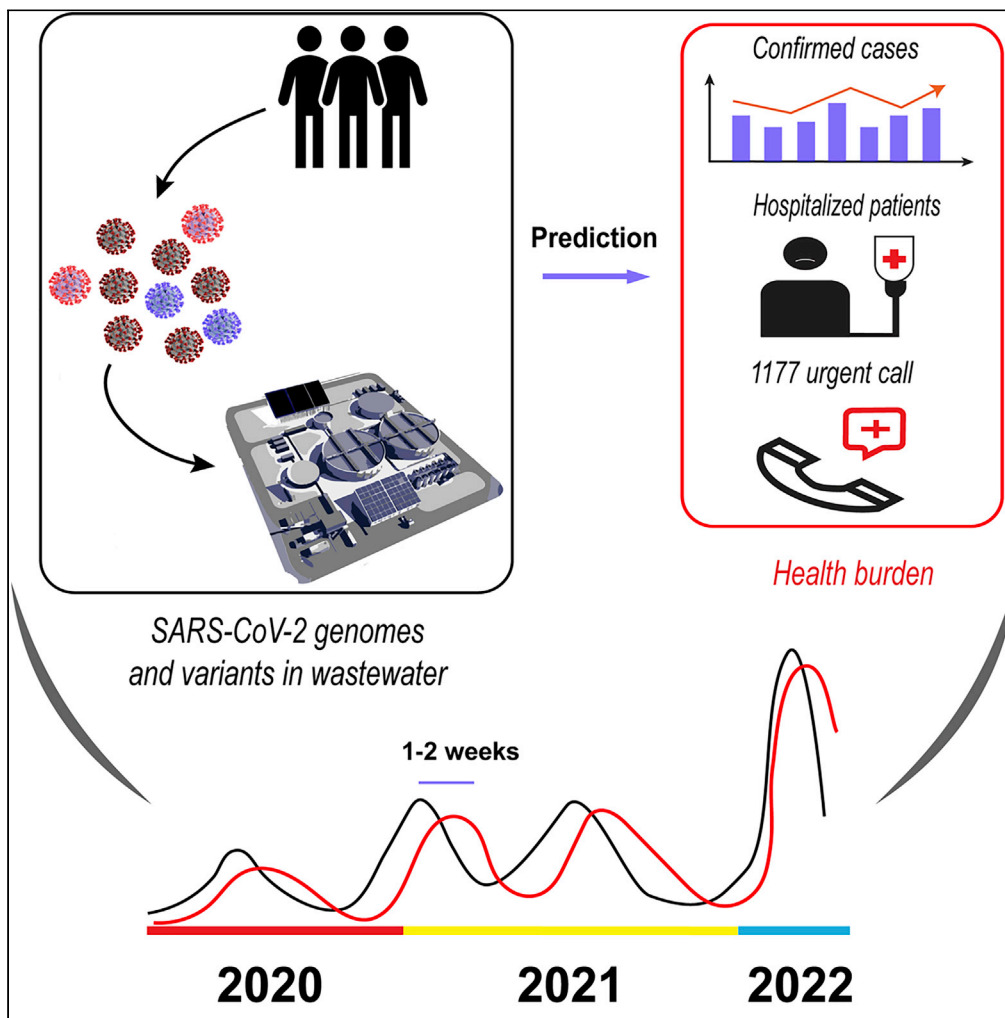


Article

The amount of SARS-CoV-2 RNA in wastewater relates to the development of the pandemic and its burden on the health system



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Highlights

The weekly variation of viral RNA preceded hospital care needs for COVID-19

Virus surveillance in wastewater could predict the burden to urgent call center

Virus surveillance in wastewater could predict the development of a pandemic

SARS-CoV-2 variants in wastewater coincided with their circulation in societies

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Article

The amount of SARS-CoV-2 RNA in wastewater relates to the development of the pandemic and its burden on the health system

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SUMMARY

Virus surveillance in wastewater can be a useful indicator of the development of the COVID-19 pandemic in communities. However, knowledge about how the amount of SARS-CoV-2 RNA in wastewater relates to different data on the burden on the health system is still limited. Herein, we monitored the amount of SARS-CoV-2 RNA and the spectrum of virus variants in weekly pooled wastewater samples for two years from mid-February 2020 and compared them with several clinical data. The two-year monitoring showed the weekly changes in the amount of viral RNA in wastewater preceded the hospital care needs for COVID-19 and the number of acute calls on adult acute respiratory distress by 1-2 weeks during the first three waves of COVID-19. Our study demonstrates that virus surveillance in wastewater can predict the development of a pandemic and its burden on the health system, regardless of society's test capacity and possibility of tracking infected cases.

INTRODUCTION

Wastewater monitoring of the occurrence, concentration, and diversity of fecal/orally transmitted pathogens is a tool for understanding changes in their spread and the emergence of new variants (Hellmer et al., 2014; Ndiaye et al., 2014; Wang et al., 2020). This approach has mainly been focused on non-enveloped viruses, such as norovirus, rotavirus, enterovirus, adenovirus, and hepatitis A and E viruses. Unlike these viruses, SARS-CoV-2, the causative agent of the COVID-19 pandemic, is an enveloped virus with a lipid membrane (Ludwig and Zarbock, 2020). It infects cells primarily in the respiratory tract and lungs by binding to the angiotensin-converting enzyme 2 (ACE2) on cell membranes (Cevik et al., 2020). It has long been assumed that enveloped viruses are susceptible to gastric acid and bile, and infectious virions are unlikely to reach the lower gastrointestinal tract. However, recent studies found that SARS-CoV-2 could also replicate in the intestine, where ACE2 is expressed at relatively high levels (Qian et al., 2021). Furthermore, COVID-19-infected individuals have been shown to excrete SARS-CoV-2 particles in their feces (Holm-Jacobsen et al., 2021; Zhang et al., 2021), which allows for the detection of the virus in wastewater (Ho et al., 2022; Lastra et al., 2022; Markt et al., 2022; Rios et al., 2021; Saguti et al., 2021; Wu et al., 2022).

Sweden adopted a moderate strategy at an early stage to combat the COVID-19 pandemic. In the beginning, testing for SARS-CoV-2 was prioritized for patients with severe symptoms, hospital staff, and the elderly at retiring homes. The Public Health Agency of Sweden and the local authorities and regions gradually increased the test capacity from June 2020 and recommended large-scale testing (Folkhälsomyndigheten, 2022a). The number of confirmed cases increased after the changes in sampling strategy. After two years of the pandemic, many countries, including Sweden, chose to lift or remove pandemic restrictions, and testing for SARS-CoV-2 was given priority to groups that need it the most, such as hospitalized patients, health care and elderly care staff, and people who physically need to be at their workplace (Folkhälsomyndigheten, 2022e). Thereby, the nationally compiled data from the Public Health Agency of Sweden did not reflect the actual circulation of SARS-CoV-2 in society.

As the COVID-19 pandemic continues to ravage the world, new variants of SARS-CoV-2 with increased transmissibility, disease severity, and/or escape from humoral immunity are emerging (Tao et al., 2021).

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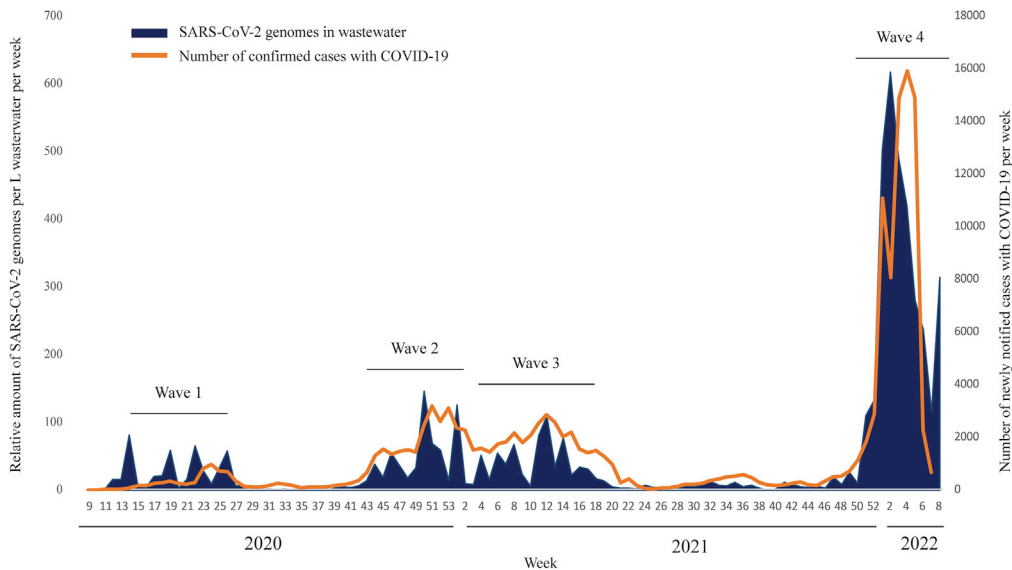


Figure 1. Relative amount of SARS-CoV-2 genomes in wastewater per week in relation to the number of new confirmed cases per week

The relative amount of SARS-CoV-2 genomes in wastewater per week was shown in the blue area, and the number of new confirmed cases was shown as orange line. There were four waves of SARS-CoV-2 RNA in the wastewater between February 10, 2020 and February 28, 2022, which was indicated in the figure.

These new variants bring additional challenges to the current prevention and control of COVID-19 and need to be monitored. Our previous pilot study showed that SARS-CoV-2 RNA monitoring in wastewater reflects the number of hospitalized patients, and the peaks for the increasing number of notified patients coincided with peaks of increasing SARS-CoV-2 RNA in the wastewater (Saguti et al., 2021). However, the knowledge about how the monitoring reflects the number of infected and circulating virus variants, which in turn will reflect the burden on health care in many respects is still limited. This two-year study was conducted to investigate whether virus surveillance in wastewater relates to different data on the burden on the health system and public health problems and if it can predict the development of the pandemic regardless of the capacity of the health system to test for SARS-CoV-2 and trace the infection.

RESULTS

Weekly variations of the amount of SARS-CoV-2 RNA in wastewater throughout the COVID-19 pandemic 2020-2022

The detection of SARS-CoV-2 genomes between week 7 and week 27, 2020, had been described in a previous pilot study (Saguti et al., 2021). The same monitoring has continued, and data until February 2022 was included in this study. There were four waves of SARS-CoV-2 RNA observed in the wastewater during the two-year surveillance (Figure 1). The first peak was from week 14 to week 26, 2020, with approximately 60-80 times more SARS-CoV-2 RNA in the wastewater than in week 11, which was the first week where SARS-CoV-2 genomes could be detected in all the subsequent weeks and it was used as the reference week in this study. The successive second and third waves were from week 43, 2020, to week 19, 2021. These two waves were not clearly distinct from each other by the wastewater monitoring, as there were probably several cluster outbreaks at different parts of the city during this period causing consistently high level of SARS-CoV-2 RNA as previously described for the first wave (Saguti et al., 2021). The fourth peak started from week 51, 2021, and peaked at week 2 in 2022. The amount of SARS-CoV-2 RNA in the wastewater during this peak was 617 times higher than that during the reference week, and 4.3 times higher than the previous highest peak at week 50 in 2020.

The temporal relationship between the amount of SARS-CoV-2 RNA in wastewater and the number of notified cases of COVID-19

The weekly relative amount of SARS-CoV-2 RNA in wastewater in relation to the number of notified COVID-19 cases from the Gothenburg area during the same time period is shown in Figure 1. Owing to different restrictions on when and who would be tested for COVID-19, the two curves showing the number of

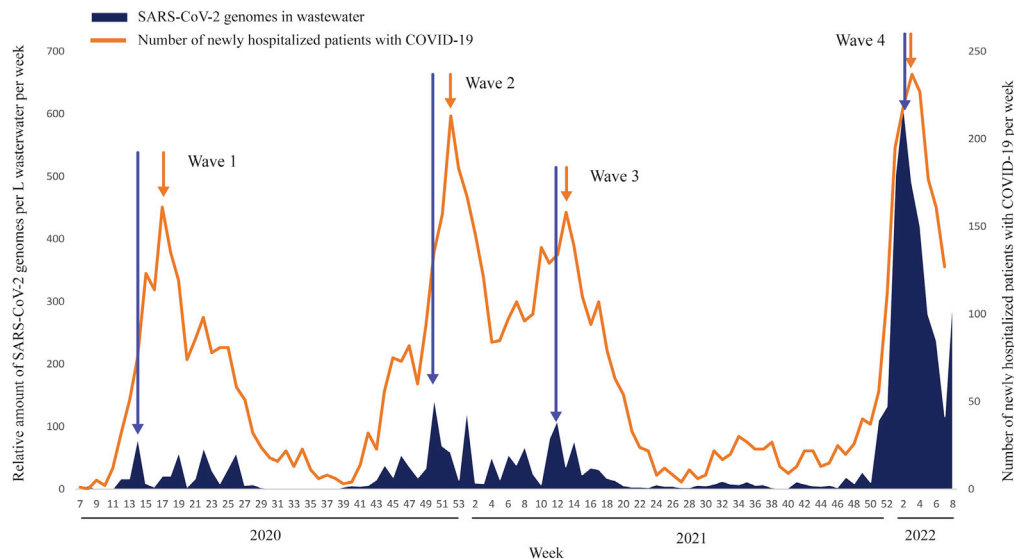


Figure 2. Relative amount of SARS-CoV-2 genomes in wastewater per week in relation to newly hospitalized patients with COVID-19 at Sahlgrenska university hospital per week

The relative amount of SARS-CoV-2 genomes in wastewater per week was shown in the blue area, and the number of newly hospitalized patients with COVID-19 was shown as orange line. The peak of SARS-CoV-2 RNA in the wastewater (blue arrow) and hospital care needs for COVID-19 (orange arrow) in each wave were indicated.

reported cases and the amount of SARS-CoV-2 RNA in wastewater did not coincide until week 13 in 2021. From this week to week 4 in 2022, anyone with symptoms could be tested for COVID-19, and the amount of SARS-CoV-2 RNA in the wastewater and the number of confirmed cases correlated simultaneously. After week 4, during the fourth wave, there was a very large number of infected persons (Table S3), which caused the testing capacity to be limited, and the testing recommendations again became more restrictive to only test hospitalized patients and elderly at retirement homes.

The temporal relationship between the amount of SARS-CoV-2 RNA in wastewater and the number of hospitalized patients

The number of weekly hospitalized patients with COVID-19 in relation to the relative amount of SARS-CoV-2 RNA in wastewater is shown in Figure 2. The previously observed delay of approximately one to two weeks between the peaks of viral genomes in the wastewater and in number of hospitalized patients (Saguti et al., 2021) was maintained throughout the two-year period (Table S3). During the fourth wave, the relation between the highest number of hospitalized patients one week after and highest relative amount of SARS-CoV-2 RNA in wastewater was 0.7, which is lower than during the previous waves, when it was 4–14 times more patients in relation to the relative amount of RNA (Table S3). The most probable reason for this is that the Omicron variant of SARS-CoV-2, which dominated both in wastewater and among the patients during this wave, caused less severe disease compared to the previous viral variants. This was also demonstrated by the fact that approximately 50% of the COVID-19 infected patients admitted to the hospital during this period were admitted for health problems other than COVID-19.

Number of urgent calls related to acute breathing difficulties throughout the COVID-19 pandemic 2020–2022

The number of telephone calls to 1177 Vårdguiden regarding acute breathing difficulties and the need for immediate care for both children and adults is shown in Figure 3. The number of 1177 calls concerning children was relatively small and stable during the pandemic, except between week 38 and week 47, 2021, where the number was about twice as high as during the other periods. In contrast, several fluctuations in the number of 1177 calls concerning adults were observed. The number of these daily calls increased from about 30 to 175 during the peak of the first wave of COVID-19 (Table S3). They also increased during the second and third waves of COVID-19 but less than during the first wave. Although there was a dramatic increase in the number of confirmed COVID-19 cases and in the amount of SARS-CoV-2 RNA in the

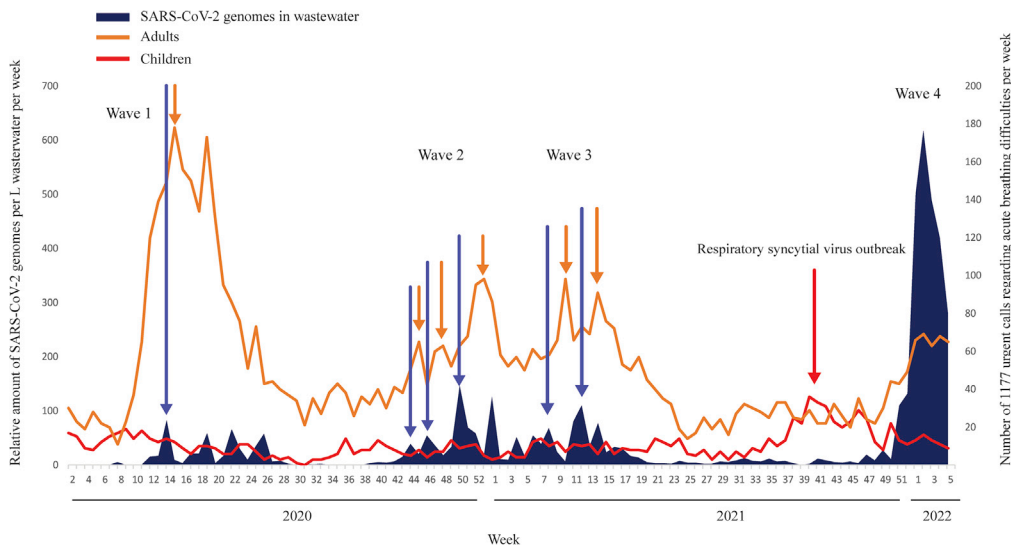


Figure 3. Relative amount of SARS-CoV-2 genomes in wastewater per week in relation to the number of telephone calls to 1177 concerning acute breathing difficulties and who need to be treated urgently

The relative amount of SARS-CoV-2 genomes in wastewater per week was shown as the blue area, and the number of telephone calls to 1177 concerning acute breathing difficulties in Gothenburg for adults was shown as orange line, and for children was shown as the red line. The peak of SARS-CoV-2 RNA in the wastewater (blue arrow) and the number of acute calls in acute shortness of breath in adults (orange arrow) in each wave were indicated.

wastewater during the fourth wave, the daily calls related to acute breathing difficulties were only about twice as high as before the first wave.

Changes in SARS-CoV-2 variants in the wastewater in relation to strains identified from patients during the corresponding time period

Thirty-three influent wastewater samples were selected for NGS analysis on the Ion Torrent S5 platform. Good coverage of the spike region was obtained for 32 samples. The mutations in the spike region that were identified in more than 20% of the sequences were selected for analysis. The mutations that were either variant of concern (VOC) related mutations or found in more than one wastewater sample are shown in Figure 4. The most common mutation in the spike region was D614G, detected in 91% (30/33) of the samples, and found in more than 99% of the sequences in 26 samples (Figure 4, Table S4). From the analysis, it was shown that some Alpha-specific mutational signatures, including del68-70, A570D, P681H, and S982A, could be detected in wastewater as early as week 8, 2020 (Figure 4). Specific mutation signatures for the Delta variant, such as T19R, del157-158, P681R, and D950N, could only be detected in wastewater after week 30 in 2021, which in time corresponded to the rapid increase of this variant among patients in Sweden (Folkhälsomyndigheten, 2022c).

Thirty-two nasopharyngeal swabs were randomly selected from patients sampled during weeks 12-15 and 38 and sequenced. The dominant SARS-CoV-2 lineages and mutations in the spike region of the strains in the patient samples are shown in Table S5. The alpha-specific mutational signatures found in the wastewater were not identified in the patient samples. In these samples, 94% (30/32) of the strains had a D614G mutation in the spike region. The second abundant mutation was D936G, which was found in 7 (24%) of the patient samples between weeks 12 and 15, 2020. Other mutations, including A846V, T859I, A1078S, D1084Y, and V1122L, could only be identified in one patient sample at a high frequency (Table S5). Several substitutions, such as D614G, K1269S, H1271D, found in patients from week 38, 2020 were common also in the strains in the wastewater during the same period (Figure 4, Table S5).

Changes in the dominant SARS-CoV-2 lineages in wastewater

The dominant lineages in 33 influent wastewater samples were also analyzed with the Pangolin classification method. The dominant SARS-CoV-2 lineages could be identified in 15 of 33 samples (Figure 4). The results showed that the B.1.1 lineage was the dominant variant in the early stage of the pandemic. From

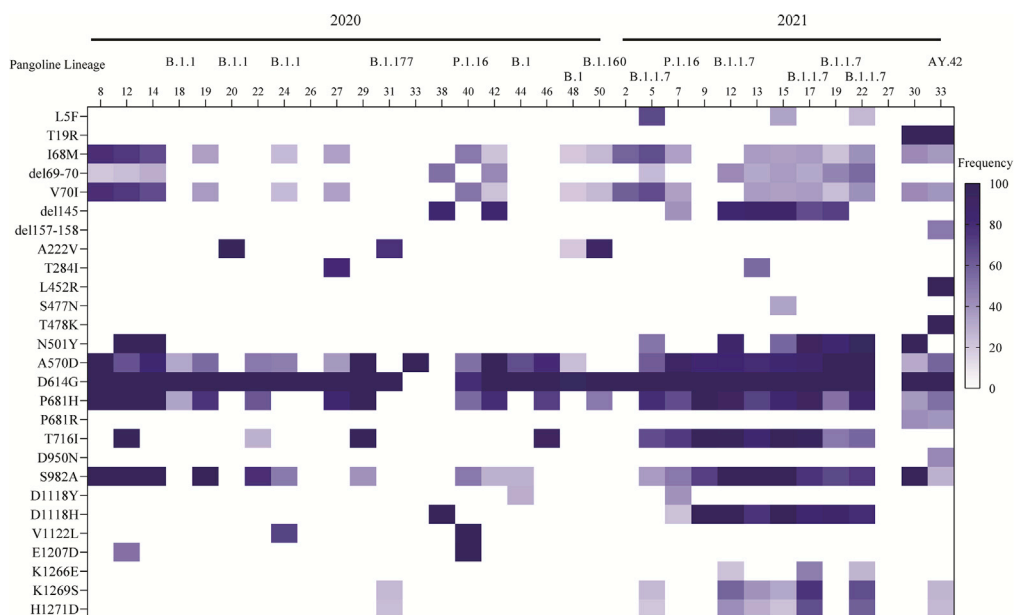


Figure 4. Selected mutations in spike regions in wastewater samples

The mutations with the frequency and the count both larger than 20 in the spike region were selected from 33 wastewater samples and the heatmap was created with GraphPad Prism 9 software. Among them, the mutations were either VOCs related mutations or have been found in more than one wastewater sample displayed in the heatmap. The SARS-CoV-2 lineages of the samples identified by the Pangolin pipeline were shown at the top of the heatmap.

summer 2020 to early winter 2021, several common European lineages, including B.1.177, B.1, B.1.160, and P.1.16, were identified in wastewater. These European lineages were replaced by the B.1.1.7 lineage (Alpha variant) during week 5 in 2021, which remained the dominant variant in wastewater until the summer of 2021. Subsequently, it was replaced by the AY.42 lineage (Delta subvariant), which remained the dominant variant until week 49 in 2021.

From week 47, 2021, we started to use in-house developed qPCR to distinguish the Omicron variant and the Delta variant in wastewater. The Omicron variant was first identified in week 49, 2021 and it only took two weeks to become dominant in wastewater (Figure 5A). From week 1, 2022, the Omicron variant accounted for about 99% of the variants in wastewater. Owing to the Omicron BA.2 variant frequently detected in clinical samples, we developed a qPCR in early February 2022 to type BA.1 and BA.2. The analysis showed BA.1 appeared in wastewater in week 2, 2022, then it was rapidly replaced by the BA.2 variant. In week 6, 2022, about 90% of the variants in wastewater was BA.2 (Figure 5B).

DISCUSSION

In this study, weekly variations of SARS-CoV-2 genomes in wastewater were monitored for two years from the beginning of the pandemic in Sweden. The variations in the amount of viral genomes were found to relate to several different sources of monitoring the pandemic, such as the number of newly confirmed cases, newly hospitalized patients with COVID-19, and the number of urgent telephone calls to 1177 Vårdguiden regarding breathing difficulties in the same region. In addition, the most common virus variants isolated from infected patients were identified earlier or simultaneously in the wastewater. This study is a follow-up to our previous pilot study conducted during the first half of 2020 (Saguti et al., 2021). The results showed that monitoring of viruses in wastewater is a good indicator of viruses circulating in the population and could be used to predict future outbreaks and increased spread of certain viruses.

There have been four waves of COVID-19 in Sweden as the pandemic began. During these waves, the changes in the amount of SARS-CoV-2 in wastewater were consistent with the changes in newly hospitalized patients with COVID-19. The time between the peak of viral genomes in wastewater and the peak of hospitalized patients was one to two weeks, which is in line with other reports, where increasing trends of viruses in wastewater preceded clinical data by 3-11 days (Lastra et al., 2022; Wu et al., 2022).

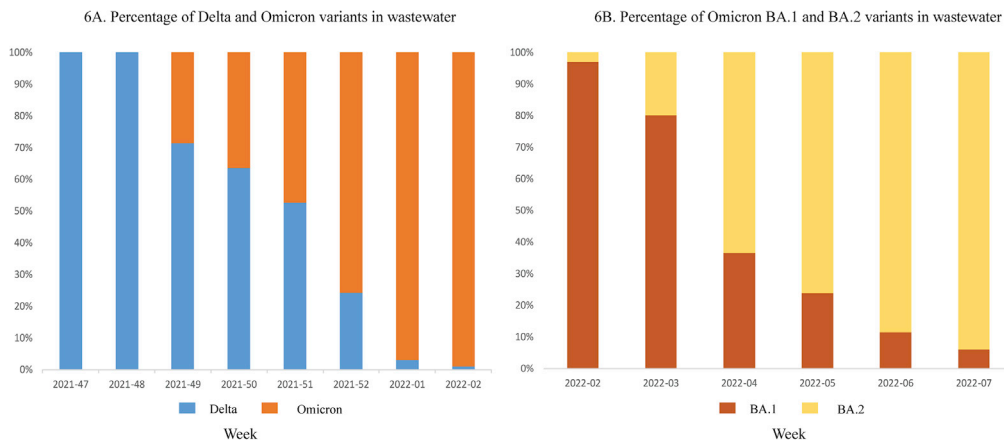


Figure 5. Changes in the SARS-CoV-2 lineages in wastewater during the fourth wave of COVID-19

(A) The percentage of Delta and Omicron variants in wastewater during week 47, 2021 and week 2, 2022.

(B) The percentage of Omicron BA.1 and BA.2 sub-variants in wastewater during week 2, 2022 and week 7, 2022.

The number of confirmed cases during the second and third waves was three times higher than that during the first wave. This may partly be owing to increased test capacity in Sweden (Folkhälsomyndigheten, 2022a). The monitoring of SARS-CoV-2 in wastewater also showed that the level of viruses during the second and third waves was nearly twice that of the first wave, indicating a higher spread. Some studies have shown that individuals infected with the Alpha variant of SARS-CoV-2 had a higher viral load and longer duration of detectable viruses in nasopharynx than those infected by previous strains (Cosentino et al., 2022; Julin et al., 2021). The viral loads in oropharyngeal swab from those infected with the Delta variants were on average $\sim 1,000$ times higher compared to those infected with the first variant (Lu et al., 2021). However, better knowledge is needed on whether certain SARS-CoV-2 variants cause higher viral load in feces than other variants. This would help in understanding if higher amounts of SARS-CoV-2 genomes in wastewater are mainly owing to increased shedding by each infected patient or to a substantial increase in infected individuals.

The number of daily urgent calls regarding acute breathing difficulties among adults may reflect the progression and severity of COVID-19 in societies. This study examined the number of daily urgent calls to 1177 Vårdguiden during a two-year period and showed that changes in the number of urgent calls regarding adults coincided with the four waves of COVID-19. This was not the case for urgent calls regarding children with breathing difficulties. Children are likely to be asymptomatic or have mild symptoms when infected with SARS-CoV-2 (Mehta et al., 2020), resulting in a low number of confirmed cases and urgent calls to 1177 Vårdguiden. The only increase in the number of urgent calls observed for children regarding breathing difficulties was during the winter of 2021, where the COVID-19 cases were at a low level. The reason for this could be that other viruses, such as respiratory syncytial virus (RSV), spread among children in Sweden during this period (Folkhälsomyndigheten, 2022b). The increase of SARS-CoV-2 RNA in wastewater coincided or preceded with approximately 1-2 weeks an increase in the number of urgent calls to 1177 Vårdguiden regarding breathing difficulties in adults. In another study from Sweden, it was shown that an increase in the number of urgent calls to 1177 preceded an increase in the number of hospitalized patients with about one to two weeks at the beginning of the pandemic (Spreco et al., 2022), as was shown also in this study. These results indicate that monitoring of viruses in wastewater could also predict an upcoming burden for the emergency medical communication centers, responsible for coordinating transports of patients and patient samples. It should be noted that it was not possible to differentiate between COVID-19 and other causes of acute breathing difficulties based on the calls to 1177 Vårdguiden.

The shift of variants of SARS-CoV-2 in the wastewater during the study period was largely consistent with the reports from the Public Health Agency of Sweden on the genetic groups of SARS-CoV-2 circulating in society (Folkhälsomyndigheten, 2022c). The genetic group B.1 and B.1.1 were early introduced in Sweden and were dominant for more than half of 2020 (Folkhälsomyndigheten, 2022d). Our monitoring showed that B.1.1 persisted about 1 month longer in the wastewater than in patient

samples, showing an undetected ongoing spread. Afterward, the B.1.1.7 (Alpha variant) became rapidly dominant and led to the third wave of COVID-19. Some specific mutational signatures for the Alpha variant, especially A570D, P681H, and S982A, were identified in wastewater as early as week 8, 2020, but not found in patients until week 52, 2020 (Folkhälsomyndigheten, 2022c). The presence of these mutations in wastewater and not in patients' samples could be a selection of virus variants during virus replication in the intestine or presence of these variants in unsampled patients. Such cryptic variants have also been observed in wastewater in New York in the USA (Smyth et al., 2022). The dominance of the Alpha variant in Gothenburg lasted for 4 months and was thereafter replaced by the Delta variant. The Delta variant did not cause another wave of COVID-19 in Sweden, although it has been shown to be more transmissible and cause more severe diseases than the Alpha variant (Thakur et al., 2022).

Our monitoring showed the Omicron variant contributed to a surge of both the SARS-CoV-2 genomes in the wastewater and the number of total confirmed cases at the end of 2021, leading to the fourth wave of COVID-19 in Sweden. Recent studies have pointed out that the Omicron variant has enhanced transmissibility compared to the previous variants (He et al., 2021; Sofonea et al., 2022). During week 2 in 2022, there was a higher increase in SARS-CoV-2 genomes in the wastewater than in any other previous week during the pandemic, which coincided with an increase in confirmed cases. Further typing of the Omicron variant showed that the BA.1 subtype was dominant until week 3, 2022 and then it was rapidly displaced by the BA.2 subtype, one week earlier than what was found in patients' samples from the Västra Götaland region. These findings showed that monitoring the virus could identify the increase and shift of SARS-CoV-2 variants in wastewater before or at the same time as the variants were identified in patient samples.

It should be noted that the dramatic increase in the number of total confirmed cases during the fourth wave of COVID-19 in Gothenburg is still underestimated. Although infection with Omicron is milder than infection with the other variants (Nealon and Cowling, 2022), the rapid spread of this variant still caused a burden on healthcare. Many health care workers or their family members were infected, leading to a shortage of staff in many regions to deal with this wave. In addition, about 50% of the patients who were hospitalized were in need of other medical treatments than for COVID-19 and had to be isolated in the wards. Furthermore, several regions had to pause or limit testing for COVID-19 owing to limited capacity in the laboratories and a shortage of test kits caused by the rapid increase of cases. As the national test capacity hit the ceiling, many people tested themselves with the antigen test kit to avoid a delay in booking time and/or a longer waiting time for PCR test results. During the fourth wave, the Public Health Agency of Sweden changed the testing criteria for SARS-CoV-2 by not including testing of persons who did not need to be physically present at work (Folkhälsomyndigheten, 2022a). Given these facts, the monitoring of the virus in wastewater probably better reflected changes in the spread of the virus in society, as the actual number of confirmed cases was underestimated during this period.

This two-year follow-up study showed that the level of SARS-CoV-2 genomes in wastewater was correlated with the number of confirmed cases in society, and an increase in the level preceded an increase in the number of imminent hospitalized patients with COVID-19. This pandemic has ravaged the world for more than two years and will last longer. Some European countries, including Sweden, have recently lifted or removed COVID-19 restrictions, and many countries may follow, which may lead to a change in test strategy and reduction of test capacity. Additionally, the willingness to test for COVID-19 will decrease. Therefore, the data of the national daily report on notified cases will not reflect the true spread. However, the continuous spread of SARS-CoV-2 in societies will still bring persistent pressure on health care. In this scenario, the quantification of SARS-CoV-2 genomes in wastewater could serve as a tool to understand the virus spread and provide an early warning of potential upcoming outbreaks. These measurements can be performed at the national level in most regions in Sweden, as recommended by the European Commission (2021), as wastewater sampling is routine at the treatment plants and there are close collaborations between the regional microbiological laboratories. Meanwhile, new variants with changed transmissibility may emerge and their impacts on the burden of health care are unknown. The detection of the newly emerged variant in wastewater will offer first-hand information about the spread of a new variant in the community. Surveillance of the virus will detect its possible increase in wastewater and can thus provide 1 to 2 weeks of preparation for the health authority before any increased burden on health care. The monitoring of SARS-CoV-2 variants together with the quantification will thus be an important supplement to the current health monitoring system.

Limitations of the study

In this study, we concentrated SARS-CoV-2 from flow-weighted weekly wastewater samples, which we used to ensure that the analysis always examines a similar amount of wastewater from each household. However, the inhibitors in wastewater were also co-concentrated with this method, likely leading to a degree of bias in the quantification of SARS-CoV-2 genomes in different weeks. Future work should also include an internal control that could be used for the normalization of wastewater monitoring to improve the reliability of the analysis. In addition, we found several mutations of the SARS-CoV-2 spike protein in wastewater appearing weeks before some of these strains were reported from patients, and other mutations were not identified in patient strains. Future work is needed to investigate if these mutations or strains are from potential animal hosts, like rats, or owing to a selection of viral strains and replication of SARS-CoV-2 in the gastrointestinal tract, as the analyzed patient strains are collected from the upper respiratory tract.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.105000>.

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AUTHOR CONTRIBUTIONS

HN conceptualized the idea of the study. LE and AJ collected the wastewater samples. LD and TB collected the clinical data. HW, MPC, TT, MA, and AK conducted the experiments. HW, KN, and HN analyzed and interpreted the results. HW drafted the article. HN, SN, and ML provided guidance and contributed to writing of the final version. All authors are involved in reviewing, editing, and approved the final article.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
QIAamp Circulating Nucleic Acid Kit	Qiagen	Cat#55114
DNeasy blood and tissue kit	Qiagen	Cat#69504
NucliSens® easyMag	bioMérieux	ref.280140
Ultra-Plex 1-Step ToughMix	Quantabio	Cat#95166-100
Super-Script VILO cDNA synthesis kit	Thermo Fisher Scientific	Cat#11754050
Ion AmpliSeq™ Library Kit 2.0	Thermo Fisher Scientific	Cat#4475345
Ion 530™ Chip Kit	Thermo Fisher Scientific	Cat#A27764
Ion S5™ System	Thermo Fisher Scientific	Cat#A27212
Oligonucleotides		
Primers and Probes	Eurofins Genomics	See Table S2
Software and algorithms		
7300 real-time PCR system software	Applied Biosystems	SDS v1.4.1
CLC Genomics Workbench 21.0.5	Qiagen	version 21.0.5
Ion Torrent Suite software	Thermo Fisher Scientific	Torrent Suite 5.16.1
Microsoft Excel	Microsoft	Excel 2016
GraphPad Prism 9	GraphPad software, Inc.	GraphPad Prism version 9
Other		
NanoCeram VS2.5-5 cartridge	Argonide	Cat#VS2.5-5
Beef extract phosphate buffer with 0.05M Glycine (pH 9.5)	Substrate unit at Microbiology (Sahlgrenska University Hospital)	Beef extract phosphate buffer
Sartorius cartridge	Sartorius	Cat# 54413007H4-CE

RESOURCE AVAILABILITY

Lead contact

Further information and requests for virus concentration, sample preparation, and results analysis should be directed to and will be fulfilled by the lead contact, Hao Wang (hao.wang@gu.se).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All wastewater monitoring data and clinical data were provided in [supplementary materials](#). Any further information and requests required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request. Additional Supplemental Items are available from Mendeley Data: <https://doi.org/10.17632/2ds6j8n5g3.1>.

METHOD DETAILS

Wastewater samples

Influent wastewater samples were collected from Rya wastewater treatment plant (WWTP), which is located in Gothenburg, Sweden. The Rya WWTP receives wastewater from more than 770,000 households and industry in Gothenburg and seven nearby municipalities. It also receives storm water and snow-melting water from some older parts of the city. The amount of wastewater from households is fairly constant during the

sampling period, while the total influent flow into the WWTP varies based on the changes of precipitation. The flow can vary up to three times on a weekly basis depending on whether it was raining or not.

The influent wastewater was collected between February 10, 2020 and February 28, 2022. A 24-h daily sample was obtained by using a fixed-site sampler that collect 30 mL per 10,000 m³ influent wastewater. Daily samples were stored at 4°C and pooled into a weekly sample as previously described (Saguti et al., 2021). Information on sampling week, volume, and influent wastewater flow is given in Table S1.

Concentration of viruses from influent wastewater samples

Viruses in influent wastewater were concentrated by our in-house developed method, which used the NanoCeram electropositive filter (Argonide, Florida, USA) as primary concentration method, and ultracentrifugation as secondary concentration method as described previously (Wang et al., 2018). The pellet obtained from ultracentrifugation was suspended in 2.4 mL Tris-HCl (pH 8.0) buffer and stored at –80°C prior to further analysis.

Detection of SARS-CoV-2 in wastewater by qPCR

Nucleic acids were extracted from 1 mL of dissolved pellet using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The in-house modified RT-qPCR was performed for the detection of SARS-CoV-2 RNA in wastewater. The 20- μ L reaction mixture contains 5 μ L extracted nucleic acids, 4 \times Ultra-Plex 1-Step ToughMix (Quantabio, Beverly, USA), 0.75 μ M of forward and reverse primer, and 0.2 μ M probe. The primers and probe have been described previously (Saguti et al., 2021). The qPCR reaction was performed with an initial reverse transcription cycle of 50°C for 10 min and 95°C for 3 min, followed by 45 cycles of 95°C for 10 s and 60°C for 1 min on a 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Each sample was analyzed in triplicate. In all qPCR runs, a 10-fold serial diluted plasmid (2 μ g/mL; Eurofins Genomics, Ebersberg, Germany) containing the target SARS-CoV-2 region was used as a positive control. Nuclease-free water (Sigma-Aldrich, St. Louis, USA) was used as a negative control.

Two in-house modified qPCRs were developed in December 2021 and February 2022 to identify SARS-CoV-2 variants in wastewater. The first qPCR was developed to distinguish between the Delta and Omicron variants, and the second qPCR was developed to further distinguish between the Omicron BA.1 and BA.2 variants. The qPCR reaction system was the same as described above except for changes of primers and probes (Table S2).

Patient samples

Nasopharyngeal swab samples from individual with COVID-19 symptoms in Gothenburg area were sent to Clinical Microbiology Laboratory at Sahlgrenska University Hospital (SU) for SARS-CoV-2 analysis. A total of 32 nasopharyngeal swab samples tested positive for SARS-CoV-2 by qPCR were selected for next generation sequencing. The samples had a Ct values between 13.5 and 26.9. Twenty-nine samples were collected between week 12 and 15 in 2020, and the other three samples were collected from week 38 in 2020.

Ethical approval

The study was approved by the Swedish Ethical Review Board (application no. 2020–03276). Written informed consent was provided by all patients upon all sampling for SARS-CoV-2. The nasopharyngeal swab samples used in this study were from the remaining materials stored in the biobank. Patients agreed to store their samples in the biobank and to be used in future research.

Next generation sequencing for influent wastewater and patient samples

Thirty-three wastewater samples between February 2020 and August 2021, which covered all peaks during this period, were selected for next generation sequencing. The 32 nasopharyngeal swab samples described above were analyzed by same process as the wastewater samples.

Due to insufficient remaining volume of the wastewater samples from the first half year of 2020, the nucleic acids extraction kit was changed to the DNeasy blood and tissue kit (Qiagen), where 200 μ L of concentrated wastewater samples were used. Nucleic acids of the nasopharyngeal swab samples were extracted using

the NucliSens® easyMag® instrument and reagents (NucliSENS easyMag; bioMérieux, France) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA with random primers using the SuperScript VILO cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). RNA libraries were then prepared on the Ion Chef platform using the Ion AmpliSeq Kit for Chef DL8 (Thermo Fisher Scientific). A final concentration of 30 pM was achieved for the library pool. The pooled library was loaded on Ion 530 Chip and sequencing was performed on the S5 platform (Thermo Fisher Scientific).

Data collection of the number of notified patients, hospitalized patients and urgent calls to 1177

The number of individuals with confirmed COVID-19 infection in Gothenburg was obtained from the Department of Communicable Disease Control in Region Västra Götaland. The notification was based on the date of SARS-CoV-2 reactivity in the patient sample. The data used in this study covered the period between week 9, 2020 and week 7, 2022. Since the 9 February 2022, the Swedish government and the Public Health Agency of Sweden began phasing out the infection control measures, including general sampling and infection tracing ceased. The number of confirmed cases of COVID-19 will not be comparable since then.

The number of daily newly hospitalized patients with COVID-19 to the hospital in Gothenburg is continuously compiled by the group for pandemic preparedness at Sahlgrenska University Hospital. The data used in this study covered the period between week 7, 2020 and week 7, 2022.

The twenty-one regions in Sweden provide healthcare information to the population through a combination of services called 1177 Vårdguiden (Healthcare Guide 1177 in English), which includes a searchable healthcare information website (www.1177.se) and a national telephone health advice line staffed by nurses (available by dialling 1177 within Sweden). The Public Health Agency of Sweden receives daily anonymized data from these services for use in national syndromic surveillance activities, including for food and water-borne diseases (Andersson et al., 2014; Bjelkmar et al., 2017), influenza-like illness (Hulth et al., 2009; Ma et al., 2015), calici virus (Edelstein et al., 2014), and tick-borne encephalitis (Martin et al., 2020).

For the pandemic time-period of week 1, 2020 through week 5, 2022, the number of calls to 1177 concerning children or adults living in the Gothenburg area suffering from acute breathing difficulties was collected from the Public Health Agency of Sweden.

QUANTIFICATION AND STATISTICAL ANALYSIS

The Ct values from qPCR were used to calculate the relative amount of viral genomes per L per week. The detailed calculation formula has been previously described (Saguti et al., 2021). All raw data from Ion Torrent sequencing were imported into CLC Genomics Workbench 21.0.5 (Qiagen). All reads were trimmed to remove short and low quality reads. The remaining reads were mapped to the reference sequence (Wuhan-Hu-1, GenBank Accession: MN908947), and the low frequency variant detection method, integrated in CLC Genomics Workbench, was applied to identify SARS-CoV-2 mutations. After CLC analysis, all identified mutations were exported to an Excel file and those mutations in the spike region were selected and compared with mutations of VOCs. Meanwhile, the SARS-CoV-2 lineages of all samples were also identified with the Pangolin pipeline (Rambaut et al., 2021) integrated into the Ion Torrent Suite software (Thermo Fisher Scientific).