



Wastewater surveillance allows early detection of SARS-CoV-2 omicron in North Rhine-Westphalia, Germany



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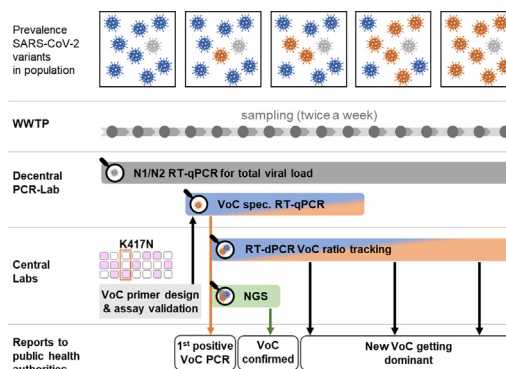
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HIGHLIGHTS

- Study reporting the first detection of SARS-CoV-2 Omicron in wastewater in NRW, Germany
- Successive workflow using RT-qPCR, dPCR and NGS sequencing
- SARS-CoV-2 Omicron detected as early as 8th December 2021
- Confirmation of Omicron by NGS in a sample obtained on 14th December 2021
- Monitoring of SARS-CoV-2 mutant fractions using digital PCR confirming Omicron as the dominant variant on 19th January 2022

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater-based epidemiology (WBE) has demonstrated its importance to support SARS-CoV-2 epidemiology complementing individual testing strategies. Due to their immune-evasive potential and the resulting significance for public health, close monitoring of SARS-CoV-2 variants of concern (VoC) is required to evaluate the regulation of early local countermeasures. In this study, we demonstrate a rapid workflow for wastewater-based early detection and monitoring of the newly emerging SARS-CoV-2 VoCs Omicron in the end of 2021 at the municipal wastewater treatment plant (WWTP) Emschermuendung (KLEM) in the Federal State of North-Rhine-Westphalia (NRW, Germany).

Initially, available primers detecting Omicron-related mutations were rapidly validated in a central laboratory. Subsequently, RT-qPCR analysis of purified SARS-CoV-2 RNA was performed in a decentral PCR laboratory in close proximity to KLEM. This decentralized approach enabled the early detection of K417N present in Omicron in samples collected on 8th December 2021 and the detection of further mutations (N501Y, Δ69/70) in subsequent biweekly sampling campaigns. The presence of Omicron in wastewater was confirmed by next generation sequencing (NGS) in a central laboratory with samples obtained on 14th December 2021. Moreover, the relative increase of the mutant

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fraction of Omicron was quantitatively monitored over time by dPCR in a central PCR laboratory starting on 12th December 2021 confirming Omicron as the dominant variant by the end of 2021.

In conclusions, WBE plays a crucial role in surveillance of SARS-CoV-2 variants and is suitable as an early warning system to identify variant emergence. In particular, the successive workflow using RT-qPCR, RT-dPCR and NGS demonstrates the strength of WBE as a versatile tool to monitor variant spreading.

1. Introduction

The coronavirus disease 2019 (COVID-19) is caused by an infection with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). The origin of SARS-CoV-2 was described in December 2019 in the Chinese metropolis of Wuhan and rapidly evolved into a global pandemic (Zhou et al., 2020; Zhu et al., 2020). Since the onset of the pandemic, over 479 million infections and a total of 6.1 million SARS-CoV-2 associated deaths (accessed 03-30-2022) have been reported globally (WHO, 2022). Due to the ongoing global vaccination shortage and the emergence of new viral variants, the pandemic continues to pose new challenges to public health care systems.

As a natural adaptation to the host, nucleotide substitutions, insertions, or deletions might arise in the SARS-CoV-2 genome. However, only certain mutations are able to change the viral properties in a significant manner affecting transmissibility, susceptibility to monoclonal antibodies (mAb) used for treatment and prophylaxis, and to antibodies from convalescent and vaccine-elicited sera (Harvey et al., 2021). It has been shown that SARS-CoV-2 Omicron exhibits a high resistance to antibody-mediated neutralization by vaccine-elicited sera as well as to sera antibodies from individuals who were both vaccinated and SARS-CoV-2 infected (Cameroni et al., 2021; Cao et al., 2021; Carreno et al., 2022; Cele et al., 2021; Dejnirattisai et al., 2022; Muik et al., 2022; Planas et al., 2021; Rossler et al., 2022; Wilhelm et al., 2021). Since mutations can also limit therapeutic options, for example with monoclonal antibodies (Cathcart et al., 2022; VanBlargan et al., 2022; Wilhelm et al., 2021), it is highly relevant to observe the spread of relevant variants on a broad scale.

The detection of SARS-CoV-2 gene fragments in wastewater was already reported shortly after the outbreak of the pandemic. A first study showed that SARS-CoV-2 gene fragments are detectable and quantifiable using molecular reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) analysis (Medema et al., 2020). Among others, our study covering wastewater treatment plants in Northrhine-Westfalia during the first COVID-19 wave in 2020 showed the non-infectivity of the SARS-CoV-2 gene fragments detected in wastewater and the principle correlation between viral load in the wastewater and the number of infections in the catchment area (Westhaus et al., 2021). Various SARS-CoV-2 variants have also been detected in wastewater samples including Omicron, utilising RT-qPCR, digital (droplet) d(d)PCR, and genomic sequencing (NGS) methods (Agrawal et al., 2022; Ahmed et al., 2022; Crits-Christoph et al., 2021; Heijnen et al., 2021; Ho et al., 2022; Izquierdo-Lara et al., 2021; Martin et al., 2020). Each of these methods has its specific advantages and limitations in terms of costs, processing time, specificity, and sensitivity (Afzal, 2020; Mardian et al., 2021). In particular, the cost- and time-effective PCR workflow allows the detection of individual VoC-characteristic mutations. In contrast to RT-qPCR, the RT-dPCR method additionally enables absolute quantification without the need of a target specific calibration curve in each run. Nevertheless, an important limitation of all PCR methods is the targeting of a single or only few pre-selected genomic substitutions. However, each SARS-CoV-2 lineage is defined by multiple unique nucleotide substitutions, which have to be covered for unambiguous lineage definition. NGS is able to provide full genomic information but is time and resource intensive and also lacks the high sensitivity compared to PCR-based method (Maljkovic Berry et al., 2020). Furthermore, specialized equipment and qualified bioinformatic personnel is needed.

In a recommendation published by the European Union commission in March 2021 (C(2021)1925), the establishment of a wastewater-based systematic surveillance of SARS-CoV-2 and its relevant variants was urged.

In particular, the monitoring of SARS-CoV-2 occurrence in wastewater was recommended as a valuable source of information about the degree of virus circulation in the community connected to a particular wastewater drainage and treatment system, complementing individual testing strategies (reviewed in Bonanno Ferraro et al., 2021; Sangkham, 2021; Thompson et al., 2020).

This study presents a successive workflow using multiple detection methods (RT-qPCR, NGS, RT-dPCR) to enable a coordinated approach for rapid detection, characterisation and tracking of the spread of the SARS-CoV-2 Omicron variant. It was developed as part of COVIDready, a German consortium for research on decentralized wastewater monitoring. In association with SARS-GenA-Seq, a German consortium focusing on NGS sequencing of SARS-CoV-2 RNA in wastewater, the aim of COVIDready is to implement the roll-out of a national wastewater monitoring system in Germany. Our step-wise coordinated workflow, using the advantages of each method, will be an important contribution in preparing for and combating future pandemic waves.

2. Material and methods

2.1. Sewage sampling

Wastewater samples were collected at the municipal WWTP Kläranlage Emschermündung (KLEM) North-Rhine Westphalia (NRW, Germany). WWTP KLEM operated by the public German water board Emschergenossenschaft is located at the mouth of river Emscher to river Rhine. KLEM has a design capacity of 1,758,965 population equivalents and had 906,222 connected residents. The wastewater flow was 348,703,426 m³ per year (m³/a) as determined for 2020. Designed originally as a river sewage treatment plant, WWTP KLEM treated the complete flow of the river Emscher until end of 2021. Since completion of the Emscher rehabilitation operating a separate sewer network, KLEM now treats undiluted municipal sewage from the connected catchment area, which is partly industrial. Using an installed autosampler, flow-proportional 24-h composite samples were collected after the grit chamber at the WWTP inlet. Samples were collected on Wednesdays and Sundays to allow surveillance of commuters and permanent residents within the catchment area. The samples were transported to the cooperative laboratory in Essen at a temperature of 4 °C within 12 h on the day of collection and processed immediately.

2.2. Decentral sample processing and RT-qPCR quantification of viral RNA

In the cooperative laboratory of Emschergenossenschaft/Lippeverband and Ruhrverband in Essen sample preparation was performed following a workflow recommended by Analytik Jena (Jena, Germany, see web reference; https://www.analytik-jena.com/fileadmin/content/applications/Automated_Extraction/InnuPure_C16_touch/AppNote_Extraction_0005_en.pdf). Accordingly, a volume of 100 ml of each sample was filtrated using electronegatively charged membrane filters (0.45 µm, MF-Millipore, Merck) placed in a stainless steel pressure filtration device at a pressure of 6 bar using pressurized N₂ gas. After filtration, the filter was cut into strips, which were placed into Lyses Tubes (Lyses Tubes J, Analytik Jena). Only four of the five provided metal beads were used in the process. We found that lyses of the filter was most effective placing two of the beads under and two beads over the cut filter material. Subsequently, one ml of DNA/RNA shield reagent (DNA/RNA Shield, Zymo Research Europe, Freiburg im Breisgau, Germany) was added and the samples were placed in a

speedmill (SpeedMill plus, Analytik Jena) for two minutes at 50 Hz using the continuous mode. To remove the debris, samples were centrifuged at 9520 xg for two minutes. The supernatant was transferred to 1.5 ml reaction tubes and used for further processing. Total RNA of the samples was isolated using the automated purification system InnuPure C16 touch (Analytik Jena, Jena) and the innuPREP AniPath DNA/RNA Kit IPC16 according to the manufacturer's instructions. Samples with added IC (IDEXX Water Internal Control, IDEXX, Kornwestheim, Germany) were included as an extraction control. Extracted RNA was used directly for RT-qPCR and subsequently stored at -80°C .

The initial screening for the presence of the Omicron variant was performed at the cooperative laboratory in Essen by analysis of the following mutations: K417N, N501Y, and $\Delta 69/70$. The proprietary primer and probes (SARS-CoV-2 Variant Panel-8 Targets) were purchased from Promega (Promega, Walldorf, Germany). For amplification, the QuantiNova Pathogen + IC Kit (Qiagen, Hilden, Germany) was used on a qTOWER³ real-time-thermocycler (Analytik Jena, Jena, Germany) according to the manufacturers' instructions.

2.3. Sample processing for variant specific digital PCR quantification

In the research laboratory at the Institute for Medical Virology of the University Hospital Frankfurt am Main, wastewater samples were processed in an optimized workflow, whose first part was adapted from the previously described 4S method for SARS-CoV-2 RNA extraction from wastewater (Whitney et al., 2021): 40 ml of wastewater samples were poured into a 50 ml tube containing 9.35 g sodium chloride and 400 μl of TE buffer (1 M Tris, 100 mM EDTA, pH: 7.2). Samples were agitated until all sodium chloride was dissolved and heat-inactivated at 70°C for 45 min. Next, each sample was filtered twice through a 5 μm filter. Repeating the filtration was particularly crucial for turbid wastewater samples to avoid downstream clogging of silica membranes. Next, 70 % ethanol was added in a 1:1 ratio and samples thoroughly agitated.

In the second part of sample processing, RNA was extracted and concentrated in two steps using an adapted protocol of the Wizard Enviro TNA Kit (Promega, Walldorf, Germany). The filtered and 70 % ethanol containing wastewater samples were directly poured into the Reservoir Extension Funnels on PureYield Binding Columns (Promega, Walldorf, Germany) that were connected to a vacuum manifold. All further steps were, unless otherwise specified, conducted according to the manufacturers protocol. During the concentration and further clean-up of 1 ml extracted RNA per sample, all steps until the first wash with Column Wash 2 (RWA) were conducted using the vacuum manifold instead of using the microcentrifuge. Finally, RNA was eluted twice in 25 μl RNA-free water for subsequent dPCR analysis.

RNA was analysed using a one-step RT-dPCR approach using the QIAcuity One-Step Viral RT-PCR Kit (Qiagen, Hilden, Germany) with a QIAcuity One Digital PCR System (Qiagen, Hilden, Germany). Primer and probe sequences (Supplementary Table 1) were obtained from Integrated DNA Technologies (IDT).

2.4. SARS-CoV-2 variant specific quantitative RT-qPCR

The initial screening for the presence of the SARS-CoV-2 Omicron variant was performed at the cooperative laboratory in Essen by analysis of the following mutations: Deletion 69/70, N501Y and K417N. The proprietary primer and probes (SARS-CoV-2 Variant Panel-8 Targets) were purchased by Promega (Promega, Walldorf, Germany).

2.5. NGS analysis

For sequencing, 200 ml of untreated wastewater was concentrated using 100-kDa Centricon Plus-70 centrifugal ultrafilters (Merck). RNA was extracted from the concentrate using the MagMAX Microbiome Ultra Nucleic Acid isolation kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Next generation sequencing (NGS) was performed of

the sample taken on 14 December 2021 on an Ion torrent platform, using the Ion AmpliSeq SARS-CoV-2 research panel (Thermo Fisher Scientific) according to a previous study (Agrawal et al., 2022). This panel consists of 237 primer pairs, resulting in an amplicon length range of 125 to 275 bp. This allows for a nearly full coverage of the SARS-CoV-2 genome. Briefly, using the Ion AmpliSeq SARS-CoV-2 research panel, the amplicon library for the sample was synthesized and sequenced using an Ion Torrent 540 chip on an Ion S5 sequencer (Thermo Fisher Scientific). The data analysis was done directly using installed software packages, i.e., the Ion Torrent Suite v5.12.2. Sequences were aligned to a SARS-CoV-2 reference genome (Wuhan-Hu-1 [GenBank accession numbers NC_045512 and MN908947.3]), using the TMAP software included in the Ion Torrent Suite and all single-nucleotide variants (SNVs) were called using Variant Caller v5.16.0.5 with default parameters.

2.6. Specificity testing using authentic SARS-CoV-2 isolates

SARS-CoV-2 isolates were obtained from nasopharyngeal swabs of travel returnees as screened by the Public Health Office of the City of Frankfurt am Main, Germany. Swab material was suspended in 1.5 ml phosphate-buffered saline (PBS) and used for viral outgrowth assays and viral stock preparations. RNA from cell culture supernatants was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. RNA was subjected to variant specific RT-qPCR genotyping and Oxford Nanopore sequencing as described previously (Wilhelm et al., 2021). As described previously SARS-CoV-2 isolates were propagated using Caco-2 and A549-AT cells, which were selected for high permissiveness to SARS-CoV-2 infection by serial dilution and passaging (Bojkova et al., 2020; Cinatl et al., 2004; Widera et al., 2021b). All cell culture work involving infectious SARS-CoV-2 was performed under biosafety level 3 (BSL-3) conditions. Sample inactivation for further processing was performed with previously evaluated methods (Widera et al., 2021a).

2.7. Epidemiological data

Epidemiological data on SARS-CoV-2 cases including the variant specific portions in NRW were obtained from the official data repository of the Federal Robert Koch Institute (RKI) in charge of public health surveillance (Federal-Robert-Koch-Institute, 2022).

3. Results

3.1. Wastewater surveillance allows early detection of SARS-CoV-2 omicron

Wastewater samples were initially analysed for total viral load (N1/N2) by RT-qPCR. Concentrations of the N1 and N2 gene fragments were between $1\text{E}+13$ and $1\text{E}+15$ copies/day, respectively (Fig. 1A). Differently high concentrations were observed for samples taken on either Wednesday or Sunday, which indicates a high fluctuation activity of commuters in addition to local residents. Congruent to the 7-day incidence, a slight decrease in SARS-CoV-2 RNA concentrations in KLEM derived wastewater was observed until Christmas, which was followed by a significant increase on the following days.

For an initial screening to detect the presence of emerging Omicron in wastewater, we used primers that were already available in early December 2021 for the detection of Omicron related mutations. A central PCR laboratory validated all primers thoroughly using several inactivated SARS-CoV-2 VoCs for subsequent RT-qPCR analysis in a decentral laboratory near the WWTP KLEM (see Section 2.6). RNA harbouring the K417N substitution characteristic for the SARS-CoV-2 Omicron variant was detected in two successive wastewater samples from the influent of KLEM on 8th of December 2021 (Fig. 1C). Further mutations (N501Y, $\Delta 69/70$) were detected in subsequent samples obtained on 19th December 2021. Simultaneously, for K417 (ct 31.2 - ct 40.6) as well as for N501 (ct 37.66 - ct 40.32) and positions 69/70 (ct 31.68 - ct 41.26), a significant increase of the ct-values could be observed, starting on December 1st and 8th, respectively, but

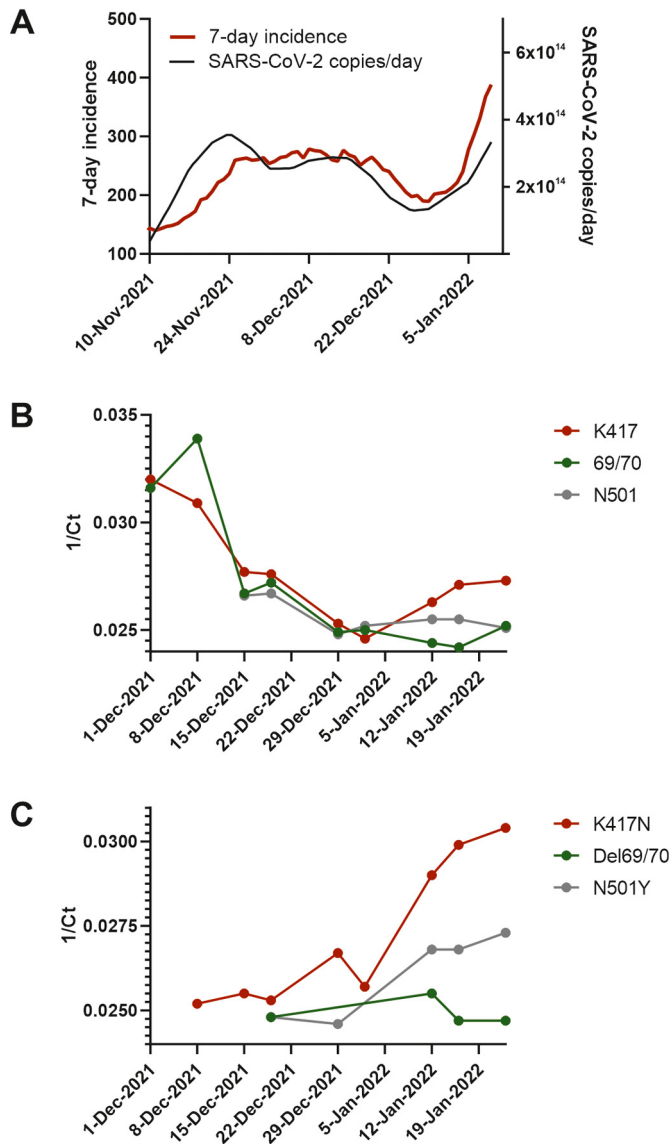


Fig. 1. Monitoring of SARS-CoV-2 viral RNA fragments in WWTP KLEM. A) Wastewater samples were analysed (RT-qPCR) for total viral load using primer pairs detecting two targets in the SARS-CoV-2 N gene (N1/N2). B and C) The presence of Omicron characteristic mutations K417N, N501Y and del69/70 in SARS-CoV-2 Spike was detected using RT-qPCR. Panel B illustrates the decline of non-Omicron amino acids K417, 69/70, and N501. Panel C shows the increase in Omicron specific substitutions K417N, del 69/70, and N501Y. The graphs on the left indicate the ct values over time. The corresponding reciprocal values are illustrated in the plot on the right.

with different dynamics. This first evidence of the non-exclusive Omicron-defining substitutions (K417N, N501Y and Δ 69/70) in conjunction with the significantly increasing ct-values for the non-Omicron-specific positions with constant total virus RNA levels, suggest a change in the ratios of circulating SARS-CoV-2 variants in the catchment area. However, whether this change in ratio might originate from the suspected Omicron variant was next determined by identification using NGS.

3.2. Confirmation of the Omicron variant by NGS

Genome sequencing was performed to determine the emergence of the SARS-CoV-2 Omicron variant in the catchment area of the WWTP KLEM. The Omicron variant has several characteristic mutations, but it shares many mutations with other variants. Therefore, it was necessary to verify the emergence of Omicron based on a complete pool of characteristic

mutations, especially for the early samples. A sample from the 14th December 2021 was sequenced, achieving an average base coverage depth of 6945 (bp) with 99 % genome coverage at 500 × depth. Overall, the following S protein mutations of the Omicron variant were detected: A67V, HV69–70del, T95I, 211del-L212I, 214EPEins, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q439K, G496S, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K and L981F (Fig. 2). Also, two N protein mutations, seven mutations in the open reading frame 1ab (ORF1ab) replication complex, and seven synonymous mutations were found. The allele frequency for most of the mutations ranged between 1 and 10 %, and the occurrence ranged between 1e + 02 - 1e + 04 reads. These results confirmed the occurrence of the SARS-CoV-2 Omicron variant in the catchment area of the WWTP KLEM as suspected from the previously PCR-based data.

3.3. Tracking the mutation fraction K417N using digital PCR

Since the assay for K417N was already available, and early experiments yielded the highest sensitivity and specificity for Omicron (Supplementary Fig. 2), position K417N was used as a surrogate marker to track the course of mutant fractions over time. Following individual testing, Omicron has become the dominant variant in Germany on a national scale and in NRW in less than four weeks (Fig. 3A, right panel). As the public available RKI data indicates, only a minor difference comparing the national and NRW specific 7-day incidence was observed during the study period (Fig. 3A, left panel). In agreement with public health data, the dPCR-based quantification of total SARS-CoV-2 RNAs (N1/N2) was precisely resembling the 7-day incidence (Fig. 3B–C). The detection of K417N clearly indicated the displacement of SARS-CoV-2 Delta as depicted in the drop of K417 and the increase in K417N (Fig. 3B right panel).

Of note, the strong increase in total SARS-CoV-2 RNA measured with a N1 and N2 specific assay strongly correlated with the rapid increase in K417N mutant fractions.

The dPCR-derived findings suggest that the Omicron variant occurred in the WWTP catchment area at a very low frequency on the 12th of December 2021. In line with public-health data derived from individual testing, the relative increase of the mutant fraction over time could be quantitatively monitored confirming Omicron as the predominant variant on 19th January 2022.

Our data on the current Omicron monitoring demonstrate the strength of decentralized wastewater monitoring. While clinical laboratories were overloaded in times of high incidences and a complete gene sequencing requires several days, a decentralized workflow followed by confirmation in independent specialized central laboratories has proven to be highly efficient. The rapid detection underscores the importance of wastewater monitoring as an integral information and surveillance system for emerging SARS-CoV-2 variants of concern. Wastewater monitoring will continue in consultation with public health authorities to determine the spread of Omicron subvariants BA.1, BA.1.1, and BA.2.

4. Discussion

As the pandemic has progressed, a variety of initiatives have developed globally for the detection of SARS-CoV-2 in wastewater, with a spectrum of analytical workflows mostly based on RT-qPCR after sample preparation and enrichment of the genetic material (La Rosa et al., 2020). In addition, the detection of viral mutations and in particular mutations found in SARS-CoV-2 VoC in wastewater is also implementable by genetic sequencing in specialized laboratories using NGS-approaches (Crits-Christoph et al., 2021). However, the sequencing-based detection of new SARS-CoV-2 variants in wastewater requires considerably higher concentrations of viral particles in comparison to PCR-approaches. Furthermore, this method is time and cost intensive and laboratory capacity is limited. In particular, during the peaks of the pandemic a lack of laboratory capacity in the public health care sector was reported bringing the focus to a decentralized wastewater testing system.

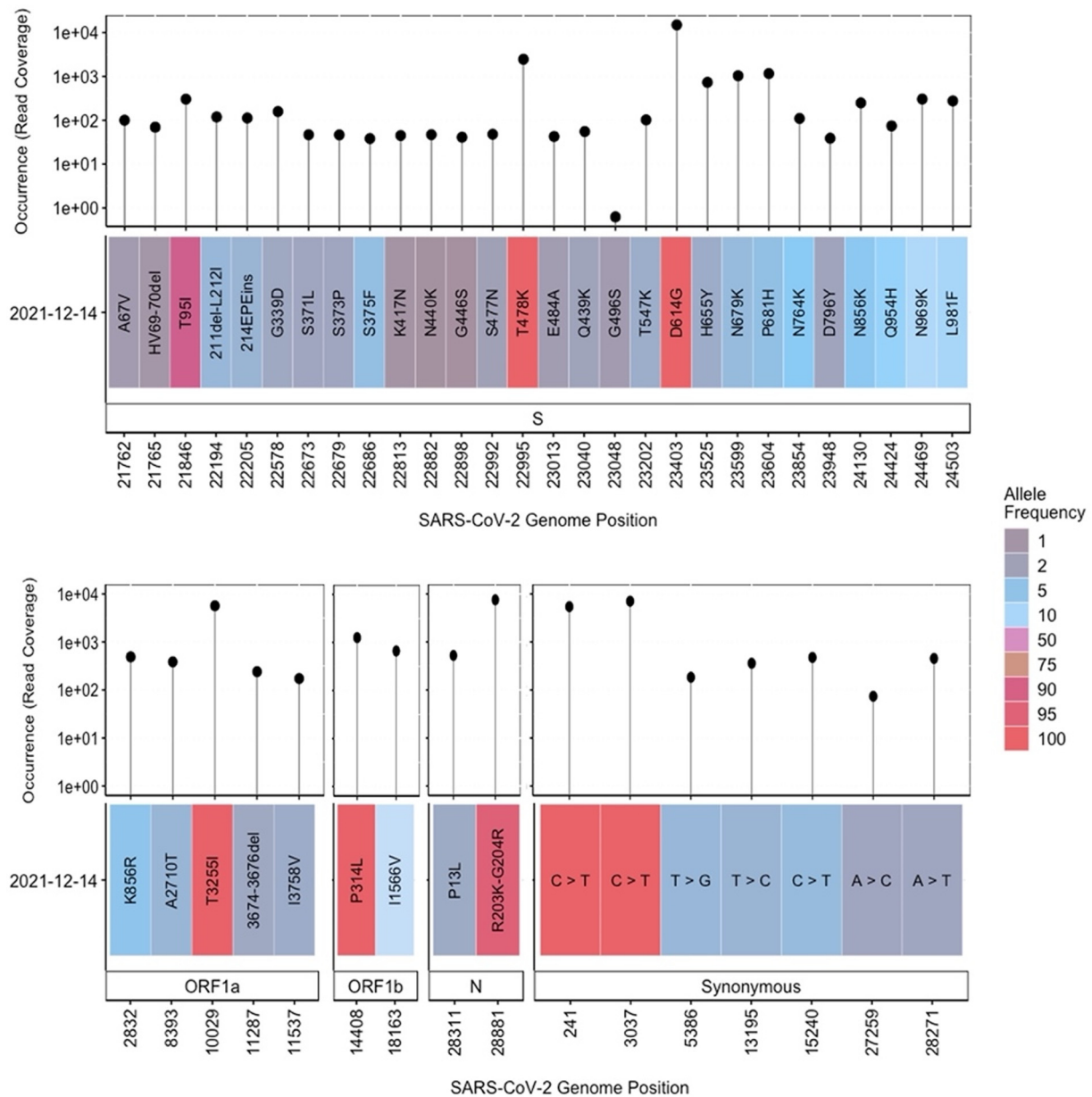


Fig. 2. Identification of the SARS-CoV-2 Omicron variant by NGS. Upper panel: Occurrence (i.e., the numbers of reads corresponding to each mutation) of characteristic S, E, M, and N protein mutations and ORF1ab mutations of the Omicron variant. Lower panels: Heatmap showing the allele frequency and the mutations corresponding to the Sars-CoV-2 genome position.

Our approach highlights that decentralized SARS-CoV-2 monitoring in WWTP laboratories equipped with PCR devices allow to cover a large part of the population and to send the analysis results within 48 h to the responsible health authorities.

SARS-CoV-2 RNA and, in particular, Omicron variant specific RNA in wastewater was investigated in two joint projects (COVIDready and SARS-GenA-Seq). In wastewater samples from the inlet of the WWTP KLEM in NRW, to which more than 900.000 residents are connected, mutations characteristic for Omicron were 1) detected by RT-qPCR, 2) confirmed by NGS sequencing, and 3) longitudinal quantified and tracked with dPCR. In the sense of an early warning system, our results confirm the feasibility of the coordinated workflow of decentralized and centralized laboratories that uses PCR for early detection and trend analyses and later confirmation by NGS. The establishment of the workflow in this study proved fast enough to detect the onset and rise of SARS-CoV-2 Omicron in the catchment areas of KLEM since the beginning of December 2021.

This early detection is in excellent agreement with the data provided by the data obtained from the official data repository of the RKI in charge of public health surveillance, which are based on the sequencing of individual samples. Of note, many of these individual samples are based on suspected cases and the data are compiled retrospectively (Federal-Robert-Koch-Institute, 2022). However, individual sample collection, testing, sequencing, bioinformatics, and the report of findings to the local public health authorities might take several days. Furthermore, in times of high incidence the lack of personnel may lead to an additional delay in data availability. In addition, public acceptance of frequent individual testing may decline as the pandemic proceeds leading to potentially biased reports that rely solely on clinical testing. The PCR-based wastewater derived data are available as early as 48 h after sample collection and might provide an initial integral picture of the pandemic situation and mutant spread even before information on individual scale is available.

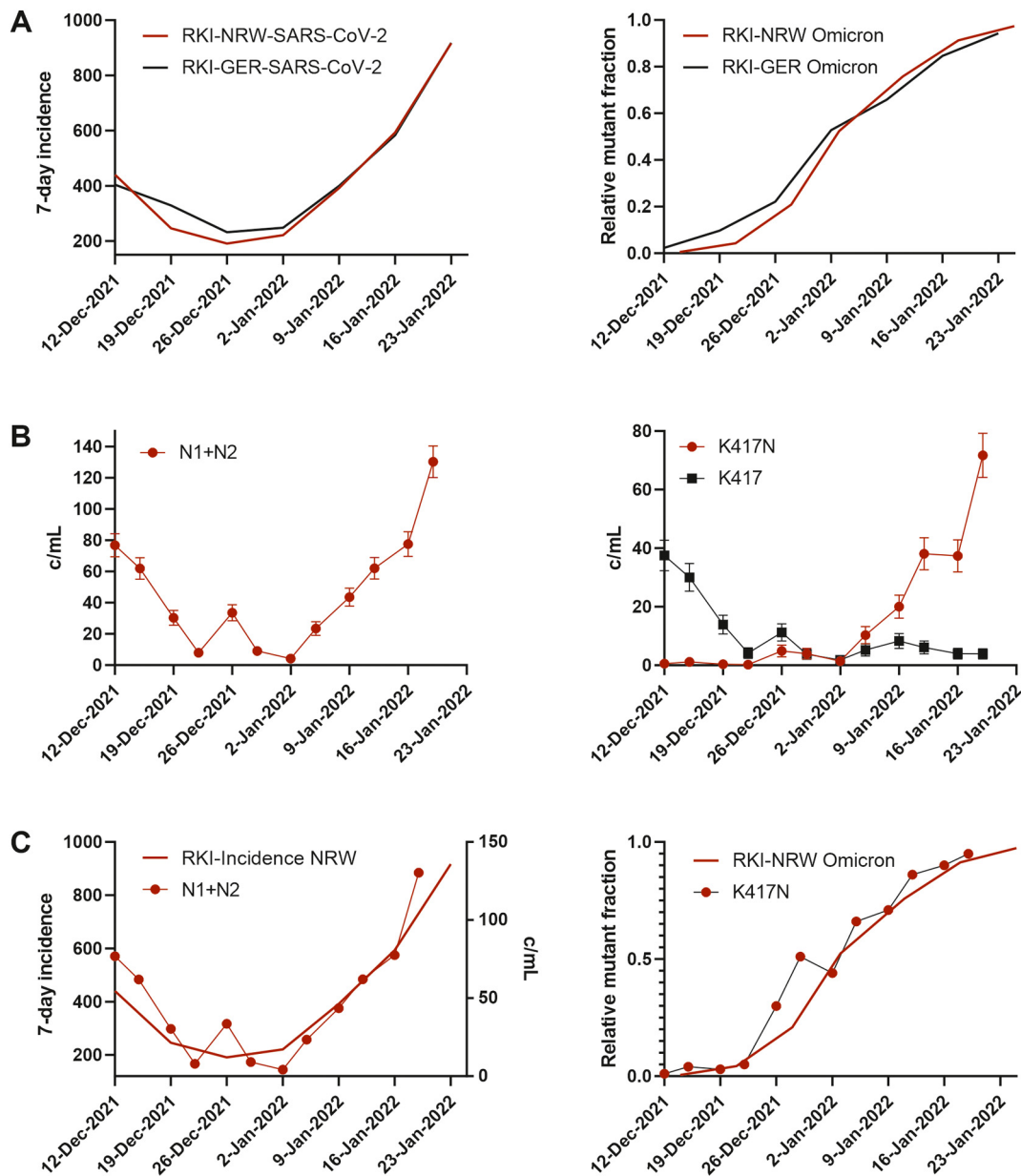


Fig. 3. Tracking of SARS-CoV-2 Omicron specific mutant fraction of K417N using digital PCR. A) 7-day incidence (left) and the Omicron mutant fraction (right) for Germany and North-Rhine-Westphalia (NRW) are indicated. Epidemiological data are based on individual testing and were obtained from official data repository of the German Federal Robert Koch Institute (RKI). B) Total SARS-CoV-2 levels (left) and the Omicron-detecting substitution K417N (right) determined in wastewater samples from WWTP KLEM. C) 7-day incidence in NRW correlates to detected SARS-CoV-2 levels in wastewater (left). The relative mutant fraction of Omicron determined by individual testing is highly comparable to the relative fraction of K417N found in wastewater (right).

In contrast to RT-qPCR, RT-dPCR allows absolute quantification without the need for a standard curve. Using the RT-dPCR method, the reaction is split into several thousand partitions with separate end-point amplifications while the number of positive partitions provides information about the quantity of molecules in the sample. Compared to RT-qPCR, this method is less susceptible to PCR inhibitors and non-specific nucleic acid amplifications, so that a higher sensitivity and specificity might be achieved. Both methods were comparable in terms of sensitivity, however, dPCR yielded more precise and reproducible data (Hindson et al., 2013). In this work, total SARS-CoV-2 levels and the time course of the relative increase in Omicron-specific mutations (K417N) could be reliably quantified using dPCR (Fig. 3). For a sensitive and specific detection of Omicron, a thorough evaluation of primers and probes used in RT-qPCR and RT-dPCR is of eminent importance (Supplementary Table 1). Using the permanently expanding library of authentic SARS-CoV VoCs archived in the BSL-3

facility of the specialized central laboratory, wastewater samples could be spiked with inactivated SARS-CoV-2 for PCR assay validation.

We suggest that the coordinated workflow established here may prove applicable also for VoC monitoring in the future. Our results confirm that accurate SARS-CoV-2 monitoring can be implemented in wastewater laboratories to support early warning of mutant SARS-CoV-2 variants. While we expect only a number of well-equipped wastewater laboratories to be in a position to establish and maintain centralized PCR analysis capacity, training of personnel, and acquisition of equipment and consumables, a decentralized approach seems to offer several advantages: (1) Full control over the workflow employed. While a comparison between inhouse-analysis and commercial laboratories for analytical cost and shipment delays is not yet possible, interlab comparison suggest, that for reliable SARS-CoV-2 trend analysis and interpretation, analytical protocols should be maintained during monitoring campaigns. Standardized ISO protocols,

which are still lacking, might help to implement comparable workflows based on common guidelines in decentral laboratories. (2) Restricting the number of wastewater samples for central dPCR and NGS analyses to decently selected samples only, optimizes limited analytical capacities during the pandemics. (3) Local expertise of WWTP operators is needed to discriminate the effects of sewage network operating conditions, including rain events, discontinuous industrial discharges, sewer infiltration water, and alteration in the connected population in the catchment area from SARS-CoV-2 infection dynamics. Thus, decentralized approaches may prove advantageous to establish close collaboration between municipal WWTP operators and municipal public health authorities for rapid reporting and data interpretation.

5. Conclusions

- Based on our findings, we conclude that wastewater-based epidemiology (WBE) can effectively support SARS-CoV-2 epidemiology complementing individual testing strategies. Particularly, at times of high incidence, WBE can be used to monitor the emergence of novel variants of concern (VoC) at early stages.
- An initial screening using already available detection assays allows early monitoring at the time of early suspicion of a possible imminent spread of a new variant. The evaluation of assays to detect Omicron related mutations using inactivated authentic SARS-CoV-2 VoCs is highly recommended.
- NGS overcomes shortcomings in the initial selectivity of SARS-CoV-2 primers and probes, which were non exclusively targeting SARS-CoV-2 Omicron RNA. Using NGS sequencing, we confirmed human SARS-CoV-2 Omicron, and propose a confirmation of positive RT-qPCR results by sequencing or other appropriate techniques in order to avoid false-positive results.
- Wastewater monitoring is conducted in exchange with local health authorities to estimate the infection incidence of SARS-CoV-2 and the spread of variants of concern in the catchment area of the WWTPs. Depending on the WWTP and catchment area with the respective ratio of commuters or permanent residents, WBE might provide time advance in reporting of several days compared to clinical findings.
- We conclude that a coordinated workflow might serve as a robust and sensitive early warning system in pandemic control. We recommend to implement a coordinated workflow in German wastewater-based epidemiology as a complementary measure in addition to individual testing strategies. Wastewater monitoring will continue in coordination with public health authorities to determine the spread of the Omicron subvariants including BA.1 and BA.2.

CRedit authorship contribution statement

Alexander Wilhelm: Investigation, Visualization, Methodology, Writing- Reviewing and Editing.

Jens Schoth: Writing- Reviewing and Editing, Visualization, Resources.

Christina Meinert-Berning: Investigation, Writing- Reviewing and Editing.

Shelesh Agrawal: Investigation, Visualization, Methodology, Writing- Reviewing and Editing.

Daniel Bastian: Visualization, Writing- Reviewing and Editing.

Laura Orschler: Investigation, Visualization, Methodology.

Sandra Ciesek: Writing- Reviewing and Editing, Supervision, Project administration.

Burkhard Teichgräber: Project administration, Supervision, Resources, Writing- Reviewing and Editing.

Thomas Wintgens: Writing- Reviewing and Editing, Supervision, Project administration.

Susanne Lackner: Writing- Reviewing and Editing, Supervision, Project administration.

Frank-Andreas Weber: Conceptualization, Writing- Reviewing and Editing, Funding acquisition, Project administration.

Marek Widera: Investigation, Visualization, Writing- Original draft preparation, Funding acquisition, Conceptualization, Supervision, Validation.

Declaration of competing interest

Alexander Wilhelm reports equipment, drugs, or supplies were provided by QIAGEN GmbH. Qiagen GmbH and Endress + Hauser are associated industry partner of the COVIDready consortium.

Jens Schoth and Burkhard Teichgräber declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Qiagen GmbH and Endress + Hauser are associated industry partner of the COVIDready consortium.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.157375>.

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