoV-2.[162] The method tangential flow filtration of concentration of sewage followed by deep sequencing used for polio surveillance by Furtak et al.[152] in Pakistan can also be employed in the monitoring of nSARS-CoV-2 from environmental samples. Likewise, sewage and wastewater monitoring can be adopted as a supplemental ecological surveillance tool to detect the prevalence and circulation of nSARS-CoV-2 like pathogens circulating in the community. This approach might help trace nSARS-CoV-2 infected large asymptomatic populations that seem to account for more than 50% of nSARS-CoV-2 infection, exhibit prolonged shedding, and possess a high risk for silent spread of the virus among the human population.

The current testing method for diagnosing nSARS-CoV-2 infection in India with a vast population is not enough for assessing the state of pandemic affairs and is constrained by cost and current given testing capacity. However, when testing of individuals preceded by a population-based screening of wastewater or sewage could serve as a valuable tool to monitor the spread of novel coronavirus in the communities and determine the burden of undiagnosed infections in India. None of India's studies have documented any stool sample testing of COVID-19 patients and reported enteric transmission. It is known that water contamination; poor sanitation, and hygiene are responsible for 4% of death and 5.7% of diseases worldwide.[163] Further, it was also reported only 19% of people worldwide wash their hands with soap and water after passing stool which is a matter of concern for fecal–oral transmission of any enteric disease.[164] It is
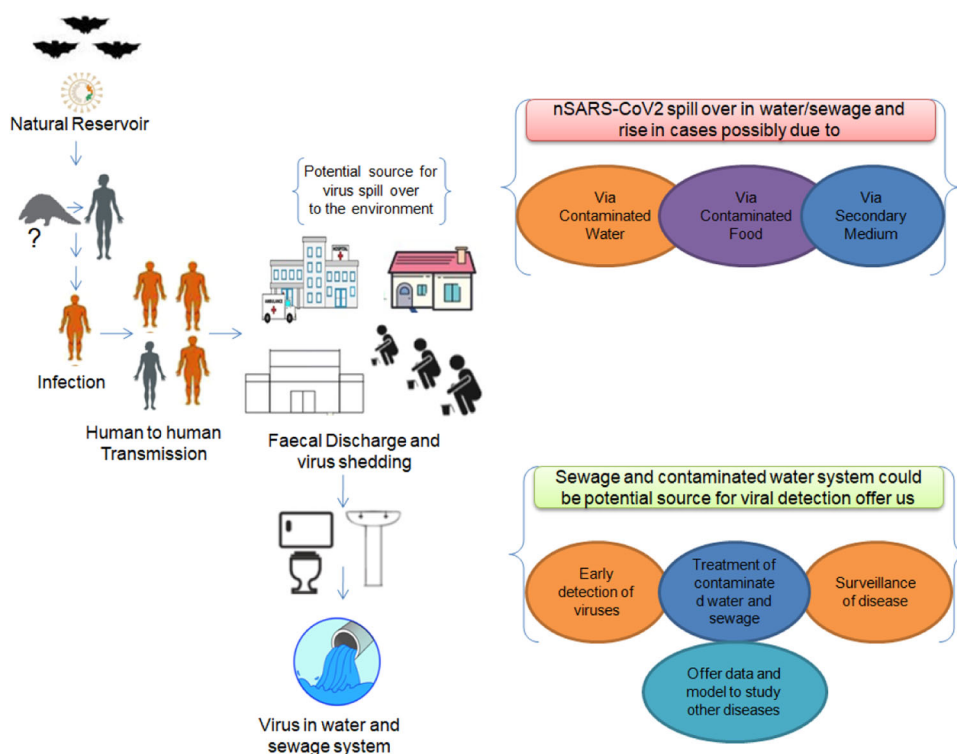
FIGURE 2 The figure provides an overview of nSARS-CoV2 infection resulting contamination of freshwater system via sewage. The figure also demonstrates a road map for nSARS-CoV2 entry to water and sewage and treatment

estimated that 2.8% of total deaths and 3.9% of total disability-adjusted life years were attributable to inadequate water, sanitation and hygiene. 60% of deaths are attributed to diarrhoea.[165] For a developing country like India with 1350 million population, 37.7 million Indians are affected by waterborne diseases, 1.5 million children die of diarrheal each year due to contamination of drinking water by fecal matter, improper sanitation of drinking water, mixing of sewage with drinking water, open defecation, poor hygiene practices after defecation, fecal–oral transmission of nSARS-CoV-2 virus will put additional pressure on the increase of positive cases because the disease is highly contagious and is spreading at an alarming rate with 5 million of positive cases in around 5–6 month only.[166] A present alarming concern of continuous increase in COVID-19 cases in India has grappled the situation with the rising possibility of spread of disease through fecal–oral transmission as the country represent the highest open defecation rates in the world, an issue having significant health implications for public health, like diarrhea, high child mortality, disease spread, malnutrition, and stunted growth.

8 | RISK OF FECAL–ORAL TRANSMISSION IN INDIA

A recent study has shown that 44% of people in rural areas from Bihar, Madhya Pradesh, Rajasthan, and Uttar Pradesh defecate in the open, and 23% of those with private toilets still defecate outside.[167] Due to the loss of jobs, many migrants have returned to their villages,

an issue of concern is the hygiene conditions to which the migrants returned and how migrants could transmit COVID-19 to those villages. The rural communities with a lack of toilets but those living in slums with a lack of access to private toilets, community toilets with the unhygienic condition, or use with improper hygiene are at serious risk with a potential for fecal–oral spread of COVID-19. Approximately 1.8 billion people worldwide use feces-contaminated as a source of drinking water; therefore, the risk of enteric spread of COVID-19 is also expected to rise by many folds unless adequate measures are followed to build a sufficient and adequate sanitation system. In the absence of basic or poor sanitation, the groundwater used as a source of drinking water in urban and rural areas is often drawn up from wells, borewells, and municipal tube wells for household use can also become contaminated with human enteric bacteria and viruses from fecal matter.[168] The endemic and epidemic transmission of numerous fecal–oral pathogens is reported in regions without safe sanitation; thus the risk of nSARS-CoV-2 transmission should be assessed, monitored, and averted in these settings.

As India's monsoons approach, there will usually be a surge in both vectorborne and waterborne diseases, including dengue, chikungunya, malaria, and typhoid. Amidst the monsoon period, the possibility of a spike in COVID-19 cases mediated through the fecal–oral transmission of nSARS-CoV-2 could not be underestimated as during monsoon season because of the flooding food supply and drinking water may become contaminated with sewage, sewer overflows carrying the virus in wastewater, in areas with poor sanitary conditions.[169] The monsoon accompanied by the

flood effect triggers many waterborne diseases including bacterial and viral. In monsoon and flood condition water quality comprised significantly affect human health and precisely in low-income countries such as India. The affected area results in poor sanitation and limited food and health supplies and services. Such conditions may lead to an outbreak of waterborne, airborne, and vectorborne communicable diseases. Most familiar diseases such as diarrhea caused due to GI by bacteria such as shigella and viruses can spread from human to human or fecal–oral route to human.[170]

9 | RISE IN VIRAL OUTBREAKS: GROWN HUMAN-WILDLIFE/ENVIRONMENT INTERACTION

In the last two decades during 2000–2020, there have been several viral outbreaks and three major were coronaviruses, that is, SARS-CoV, MERS-CoV, and nSARS-CoV2. Based on research findings, it is evident the natural reservoir of these viruses is wildlife and close proximity of domestic animals and wildlife allowed a passage to the human. Pathogens shared between people and animals result in millions of deaths annually and a single outbreak may cause economic losses amounting to tens of billions of dollars. Such tremendous losses are incurred mostly by viruses and because responses to viral outbreaks are often late. These diseases are particularly major public health risks to developing countries like India and require immediate intervention. Therefore, early detection and prevention of zoonotic spillover and spread are critical to reducing the impact of epidemics and pandemics. Emerging infectious diseases pose substantial threats to the health of animals, people, and economies globally.[171] Zoonotic pathogens shared with wild or domestic animals account for the majority of EIDs, and viruses comprise 25%–44% of these emerging and re-emerging pathogens. Over the past decade, attempts to control deadly zoonotic viruses, like SARS and MERS coronaviruses and highly pathogenic avian influenza viruses, have been, out of necessity, almost entirely reactionary and, consequently, prohibitively expensive.[172] Alternatively, such pandemics could likely be controlled if we could predict their occurrences. The strategy requires a multi-pronged approach where

1. Ecology of novel zoonotic viruses are better understood,
2. High-risk interfaces between wildlife, livestock, and people are identified, and targeted surveillance and monitoring for viral spillover is established,
3. Risky human behaviors that may facilitate spill over and spread of such zoonotic viruses are characterized and used to inform surveillance and intervention strategies, and finally, and
4. Laboratory systems to rapidly detect and respond to EIDs are strengthened. Since the world is much more connected today than it used to be decades ago, the success of these strategies impinge upon their implementation both at the country level to impede local outbreaks, and at the global levels to control transfer of viruses between countries that may cause pandemics.

10 | INDIAN GEOGRAPHY AND VIRAL OUTBREAK

The Indian subcontinent is known for its extraordinary biodiversity. Also, vast numbers of Indians still live within modified natural habitats and regularly interact with wildlife both directly and indirectly through their livestock. Modified landscapes often create interfaces between humans and wildlife that can promote spillover of zoonotic viruses.[171] Porous borders that allow human and animal movement between neighboring countries often via livestock markets or value chains—may extend the risk of spillover and outbreaks of zoonotic disease to broader regions where outbreaks may go unnoticed due to lack of surveillance. It is evident from the literature that there are regions in north India that have witnessed long experienced severe outbreaks of zoonotic diseases.[172–174] These regions also have a porous border which makes them a potential corridor of trans-boundary transmission of EIDs.[175] Additionally, these are also home to diverse wildlife including bats, nonhuman primates, and rodents that are known to be key reservoirs of zoonotic viruses. These animals often co-occur with humans. Therefore, targeted screening of animals and humans at high-risk interfaces is required where people and animals have high levels of contact, for novel viruses with zoonotic potential such as viruses from families that include high-consequence pathogens (e.g., coronaviruses, Influenza viruses, flaviviruses, filoviruses, and paramyxoviruses), may help identify points of spillover and amplification of zoonotic viruses. This "One Health" approach will be helpful for the discovery of novel viruses that may be the cause of important clinical syndromes such as acute encephalitis, severe acute respiratory disease, and hemorrhagic fevers whose etiology are often unknown, and the strengthening of public health systems. Moreover, if viral discovery should be complemented by (1) the identification of animal reservoirs, (2) characterization of (a) the pathways by which the viruses spill over to humans, and (b) the high-risk human behaviors that promote such spillovers. Taken together, these benefits will potentially reduce the time between detection and response to an outbreak caused by a novel virus. Thus, to implement the One-Health surveillance approach efforts should be focused on the highest risk locations and interfaces, where animals and people share changing landscapes, and diseases of unknown origin continue to take a significant toll.

11 | FUTURE

As GIs, floods have also documented the infectious diseases in the respiratory tract such as acute respiratory infections (ARIs), a primary reason of morbidity and mortality reported among the people displaced by natural disaster.[31,78,84,176,177] Therefore, the monsoon's potential consequences on the spread of nSARS-CoV-2 in the ongoing pandemic COVID-19 situation are a considerable cause of concern that should not be underestimated but needs to be further studied, high risk of the rapid spread of COVID-19 during the rainy/monsoon season in India. Better characterization and monitoring of

nSARS-CoV-2 in wastewater and accumulated human waste for assessment of viral pathogen diversity, including the emerging coronavirus among the circulating strains by means of phylogenetic analysis, allow for the comparisons among different areas with/without safely managed sanitation during the pre-pandemic/epidemic, on-going pandemic and post-pandemic situation and would also help evaluate the evolution in the virus genome over time as previously shown for enteric viruses.[15,178–181] These efforts will prevent virus entry into household water sources and water distribution systems, subsequently preventing public health from possible waterborne infections. Thus, with the dense population, where most people do not have access to proper sanitation, clean drinking water, and hygiene practices, the spread of the virus infection by enteric transmission cannot be ignored. Therefore, there is a foremost requirement to address the fecal–oral transmission of nSARS-CoV-2 for countries like India and others to establish immediate measures to mitigate the exposure of people living in the most vulnerable condition.[182]

In this regard, India should adopt sewage epidemiology/wastewater-based epidemiology (WBE), the best method to identify the novel coronavirus presence in the water. This approach will be helpful for (1) early detection of asymptomatic viral infection in a community, (2) development of early detection tool for identification of COVID-19 spread, (3) useful in early detection and identification of high-risk areas, will have the epidemiologic potential for heavily populated areas, where the door to door sampling, testing and tracing for COVID-19 cases are not practically feasible and worth for implementation, lockdown, implementation of social isolation and evacuation strategies for community found to be vulnerable for further spread of the virus.

12 | CONCLUSIONS

At the end of 2019, the nSARS-CoV2 was transmitted from Wuhan, China to the entire world in less than 3 months. The mode of transmission remains airborne (droplets containing active viruses), surface, and physical contact. Among these transmission modes, the infection and viability of nSARS-CoV2 were highest in airborne and physical contact. In the year 2020, research evidence demonstrated other important modes of nSARS-CoV2 enhanced infection rate and transmission of the pathogen. We reviewed here the fecal mode of nSARS-CoV2 and higher COVID19 cases around the globe. In conclusion, in addition to respiratory samples, fecal, wastewater, and sewage can be a vital source of information for diagnosing nSARS-CoV-2 in symptomatic and asymptomatic patients. These samples can be used as a marker of community transmission of COVID-19 disease. The fecal matter containing nSARS-CoV-2 also contaminates water resources. Additionally, rapid mutations in nSARS-CoV-2 also result in a higher prevalence of circulating viruses. The low-income countries, including India, where personal hygiene and sanitation remain compromised, risk viral infections. The reoccurrence of viral outbreaks, including nSARS-CoV2, remains associated with fecal matter and sewage/wastewater. The fecal route of virus transmission (nSARS-CoV2) is one of the most crucial modes and investigated least compared to other modes such as airborne, droplets, and physical contact. The fecal route-based findings help to build policies to control viral spread in the community via securing water contamination. It becomes even more critical in the case of India and other low-income countries where population density is high and habitat in a setup where sanitation and hygiene remain poor. Such a study also provides an urgency of water treatment plants. Educational programs on the fecal route of nSARS-CoV2 transmission would help in the prevention of viral spread in a country with an overwhelming population with compromised medical care facilities.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Rashmi Chowdhary: Conceptualization and analysis; Harjeet S. Maan: Writing and final, compilation; Deepti Chaurasia: Proofreading and editing; Debasis Biswas: Critical analysis and editing; Garima Kapoor: Review and editing; Lokendra Dave: Design and writing; Arshi Siddiqui: Collection and retrieval of scientific evidences; Savita Pal: Critical review and editing; Hari O. Singh: Critical review.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analyzed during the current study.

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