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Wastewater monitoring of SARS-CoV-2 shows high correlation with COVID-19 case numbers and allowed early detection of the first confirmed B.1.1.529 infection in Switzerland: results of an observational surveillance study

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Summary

AIMS OF THE STUDY: Wastewater-based epidemiology has contributed significantly to the comprehension of the dynamics of the current COVID-19 pandemic. Its additional value in monitoring SARS-CoV-2 circulation in the population and identifying newly arising variants independently of diagnostic testing is now undisputed. As a proof of concept, we report here correlations between SARS-CoV-2 detection in wastewater and the officially recorded COVID-19 case numbers, as well as the validity of such surveillance to detect emerging variants, exemplified by the detection of the B.1.1.529 variant Omicron in Basel, Switzerland.

METHODS: From July 1 to December 31, 2021, wastewater samples were collected six times a week from the inflow of the local wastewater treatment plant that receives wastewater from the catchment area of the city of Basel, Switzerland, comprising 273,075 inhabitants. The number of SARS-CoV-2 RNA copies was determined by reverse transcriptase-quantitative PCR. Spearman's rank correlation coefficients were calculated to determine correlations with the median seven-day incidence of genome copies per litre of wastewater and official case data. To explore delayed correlation effects between the seven-day median number of genome copies/litre wastewater and the median seven-day incidence of SARS-CoV-2 cases, time-lagged Spearman's rank correlation coefficients were calculated for up to 14 days. RNA extracts from daily wastewater samples were used to genotype circulating SARS-CoV-2 variants by next-generation sequencing.

RESULTS: The number of daily cases and the median seven-day incidence of SARS-CoV-2 infections in the catchment area showed a high correlation with SARS-CoV-2 measurements in wastewater samples. All correlations between the seven-day median number of genome copies/litre wastewater and the time-lagged median seven-day incidence of SARS-CoV-2 cases were significant ($p<0.001$) for the investigated lag of up to 14 days. Correlation coefficients declined constantly from the maximum of 0.9395 on day 1 to the minimum of 0.8016 on day 14. The B.1.1.529 variant Omicron was detected in wastewater samples collected on November 21, 2021, before its official acknowledgement in a clinical sample by health authorities.

CONCLUSIONS: In this proof-of-concept study, wastewater-based epidemiology proved a reliable and sensitive surveillance approach, complementing routine clinical testing for mapping COVID-19 pandemic dynamics and observing newly circulating SARS-CoV-2 variants.

Introduction

Wastewater-based epidemiology has contributed significantly to the comprehension of the dynamics of the current COVID-19 pandemic, as shown by early reports [1–3]. A two-month lead of the first detection of SARS-CoV-2 in wastewater compared to reported clinical cases has been demonstrated [4]. Thus, the additional value of waste-

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water-based epidemiology to monitor SARS-CoV-2 circulation in the population [5, 6] and identify newly arising SARS-CoV-2 variants independently of diagnostic testing [7] is now undisputed. As a proof of concept, we report here correlations between SARS-CoV-2 detection in wastewater and the officially recorded COVID-19 case numbers, as well as the validity of such surveillance to detect emerging variants, exemplified by the detection of the B.1.1.529 variant Omicron (including sublineages) in a sample from November 21, 2021, thus contributing to our understanding of the global epidemiology of SARS-CoV-2.

Material and methods

Sample collection and analysis

Wastewater samples were collected from the local wastewater treatment plant (ProRheno AG, Basel, Switzerland), which receives wastewater from the catchment area of the city of Basel, Switzerland, comprising 273,075 inhabitants (including 201,971 of the political district of Canton of Basel-Stadt and 67,388 of the Canton Basel-Landschaft in Switzerland, as well as 3,716 of parts of three municipalities in Germany and France). From July 1, 2021, until December 31, 2021, 24-hour composite samples from the wastewater inflow were collected daily (except for two days, generally on weekends, when 48-hour composite samples were taken). Until further processing, samples were stored at 4 ºC for a maximum of 72 hours. Total nucleic acid was concentrated and extracted from 40 mL of wastewater using the Maxwell® RSC Environ Wastewater TNA Kit (Promega, Madison, USA). The number of SARS-CoV-2 RNA copies was determined by Wastewater SARS-CoV-2 RT-qPCR Systems (Promega) of the Envelope (E) gene and N1 and N2 regions of the Neocapsid (N) gene. The calculated means of two of three E, N1 and N2 amplifications, each consisting of two technical replicates of RT-qPCR measurements of each composite wastewater sample, were used for visualisations and correlations. In each reaction, the number of pepper mild mottle virus (PMMoV), a robust nucleic acid faecal biomarker in wastewater [8, 9], was also quantified. Thus, PMMoV-normalised SARS-CoV-2 genome copies were calculated by dividing the absolute (non-normalised) SARS-CoV-2 genome copies by the number of PMMoV genome copies in each sample. In addition, SARS-CoV-2 genome copies were normalised by inflow-volume of the wastewater treatment plant to control for the variance of wastewater volume and concentration [10]. For this purpose, the number of SARS-CoV-2 genome copies per L wastewater was multiplied by the total daily wastewater volume reaching the wastewater treatment plant (inflow-volume). The number of SARS-CoV-2 RNA copies inflow-volume-normalised compared with the number of active COVID-19 cases is available online via https://data.bs.ch/explore/dataset/100187. A study protocol was not made publically available. Because this study did not involve human research, no ethical consent was sought.

COVID-19 case data

Epidemiological data was provided from the COVID-19 dashboard Basel-Stadt (data.bs.ch) and the Health Departments of Basel-Stadt and Basel-Landschaft. Case data is reported by the cantonal authorities and is based on mandatory reporting of all PCR- or antigen-test-confirmed results as further specified by federal law [11]. During the study period, local and national recommendations advised low-threshold testing for all people experiencing symptoms consistent with SARS-CoV-2 infection. In addition, wide-scale regular testing was performed by different employers, schools and healthcare systems and was encouraged by cantonal authorities. Multiple testing was corrected for by the cantonal authorities.

Correlation analysis

Because our data were non-normally distributed, Spearman's rank correlation coefficients were calculated for samples collected from July 6, 2021, to December 31, 2021 (n = 179) to determine correlations with the median seven-day incidence of genome copies per litre of wastewater and case data. To explore delayed correlation effects between the seven-day median number of genome copies/litre wastewater and the median seven-day incidence of SARS-CoV-2 cases, time-lagged Spearman's rank correlation coefficients were calculated for up to 14 days. In general, we considered values between 0.8 and 1 indicative of a very strong correlation. All statistical analyses were performed using STATA 16.0 (Stata Corp., College Station, Texas, USA).

Sequencing

From October 6 until November 15, 2021, weekly or bi-weekly (4 samples) RNA extracts from daily wastewater samples were used to genotype circulating SARS-CoV-2 variants by next-generation sequencing (NGS). From November 15 until December 31, 2021, due to the newly emerged B.1.1.529 variant, samples were analysed daily. From the 27 RNA wastewater extracts, cDNA transcripts were generated and analysed by NGS using the workflow described by Jahn et al. [7] including the optimised ARTIC v4 protocol [12].

Coordinated surveillance of circulating strains by sequencing was set up in Switzerland. After the emergence of Omicron and the partial failure of some PCR tests to detect this new variant (due to S gene dropout, as indicated by the WHO), the sequencing rate was accelerated to circumvent this problem. This applied to the first COVID-19 Omicron cases.

Mutation calling

NGS analysis of wastewater data was performed using V-pipe ([13]; https://github.com/cbg-ethz/V-pipe, Apach2-2.0 license), a bioinformatics pipeline for end-to-end analysis of viral sequencing reads obtained from mixed samples. Individual mutation calling is based on base count per position queried from the alignments generated from V-pipe. The prevalence of these individual mutations among variants was determined using COV-spectrum [14]. To enhance the signal strength of variants in each sample, co-occurring signature mutations on the same pair were also considered. This was performed via mutation co-occurrence using the software package Cojac (https://github.com/cbg-ethz/cojac, GPL-3.0 license) as

described previously [7]. A detailed description of the procedure for analysing the wastewater samples, together with links to exact versions of software used and additional notebooks, can be found at https://github.com/cbg-ethz/cowwid; the last version used for this publication is 0.7. Information about the current variant prevalence is available online via https://bsse.ethz.ch/cbg/research/computational-virology/sarscov2-variants-wastewater-surveillance.html.

Results

Detection of SARS-CoV-2 RNA in wastewater

Since the initiation of wastewater monitoring in the catchment area of the city of Basel, Switzerland, two peaks of SARS-CoV-2 concentration occurred. The first revealed a maximal absolute SARS-CoV-2 RNA titre of 1.99 $\times 10^5$ genome copies (gc)/L wastewater (figure 1A; 5.20 $\times$ 10^{12} gc/day and 100,000 inhabitants; normalised for the wastewater influx volume of 100,000 inhabitants; data not shown) on August 19, 2021. The pepper mild mottle virus–normalised curve reached a maximum of 1.41 $\times$ 10^{-2} on July 30, 2021 (data not shown). The second increase in SARS-CoV-2 concentration began on November 11, 2021, with the maximum outside the reported timeframe. Until December 31, 2021, the highest levels were measured on December 7, 2021, with a SARS-CoV-2 RNA titre of 4.13 $\times$ 10^5 gc/L wastewater and 1.08 $\times$ 10^{13} gc/100,000 inhabitants, and on December 23–26, 2021 (pooled sample) with a pepper mild mottle virus–normalised SARS-CoV-2 3.98 $\times$ 10^{-2}, respectively.

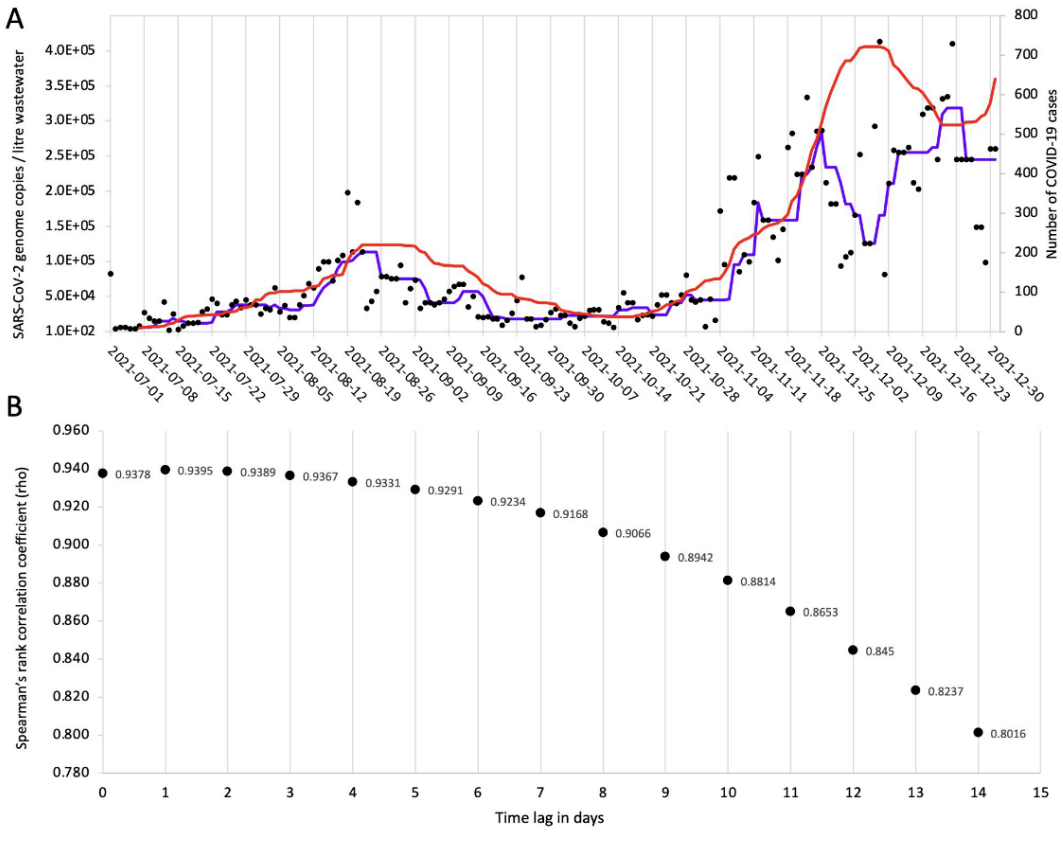
Correlation with epidemiological data

The number of daily cases and the median seven-day incidence of SARS-CoV-2 infections in the catchment area showed a high correlation with SARS-CoV-2 measurements in wastewater samples as measured by the Spearman's rank correlation coefficients (figure 1A and table 1). All correlations between the seven-day median number of gc/L wastewater (non-normalised, PMMoV–normalised and inflow-volume normalised) and time-lagged median seven-day incidence of SARS-CoV-2 cases were significant ($p < 0.001$) for the investigated lag of up to 14 days. Correlation coefficients declined constantly from the maximum of 0.9395 on day 1 to the minimum of 0.8016 on day 14 (figure 1B).

High sensitivity in low-incidence periods

The SARS-CoV-2 RNA signal was detectable even in very low COVID-19 incidence periods at the beginning of the regular sampling. Between July 1 and July 8, 2021, when we observed the lowest SARS-CoV-2 RNA signal in wastewater (4 – 165 gc/PCR reaction, 3.79 $\times$ 10^3 – 8.25 $\times$

Figure 1: Detection of SARS-CoV-2 RNA in wastewater in relation to COVID-19 case numbers in the catchment area of the city of Basel during the indicated period A) The number of SARS-CoV-2 genome copies of each composite wastewater sample (dots) and their seven-day median (blue line) are shown per litre of wastewater (non-normalised). The number of newly reported COVID-19 cases is represented by the median seven-day incidence (red line). B) The seven-day median number of SARS-CoV-2 genome copies/litre wastewater was correlated with the time-lagged (zero to 14 days; x-axis) median seven-day incidence of SARS-CoV-2 cases, and Spearman's rank coefficients values are given.



10^4 gc/L wastewater), were between one to 10 newly reported COVID-19 cases per day (figure 1).

Detection of SARS-CoV-2 variant Omicron before reported cases

The signature mutations defining the B.1.617.2 Delta variant of SARS-CoV-2 were predominantly present in all samples sequenced before December 29, 2021 (frequency estimates >88%; figure 2). From November 21, 2021, mutations associated with the B.1.1.529 variant were traceable (frequency estimates 0.335%). Although only a few mutated reads were associated with Omicron, the detection was robust due to the co-occurrence of mutations on three amplicons (75, 77 and 78) that are all characteristic of Omicron (and two of these amplicons contain mutations that individually are characteristic of Omicron; sample 2021-11-21, see supplementary table in appendix). All mutation co-occurrences on the reported amplicons were mainly found in Omicron samples (<1% for other variants), including amplicon 78, whose mutations can be found (>1%) in other variants, but their co-occurrence is Omicron-specific.

The wastewater sample in which B.1.1.529 Omicron was first detected was collected one day before the first confirmed clinical cases were recorded on November 22, 2021. The increasing prevalence of Omicron in wastewater thereafter relates to a logistic growth rate of 0.17 (0.13–0.21) per day, corresponding to a maximum doubling time of 4.1 (3.3–5.3) days in this period.

Discussion

The officially reported case numbers showed a high correlation with daily SARS-CoV-2 measurements in wastewater samples collected in this central European city. This finding is in agreement with previous reports, for example from Australia, the Netherlands and the United States [1–3]. Correlations between median seven-day incidence as determined by wastewater measurements and case data remained significant and very strong over 14 days, supporting the potential of wastewater-based epidemiology as an early warning system [5, 6]. Correlation estimates based on either values normalised by measurements of PMMoV or inflow-volume to the wastewater treatment plant were similar to non-normalised estimates. However, further studies on larger and independent datasets are needed to confirm these findings before drawing conclusions on the utility of such normalisation efforts.

The analysis of wastewater samples for SARS-CoV-2 RNA proved very sensitive. As few as 10 newly confirmed COVID-19 cases amongst 273,075 inhabitants of the catchment area were detectable during a low-incidence period at the beginning of July 2021. Previously confirmed COVID-19 cases staying in home isolation and asymptomatic and otherwise unreported cases may be contributing equally to the daily shedding of SARS-CoV-2 into wastewater. The overall rate of asymptomatic infections comprises approximately a fourth, according to a meta-analysis [15]. These individuals supposedly shed as much SARS-CoV-2 in faeces as symptomatic patients [16, 17]. However, these reports also document non-shedders having an opposite effect on detectability. The heterogeneous viral load per gram of faeces further hinders the estimation of the minimal daily infection number essential for detection in wastewater [17–19]. For more precise estimates of the sensitivity of wastewater surveillance, a zero baseline of infections would be needed. A promising approach could be to combine clinical observations, COVID-19 test results and wastewater data [19], thus overcoming the heterogeneous viral load shed and shedding durations.

A strong indicator of the high sensitivity of the wastewater-based epidemiology approach reported here is the identification of the SARS-CoV-2 B.1.1.529 variant by NGS before the recognition of the first clinical case in Switzerland. Recently, Kirby et al. reported similar early detection in wastewater in the United States [20]. This confirms genotyping of viral strains from wastewater as an effective tool to gain insights into virus variants circulating in the community [7, 21–24]. Since the first two detected individuals with an Omicron infection in Basel had no epidemiological link to South Africa and no travel history, our wastewater analysis supports that Omicron must have arrived in late November in our catchment area before its official acknowledgement by the WHO on November 22, 2021.

Conclusion

In this proof-of-concept study, wastewater-based epidemiology proved a reliable and sensitive surveillance approach, complementing routine clinical testing for mapping the COVID-19 pandemic dynamics and observing newly circulating SARS-CoV-2 virus variants. This is particularly important when testing regimes are hampered for sociopsychological or socioeconomic reasons or will be downscaled soon. The validity of this method was further confirmed by its detecting the most recently arising SARS-CoV-2 B.1.1.529 variant in a wastewater sample collected before the first COVID-19 confirmed infections. Wastewater-based epidemiology can serve as an important and cost-effective instrument beyond the current pandemic for

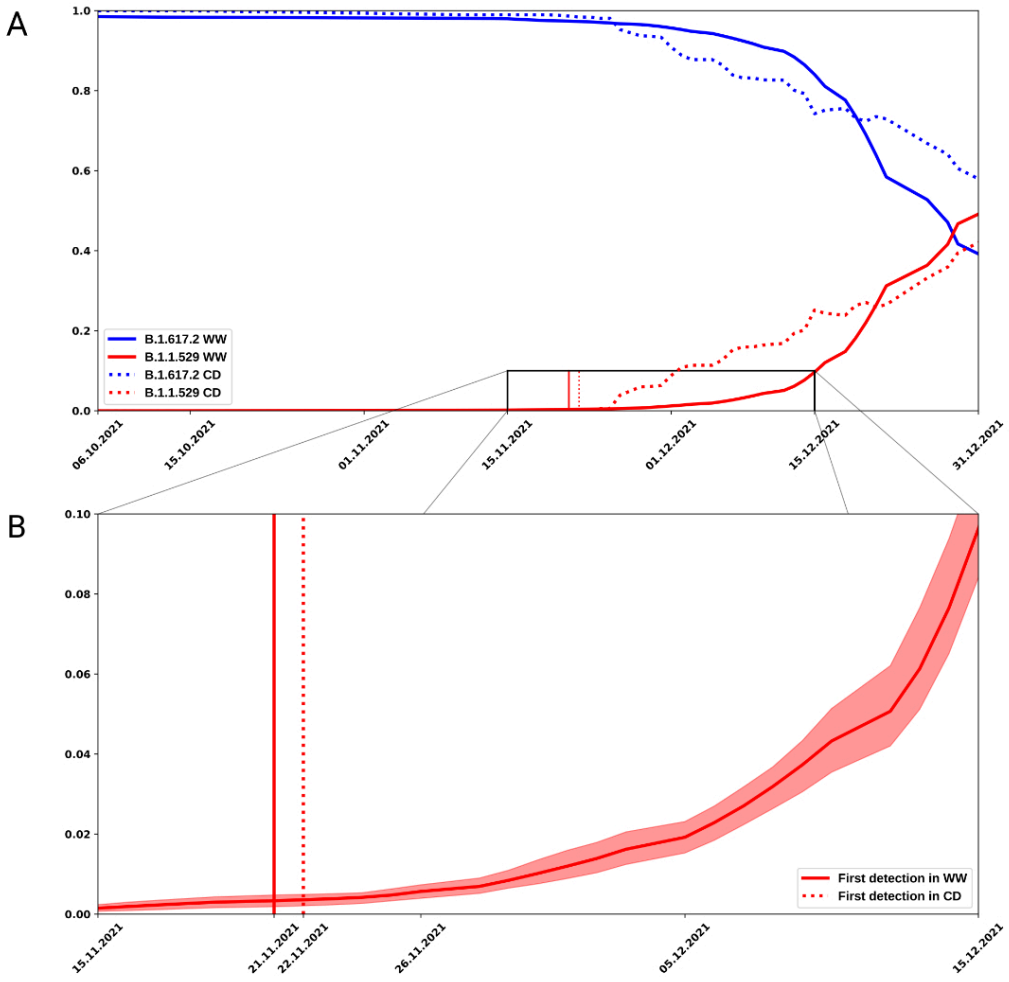
Table 1:
Correlations between different wastewater measures of SARS-CoV-2 viral loads and reported case numbers of SARS-CoV-2 infections (n = 179)

Reported case numbers of SARS-CoV-2 infections	Wastewater-measurements (n = 179*)	Spearman's rho	p-value
Number of daily cases	Daily genome copies / litre wastewater	0.821	<0.001
	Daily genome copies / litre wastewater, PMMoV**-normalised	0.798	<0.001
	Daily genome copies / litre wastewater, inflow-volume-normalised	0.828	<0.001
Seven-day incidence (median)	Seven day incidence of genome copies / litre wastewater	0.938	<0.001
	Seven day incidence of genome copies / litre wastewater, PMMoV-normalised	0.903	<0.001
	Seven day incidence of genome copies / litre wastewater, inflow-volume-normalised	0.931	<0.001

*The data used for the correlation calculation included the daily number of SARS-CoV-2 genome copies in wastewater (non-normalised, PMMoV-normalised and inflow-volume normalised) of 179 samples collected from July 6, 2021 to December 31, 2021 (n = 179) and the number of daily cases and seven-day incidence (median), respectively.

**PMMoV Pepper Mild Mottle Virus

Figure 2: Prevalence of B.1.617.2 (Delta) and B.1.1.529 (Omicron) variants of SARS-CoV-2 in wastewater samples and confirmed cases A) Estimated prevalence of B.1.617.2 (Delta; blue) and B.1.1.529 (Omicron; red) variants of SARS-CoV-2 in wastewater in the city of Basel (ridge regression and LOESS smoothing; solid lines; WW wastewater) and among confirmed cases (from clinical swab test; rolling average of 20 days; dashed lines; CD case data) are shown. These curves were generated using data from samples spanning from July 1, 2021, until January 13, 2022. The boxed region is depicted in more detail in panel B. B) Zoomed-in prevalence focusing on the period November 15 to December 15, 2021, and fraction range [0;0.1] of initial increase of B.1.1.529 (Omicron) after the first detection in wastewater on November 21, 2021, and confirmed cases on November 22, 2021, with confidence bands. Curves were estimated by performing ridge regression followed by LOWESS smoothing on the individual SNVs over the considered period. Mutation proportions were deconvolved into variant proportions by ridge regression and temporally smoothed using LOWESS.



detecting the dynamics of other circulating viruses or newly arising pathogens [25–27].

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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Appendix

Table S1:
Early detection of B.1.1.529 (Omicron) variants of SARS-CoV-2 in wastewater samples with amplicons co-occurence.

Date of sample collection(Year-month-day)	Amplicon 75 [22428-22785] omicron B.1.1.529 22578A*, 22673CTC*, 22679C	Amplicon 76 [22677-23028] omicron B.1.1.529 22679C, 22813T, 22882G*, 22898A*, 22992A, 23013C	Amplicon 77 [22974-23327] omicron B.1.1.529 22992A, 23013C, 23040G*, 23048A*, 23055G*, 23202A*	Amplicon 78 [23246-23611] omicron B.1.1.529 23525T, 23599G	Amplicon 88 [26277-26635] omicron B.1.1.529 26530G*, 26577G
2021-11-15	0 / 756 0.00%	0 / 30 0.00%	0 / 2650 0.00%	1 / 66395 0.00%	0 / 3132 0.00%
2021-11-16	0 / 1605 0.00%	0 / 53 0.00%	0 / 5601 0.00%	0 / 173574 0.00%	0 / 6650 0.00%
2021-11-17	0 / 1649 0.00%	0 / 39 0.00%	0 / 8804 0.00%	1 / 228000 0.00%	0 / 12211 0.00%
2021-11-18	0 / 176 0.00%	0 / 4 0.00%	0 / 876 0.00%	0 / 45134 0.00%	0 / 803 0.00%
2021-11-19	0 / 3549 0.00%	0 / 196 0.00%	0 / 19599 0.00%	1 / 333395 0.00%	0 / 10995 0.00%
2021-11-21	11 / 675 1.63%	0 / 23 0.00%	55 / 1763 3.12%	1655 / 80628 2.05%	0 / 1516 0.00%
2021-11-22	28 / 932 3.00%	0 / 152 0.00%	314 / 13050 2.41%	0 / 240207 0.00%	0 / 11491 0.00%
2021-11-23	0 / 237 0.00%	0 / 9 0.00%	0 / 999 0.00%	0 / 36861 0.00%	0 / 781 0.00%
2021-11-25	0 / 824 0.00%	0 / 6 0.00%	0 / 1939 0.00%	0 / 39353 0.00%	0 / 924 0.00%
2021-11-26	23 / 775 2.97%	0 / 57 0.00%	0 / 5681 0.00%	0 / 113299 0.00%	0 / 2342 0.00%
2021-11-28	0 / 0 NA	0 / 5 0.00%	0 / 0 NA	429 / 32761 1.31%	0 / 618 0.00%
2021-11-29	0 / 1174 0.00%	0 / 2 0.00%	0 / 1264 0.00%	0 / 334641 0.00%	0 / 8022 0.00%
2021-11-30	9 / 303 2.97%	0 / 11 0.00%	37 / 1911 1.94%	0 / 49833 0.00%	0 / 1663 0.00%
2021-12-02	1 / 91 1.10%	0 / 9 0.00%	3 / 600 0.50%	0 / 17460 0.00%	0 / 479 0.00%
2021-12-03	27 / 1938 1.39%	0 / 75 0.00%	0 / 10933 0.00%	0 / 194015 0.00%	0 / 6502 0.00%
2021-12-05	19 / 1139 1.67%	0 / 4 0.00%	63 / 3660 1.72%	1409 / 69959 2.01%	0 / 1596 0.00%
2021-12-06	43 / 3951 1.09%	0 / 195 0.00%	0 / 27869 0.00%	3261 / 218885 1.49%	0 / 10835 0.00%
2021-12-07	1 / 212 0.47%	0 / 6 0.00%	17 / 1234 1.38%	233 / 30697 0.76%	0 / 608 0.00%
2021-12-08	22 / 477 4.61%	0 / 16 0.00%	40 / 1436 2.79%	1 / 140567 0.00%	0 / 7642 0.00%
2021-12-09	0 / 233 0.00%	0 / 23 0.00%	0 / 27 0.00%	0 / 413 0.00%	0 / 318 0.00%
2021-12-10	0 / 2136 0.00%	0 / 182 0.00%	0 / 195 0.00%	0 / 3998 0.00%	0 / 2634 0.00%
2021-12-12	0 / 1435 0.00%	0 / 138 0.00%	0 / 161 0.00%	0 / 3458 0.00%	0 / 2250 0.00%
2021-12-13	0 / 257 0.00%	0 / 14 0.00%	0 / 15 0.00%	41 / 449 9.13%	0 / 429 0.00%
2021-12-14	0 / 298 0.00%	0 / 15 0.00%	0 / 12 0.00%	26 / 554 4.69%	0 / 349 0.00%
2021-12-15	0 / 1486 0.00%	0 / 91 0.00%	0 / 104 0.00%	106 / 1737 6.10%	0 / 1991 0.00%
2021-12-16	0 / 11 0.00%	0 / 2 0.00%	24 / 1308 1.83%	5255 / 79680 6.60%	0 / 990 0.00%
2021-12-18	0 / 5 0.00%	0 / 1 0.00%	12 / 359 3.34%	1202 / 29999 4.01%	0 / 277 0.00%

Co-occurrence signals (defined as ≥5 read pairs and ≥0.1% frequency) are highlighted with grey background. The earliest detection was in sample from collection date November 21, 2021 (2021-11-21) on amplicons 75, 77 and 78. Individual mutations that are mainly found in Omicron (<1% in other variants) are indicated with an asterisk (*). Notice that amplicon 76 and 88 have poor coverage with Omicron due to several mutations in regions targeted by primers of the ARTIC V4 protocol.