Understanding COVID-19 Situation in Nepal and Implications for SARS-CoV-2 Transmission and Management

Environmental Health Insights
Volume 16: 1–12
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11786302221104348
SAGE

Prabin Dawadi[1,2], Gopiram Syangtan[2,3], Bhupendra Lama[2], Sushil R. Kanel[4], Dev Raj Joshi[2], Lok R. Pokhrel[5], Rameshwar Adhikari[6], Hem R. Joshi[7] and Ioana Pavel[8]

[1]Biological Resources Unit, Nepal Academy of Science and Technology, Lalitpur, Bagmati, Nepal. [2]Central Department of Microbiology, Tribhuvan University, Kathmandu, Bagmati, Nepal. [3]Shi-Gan International College of Science and Technology, Tribhuvan University, Kathmandu, Bagmati, Nepal. [4]Department of Chemistry, Wright State University, Dayton, OH, USA. [5]Department of Public Health, The Brody School of Medicine, East Carolina University, Greenville, NC, USA. [6]Research Center for Applied Science and Technology, Tribhuvan University, Kathmandu, Nepal. [7]Department of Mathematics, Xavier University, Cincinnati, OH, USA. [8]Department of Physical and Environmental Sciences, Texas A&M University at Corpus Christi, Corpus Christi, TX, USA.

ABSTRACT

BACKGROUND: The pandemic of Coronavirus Disease 2019 (COVID-19), one of the most infectious diseases in the modern history, is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and has had a profound health and economic toll, globally. This paper identifies the overall health status associated with COVID-19 pandemic in all 7 provinces of Nepal, a developing country in South Asia, analyzing data from January 2020 to February 2022. It focuses on the SARS-CoV-2 prevalence, transmission through wastewater and other routes, diagnostics, treatment options, and alternative medicines, thereby offering key perspectives for its management.

MATERIALS AND METHODS: Studies regarding coronavirus spanning the 2017 to 2022 period were searched on the web, Nepalese database, and Web of Science. Refined criteria included SARS-CoV-2 in wastewater of Nepal or worldwide. Demographic data (sex, age-group, and geographic location) were also obtained from websites and relevant reports of the Ministry of Health and Population (MOHP) of Nepal, ranging from January 2020 to February 2022. Moreover, trends concerning lockdown, business, and border activities in Nepal between February 2020 and October 2020 were evaluated. The viral dissemination pathways, diagnosis, and available treatment options, including the Ayurvedic medicine, were also examined.

RESULTS: Aerosols generated during the hospital, industrial, recreational, and household activities were found to contribute to the propagation of SARS-CoV-2 into environmental wastewater, thereby putting the surrounding communities at risk of infection. When lockdown ended and businesses opened in October 2020, the number of active cases of COVID-19 increased exponentially. Bagmati Province had the highest number of cases (53.84%), while the remaining 6 provinces tallied 46.16%. Kathmandu district had the highest number of COVID-19 cases (138,319 cases), while Manang district had the smallest number of infections (81 cases). The male population was found to be predominantly infected (58.7%). The most affected age groups were the 31 to 40 years old males (25.92%) and the 21 to 30 years old females (26.85%).

CONCLUSION: The pandemic impacted the public health and economic growth in our study duration. SARS-CoV-2 was prevalent in the wastewater of Nepal. The Terai districts and the megacities were mostly affected by SARS-CoV-2 infections. Working-age groups and males were identified as the highest risk groups. More investigations on the therapeutic and alternative cures are recommended. These findings may guide the researchers and professionals with handling the COVID-19 challenges in developing countries such as Nepal and better prepare for future pandemics.

KEYWORDS: COVID-19, SARS-CoV-2, wastewater surveillance, Nepal, pandemic

RECEIVED: March 26, 2022. **ACCEPTED:** May 14, 2022.

TYPE: Short Report

FUNDING: The author received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHORS: Dev Raj Joshi, Central Department of Microbiology, Tribhuvan University, Kathmandu, Bagmati 44601, Nepal. Email: dev.joshi@cdmi.tu.edu.np

Lok R. Pokhrel, Department of Public Health, The Brody School of Medicine, East Carolina University, 2233 ECHI Bldg., 115 Heart Dr., Greenville, NC 27834, USA. Email: pokhrell18@ecu.edu

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

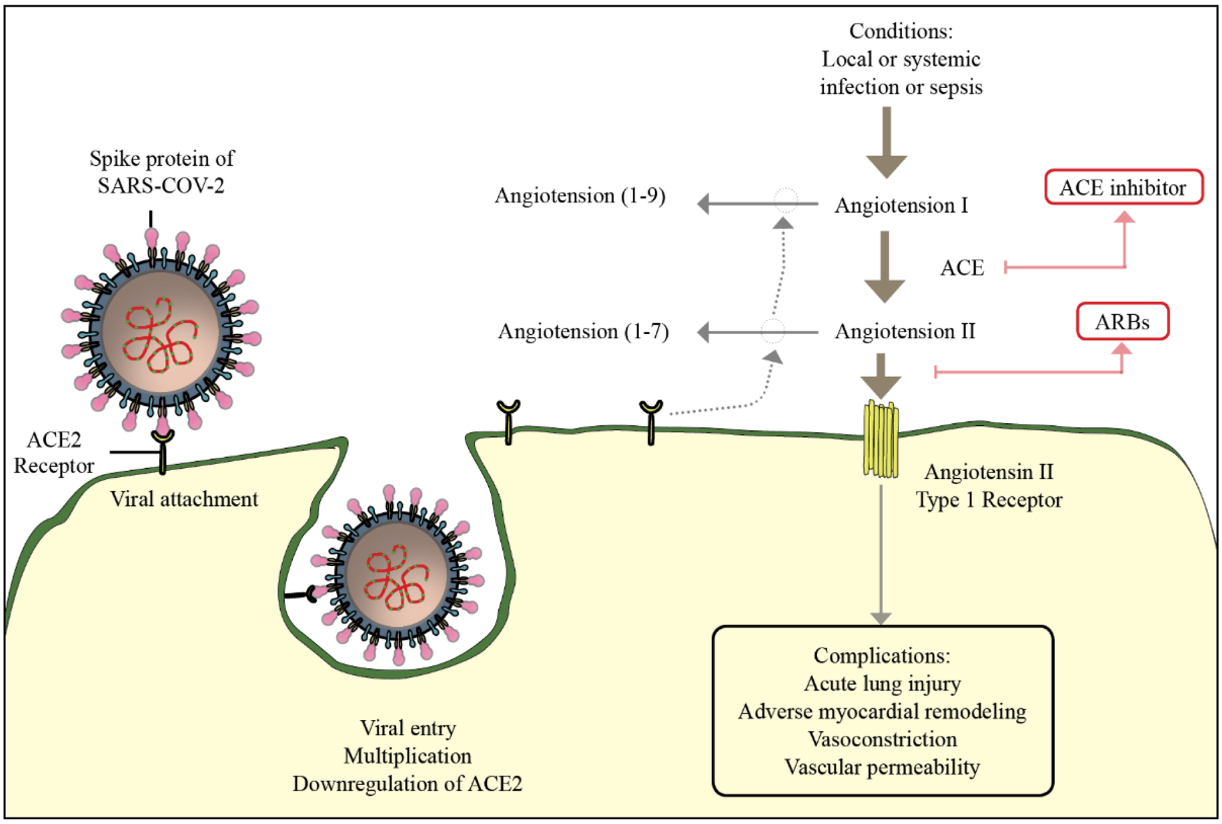

Figure 1. Schematic depicting SARS-CoV-2 structure and its pathogenesis.

Background

Emerging infectious diseases (EIDs) present one of the greatest challenges to public health in the 21st century. An emerging virus, depending on its potential to spread among humans, may cause individual or sporadic cases, culminating in a localized outbreak requiring public health intervention, or, in the worst-case scenario, a widespread epidemic, or worldwide pandemic.[1] The novel Coronavirus Disease 2019 (COVID-19) is a new respiratory disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) that is causing worldwide public health and economic challenges and has been recognized as a pandemic by the World Health Organization.[2] The virus was first reported in Wuhan, Hubei Province, China, in December 2019.[2,3] SARS-CoV-2 is an enveloped and positive single-stranded RNA virus belonging to the ß-coronavirus genus.[2-4] SARS-CoV-2 holds high homology with SARS-CoV and targets angiotensin-converting enzyme receptor-2 (ACE2) for the viral attachment.[4] A schematic depicting SARS-CoV-2 structure and pathogenesis is presented in Figure 1.[5] There are very few studies on the transmission of SARS-CoV-2 through treated or untreated wastewater from advanced countries; however, COVID-19 surveillance of wastewater in developing countries has not been reported adequately.[6] There is thus a need to study the presence of SARS-CoV-2 in wastewater in the developing countries like Nepal.

On 24th January 2020, the first case of COVID-19 was reported in a Nepalese student, who had recently returned from China to Nepal, and the second case was identified about 2 months later in a person returning from France.[7] A complete genome sequence of SARS-CoV-2 strain from a Nepalese patient with COVID-19 showed 99.6% identity with SARS-CoV-2 reference genome and the full-genome comparison of the isolate revealed >99.99% identity with 2 previously sequenced genomes available at GenBank (MN988668 and NC045512) for SARS-CoV-2 from Wuhan, China, and >99.9% with 7 additional sequences: MN938384.1, MN975262.1, MN985325.1, MN988713.1, MN994467.1, MN994468.1, and MN997409.[8,9] The majority of COVID-19 cases were reported to be asymptomatic. The likelihood of COVID-19 outbreak was noticeably underestimated in Nepal during the early phase of the pandemic and there was a subsequent rise in cases over time.[10]

Notably, the healthcare system in Nepal was not prepared to manage such an outbreak in terms of physical facilities in the hospitals, availability of health care professionals, and arrangement of diagnostic as well as safety materials for the frontline healthcare professionals. There were misconceptions spread in the society that the Nepalese people are resistant against the COVID-19 for some unknown reasons. They also believed that their culinary practice and traditional medicine were effective against the disease, albeit with no scientific evidence. On one

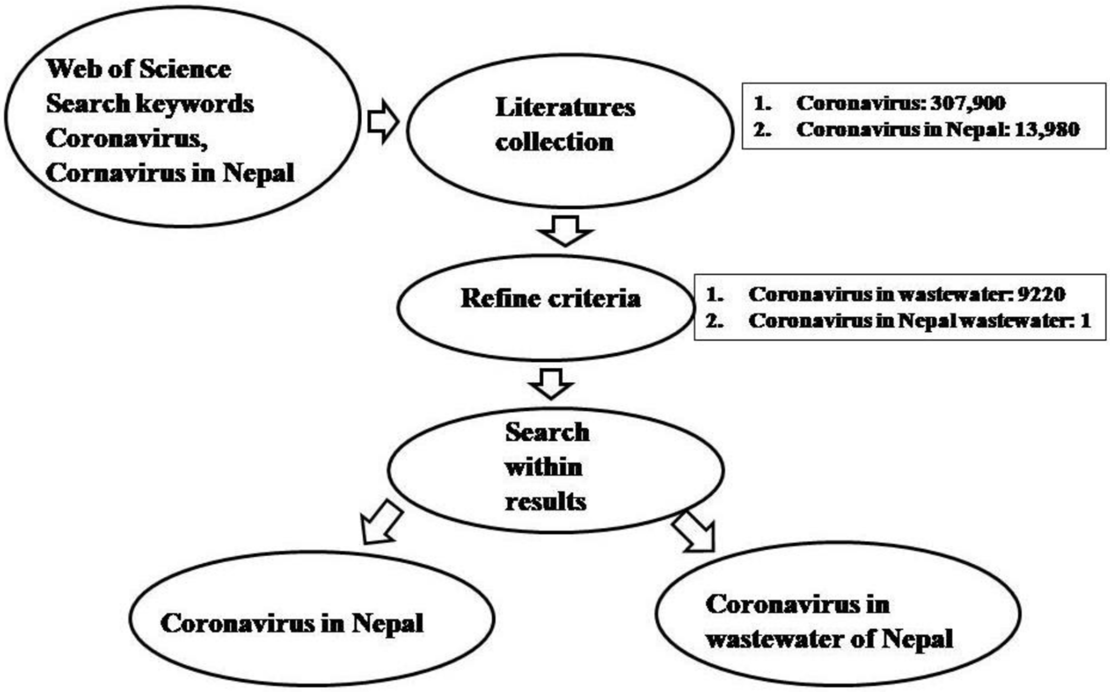

Figure 2. Main search criteria used for the selection and categorization of published literature on coronavirus in Nepal.

hand, there has been a challenge, particularly, for healthcare professionals in tackling COVID-19 with utmost precautions and with limited resources. On the other hand, there was a challenge for the scientific community to carry out a comprehensive analysis of the trend of the disease outbreak and recommend the government for efficient strategy formulation.

The population living under the absolute poverty line is 18.7% of the total population according to the Department of Information, Nepal. The population covered by health insurance in the base year 2018/19 was reported to be 7%.[11] The present health system's capacity to respond to COVID-19 is inadequate. According to the Ministry of Health and Population Nepal in 2020, there were 26 930 hospital beds, 1595 ICU beds, 840 ventilators, 194 hospitals with ICU facilities, 111 hospitals with COVID clinics, 13 Level-1 COVID hospitals, 12 Level II COVID hospitals, 3 Level III COVID hospitals, and 3076 isolation beds.[12] Some hospitals were designated to treat COVID-19 cases in all 7 provinces of Nepal. But there was negligence to extend strategies to trace, isolate, test, and treat since efforts were deficient to standardize testing facilities and manage isolation centers for COVID-19 patients. The panic and psychological impact regarding COVID-19 led to losses of lives by non-COVID-related diseases in the early period of the pandemic[13] as well as an increase in suicide rates.[14] Later, the deaths continued since the strategies were inadequate to manage COVID-19 and non-COVID-related diseases in those hospitalized. People feared visiting the hospital to treat minor illnesses, which might be attributed to the increased severity of COVID-19 patients with comorbidities.

Herein, this study summarizes the overall status of COVID-19 in a developing country, Nepal, with SARS-CoV-2 transmission through wastewater and other routes; disease cases in all 7 provinces; diagnostics, treatment options, and alternative medicines; and offers perspectives in managing the disease and any future pandemics. The systematic literature review was based on the search criteria consisting of keywords: "coronavirus," "coronavirus in Nepal," and "coronavirus in wastewater," and the publications from 2017 to 2022 were included (Figure 2), which was further refined to "coronavirus in wastewater" and "coronavirus in wastewater of Nepal": the 2 broader categories in the Web of Science. Also, data reported from January 2020 to February 2022 from the Ministry of Health and Population, Nepal, were included to determine the impact of pandemic on different age-groups, sexes, and by geography. Moreover, data ranging from February 2020 to October 2020 were used to evaluate trends related with lockdown, business, and border activity. The data were visualized in the form of graphs (GraphPad prism 8.4.3) and tables, the conceptual figures were created in Adobe Illustrator 2020, the geographic maps were created using AcrGIS, and the statistical calculations were performed in MS-Excel 2007. The findings are novel and may guide researchers and professionals working on managing COVID-19 in a developing country Nepal for better risk assessment and management.

SARS-CoV-2 in Wastewater

Wastewater surveillance is an approach to monitoring diseases via wastewater effluent.[15] SARS-CoV-2 can disseminate

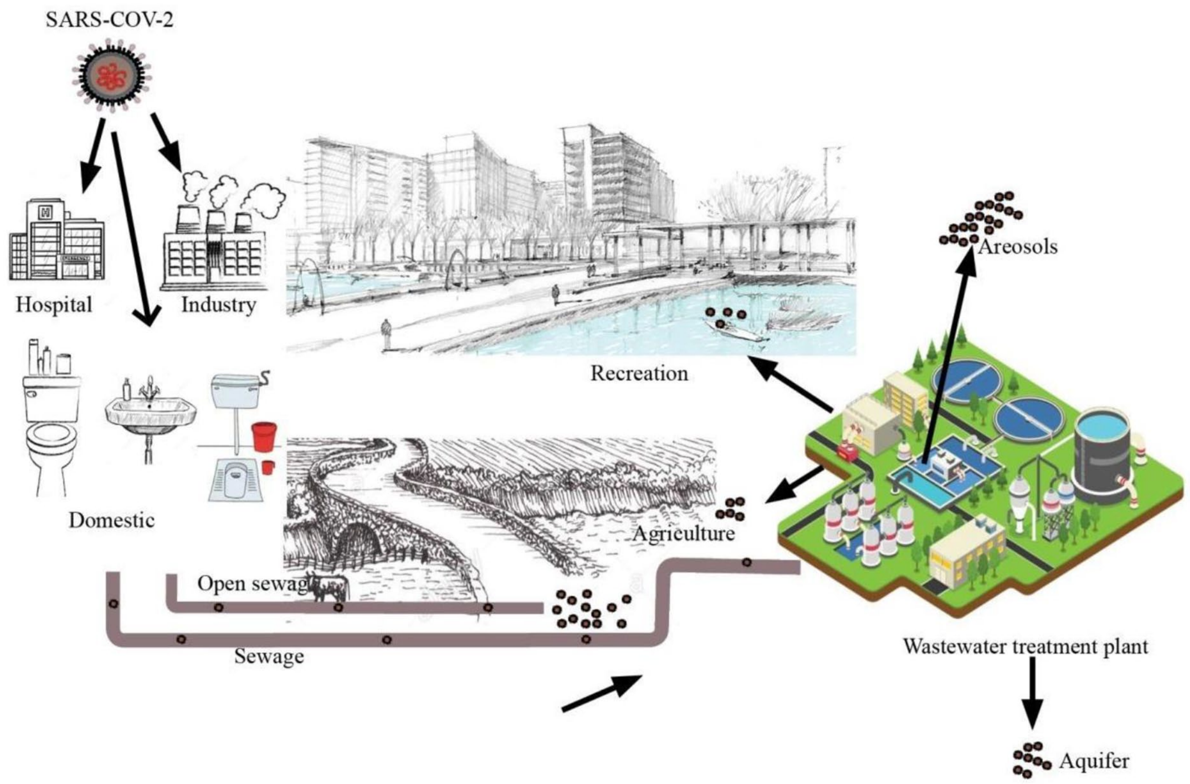

Figure 3. Schematic depicting dissemination pathways of SARS-CoV-2 from household activities, hospitals, and industries to the environment.

through water and wastewater, leading to potential environmental transmission as shown in Figure 3.[16] The potential harbored by SARS-CoV-2 for transmission via fecal-oral and aerosol routes poses an imminent challenge to comprehend the survival of the virus circulating in the environment.[17] The presence and evolution of SARS-CoV-2 in waters, soils, and other environmental compartments may pose a public health risk.[18] SARS-CoV-2 enters wastewater through the residential, industrial, and quarantine (isolation) facilities with COVID-19 patients.[19]

In Nepal, SARS-CoV-2 ribonucleic acid (RNA) was found in 60% (50/84 samples) of wastewater and river water samples analyzed.[20] Different studies have documented the presence of SARS-CoV-2 RNA in wastewater from different countries.[21-37] In the neighboring country, India, the viral genome was detected in the wastewater system.[38] Viral shedding through the digestive route seems to last longer than the shedding through the respiratory tract.[39] The impact of lockdown on SARS-CoV-2 dynamics was assessed using viral genome quantification in Paris wastewater and a significant decrease in the number of genome units was recorded, which coincided with the expected decline in the number of new COVID-19 cases with the length of lockdown.[40] The possibility of secondary transmission via wastewater should not be overlooked, as the virus has been found in human feces and wastewater samples from many countries, with possible cases of transmission still being debated.[41] Some coronaviruses can potentially survive in the gastrointestinal tract and spread via fecal-oral route or via inhalation of contaminated wastewater droplets.[42]

The design encompassing wastewater plumbing system could allow harboring pathogenic microorganisms and has been suggested to hold the potential for enabling airborne transmission of the viruses, such as SARS-CoV-2, upon aerosol generation. Further, self-isolation and official quarantine centers for infected people could serve as a hotspot for virus shedding into the system.[43,44] The possibility of extended duration of viral shedding in feces, for nearly 5 weeks after the patients' respiratory samples tested negative for SARS-CoV-2 RNA and the virus remaining viable for days in feces, may contribute to fecal-oral transmission.[45,46] The presence of SARS-CoV-2 in the infected person's feces and urine, even after viral clearance in the respiratory tract, as well as its presence in untreated wastewater, may elevate the likelihood of future fecal-oral transmission to potential intestinal infection.[6,47] However, the presence of viral genetic materials in stool does not always mean that viable infectious virions are present in feces or that the virus can or has spread by fecal-fomite, fecal-oral, or fecal-aerosol/droplet transmission.[48,49]

Wastewater surveillance includes the concentration of SARS-CoV-2 RNA from wastewater in a catchment or sampling point and enumeration of viral RNA copies using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).[36] However, the problems associated with biomedical wastewater treatment and disposals are of public health concern, particularly in developing countries like Nepal

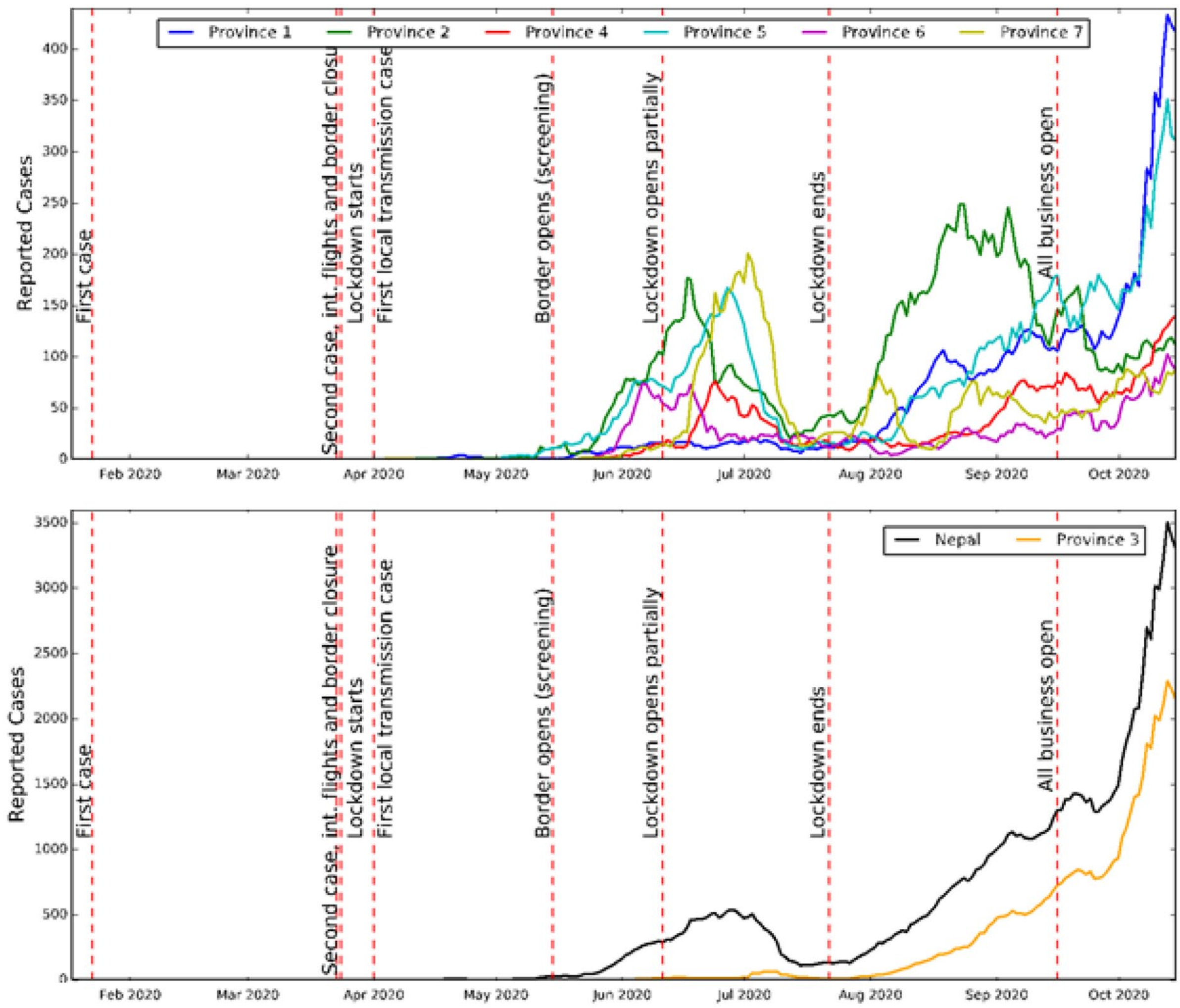

Figure 4. Reported cases in all 7 provinces of Nepal concerning lockdown, and business and border activities.

where hazardous waste landfills are absent.[50] Longitudinal analysis of wastewater can provide population-level estimates of the burden of SARS-CoV-2 where in-person or at-home testing may not be available.[51] SARS-CoV-2 in wastewater may reflect a potential health threat to individuals and has the potential to spread through aerosol inhalation or ingestion when the virus remains infectious in wastewater. Various coronaviruses could be cultured for a few days from wastewater.[52,53] Also, the enteric transmission of SARS-CoV-2 may be possible. Environmental surveillance of SARS-CoV-2 could serve as a data source and indicate whether the virus is circulating in the community or not.[33,54]

SARS-CoV-2 Transmission and Cases in Nepal

While the source of SARS-CoV-2 is still unknown, bats and pangolins have been suspected, crossing the species barrier, and rapid human-to-human airborne transmission has been established.[55-57] Viral transmission could also occur through other routes including fomite, fecal-oral, blood-borne, mother-to-child, and animal-to-human routes.[58-60]

Nepal is not an exception to COVID-19 and encountered challenges to prevent the spread of infection. Nepal was under complete lockdown from March 24, 2020, for several months in an attempt to control COVID-19 and prevent its spread into the community.[61] At the same time, a large number of Nepalese citizens returned from highly infected areas like India and China through open borders and entered different parts of the country without quarantining. When the lockdown ended and people returned to their normal schedule in October 2020, the cases increased exponentially (Figure 4). Gradually, COVID-19 spread all over Nepal, with an increase in the number of new cases and deaths. Among them, most of the infected patients were reported from Bagmati Province. In Nepal, research documented a possible link between COVID-19 and temperature indicators, showing increased transmission of the disease in winter.[62] This created an alarming scenario in a low-income country Nepal with an inadequate healthcare system. The data from January 2020 to February 2022 demonstrated the highest numbers of cases for Kathmandu district, while the lowest occurred in Manang district (Table 1). In Nepal, the total number of cases until February 17, 2021, was 974 493. The highest total cases were found in Bagmati Province (53.84%) followed by Province 1 (13.07%), Lumbini province (11.18%), and Gandaki province (9.55%), respectively. The cases were low

Table 1. District-wise highest and lowest cases of COVID-19 in 7 provinces of Nepal (January 24, 2020 through February 17, 2022).

PROVINCES	HIGHEST (NUMBER/%)	LOWEST (NUMBER/%)	TOTAL CASES IN PROVINCES
Province no. 1	Morang (47 735/37.51%)	Sankhuwashaba (776/0.61%)	127 251
Madhesh province	Dhanusa (11 200/21.18%)	Bara (5618/10.62%)	52 865
Bagmati province	Kathmandu (138 319/61.41%)	Rasuwa (1334/0.26%)	524 896
Gandaki province	Kaski (44 968/48.29%)	Manang (81/0.09%)	93 133
Lumbini province	Banke (20 392/18.71%)	Rukum East (284/0.26%)	108 998
Karnali province	Surkhet (127 111/53.40%)	Humla (140/0.58%)	23 803
Sudurpashchim province	Kailali (7416/47.93%)	Bajura (244/1.96%)	14 238
			Total: 945 184

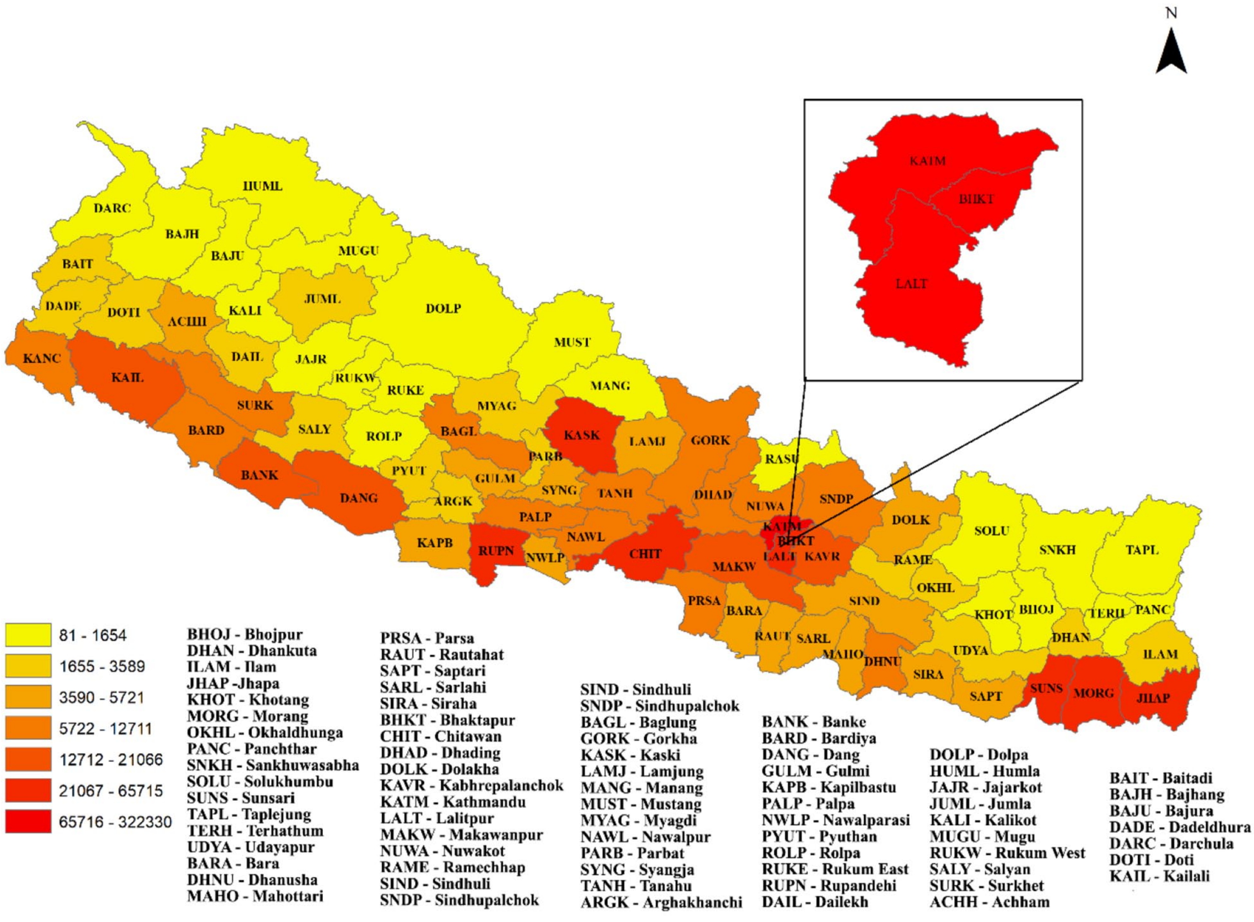

Figure 5. Distribution of the total number of positive cases in 7 provinces and 77 districts in Nepal (January 24, 2020 through February 17, 2021).

in Karnali Province (2.44%), Sudurpashchim Province (4.5%), and Madhesh Province (5.42%). Figure 5 shows the COVID-19 cases trend district-wise and province-wise for Nepal for the period January 24, 2020 through February 17, 2021. The Terai region, including the Kathmandu valley, had a significant number of cases. This could be linked to Nepal's open border with India on 3 sides: east, south, and west, as well as influx from COVID-19 affected areas nearby.[61] Also, megacities may have a greater number of cases because of dense populations.[63]

The gender-wise distribution of COVID-19 cases is presented in Figure 6. Data showed that males were predominantly infected (58.7%) compared to females (41.23%) (Figure 6). The age group between 31 and 40 years old males were infected the most (25.92%), while the females aged 21 to 30 years old were infected the most (26.85%) (Figure 7). The reason may be these age groups belong to the active working population in Nepal. The age group 80+ were the least infected, likely due to the low number of elderly population above 80 tested. Males were more

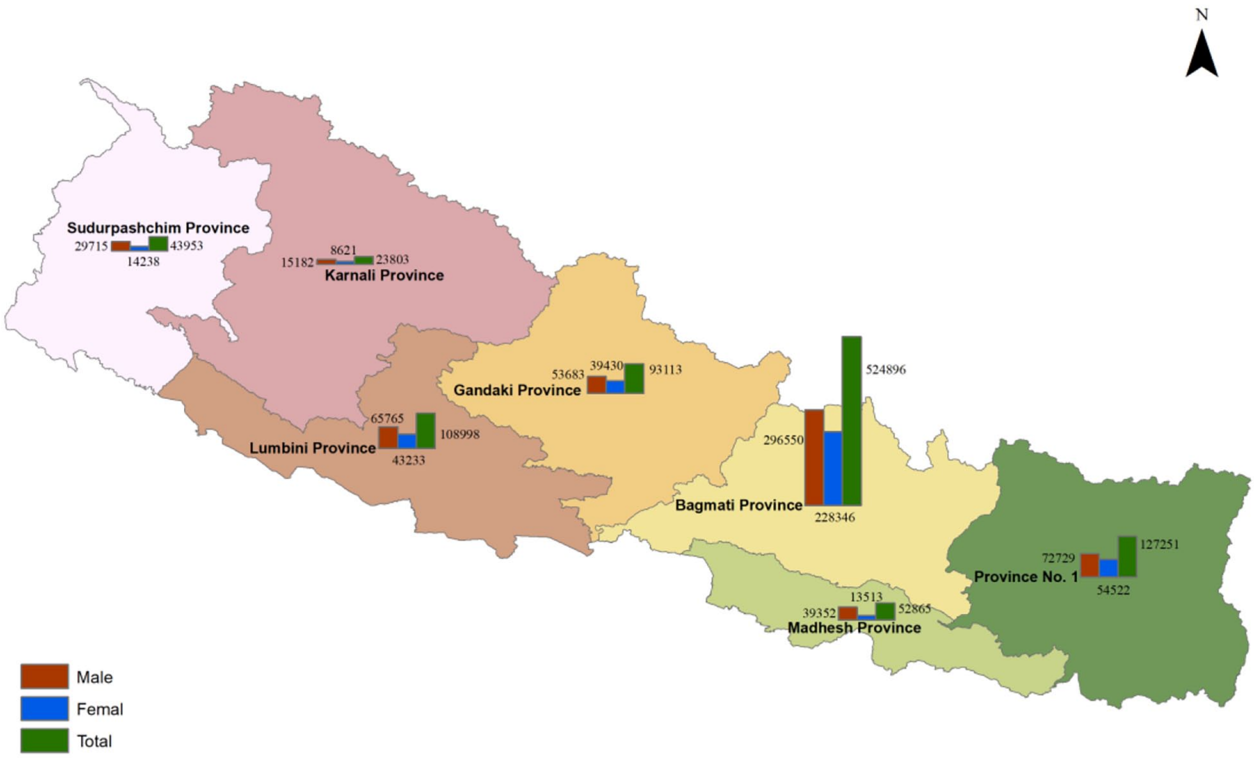

Figure 6. Gender-wise distribution of COVID-19 cases in Nepal (January 24, 2020 through February 17, 2022).

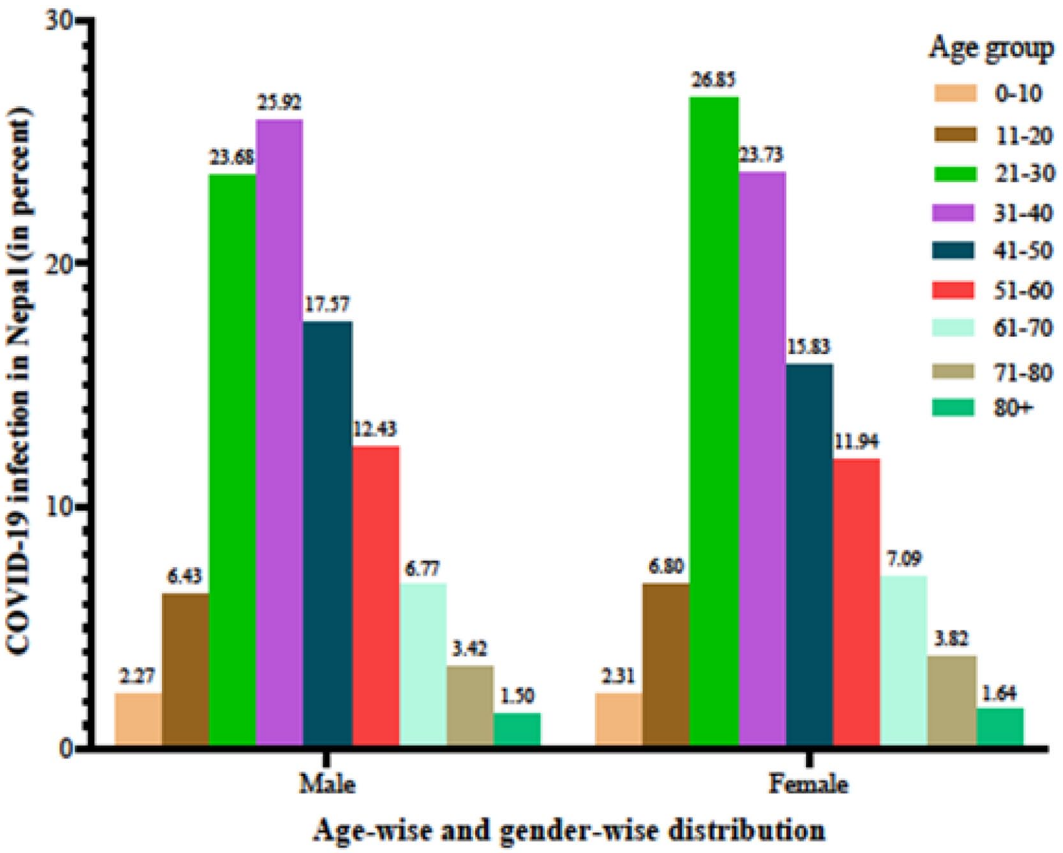

Figure 7. Gender- and age-wise distributions of COVID-19 infections in Nepal (January 24, 2020 through February 17, 2022).

symptomatic than females. A meta-analysis further corroborated that females could present COVID-19 cases in asymptomatic form compared to males.[64]

The first death in Nepal was a 29-year-old new mother with an unknown mode of transmission on May 16, 2020, and by June 21, there were 23 deaths reported. Surprisingly, most of the deaths happened outside of hospitals, and COVID-19 was confirmed postmortem.[65]

Laboratory Diagnosis to Detect SARS-CoV-2 in Nepal

As a low-resource country, Nepal has had suffered a profound impact on the clinical microbiology laboratories over the course of this pandemic.[66] Not only clinically, but also technologically and logistically, rapid and accurate detection of this novel virus offers considerable challenges. Before laboratory diagnosis, to categorize the suspected patients of COVID-19, the clinicians should observe the following symptoms: fever or symptoms of lower respiratory infection, such as cough or shortness of breath, fatigue, dyspnea, sore throat, headache, conjunctivitis, and/or gastrointestinal issues.[67-69]

The gold standard for the detection of SARS-CoV-2 is the identification of the viral genome targets by nucleic acid amplification test (NAAT), such as real-time reverse transcription-polymerase chain reaction (rRT-PCR), used globally for the diagnosis of COVID-19 in the upper respiratory samples during the first week of infections. It uses the TaqMan fluorogenic probe-based chemistry and 5′-nuclease activity of Taq DNA polymerase.[70]

Nonetheless, rapid diagnostic tests (RDTs), such as antigen-detecting RDTs (Ag-RDT) and antibody-detecting RDTs, virus isolation (culture method), electron microscopic examination, complete genomic sequencing technique (Meta-genomics, next-generation sequencing), and isothermal-CRISPR-based diagnostics have been used for the detection of SARS-CoV-2.[71-73] However, each method presents its limitations. Comparative study of different diagnostics molecular technologies has revealed that CRISPR-COVID had a 100% specificity and 40 minutes as the reaction turn-around time (TAT); RT-PCR-COVID with 90.4% specificity and 1.5 hours TAT; and NGS with 100% specificity and approximately 20 hours TAT.[71] Whereas antigen and antibody-based rapid immunoassays with a wide range of sensitivity and specificity are also available in the market.[72]

In Nepal, confirmatory test for COVID-19 and/or detection of genomic material (i.e., RNA) of SARS-CoV-2 involves the rRT-PCR method. However, serology-based immunoglobulin (IgM and IgG) antibody tests and antigen tests are also being introduced as supplementary and seroprevalence tools for community surveillance.[74] PCR assays were rapidly deployed in the country during the early stages of the pandemic and have formed the cornerstone of detection. The central government agencies also rapidly deployed funding mechanisms for developing novel testing strategies. The RDTs didn't exhibit enough reliable performance (sensitivity and specificity) and were not recommended for stand-alone use to guide decision-making in any setting. A similar response was found in her southern neighbor, India.[75]

Upon meeting the national testing guidelines, specimens collected from the upper respiratory tract include nasopharyngeal (NP) swab and oropharyngeal swab: synthetic fiber swabs with plastic shafts are preferred over calcium alginate swabs or cotton-tipped swabs with wooden shafts, for molecular diagnosis. The swabs are immediately placed in sterile tubes containing 2 to 3 mL of viral transport media (VTM). However, other possible samples include bronchoalveolar lavage, tracheal aspirate, NP aspirate, nasal wash, saliva, sputum, blood/paired serum, urine, and stool for detection of the viral RNA.[76-79] Clinicians are advised to wear proper personal protective equipment (PPE) during specimen collection, pack the specimens in a triple packaging system, and maintain the cold chain for the transport of specimens in VTM before processing.[80,81] However, many affluent countries have also encountered challenges in the test delivery, specimen collection and transport, and limited testing. These challenges persisted greater in low-resource settings as in Nepal.[81,82]

Tests are performed in designated laboratories for patients meeting the case definition of COVID-19 following clinical observations and national guidelines. Diagnosis of COVID-19 is ultimately confirmed by rRT-PCR.[83,84] Although RT-PCR is considered the standard laboratory test for the diagnosis of COVID-19, it may also yield a false negative/positive result in some cases.[85] In the early stage of the disease, several cases with false-negative/positive RT-PCR results were reported probably because of inadequate viral loads in the sample and/or technical issues during nucleic acid extraction.[86]

Molecular tests form the basis for confirming COVID-19, whereas computed tomography (CT) scan may support the diagnosis[87] but serological tests for SARS-CoV-2 that are also widely available play an increasingly important role in understanding the epidemiology of the virus and in identifying populations at higher risk for infection.[66,74,88,89] In cases with typical clinical manifestations, chest CT may prove to be an invaluable asset because it may show characteristic features of the disease even when the RT-PCR screening test is negative.[90,91]

Treatment of COVID-19 in Nepal

The antiviral therapeutics used globally against SARS-CoV-2 infection were not particularly designed to act against SARS-CoV-2. Camostatmesilate (Foipan™) and Nafamostatmesilate (Buipel™) are serine protease inhibitors, which target TMPRSS2.[92,93] An antimalarial drug Chloroquine phosphate (Resochin™) targets ACE2,[94,95] and hydroxychloroquine (Quensyl™, Plaquenil™, Hydroquin™, Dolquine™, Quinoric™) acts against endosome and pH.[95-97] Remdesivir is an adenine nucleotide analog targeting viral RdRp.[98-100] Favipiravir (Avigan™) also targets RdRp.[101] Lopinavir/Ritonavir (Kaletra™) targets viral proteases.[102,103] Umifenovir

(Arbidol™) targets membrane fusion and clathrin-mediated endocytosis.[104] These drugs are under various phases of clinical trials against SARS-CoV-2 infection.

In Nepal, Remdesivir is the major potential drug under evaluation. Further, plasma therapy is also under trial. On 9th August 2020, the Government of Nepal granted permission to use Remdesivir in COVID-19 patients as an experimental drug. The Ministry of Health and Population (MoHP) and the Department of Drug Administration (DDA) of Nepal authorized the import and usage of Remdesivir for treating COVID-19 and delegated authority to Nepal Health Research Council (NHRC) to oversee its administration as an experimental use drug (Nepal Health Research Council).[105]

However, it was found that Remdesivir use was not statistically associated with a difference in time to clinical improvement in SARS-CoV-2 infected patients but was found effective based on individuality.[106] Patients receiving Remdesivir presented complications, including hypersensitivity reactions such as anaphylactic and infusion-related reactions.[74] The drug has displayed a mixed result in COVID-19 patients and has side effects to the level of acceptance.[107]

Treatment Options Against SARS-CoV-2 Infection in Nepal

Remdesivir

Remdesivir (Veklury) was approved by the US Food and Drug Administration (FDA) for use against mild-to-severe COVID-19.[108] The median time to recovery was significantly reduced by 5 days in patients that received Veklury (10 days for the recovery for Veklury group compared to 15 days for the placebo group). The odds of clinical improvement at day-15 were also statistically significantly higher in the Veklury group compared to the placebo group. The overall 29-day mortality was 15% for the placebo group and 11% for the Veklury group; this difference was not statistically significant.[104] In a large study conducted under the SOLIDARITY trial (a World Health Organization-sponsored, open-label, randomized trial), that included 12 000 patients in 500 hospital sites in over 30 countries, the study did not find a statistically significant difference in mortality between the Veklury group and the standard-of-care group.[106] Remdesivir has been shown to speed up the recovery rate in hospitalized patients requiring supplemental oxygen but the drug alone is not adequate to solve the issues arising from the pandemic.[109]

Convalescent plasma therapy

Clinical trials on the use of convalescent plasma therapy against SARS-CoV-2 infection have been conducted in Nepal.[110] Immune-based therapy consists of convalescent plasma and immunoglobulins, interleukin-1 (IL-1) inhibitors, interleukin-6 (IL-6) inhibitors, and other immuno-modulators. Blockage of IL-6 and IL-1 and inhibition of Janus Kinase (JAK) may lead to treating systemic inflammation associated with severe COVID-19.[111] Convalescent blood products consist of convalescent whole blood or convalescent plasma or convalescent serum, pooled human immunoglobulin for intravenous or intramuscular administration, high-titer human immunoglobulin, and polyclonal or monoclonal antibodies.[112] Previous studies in the United States[113] and China[114-117] reported plasma therapy as an option for treatment against severe COVID-19 but more clinical trials are needed to confirm its proposed efficacy. In Nepal, convalescent plasma showed beneficial effects against COVID-19, but larger, randomized controlled trials are required to confirm its efficacy.[118]

Ayurveda and alternative treatments in Nepal

The Ayurvedic medical system has its origin in the Indian subcontinent.[119] Research published over the years in Ayurvedic and traditional Chinese medicine (TCM) have demonstrated that herbs and/or TCM can limit viral replication, limit virus entry and attachment to the host cell, and promote the patient immune system. For example, medicinal herbs with immuno-modulatory and antioxidant characteristics, such as ashwagandha (*Withania somnifera*), have been documented to enhance immune response and reduce viral replication.[120] Tulsi, haldi (turmeric), giloy, black pepper, ginger roots, cloves, cardamom, lemon, and ashwagandha were among the phytochemical and antiviral compounds evaluated in a recent study in the hopes of finding a cure for COVID-19.[121] To investigate the antiviral effect of phytochemical components and bioactive compounds found in herbs, researchers docked them with distinct coronavirus target proteins such as viral capsid spike and protease. The study indicated that certain phytochemicals used in traditional medicine had a high affinity for viral proteins, making them potential candidates for target drug design.[122]

A research compared the Ayurvedic protocols suggested by the governments of Nepal and India.[123] There is a lot of evidence that the Ayurvedic and traditional systems of medicines offer excellent potential in dealing with COVID-19 pandemic and other epidemics that the society may encounter in the future. Altogether, during this pandemic about 60 medicinal plants belonging to 36 families were utilized in Nepal.[124] There is, thus, a need to explore and utilize the traditional Ayurvedic knowledge vis-à-vis the state-of-the-art technologies to address the ongoing pandemic and prepare for any future respiratory viral disease outbreaks.

Role of Sanitation in Public Health Protection

Inadequate hygiene, sanitation, and disinfection approaches in healthcare facilities, as well as dwellings without proper wastewater disposal and management, may expose individuals to the circulating virus particles.[6] Overuse of non-biodegradable plastics during the epidemic has exacerbated plastic pollution, posing a considerable health threat to land and aquatic ecosystems in Nepal.[125] Access to safe water, nutritious food, and lack of sanitation and hygiene remain a challenge in most rural communities and mountain regions due to geographical challenges and lack of effective people-focused programs.[126,127] Apparently healthy people were infected as a result of poorly handled quarantine and hospital waste across the country during the pandemic, as reported on national news media.[128]

Individuals who believe they are at risk and are aware of the seriousness of COVID-19 implications are more likely to exercise caution.[129] Hand washing has been recommended as a preventative measure against the circulating and emerging SARS-CoV-2 strains.[130] The survival duration of coronavirus in water environments is highly influenced by temperature, water properties, suspended solids, and organic matter concentrations, solution pH, and disinfectant dose, with the advantage that the current drinking water disinfection process effectively inactivates most bacterial and viral pathogens present in water, including SARS-CoV-2.[131] The environmental discharge of inappropriately treated wastewater might expose public to coronavirus infection, underscoring the importance of proper wastewater treatment and management in the developing county Nepal.[132]

Conclusion and Future Perspective

The pandemic has had an impact on public health and economic growth in Nepal. SARS-CoV-2 was detected in wastewater in Nepal. SARS-CoV-2 infections were particularly common in Terai areas and megacities. Working-age groups and males were identified as the most exposed groups. With limited available healthcare resources in Nepal, it has been a significant challenge managing the growing influx of COVID-19 patients. It is, thus, critical for the hospitals and clinics in all (7) provinces to pool their resources and develop a central coordination mechanism to mobilize the available resources to effectively manage and care for COVID-19 patients. Quarantine facilities should be properly managed, staffed, and surveilled ensuring the health and safety of those that are quarantined. The coordination mechanism of the hub and satellite hospitals might be an area to work on to effectively address the issues related to limited resources and capacity building in Nepal with far limited resources to address the ongoing pandemic and prepare for any future emerging infectious diseases of epidemic and pandemic potential.

Poor sanitation and mismanagement of wastewater might serve as possible environmental factors contributing to COVID-19 transmission in Nepal; however, more focused research is needed to better understand how wastewater surveillance might help predict early transmission of SARS-CoV2 in the community and help mitigate COVID-19 spread.

Acknowledgements

We would like to acknowledge the Central Department of Microbiology, Tribhuvan University, Nepal, and the Nepal Academy of Science and Technology, Lalitpur, Nepal for their support to researchers during the COVID-19 pandemic.

Author Contributions

Dev R Joshi, Sushil R. Kanel, and Lok R. Pokhrel: conceived the study design and contributed to writing and editing the manuscript. Prabin Dawadi: contributed to study design, literature search, results interpretation, data analysis, statistical output interpretations, manuscript writing. Gopiram Syangtan: literature search, results interpretation, data analysis, manuscript writing. Bhupendra Lama: data analysis, statistical output interpretations, results interpretation, manuscript writing. Rameshwar Adhikari: Commented on the manuscript. Hem R. Joshi: Statistical trend analysis. Ioana Pavel: Commented on the manuscript.

Availability of Data and Materials

All data generated for this study are included in this article. The data are also available from the corresponding author upon reasonable request.

REFERENCES

1. Grubaugh ND, Ladner JT, Lemey P, et al. Tracking virus outbreaks in the twenty-first century. *Nat Microbiol*. 2019;4:10-19.
2. WHO. WHO announces COVID-19 outbreak a pandemic. 2020. Accessed February 15, 2022. http://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic
3. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med*. 2020;26:450-452.
4. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med*. 2020;46:586-590.
5. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N Engl J Med*. 2020;382:1653-1659.
6. Pandey D, Verma S, Verma P, et al. SARS-CoV-2 in wastewater: challenges for developing countries. *Int J Hyg Environ Health*. 2021;231:113634.
7. Pun SB, Mandal S, Bhandari L, et al. Understanding COVID-19 in Nepal. *J Nepal Health Res Counc*. 2020;18:126-127.
8. Sah R, Rodriguez-Morales AJ, Jha R, et al. Complete genome sequence of a 2019 novel coronavirus (SARS-CoV-2) strain isolated in Nepal. *Microbiol Resour Announc*. 2020;9:e00169-20.
9. Bastola A, Sah R, Rodriguez-Morales AJ, et al. The first 2019 novel coronavirus case in Nepal. *Lancet Infect Dis*. 2020;20:279-280.
10. Marahatta SB, Paudel S, Aryal N. Tackling COVID-19 in Nepal: opportunities and challenges. *J Karnali Acad Health Sci*. 2020;3:1-12.
11. National Planning Commission. The fifteenth plan. Government of Nepal National Planning Commission. 2020. Accessed January 30, 2022. https://npc.gov.np/images/category/15th_plan_English_Version.pdf
12. Government of Nepal Ministry of Health. Population hospital-based interventions public health and social measures management and oversight health sector emergency response plan COVID-19 pandemic. 2020.
13. Nepal R, Bhattarai B. The grim reality of health system uncovered with COVID-19 pandemic in Nepal. *J Nepal Health Res Counc*. 2020;18:569-571.
14. Acharya B, Subedi K, Acharya P, Ghimire S. Association between COVID-19 pandemic and the suicide rates in Nepal. *PLoS One*. 2022;17:e0262958.
15. Carducci A, Federigi I, Liu D, Thompson JR, Verani M. Making waves: coronavirus detection, presence and persistence in the water environment: state of the art and knowledge needs for public health. *Water Res*. 2020;179:115907.
16. Bogler A, Packman A, Furman A, et al. Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic. *Nat Sustain*. 2020;3:981-990.
17. Arslan M, Xu B, Gamal El-Din M. Transmission of SARS-CoV-2 via fecal-oral and aerosols-borne routes: environmental dynamics and implications for wastewater management in underprivileged societies. *Sci Total Environ*. 2020;743:140709.
18. Núñez-Delgado A. What do we know about the SARS-CoV-2 coronavirus in the environment? *Sci Total Environ*. 2020;727:138647.
19. Nghiem LD, Morgan B, Donner E, Short MD. The COVID-19 pandemic: considerations for the waste and wastewater services sector. *Case Stud Chem Environ Eng*. 2020;1:100006.

20. Tandukar S, Sthapit N, Thakali O, et al. Detection of SARS-CoV-2 RNA in wastewater, river water, and hospital wastewater of Nepal. *Sci Total Environ*. 2022;824:153816.
21. Ahmed W, Angel N, Edson J, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci Total Environ*. 2020;728:138764.
22. Collivignarelli MC, Collivignarelli C, Carnevale Miino M, Abbà A, Pedrazzani R, Bertanza G. SARS-CoV-2 in sewer systems and connected facilities. *Process Saf Environ Prot*. 2020;143:196-203.
23. D'Aoust PM, Mercier E, Montpetit D, et al. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. *Water Res*. 2021;188:116560.
24. Fongaro G, Stoco PH, Souza DSM, et al. The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina, Brazil, November 2019. *Sci Total Environ*. 2021;778:146198.
25. Gerrity D, Papp K, Stoker M, Sims A, Frehner W. Early-pandemic wastewater surveillance of SARS-CoV-2 in southern Nevada: methodology, occurrence, and incidence/prevalence considerations. *Water Res X*. 2021;10:100086.
26. Hata A, Honda R, Hara-Yamamura, et al. Detection of SARS-CoV-2 in wastewater in Japan by multiple molecular assays-implication for wastewater-based epidemiology (WBE). *MedRxiv2020.06.09.20126417*. 2020.
27. Izquierdo-Lara R, Elsinga G, Heijnen L, et al. Monitoring SARS-CoV-2 circulation and diversity through community wastewater sequencing, the Netherlands and Belgium. *Emerg Infect Dis*. 2021;27:1405-1415.
28. La Rosa G, Iaconelli M, Mancini P, et al. First detection of SARS-CoV-2 in untreated wastewaters in Italy. *Sci Total Environ*. 2020;736:139652.
29. Randazzo W, Cuevas-Ferrando E, Sanjuán R, Domingo-Calap P, Sánchez G. Metropolitan wastewater analysis for COVID-19 epidemiological surveillance. *Int J Hyg Environ Health*. 2020;230:113621.
30. Alpaslan-Kocamemi B, Kurt H, Sait A, et al. SARS-CoV-2 detection in Istanbul wastewater treatment plant sludges. *medRxiv*. 2020. https://doi.org/10.1101/2020.05.12.20099358
31. Tanhaei M, Mohebbi SR, Hosseini SM, et al. The first detection of SARS-CoV-2 RNA in the wastewater of Tehran, Iran. *Environ Sci Pollut Res Int*. 2021;28:38629-38636.
32. Westhaus S, Weber F-A, Schiwy S, et al. Detection of SARS-CoV-2 in raw and treated wastewater in Germany – suitability for COVID-19 surveillance and potential transmission risks. *Sci Total Environ*. 2021;751:141750.
33. Lodder W, de Roda Husman AM. SARS-CoV-2 in wastewater: potential health risk, but also data source. *Lancet Gastroenterol Hepatol*. 2020;5:533-534.
34. Haramoto E, Malla B, Thakali O, Kitajima M. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. *Sci Total Environ*. 2020;737:140405.
35. Peccia J, Zulli A, Brackney DE, et al. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *medRxiv2020.05.19.20105999*. 2020.
36. Wu F, Zhang J, Xiao A, et al. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *mSystems*. 2020;5:e00614-20.
37. Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res*. 2020;181:115942.
38. Kumar M, Patel AK, Shah AV, et al. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. *Sci Total Environ*. 2020;746:141326.
39. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol*. 2020;5:434-435.
40. Wurtzer S, Marechal V, Mouchel J-M, et al. Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in waste water, Greater Paris, France, 5 March to 23 April 2020. *Euro Surv*. 2020;25:1-14.
41. Liu D, Thompson JR, Carducci A, Bi X. Potential secondary transmission of SARS-CoV-2 via wastewater. *Sci Total Environ*. 2020;749:142358.
42. Elsamadony M, Fujii M, Miura T, Watanabe T. Possible transmission of viruses from contaminated human feces and sewage: implications for SARS-CoV-2. *Sci Total Environ*. 2021;755:142575.
43. Casanova L, Rutala WA, Weber DJ, Sobsey MD. Survival of surrogate coronaviruses in water. *Water Res*. 2009;43:1893-1898.
44. Gormley M, Aspray TJ, Kelly DA. COVID-19: mitigating transmission via wastewater plumbing systems. *Lancet Glob Health*. 2020;8:e643.
45. Goh GKM, Dunker AK, Foster JA, Uversky VN. Shell disorder analysis predicts greater resilience of the SARS-CoV-2 (COVID-19) outside the body and in body fluids. *Microb Pathog*. 2020;144:104177.
46. Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal–oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol*. 2020;5:335-337.
47. Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol*. 2021;18:269-283.
48. Amirian ES. Potential fecal transmission of SARS-CoV-2: current evidence and implications for public health. *Int J Infect Dis*. 2020;95:363-370.
49. de Graaf M, Beck R, Caccio SM, et al. Sustained fecal-oral human-to-human transmission following a zoonotic event. *Curr Opin Virol*. 2017;22:1-6.
50. Kataki S, Chatterjee S, Vairale MG, Sharma S, Dwivedi SK. Concerns and strategies for wastewater treatment during COVID-19 pandemic to stop plausible transmission. *Resourc Conservat Recycl*. 2020;164:105156.
51. Ahmed W, Bertsch PM, Bivins A, et al. Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. *Sci Total Environ*. 2020;739:139960.
52. Gundy PM, Gerba CP, Pepper IL. Survival of coronaviruses in water and wastewater. *Food Environ Virol*. 2008;1:10-14.
53. Naddeo V, Liu H. Editorial perspectives: 2019 novel coronavirus (SARS-CoV-2): what is its fate in urban water cycle and how can the water research community respond? *Environ Sci Water Res Technol*. 2020;6:1213-1216.
54. Gudra D, Dejus S, Bartkevics V, et al. Detection of SARS-CoV-2 RNA in wastewater and importance of population size assessment in smaller cities: an exploratory case study from two municipalities in Latvia. *Sci Total Environ*. 2022;823:153775.
55. Han GZ. Spotlight pangolins harbor SARS-CoV-2-related coronaviruses. *Trends Microbiol*. 2020;28:515-517.
56. Oreshkova N, Molenaar R-J, Vreman S, et al. *SARS-CoV2 Infection in Farmed Mink. bioRxiv 2020, 2020.05.18.101493*. 2020.
57. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. *J Adv Res*. 2020;24:91-98.
58. Ferretti L, Wymant C, Kendall M, et al. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. *Science*. 2020;368:0-8.
59. Naddeo V. Environmental science water research & technology editorial perspectives: 2019 novel coronavirus. Published online 2020. doi:10.1039/d0ew90015j
60. Yesudhas D, Srivastava A, Gromiha MM. COVID-19 outbreak: history, mechanism, transmission, structural studies and therapeutics. *Infection*. 2021;49:199-213.
61. Piryani RM, Piryani S, Shah JN. Nepal's response to contain COVID-19 infection. *J Nepal Health Res Counc*. 2020;18:128-134.
62. Tandukar S, Bhandari D, Rajani Ghaju S, et al. Association between climatic and nonclimatic parameters and transmission of SARS-CoV-2 infection in Nepal. *Environ Dis*. 2021;6:38-44.
63. Dhakal S, Karki S. Early epidemiological features of COVID-19 in Nepal and public health response. *Front Med*. 2020;7:524.
64. Syangtan G, Bista S, Dawadi P, et al. Asymptomatic SARS-CoV-2 carriers: a systematic review and meta-analysis. *Front Public Health*. 2020;8:587374.
65. Panthee B, Dhungana S, Panthee N, Paudel A, Gyawali S, Panthee S. COVID-19: the current situation in Nepal. *New Microbes New Infect*. 2020;37:100737.
66. Tang B, Bragazzi NL, Li Q, Tang S, Xiao Y, Wu J. An updated estimation of the risk of transmission of the novel coronavirus (2019-nCov). *Infect Dis Model*. 2020;5:248-255.
67. Cohen PA, Hall LE, John JN, Rapoport AB. The early natural history of SARS-CoV-2 infection: clinical observations from an urban, ambulatory COVID-19 clinic. *Mayo Clin Proc*. 2020;95:1124-1126.
68. Deng S-Q, Peng H-J. Characteristics of and public health responses to the coronavirus disease 2019 outbreak in China. *J Clin Med*. 2020;9:575.
69. Mei X, Zhang Y, Zhu H, et al. Observations about symptomatic and asymptomatic infections of 494 patients with COVID-19 in Shanghai, China. *Am J Infect Control*. 2020;48:1045-1050.
70. Goudouris ES. Laboratory diagnosis of COVID-19. *J Pediatr*. 2021;97:7-12.
71. Hou C, Hua Z, Xu P, et al. Estimating the prevalence of hepatitis B by wastewater-based epidemiology in 19 cities in China. *Sci Total Environ*. 2020;740:139696.
72. Kumar M, Manish K, Arbind Kumar P, et al. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. *Sci Tot Environ*. 2020;746:141326.
73. Udugama B, Kadhiresan P, Kozlowski HN, et al. Diagnosing COVID-19: the disease and tools for detection. *NLM (Medline)*. 2020;14:3822-3835.
74. Nepal Health Research Council (NHRC). Comparative evaluation of commercially available rapid diagnostic test kits for the use of screening of suspected cases of novel corona virus infection in Nepal. Accessed January 15, 2022. http://nhrc.gov.np/comparative-evaluation-of-commercially-available-rapid-diagnostic-test-kits-for-the-use-of-screening-of-suspected-cases-of-novel-corona-virus-infection-in-nepal/
75. Moorthy M, Fletcher J. SARS-CoV-2 laboratory testing in India's pandemic response: a public health perspective. *Indian J Public Health*. 2020;64:S128-S131.
76. Grassly NC, Pons-Salort M, Parker EPK, et al. Comparison of molecular testing strategies for COVID-19 control: a mathematical modelling study. *Lancet*. 2020;20:1381.
77. Peng L, Liu J, Xu W, et al. SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. *J Med Virol*. 2020;92:1676-1680.

78. Tan SH, Allicock O, Armstrong-Hough M, Wyllie AL. Saliva as a gold-standard sample for SARS-CoV-2 detection. *Lancet Respir Med*. 2021;9:562-564.
79. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med*. 2020;383:1283-1286.
80. Nayak N, Rai S. Novel coronavirus disease (COVID-19): an update. *Nepal Med Coll J*. 2020;22:93-98.
81. Shrestha LB, Pokharel K. Standard operating procedure for specimen collection, packaging and transport for diagnosis of SARS-CoV-2. *J Nepal Med Assoc*. 2020;58:627-629.
82. Bhutta ZA, Basnyat B, Saha S, Laxminarayan R. Covid-19 risks and response in South Asia. *BMJ*. 2020;368:m1190.
83. Long C, Xu H, Shen Q, et al. Diagnosis of the coronavirus disease (COVID-19): rRT-PCR or CT? *Eur J Radiol*. 2020;126:108961.
84. Caruana G, Croxatto A, Coste AT, et al. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. *Clin Microbiol Infect*. 2020;26:1178-1182.
85. Chen H-B, Guo J-Y, Shu Y-C, Lee YH, Chang FH. Improvement of sensitivity of pooling strategies for COVID-19. *Comput Math Methods Med*. 2021;2021:e6636396.
86. Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. *Lancet Respir Med*. 2020;8:1167-1168.
87. Pascarella G, Strumia A, Piliego C, et al. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med*. 2020;288:192-206.
88. Fang FC, Naccache SN, Greninger AL, et al. The laboratory diagnosis of coronavirus disease 2019—frequently asked questions. *Clin Infect Dis*. 2020;71:2996-3001.
89. Jorfi M, Luo NM, Hazra A, et al. Diagnostic technology for COVID-19: comparative evaluation of antigen and serology-based SARS-CoV-2 immunoassays, and contact tracing solutions for potential use as at-home products. *medRxiv*. doi:10.1101/2020.06.25.20140236
90. Ghosh S, Deshwal H, Saeedan MB, Khanna VK, Raoof S, Mehta AC. Imaging algorithm for COVID-19: a practical approach. *Clin Imaging*. 2021;72:22-30.
91. Saeed GA, Gaba W, Shah A, et al. Correlation between chest CT severity scores and the clinical parameters of adult patients with COVID-19 pneumonia. *Radiol Res Pract*. 2021;2021:e6697677.
92. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181:271-280.e8.
93. Sai JK, Suyama M, Kubokawa Y, Matsumura Y, Inami K, Watanabe S. Efficacy of camostat mesilate against dyspepsia associated with non-alcoholic mild pancreatic disease. *J Gastroenterol*. 2010;45:335-341.
94. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14:72-73.
95. Stahlmann R, Lode H. Medication for COVID-19—an overview of approaches currently under study. *Deutscher Arzte-Verlag GmbH*. 2020;117:213.
96. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;56:105949.
97. Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;71:732-739.
98. Grein J, Ohmagari N, Shin D, et al. Compassionate use of remdesivir for patients with severe Covid-19. *N Engl J Med*. 2020;382:e101.
99. Mulangu S, Dodd LE, Davey RT, et al. A randomized, controlled trial of ZMapp for Ebola virus infection. *N Engl J Med*. 2016;375:1448-1456.
100. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med*. 2017;9:eaal3653.
101. Sissoko D, Laouenan C, Folkesson E, et al. Experimental treatment with favipiravir for Ebola virus disease (the JIKI trial): a historically controlled, single-arm proof-of-concept trial in Guinea. *PLoS Med*. 2016;13:e1001967
102. Cao B, Wang Y, Wen D, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe COVID-19. *N Engl J Med*. 2020;382:1787-1799.
103. Chu CM, Cheng VC, Hung IF, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax*. 2004;59:252-256.
104. Deng L, Li C, Zeng Q, et al. Arbidol combined with LPV/r versus LPV/r alone against corona virus disease 2019: a retrospective cohort study. *Infect J*. 2020;81:e1-e5.
105. Nepal Health Research Council (NHRC). Interim guidelines for use of remdesivir as a study drug. 2020. Accessed January 22, 2021. http://nhrc.gov.np/wp-content/uploads/2020/09/Interim-Guidelines-for-Use-of-Remdesivir_20200923_0001.pdf
106. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323:1843-1844.
107. Singh AK, Singh A, Singh R, Misra A. Remdesivir in COVID-19: a critical review of pharmacology, pre-clinical and clinical studies. *Diabetes Metab Syndr Clin Res Rev*. 2020;14:641-648.
108. Pirzada RH, Haseeb M, Batool M, Kim M, Choi S. Remdesivir and ledipasvir among the FDA-approved antiviral drugs have potential to inhibit SARS-CoV-2 replication. *Cells*. 2021;10:1052.
109. Malin JJ, Suárez I, Priesner V, Fätkenheuer G, Rybniker J. Remdesivir against COVID-19 and other viral diseases. *Clin Microbiol Rev*. 2020;34:1-21.
110. Gyanwali P, Sharma S, Pant S, et al. Safety and efficacy of different therapeutic interventions on prevention and treatment of COVID-19. *J Nepal Health Res Counc*. 2020;18:151-158.
111. Beigel JH, Tomashek KM, Dodd LE. Remdesivir for the treatment of COVID-19 – preliminary report. Reply. *N Engl J Med*. 2020;383:994.
112. Marano G, Vaglio S, Pupella S, et al. Convalescent plasma: new evidence for an old therapeutic tool? *SIMTI Servizi Sri. Blood Transfus*.2016;14:152.
113. Liu STH, Lin H-M, Baine I, et al. Convalescent plasma treatment of severe COVID-19: a propensity score-matched control study. *Nat Med*. 2020;26:1708-1713.
114. Ahn JY, Sohn Y, Lee SH, et al. Use of convalescent plasma therapy in two covid-19 patients with acute respiratory distress syndrome in Korea. *J Korean Med Sci*. 2020;35:e149.
115. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA* 2020; 117: 9490–9496.
116. Li T. Diagnosis and clinical management of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection: an operational recommendation of Peking Union Medical College Hospital (V2.0). *Emerg Microbes Infect*. 2020;9:582-585.
117. Ye M, Fu D, Ren Y, et al. Treatment with convalescent plasma for COVID-19 patients in Wuhan, China. *J Med Virol*. 2020;92:1890-1901.
118. Das SK, Ranabhat K, Bhattarai S, et al. Combination of convalescent plasma therapy and repurposed drugs to treat severe COVID-19 patient with multimorbidity. *Clin Case Rep*. 2021;9:2132-2137.
119. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J Ethnopharmacol*. 2006;103:25-35.
120. Tillu G, Chaturvedi S, Chopra A, Patwardhan B. Public health approach of Ayurveda and yoga for COVID-19 Prophylaxis. *J Altern Complement Med*. 2020;26:360-364.
121. Maurya DK, Sharma D. Evaluation of traditional ayurvedic preparation for prevention and management of the novel coronavirus (SARS-CoV-2) using molecular Docking approach. *J Biomol Struct Dyn*. Published online 2020. doi:10.1080/07391102.2020.1852119
122. Bhattacharya R, Dev K, Sourirajan A. Antiviral activity of bioactive phytocompounds against coronavirus: an update. *J Virol Methods*. 2021;290:114070.
123. Pandit RD, Singh RK. COVID-19 ayurveda treatment protocol of governments of Nepal and India: a review and perspective. *Appl Sci Technol Ann*. 2020;1:72-80.
124. Khadka D, Dhamala MK, Li F, et al. The use of medicinal plants to prevent COVID-19 in Nepal. *J Ethnobiol Ethnomed*. 2021;17:26.
125. Islam A, Kalam MA, Sayeed MA, et al. Escalating SARS-CoV-2 circulation in environment and tracking waste management in South Asia. *Environ Sci Pollut Res*. 2021;28:61951-61968.
126. Wang C, Pan J, Yaya S, Yadav RB, Yao D. Geographic inequalities in accessing improved water and sanitation facilities in Nepal. *Int J Environ Res Public Health*. 2019;16:1269.
127. Shrestha A, Six J, Dahal D, Marks S, Meierhofer R. Association of nutrition, water, sanitation and hygiene practices with children's nutritional status, intestinal parasitic infections and diarrhoea in rural Nepal: a cross-sectional study. *BMC Public Health*. 2020;20:1241.
128. The Kathmandu Post. Poorly handled Covid-19 garbage could give rise to hazardous waste crisis. September 29, 2021. Accessed January 30, 2022. https://kathmandupost.com/health/2021/09/29/poorly-handled-covid-19-garbage-could-give-rise-to-hazardous-waste-crisis
129. Vally Z. Public perceptions, anxiety and the perceived efficacy of health-protective behaviours to mitigate the spread of the SARS-Cov-2/COVID-19 pandemic. *Public Health*. 2020;187:67-73.
130. Mukherjee S, Vincent CK, Jayasekera HW, Yekhe AS. Personal care formulations demonstrate virucidal efficacy against multiple SARS-CoV-2 variants of concern: implications for hand hygiene and public health. *PLoS Glob Public Health*. 2022;2:e0000228.
131. Tran HN, Le GT, Nguyen DT, et al. SARS-CoV-2 coronavirus in water and wastewater: a critical review about presence and concern. *Environ Res*. 2021;193:110265.
132. Bhatt A, Arora P, Prajapati SK. Occurrence, fates and potential treatment approaches for removal of viruses from wastewater: a review with emphasis on SARS-CoV-2. *J Environ Chem Eng*. 2020;8:104429.