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Brief Report

Rapid Spread of Omicron Sub-Lineage as Evidence by Wastewater Surveillance

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Abstract: The search for better tools for interpreting and understanding wastewater surveillance has continued since the beginning of the coronavirus disease 2019 (COVID-19) pandemic. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has continued to mutate, thus complicating the interpretation of surveillance results. We assessed the Omicron variants (BA.1, BA.2, and BA.5) associated with wastewater-derived SARS-CoV-2 RNA trends by estimating the effective reproduction number (R_{eff}) using an epidemic model that integrates explicitly the SARS-CoV-2 N2 gene concentration detected in wastewater through rt-qPCR quantitative analysis. The model inferred COVID-19 cases based on wastewater data and compared them with the ones reported by clinical surveillance. The variant of the SARS-CoV-2 associated with the wastewater-derived viral RNA was monitored through wastewater whole-genome sequencing. Three major waves between January and September 2022 were associated with the Omicron subvariants (BA.1, BA.2, and BA.5). This work showed that disease trends can be monitored using estimates of the effective reproduction number which is simple and easy to understand.

Keywords: effective reproduction number; wastewater surveillance; omicron waves; transmissible mutation; BA.1; BA.2; BA.5; whole genome sequencing

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the COVID-19 pandemic, has acquired mutations that have given rise to variants with distinguishing characteristics [1]. Variants of concern (VOCs) that have enhanced immune evasion abilities are more transmissible and can cause more severe infections [2]. Hence, they pose a greater threat to public safety. VOCs are classified according to the Greek alphabet and have included Alpha (i.e., B.1.1.7), Beta (i.e., B.1351), Delta (i.e., B.1617.2), and since December 2021, Omicron (i.e., B.1.1.529) [1,3]. In addition, multiple sublineages have emerged and are categorized as variants of interest or variants under monitoring

depending on the risk they pose to public health. Since early 2022, the major Omicron subvariant BA.2 has completely taken over from BA.1 in most countries (especially in the United Kingdom, United States, and Canada), and its many sublineages, including BA.3, BA.4, and BA.5 [1], have different health implications [4–6]. Wastewater has the potential to characterize the virus's community infection rate and the subvariants circulating [7–9].

Viral RNA can be shed from those infected by SARS-CoV-2 and its variants into fecal matter. Also, it can enter into wastewater through the secretion of waste from respiratory sources. Therefore, wastewater surveillance is an ideal complementary method for detecting and measuring SARS-CoV-2 spread on a community scale without relying on individual clinical testing [10]. Wastewater surveillance can also be used to estimate the effective reproductive number (R_{eff}), which describes the time-varying average of new infections caused by an infected individual and can be a key indicator of whether a pandemic is diminishing or growing [11–14]. An earlier study has shown that Reff from wastewater works well on data from Zurich, Switzerland, and San Jose, California, USA [12]. Similarly, Reff derived from wastewater and clinical data was reported to have a good correlation [12]. Therefore, wastewater R_{eff} represents an alternate method to describe how SARS-CoV-2 infection is spreading in a given community. A framework to measure the reproductive advantage of variants of concern by calculating wastewater-based variant-specific reproduction numbers was developed [15], comparing the respective effective reproductive numbers between the subvariants of Omicron. This paper aims to advance the understanding of how wastewater can be utilized to better understand the transmission of SARS-CoV-2 variants and help direct public health responses and interventions. Also, this paper aims to determine how wastewater derived effective reproduction numbers related to the emergence of different subvariants of Omicron detected in wastewater. This work showed that transforming the raw wastewater data to an effective reproduction number makes the interpretation of wastewater data easier for the end user to comprehend.

2. Materials and Methods

2.1. Study Areas

This study was carried out in Saskatoon, the largest city in Saskatchewan, Canada. It hosts a population of approximately 300,000. Raw wastewater was received three times a week from the wastewater treatment plant between January and September 2022. The wastewater was a composite sample collected every 15 min over 24 h. Samples were transported on ice and held at 4 °C before analysis. Samples (100 mL) were sent to the National Microbiology Laboratory in Winnipeg for whole genome sequencing [7,8].

2.2. Wastewater Environmental RNA Extraction and SARS-CoV-2 RNA Quantification

A detailed methodology for wastewater environmental RNA extraction and quantification can be found in our previous reports [7,8]. Briefly, viruses in whole wastewater were concentrated using a polyethylene glycol (PEG) centrifugation method. RNA was immediately extracted from pellets using RNeasy Power Microbiome kits following the manufacturer's protocol (Qiagen, Germantown, MD, USA). RNA was quantified using a reverse transcription-quantitative polymerase chain reaction (rt-qPCR), and the RNA concentrations were determined by comparing with the ATCC standard (Tables S1–S3), which has eight points, followed by quantitative SARS-CoV-2 RNA calculation according to the following equation:

$$C_i = \frac{C_{i,\,RNA} \times V_{RNA-elution,\,i}}{V_{Template,i} \times V_{WW,i}} \times 100 \quad (1)$$

where C_i = quantitative SARS-CoV-2 RNA (N2 gene copies (gc)/100 mL wastewater); i = sample ID; $C_{i,RNA}$ = concentration of viral RNA in the RNA elution (GC/reaction); $V_{RNA\text{-}elution,i}$ = volume of buffer AVE for RNA elution (µL), $V_{Template,i}$ = volume of RNA added to the rt-qPCR reaction mixture (µL/reaction); $V_{ww,i}$ = volume of wastewater processed (mL).

Viral RNA recovery was determined for the entire process with noncontagious, artificial, armored viral particles (AQHRPs; Armored RNA Quant RNase P, Asuragen, TX, USA) utilized as internal spiking positive controls (internal process control for the whole process; IPCW). A 30 µL aliquot of IPCW was extracted as an external positive control (EPC) for the recovery ratio (RR) for each batch (Equation (2)), and then, RR was used to calculate the efficiency-adjusted SARS-CoV-2 RNA ($C_{adj\text{-}RR,i}$) (Equation (3)).

$$RR_{i,k} = \frac{C_{AQHRP,i,k}}{C_{AQHRP,EPC,k}} \quad (2)$$

$$C_{adj-RR,i} = \frac{C_i}{RR_{i,k}} \quad (3)$$

where i = sample ID, k = batch ID, $RR_{i,k}$ = RR estimation of sample i for batch k, $C_{AQHRP,i,k}$ = concentration of the IPCW of sample i for batch k, $C_{AQHRP,EPC,k}$ = concentration of the EPC of batch k.

2.3. Effective Reproduction Number (R_{eff})

Estimates of R_{eff} were calculated using the wastewater epidemic model (WEM) R package [16]. The WEM package uses a SEIR compartmental model to simulate SARS-CoV-2 transmission in a community. In this model, individuals are placed in various compartments that reflect their disease state and outcome. Within each compartment are parameters that represent the approximate duration of infectiousness and the approximate duration of fecal shedding. These parameters, along with vaccination effectiveness, are used to model SARS-CoV-2 transmission. The model can use both clinical data (reported cases) and wastewater concentration data (sampled N2 gene copies per milliliter) to estimate R_{eff} and forecast future cases. The WEM package is publicly available with more details on the model in Nourbakhsh et al. [16] on its GitHub repository (https://github.com/phac-nml-phrsd/wem, accessed on 1 October 2022). The data used to estimate R_{eff} are in Supplementary File SI.

When using the wastewater concentration data exclusively to calibrate unobserved model parameters, the model estimates a "wastewater-based" R_{eff}. When available, we also use clinical reports of COVID cases from the city of Saskatchewan (https://dashboard.saskatchewan.ca/health-wellness/covid-19/cases, accessed on 1 October 2022) to estimate a "clinical-based" R_{eff}. Using the wastewater data, the model also estimates the number of "reportable" cases that would have been reported should the clinical surveillance efforts be kept at the same level as during the COVID waves prior to Omicron. As with many other jurisdictions in Canada (and worldwide), a significant decrease in clinical surveillance occurred after the first Omicron wave in Saskatoon. An approximate Bayesian computation (ABC) algorithm [17–19] within the WEM package was used to fit the data (using 10,000 ABC iterations and 50 posterior distribution samples) to obtain the posterior distribution of R_{eff} (whether clinical or wastewater-based).

3. Results

Wastewater surveillance has been helpful throughout the pandemic, and the need to make the interpretation of results simpler continues to concern the academic community. A relationship could be generated from clinical cases and wastewater data; for example, Figure 1 shows the inferred number of reportable cases in Saskatoon (Figure 1A) based on wastewater surveillance, the concentration of the N gene of SARS-CoV-2 in wastewater (Figure 1B), as well as the derived effective reproduction numbers (Figure 1C). The circulating VOC was also indicated in the plots. The raw data from which Figure 1 is generated is plotted in Figure S1. The breadth of coverage varied between 97.6 and 99.57%, and the average frequencies of reads varied between 0.64 and 0.98 (Table S4).

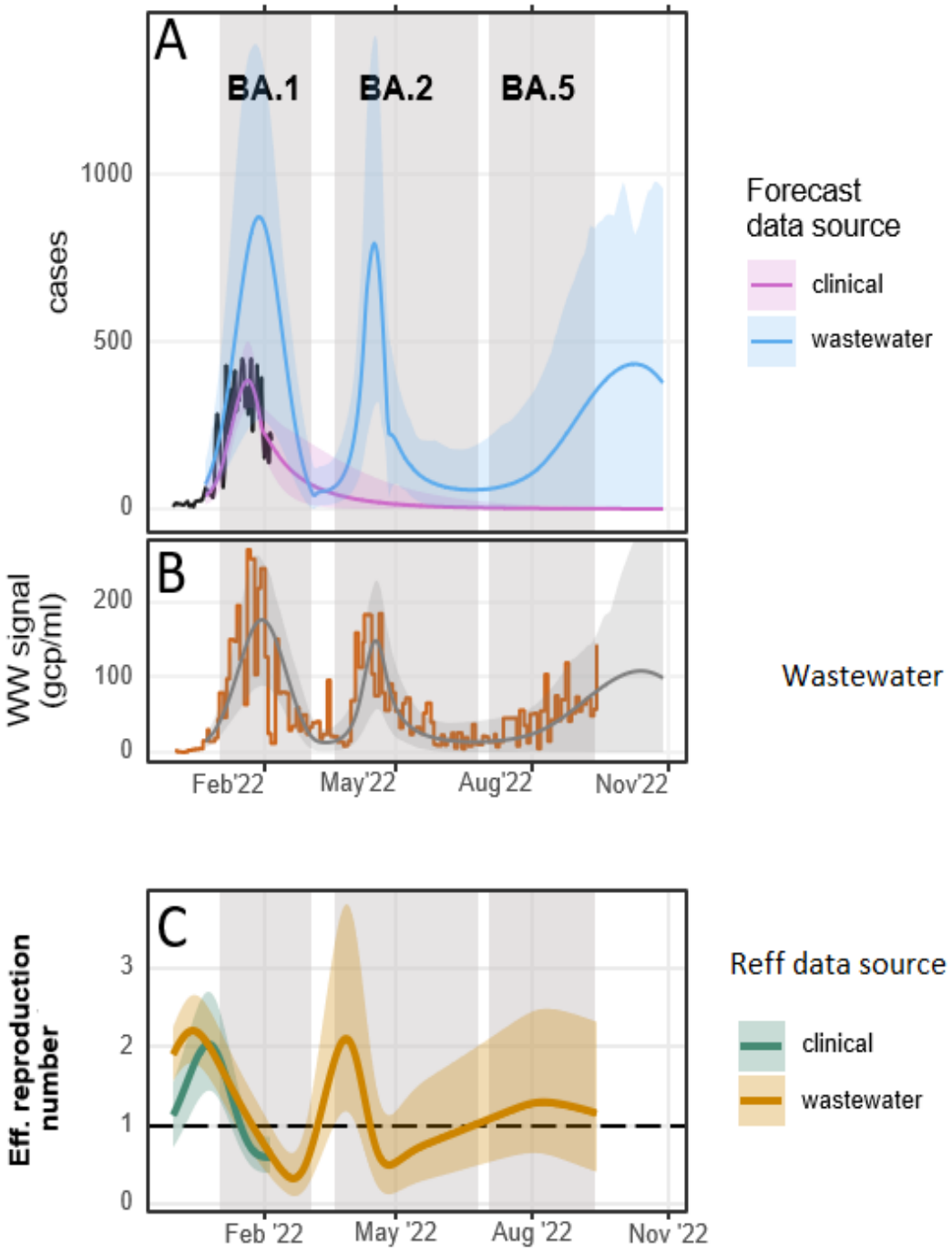

Figure 1. A model showing the prediction from wastewater SARS-CoV-2 RNA (**A**) COVID-19 cases, (**B**) wastewater signal (orange line is the observed wastewater signal, while the dark gray line is the model-fitted signal), (**C**) effective reproduction number derived from clinical (green curve) and wastewater (brown curve) data. The vertical gray shaded regions in each plot reflect the period of when the BA.1, BA.2, and BA.5 Omicron sublineages were dominant. The curves (panels A and B) displayed beyond the last observation point (left end of BA.5 shaded region) represent the forecasted values by WEM. The colored shaded regions around the curves represent the 95% credible intervals of the estimates. Clinical data were not available beyond February 2022. gcp/mL: gene copies of SARS-CoV-2 N2 gene per milliliter of sampled wastewater.

4. Discussion

The effective reproduction number represents changes in COVID-19 infections from January to September 2022, which may reflect changes in the population's immune status,

government policy, seasonality, emergence of new variants, and individual behaviors [20]. When the clinical data were available in January 2022, we observed a strong correlation (r = 1.000, $p < 0.0001$) between wastewater-based Reff and clinical-based Reff (Figure 1, panel A). Similarly, a strong correlation (r = 0.7749, $p < 0.0001$) has been reported between wastewater studies and clinical cases elsewhere [21]. Considering the strong correlation between clinical and wastewater-based R_{eff}, it would have been expected that a 100% match would be observed, but the wastewater model was designed to account for both symptomatic and asymptomatic cases. Another study also showed a strong correlation between clinical and wastewater samples in terms of estimated prevalence, then concluded that when there is any difference, the mismatch will be related to movement within and outside of the city [22]. Nevertheless, shedding rate differences between variants has been anticipated to be a possible limitation to applications of the same model to all variants.

The effective reproduction number during the BA.5 wave (July–September 2022) showed a weaker increasing phase of the wave when compared to the BA.2 and BA.1 driven waves, which suggests a potential decline in severity and/or transmissibility for BA.5 (transmissibility can be affected by intrinsic feature of the virus or by external forces, like public health interventions) or lower fecal shedding for this variant. While it is possible to estimate the trend of the virus using wastewater data, it is more challenging to assess the change in virulence for different SARS-CoV-2 lineages using this data source alone. Interestingly, a study has shown that there is no difference in severity for BA.5 compared to BA.1 infections [23]. Another study has shown that BA.5 infections were associated with an 18% higher risk of hospitalization compared to BA.1 and BA.2 [24]. Nevertheless, the effects of vaccination on the shedding rate are yet to be understood. There is evidence that previous infection and vaccination strongly protected against severe cases [23]. Increased hospitalization during the BA5 wave might also be responsible for lower wastewater-derived R_{eff} because patients in intensive care units contribute little or no waste to wastewater treatment plants, as most of the time, they could be bedridden. Similarly, booster vaccinations and previous infection might be responsible for the lower R_{eff} during BA.5 compared to BA.1 and BA.2.

To understand COVID-19 community infection trends, R_{eff} can be an important indicator showing increasing (R_{eff} greater than 1) or decreasing (R_{eff} less than 1) case numbers [25,26]. Thus, wastewater-based R_{eff} was able to effectively differentiate between waves caused by different VOCs. This study has shown that viral concentration measured in wastewater can be used to infer infection prevalence in a community and to infer changes in trends of transmissions (wastewater-based R_{eff}). This is particularly useful when clinical surveillance is minimal (or non-existent) because it allows us to continue monitoring the epidemic trajectory and potentially trigger public health interventions. Finally, the WEM's forecast of the wastewater signal (Figure 1A) followed the same trend as the actual wastewater signal (Figure S1). An earlier study has also shown that a case report for hospitalization and death correlated with R_{eff} estimates [20]. Since clinical testing is declining for COVID-19, wastewater-based R_{eff} will be a good tool to know what is happening at the community level. This work supported the idea that wastewater data can be translated into meaningful epidemiological data such as total community infection cases and reproduction numbers.

Three peaks (or waves), which are associated with different sub-variants of Omicron, were distinguished in the plot. The first peak in wastewater was associated with BA.1 and BA.1.1, but after the peak declined, another wave, which started in March and was associated with BA.2, BA.2.3, and BA.2.12.1 (Table S4), was apparent. In June 2022, another wave, corresponding to BA.5, BA.5.2, and BA.5.2.1, was evident (Table S4). Another study from South Africa showed a similar trend where variants spread rapidly, with the prevalence of Omicron-positive wastewater samples rising to >80% by 10 January 2022 with BA.2 as the predominant sublineage by 10 March 2022, whilst on 18 April 2022, BA.4 and BA.5 were dominant [21]. In Saskatoon wastewater, it was evident that BA5 was the

major sublineage by August 2022, and this reflects the worldwide dominance of BA5 in clinical cases [27].

A lineage generally shares a common ancestor genome, which might have some closely related variations, and when they start having distinct mutations, they are said to be sublineages. At every time during the pandemic, more than one variant was always in circulation, and they were always classified either into a variant of concern, a variant of interest (VOI), or a variant under monitoring (VUM) depending on the effect they had on public health. During each of these waves, monitoring subvariants using sequencing was very powerful and gave insights into the emergence of new variants and sublineages [7]. Since variants in circulations may be more than what is known, this limits the ability of rt-PCR to monitor them. Hence, there is a need to use whole genome sequencing, which could potentially show all circulating variants. Whole genome sequencing can potentially group identified variants into consensus and non-consensus sequencing based on the percentage breadth of coverage, average depth of coverage, and frequency of reads with greater than 30 coverage [7]. This sub-consensus VOC is very important because it might give an indication of VOCs that will possibly replace the existing dominant VOC. For instance, although some of the lineages detected did not thrive, between January and March 2022, BA.2 was evident in wastewater and, after some time, became the dominant VOC. Thus, to understand all the variants in a particular territory, monitoring wastewater for both consensus and sub-consensus sequencing through whole genome sequencing is essential.

The ability of a VOC to replace the existing dominant one is a function of how its mutations permit attachment to host cells effectively and allow it to evade the body's immune responses [28–30]. For instance, BA.2 shares 32 mutations with BA.1 but has 28 distinct mutations [29,31]; thus, it has the potential to reinfect patients originally infected with BA.1, thereby allowing BA.1 to displace it as the dominant VOC. Similarly, BA.5 shares a common genetic origin with BA.2 except for the additional L452R and F486V mutations, which enabled it to replace BA.2 [25]. The type of mutations present in each VOC or its sublineages have played a crucial role in their stability and transmissibility [30]; hence, these might be some of the key factors affecting their ability to replace the existing VOC.

Some variants, such as BA.2.9, BA.4.1, and BA.5.3, identified in sub-consensus sequencing failed to become dominant VOCs because they did not have distinct mutations that were stable enough or transmissible enough to replace the existing VOC. For instance, BA.3 shared most of its mutations with BA.1 and BA.2, except for one, which is not known for transmissibility [31]; hence, it has no potential to replace BA.2. Therefore, the nature of the unique mutations of each of the sub-consensus variants will determine their potential of becoming the dominant VOC.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16020318/s1, Table S1: Sequences of primers and probes; Table S2: Recipe, quantitative RNA standard, range of standard curve, and settings for threshold and baseline of the RT-qPCR assays; Table S3: Performance of TaqMan RT-qPCR assays; Figure S1: Trend of wastewater SARS-CoV-2 RNA between January and September 2022; Table S4: Distribution of Omicron sub-variants in Saskatoon between January and September 2022. File SX: CSV file of the wastewater concentrations and clinical reports.

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