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Rapid transition between SARS-CoV-2 variants of concern Delta and Omicron detected by monitoring municipal wastewater from three Canadian cities

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HIGHLIGHTS
• Omicron supplanted Delta in two weeks.
• The largest city was the first to have a new variant of concern.
• AY.12, AY.25, AY.27, and AY.93 were the common Delta sublineages.
• BA.1 and BA.1.1 were the consensus sequence in January 2022.
• Sub-consensus sequences showed lineages at the trace level.

GRAPHICAL ABSTRACT

ABSTRACT

Monitoring the communal incidence of COVID-19 is important for both government and residents of an area to make informed decisions. However, continuous reliance on one means of monitoring might not be accurate because of biases introduced by government policies or behaviours of residents. Wastewater surveillance was employed to monitor concentrations of SARS-CoV-2 RNA in raw influent wastewater from wastewater treatment plants serving three Canadian Prairie cities with different population sizes. Data obtained from wastewater are not directly influenced by government...
regulations or behaviours of individuals. The means of three weekly samples collected using 24 h composite auto-

sampler were determined. Viral loads were determined by RT-qPCR, and whole-genome sequencing was used to
characterize variants of concern (VOC). The dominant VOCs in the three cities were the same but with different propor-
tions of sub-lineages. Sub-lineages of Delta were BA.1, BA.25, BA.27 and BA.93 in 2021, while the major sub-lineage of
Omicron was BA.1 in January 2022, and BA.2 subsequently became a trace-level sub-variant then the predominant
VOC. When each VOC was first detected varied among cities; However, Saskatoon, with the largest population, was
always the first to present new VOCs. Viral loads varied among cities, but there was no direct correlation with popu-

lation size, possibly because of differences in flow regimes. Population is one of the factors that affects trends in on-
set and development of local outbreaks during the pandemic. This might be due to demography or the fact that larger pop-
ulations had greater potential for inter- and intra-country migration. Hence, wastewater surveillance data from larger
cities can typically be used to indicate what to expect in smaller communities.

1. Introduction

Wastewater surveillance has been a useful tool to inform public health
oficials and the general public within a geographical region about the state of the pandemic prevalence in their location (Pecchia et al., 2020). Over the past year, wastewater surveillance developed for SARS-CoV-2 has evolved to monitor for several variants of concern (VOCs), including Alpha, Beta, Gamma, Delta and Omicron (Grabier et al., 2021; Yaniv et al., 2021; Mishra et al., 2021; Ahmed et al., 2021; Izquierdo-Lara et al., 2021; Peterson et al., 2022; Johnson et al., 2022) and sub-lineages of the Delta and Omicron VOCs.

Differences in transmissibility have resulted in increased rates of infec-
tion compared to the ancestral strain and other more recent VOCs (Callaway, 2021; Li et al., 2022; Papanikolaou et al., 2022). The Delta VOC is characterized by multiple mutations, which sparked concern about the possibility of partial escape of immune defences from previous infections or the effectiveness of vaccination (Misra et al., 2021; Ahmed et al., 2022; Eyre et al., 2022). Nonetheless, frequencies of severe out-
comes and mortalities in fully vaccinated and boosted populations have been less compared to unvaccinated or partially vaccinated populations (Papanikolaou et al., 2022). However, these variants have still resulted in increased numbers of symptomatic individuals, who in some cases have re-
quired hospitalization and therapeutic interventions. For instance, a study of Corona Virus disease (COVID-19) case data for Ontario, Canada, from February to June 2021, showed that the emergence of the Delta VOC re-
sulted in greater rates of hospitalization, admission to intensive care (ICU), and death (Fisman and Tuite, 2021).

Clinical data from polymerase chain reaction (PCR) and whole-genome sequencing of individual patients was previously the main metric for moni-
toring case numbers and emerging variants. However, it is difficult to ob-
tain reliable information on community transmission due to policy changes across Canada, including decreased availability of clinical testing of individuals and a move away from PCR testing to rapid antigen tests. Re-

sults of rapid antigen tests are not centrally collected. Also, there has been an increased number of asymptomatic COVID-positive patients due to in-
creased vaccination rates (Karmi and Karmi, 2021). Therefore, efficient and accurate monitoring for emerging variants and overall viral loads in wastewater are useful for understanding the prevalence of SARS-CoV-2 in communities. This is particularly true because access to clinical testing and analytical facilities are prioritized for certain populations at greater risk for severe outcomes due to infection with COVID-19 (SHA, 2022; Arts et al., 2022). Hence, accessibility to clinical testing is limited to certain segments of the population (Covantes-Rosales et al., 2022). Therefore, to accurately identify and quantify prevalence of SARS-CoV-2 in a population, including variants of concern, wastewater surveillance appears to be the most promising public health alternative to individual mass testing. It pro-

vides a rapid, efficient, cost-effective and non-invasive integrative measure of the entire population, and there are no issues of privacy when samples are collected from municipal wastewater plants. Previous work has shown that clinical and wastewater data follow the same trends for Saska-
toon, the largest urban center in Saskatchewan (Xie et al., 2022). Similarly, the major consensus SARS-CoV-2 genotypes detected in the wastewater were earlier identical to clinical genomes from the same area (Cris-

Christoph et al., 2021). Therefore, sequencing of VOCs in wastewater is im-
portant where complete sequencing of all clinical cases is not possible.

Here we present the results of monitoring total SARS-CoV-2 RNA in wastewaters of three cold-region Prairie cities in Saskatchewan of various sizes, including Saskatoon, Prince Albert and North Battleford, dating a time when clinical testing became increasingly unavailable to the general population. A time lag between the arrival of the Omicron VOC among the three cities provided reliable monitoring to describe progressions of the fourth and fifth waves of SARS-COV-2 in Saskatchewan that were driven by the Delta and Omicron VOCs, respectively.

2. Material and methods

2.1. Study area overviews

Three cities in Saskatchewan, Canada, were studied, including Saskatoon (the most populated city with ~300,000 people), Prince Albert (~43,000 people), and North Battleford (~19,300 people). These are the first, third, and seventh largest cities in the Province of Saskatchewan, with a population of approximately 1,181,000. Municipal wastewaters are received and treated by local municipal wastewater treatment plants (WWTPs) before being re-

leased to the South Saskatchewan (Saskatoon) or North Saskatchewan (Prince Albert and North Battleford) Rivers. The Saskatoon, Prince Albert, and North Battleford WWTPs received an average flow of 78, 12, and 4 mil-

lion litres per day, respectively, during the study period (Table S1). One-litre composite wastewater samples were collected from the primary clarifier influ-

uent using an auto-sampler (60 mL every 15 min over 24 h) and maintained at 4 °C. The collected samples were transported on ice to the Toxicology Laboratory at the University of Saskatchewan to be heat-inactivated at 65 °C for 30 min and RNA extracted for qPCR analysis within 24 h. Overall, samples were received three times a week from each WWTP between August 2021 and January 31, 2022.

2.2. Sample pre-processing, viral enrichment, and wastewater environmental RNA (weRNA) extraction

Samples were enriched, concentrated, and RNA extracted as described previously (Xie et al., 2022) with slight modifications. Briefly, to assess re-

covery during the whole process, synthetic armoured viral particles (AQHRP; Armoured RNA Quant RNase P, Asuragen, TX, USA) were used as an internal spiking control for the whole process (Xie et al., 2022). A freshly diluted 10-μL aliquot (1.0 × 10⁶ gene copies, gc) of the synthetic viral particles was mixed with 70 mL of raw influent sample. Virus particles were enriched using the PEG-8000 precipitation method by adding 7 g PEG-8000 and 1.6 g NaCl to the 70 mL influent sample (Ahmed et al., 2020a and b). Enriched samples were agitation overnight (12–14 h) at 4 °C on an orbital shaker, with a speed of 10 rpm and angle of 360° and then centrifuged at 12,000 × g at 4 °C for 1 h to pellet the virus. After 1 h centrifuge, the supernatants were removed as much as possible. After pre-

processing, weRNA was immediately extracted from pellets using RNeasy Power microomi kits following the manufacturer's protocol (Qiagen, USA). The RNA was eluted with 100 μL AVE buffer (Xie et al., 2022).

The
recovery ratio (RR, Eq. (1)) was calculated to assess the practical performance of each batch (Eq. (1)).

\[
RR_{ij} = \frac{C_{AQRPH_i,j}}{C_{AQRPH,EPC,j}} \times 100
\]

(1)

where \(i\) and \(j\) represent sample and batch IDs, respectively; \(C_{AQRPH_i,j}\) is the concentration of synthetic viral particles of sample \(i\) for batch \(j\); \(C_{AQRPH,EPC,j}\) is the concentration of external positive control (EPC) of batch \(j\). The RR for this study was approximately 9.60 ± 8.04 %, consistent with RR reported earlier (Xie et al., 2022).

2.3. RT-qPCR assays for detection of viruses and variants of concern (VOCs)

Concentrations of SARS-CoV-2 and AQRPH were quantified by TaqMan RT-qPCR assays. Quantitative VOC assays were adopted based on Twist® Synthetic (Twist Bioscience, CA, USA) SARS-CoV-2 RNA standards for the detection of Omicron via N200 assays (Puzzen et al., 2022) and Delta lineages via P681R assay (Thermofisher, CA, USA) (Xie et al., 2022). Synthetic quantitative RNA standards were confirmed by digital droplet PCR in eight replicates following the manufacturer’s protocol (dPCR, Bio-Rad Laboratories, CA, USA). Sequences of primers and probes are shown in Table S2; similarly, the operation of the RT-qPCR is shown in Table S3 and S4, as reported earlier (Xie et al., 2022; Puzzen et al., 2022).

2.4. Next-generation sequencing

Whole-genome sequencing (WGS) of wastewater samples were done at the Division of Enteric Diseases, National Microbiology Laboratory, Public Health Agency of Canada (Winnipeg, MB, Canada). The method for sequencing was described in https://www.medrxiv.org/content/10.1101/2021.03.11.21253409v1.full.pdf (Landgraff et al., 2021). cDNA was synthesized using the SuperScript IV First-Strand Synthesis System (Invitrogen, USA). Tiled amplicons were amplified according to the ArticV3 protocol. Tiled amplicons were sequenced with MiSeq 300PE V3 chemistry (Illumina, USA). Mutations were identified on mapping files generated by SAMtools v 1.7 against a SARS-CoV-2 reference sequence (MN908947.3) (Danecek et al., 2021; Landgraff et al., 2021). SARS-CoV-2 lineage was assigned based on coverage of consensus mutations following the Pango Nomenclature proposal (Xie et al., 2022; Landgraff et al., 2021).

2.5. Statistical analyses

Plots were generated using Origin Pro 2021C (OriginLab Corporation USA). Viral loads were normalized to concentrations of the artificial sweetener, ascesulfame (6-methyl-2,2-dioxooxathiazin-4-one; CAS 33665-90-6) by dividing numbers of copies of target genes by concentrations of ascesulfame. Daily influent flow rates were used to normalize viral load by multiplying the gene copies by daily flow rates to obtain gene copies per day. A normality test was done using Shapiro-Wilk at 5 % decision level. Correlation coefficients were determined using Pearson correlation (r) or Spearman correlation depending on if the normality test was accepted or rejected.

3. Results and discussion

The load of SARS-CoV-2 viral RNA in wastewater can be used to follow the trajectory of COVID-19 infections in the cities studied (Fig. S1) (Crisis-Christoph et al., 2021; Arts et al., 2022; Xie et al., 2022; Yu et al., 2022). Delta became the only VOC in the cities studied after it had replaced the Alpha VOC in June 2021 (Xie et al., 2022), which occurred before the current study. The Delta VOC was the only observed variant, with its sub-lineages detected throughout August until the first week of December 2021 (Fig. 1). Since several sub-lineages of Delta emerged, which were monitored through whole-genome sequencing (Tables 1–3), it became clear that the specific sub-lineages circulating in various communities might be related to how frequently new people visited the community, which is a function of the total population and demography of the area. Migration has already been shown to play an important role in the spread of SARS-CoV-2 (Chen et al., 2020). Some studies also showed how new VOCs could enter communities through migration by detecting new VOCs in wastewaters of airplanes (Ahmed et al., 2022a and b). The emerging and the dominant, circulating VOCs in each community were monitored cost-effectively, with limited biases by wastewater surveillance (Ai et al., 2021). Wastewater surveillance has been a useful tool for monitoring overall SARS-CoV-2 RNA and understanding the specific VOCs dominating each community (Yu et al., 2022).

VOC sub-lineages (Tables 1–3) demonstrated a pattern of occurrence of the virus lineages and the timing when new sub-lineages became apparent. Delta VOC and its AY lineages were the dominant VOC in wastewater, driving the fourth wave. The dominance could be because >75 % of the spike proteins in the Delta VOC are capable of invading human cells (Wang and Han, 2022). Delta has 15 mutations of the spike protein, while Omicron has 32 (CDC, 2022). Hence, it took, Omicron 7 days to replace Delta in Saskatoon, 15 days in Prince Albert, and it became the dominant VOC on the first day it was detected in North Battleford. Similarly, it has been shown that Omicron displaced Delta in Ontario province-wide analysis of both clinical and wastewater surveillance in only 2 weeks following its first detection in travellers through clinical genomic surveillance (Arts et al., 2022). Overall, Delta was displaced by Omicron VOC in less than a month because of the additional mutations and deletions. The additional mutations and deletions allow Omicron to have increased transmissibility (Tables 1–3) (Karim and Karim, 2021).

The total viral load detected by RT-qPCR for the Omicron VOC was 2-fold greater than that observed for the Delta VOC. The maximum viral loads for Delta VOC were approximately 125,000, 110,000, and 182,000 gene copies/100 mL for Saskatoon, Prince Albert, and North Battleford, respectively, while the greatest viral loads for Omicron were 201,000, 340,000, and 190,000 gene copies/100 mL for Saskatoon, Prince Albert, and North Battleford, respectively. Another study has shown that the viral load associated with Omicron was very high compared to the earlier viral
Interestingly, some studies have found that vaccination efficacy might be reduced due to its ability to infect vaccinated people. A large load of virus particles observed for Omicron could directly relate to the increased rate of infection because of its ability to infect vaccinated people (Covantes-Rosales et al., 2022). This might soon become the main lineages (Crits-Christoph et al., 2021). The trace amounts of BA.2 were detected infrequently and identified by sub-consensus sequences. Therefore assignments could be made to either BA.1 or BA.2. In January 2021, the major subvariants of Omicron were BA.1 and BA.1.1, although trace amounts of BA.2 were detected infrequently and identified by sub-consensus sequence (Tables 1–3). The mutations with coverage >30% were considered consensus (Izquierdo-Lara et al., 2021). The consensus sequence represented the predominant lineages in the study areas but might not give the complete picture of other lineages in trace amounts, which were considered consensus (Izquierdo-Lara et al., 2021). The consensus sequence helped identify lineages present in trace amounts as they were not seen by consensus (Izquierdo-Lara et al., 2021). The sub-consensus sequence helped identify lineages present in trace amounts as they were not seen in the consensus sequence. Thus, the BA.2 sub-lineage was too rare to be seen in the consensus sequence. Therefore assignments could be made to either BA.1 or BA.2. In January 2021, the major subvariants of Omicron were BA.1 and BA.1.1, although trace amounts of BA.2 were detected infrequently and identified by sub-consensus sequence (Tables 1–3). The mutations with coverage >30% were considered consensus (Izquierdo-Lara et al., 2021). The consensus sequence represented the predominant lineages in the study areas but might not give the complete picture of other lineages in trace amounts, which might soon become the main lineages (Crits-Christoph et al., 2021). The breadth of coverage was generally >97%, except on a few occasions (Table 1–3), but 100% breadth of coverage was needed to have complete consensus viral genomes (Crits-Christoph et al., 2021). The sub-consensus sequence helped identify lineages present in trace amounts as they were too rare to be seen in the consensus sequence. Thus, the BA.2 sub-lineage and AY-lineages, which were not seen by consensus, were detected at trace, low, and moderate prevalence.

Monitoring the sub-lineage circulating is important because it might help understand the risk of hospitalization or mortality (Nyberg et al., 2022). Several sub-lineages of the Delta VOC were evident in the three cities (Tables 1–3). >250 AY lineages have been detected worldwide.
The week and duration of the dominance of each of the AY-lineages are summarized in Tables 1–3. Each of these AY-lineages is typical to a particular area or country; for example, AY.5.2 is called the Portugal lineage even though the percentage in most countries are as follows: Belgium 43.0 %, Portugal 38.0 %, United Kingdom 6.0 %, France 3.0 %, Netherlands 2.0 % (CDC, 2022). It is not surprising that AY.25 and AY.27 are present in North Battleford, though the percentage in most countries are as follows: Belgium 43.0 %, Portugal 38.0 %, United Kingdom 6.0 %, France 3.0 %, Netherlands 2.0 % (CDC, 2022). It is not surprising that AY.25 and AY.27 are present in North Battleford, but their number of reads was too low to be considered as possible VOCs or sub-lineages in either consensus or sub-consensus. For example, AY.33, AY.21, and AY.15 had 2, 1, and 1 number of mutations, respectively, but their number of reads was too low to be considered.

Considered to consist of coverage, the number of mutations, and the read frequencies were all comparable. For example, AY.33, AY.21, and AY.15 had 2, 1, and 1 number of mutations, respectively, but their number of reads was too low to be considered.

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report where two large Alberta cities, Calgary and Edmonton, exhibited a more rapid emergence of Omicron relative to the smaller and more remote Alberta municipalities of Brooks and Taber (Hubert et al., 2022). Therefore, larger cities with a greater influx of travellers and greater population densities have a greater risk of observing new lineages or variants (Arts et al., 2022).

When viral loads were normalized to concentrations of the artificial sweetener “acesulfame”, no changes were observed in the trend of the viral load (Fig. 3). The Spearman correlation coefficients (r) between normalized data and the unnormalized data and the gene copies normalized by acesulfame were r = 0.986 (p < 0.0001), 0.975 (p < 0.0001), and 0.978 (p < 0.0001) for Saskatoon, Prince Albert, and North Battleford, respectively. This lack of changes in trend might be because acesulfame was stable during the study period (Fig. S2). Acesulfame was better than pepper mild mottle virus or concentrations of creatinine or ammonia as indicators of population contributions contributing wastes to WWTPs and can be used to correct for effects of dilution (Xie et al., 2022). Hence, the population served by the WWTPs during the study period was relatively constant. Thus, normalization with acesulfame helped to ascertain that there were no variations in the numbers of persons contributing waste to the WWTPs. It also helps to correct the effects of dilution between sampling days, if any. Like the gene copies per acesulfame, the gene copies per day correlated significantly between August 2021 and January 2022 (Table S1).

## Table 3
Data obtained from whole genome sequencing of wastewater environmental RNA from August 2021 to January 2022 for North Battleford.

| Date Collected | Nextclade (consensus) | Pangolin | % Breadth of coverage (≥ 5x depth) | Average depth of coverage | Median Depth of Coverage | VOC Detected (consensus) | Number of VOC mutations (consensus) | Frequencies of reads (> cov 30) with VOC mutation (consensus) | Number of mutations supporting presence of additional VOC (subconsensus) | Additional Delta sublineages or VOCs, VOIs detected in subconsensus sequences |
|----------------|-----------------------|----------|-----------------------------------|--------------------------|-------------------------|--------------------------|-------------------------------------|------------------------------------------------|------------------------------------------------|---------------------------------------------------------------|
| 06/08/2021     | 20B                   | None     | 49.54                             | 1176.78                  | 2                       | None detected            | n/a                                 | n/a                                           | B.1.1.7 = 4 (0.21), B.1.617.2 = 2 (<0.1)               | Possible B.1.1.7 (Alpha)                                 |
| 20/08/2021     | 21A (Delta)           | B.1.617.2| 98.98                             | 4986.72                  | 5603                    | B.1.617.2 (Delta) sublineage AY.6 | 10/12                               | 0.84                                           | <4                                                  |                                                 |
| 30/08/2021     | 21A (Delta)           | B.1.617.2| 99.6                              | 6319.17                  | 6650                    | B.1.617.2 (Delta)         | 13/13                               | 0.99                                           | AY.25 = 2 (0.14), AY.21 = 1 (0.18)                | AY.25 (weak signal)                                    |
| 03/09/2021     | 21A (Delta)           | B.1.617.2| 99.6                              | 4648.12                  | 3777                    | B.1.617.2 (Delta)         | 11/13                               | 0.84                                           | AY.25 = 2 (0.1)                                     | AY.25 (weak signal)                                    |
| 09/09/2021     | 21A (Delta)           | AY.12    | 97.71                             | 5627.37                  | 5480                    | Delta sublineage AY.12   | 11/13                               | 0.84                                           | AY.25 = 4 (0.13)                                     | AY.25 (weak signal)                                    |
| 16/09/2021     | 21I (Delta)           | B.1.617.2| 98.72                             | 5194.09                  | 4595                    | B.1.617.2 (Delta)         | 11/13                               | 0.84                                           | AY.25 = 2 (0.11), AY.27 = 1 (<0.1)              | AY.25                                                  |
| 03/10/2021     | 21I (Delta)           | B.1.617.2| 99.6                              | 6164.71                  | 6232                    | B.1.617.2                | 13/13                               | 1                                              | AY.25 = 4 (0.16), AY.27 = 3 (0.15)               | IE                                                   |
| 09/10/2021     | 21I (Delta)           | AY.39    | 99.52                             | 5031.35                  | 5945                    | AY.39                    | 11/13                               | 0.85                                           | AY.25 = 2 (0.19), AY.27 = 3 (0.68)               | AY.25, AY.27                                          |
| 22/10/2021     | 21I (Delta)           | B.1.617.2| 99.57                             | 4949.38                  | 5961                    | B.1.617.2                | 11/13                               | 0.84                                           | AY.25 = 2 (0.18), AY.27 = 3 (0.22)               | AY.25, AY.27                                          |
| 12/11/2021     | 21A (Delta)           | AY.70    | 85.12                             | 3017.02                  | 1311                    | AY.70                    | 7/13                                | 0.54                                           | AY.25 = 1 (0.5), AY.103 = 1 (0.25)             | AY.4.1, AY.103                                        |
| 26/11/2021     | 21I (Delta)           | AY.74    | 91.33                             | 6001.55                  | 2523                    | AY.74                    | 11/13                               | 0.85                                           | AY.4.1 = 1 (0.5)                                   | AY.25, AY.27                                          |
| 03/01/2022     | 21I (Delta)           | AY.27    | 99.6                              | 16,478.08                | 7917                    | AY.27 (Delta)            | 13/13                               | 0.73                                           | BA.2 = 27 (0.42), Moderate presence of BA.2          | Moderate presence of Delta                             |
| 21/01/2022     | 21K (Omicron)         | BA.1     | 98.69                             | 98.69                    | 98.69                   | BA.1 (Omicron)           | 50/51                               | 0.9                                            | Moderate presence of BA.2                          | Trace presence of Delta                                 |
| 23/01/2022     | 21K (Omicron)         | BA.1     | 98.65                             | 12,386.05                | 7772                    | BA.1 (Omicron)           | 50/51                               | 0.77                                           | BA.2 = 27 (0.42), Moderate presence of BA.2          | Moderate presence of BA.2                             |

Fig. 2. Comparisons of AY lineages sequenced in the three study cities between August 2021 and December 2022.
is reported. The city with the largest population, Saskatoon, was the first to become Omicron VOC dominated. The AY-lineages associated with the USA (AY.25) and Canada (AY.27) were the major AY-lineages detected when Delta was the dominant VOC. Omicron displaced Delta variants within a week in Saskatoon as the major VOC because of its higher transmissibility due to having over 50 mutations. BA.1 and BA.1.1 were the dominant Omicron sub-lineages in January 2022, with only trace levels found for BA.2. The viral load in the fifth wave driven by Omicron was greater than that observed during the fourth wave, when Delta was the dominant VOC, likely due to the possibility of re-infection with Omicron by those already recovered from SARS-CoV-2 infections. There were strong correlations (>0.9) between normalized and unnormalized samples in the three cities because the population indicator was relatively stable during the study period. Also, inflow normalized viral load significantly correlated with acesulfame normalized viral load.

4. Conclusions

The SARS-CoV-2 viral load of three cities with different population sizes is reported. The city with the largest population, Saskatoon, was the first to exhibit the Omicron VOC in wastewater and the first city to become Omicron VOC dominated. The AY-lineages associated with the USA (AY.25) and Canada (AY.27) were the major AY-lineages detected when Delta was the dominant VOC. Omicron displaced Delta variants within a week in Saskatoon as the major VOC because of its higher transmissibility due to having over 50 mutations. BA.1 and BA.1.1 were the dominant Omicron sub-lineages in January 2022, with only trace levels found for BA.2. The viral load in the fifth wave driven by Omicron was greater than that observed during the fourth wave, when Delta was the dominant VOC, likely due to the possibility of re-infection with Omicron by those already recovered from SARS-CoV-2 infections. There were strong correlations (>0.9) between normalized and unnormalized samples in the three cities because the population indicator was relatively stable during the study period. Also, inflow normalized viral load significantly correlated with acesulfame normalized viral load.

CRediT authorship contribution statement

JPG, MA, KNM, PDJ: provided funding, project conceptualization and management, method development; data collection and curation; wrote and edited the final manuscript.

Declaration of competing interest

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 paper.

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