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# Metagenomics of Wastewater Influent from Wastewater Treatment Facilities across Ontario in the Era of Emerging SARS-CoV-2 Variants of Concern


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# Metagenomics of Wastewater Influent from Wastewater Treatment Facilities across Ontario in the Era of Emerging SARS-CoV-2 Variants of Concern

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**ABSTRACT** We report metagenomic sequencing analyses of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in composite wastewater influent from 10 regions in Ontario, Canada, during the transition between Delta and Omicron variants of concern. The Delta and Omicron BA.1/BA.1.1 and BA.2-defining mutations occurring in various frequencies were reported in the consensus and subconsensus sequences of the composite samples.

The emergence and global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (genus *Betacoronavirus*, family *Coronaviridae*) variants of concern (VOCs) pose a significant threat to global health (1, 2). SARS-CoV-2 is shed in human fecal matter and less so in urine by infected individuals, and its detection in wastewater triggered global interest in tracking the dissemination of its VOCs (3, 4). The province of Ontario and other jurisdictions have adopted genomic surveillance of wastewater for monitoring known and emerging SARS-CoV-2 VOCs in the community (5–12).

We collected 24-h composite samples of raw influent from 28 wastewater treatment plants in 10 regions across Ontario between November 2021 and February 2022 ( $n = 48$ ) (Table 1) as part of the COVID-19 Regional Genomic Initiative (CORGI) in Ontario. Nanotrap magnetic virus particles (Ceres Nanosciences) were added to 50-mL wastewater samples to capture and concentrate the virus, followed by RNA extraction with the QIAamp viral RNA minikit (Qiagen) according to the manufacturer's instructions. The CDC N1 and N2 regions were detected in the RNA samples with one-step reverse transcriptase quantitative PCR (RT-qPCR) performed on QuantStudio 5 (Thermo Fisher Scientific) (13) by using the 2019-nCoV CDC RUO kit (IDT, Coralville, USA) and TaqPath master mix (Thermo Fisher Scientific) as described (14). Wastewater samples with a cycle threshold of  $\leq 35$  were sequenced. For genomic sequencing, cDNA synthesis was performed using the SuperScript IV first-strand

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**TABLE 1** Summary of sequencing data of the samples

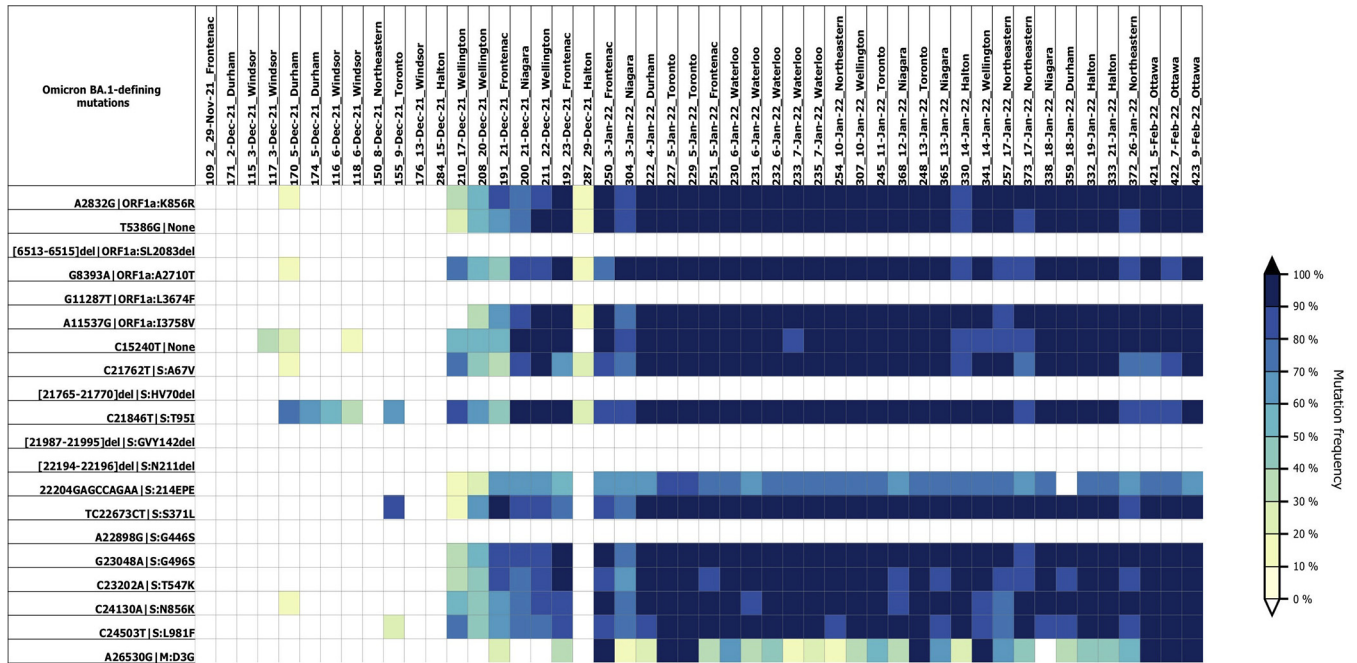
| Sample ID | Date of collection (day-mo-yr) <sup>a</sup> | Sampling point          | Wastewater sample location ID                        | Region           | GPS coordinates                           | No. of input reads | % mapped reads | % breadth of coverage | Variant detected (consensus) | Variant detected (subconsensus)         | SRA accession no. |
|-----------|---|-------------------------|--|------------------|---|--------------------|----------------|-----------------------|------------------------------|---|-------------------|
| 109_2     | 29-Nov-21                                   | Frontenac WWTP-1        | Kingston/Ravensview WWTP                             | Frontenac        | 44,241,251, -76,420,616                   | 3,859,510          | 80.39          | 99.08                 | Delta sublineage AY.25       | None                                    | SRR18762211       |
| 171       | 2-Dec-21                                    | Durham WWTP-1           | Midland/S3.2.12.21                                   | Durham           | 44,756,950,03 -79,874,838,45              | 2,990,080          | 55.65          | 96.74                 | Delta sublineage AY.25.3     | None                                    | SRR18680489       |
| 115       | 3-Dec-21                                    | Windsor WWTP-1          | Windsor Lou Romano WRP 172                           | Windsor          | 42,283,84, -83,085,27                     | 2,728,910          | 58.49          | 98.18                 | Delta B.1.617.2              | None                                    | SRR18680486       |
| 117       | 3-Dec-21                                    | Windsor WWTP-2          | Leamington PCC 148                                   | Windsor          | 42,034,43, -82,588,50                     | 2,794,614          | 43.32          | 98.17                 | Delta sublineage AY.103      | None                                    | SRR18680474       |
| 170       | 5-Dec-21                                    | Durham WWTP-2           | Ajax/F07.5.12.21                                     | Durham           | 43,838,313,55, -79,041,841,9              | 2,828,286          | 90.16          | 96.66                 | Delta sublineage AY.121      | Traces of Omicron BA.1                  | SRR18680490       |
| 174       | 5-Dec-21                                    | Durham WWTP-3           | Orillia/S4.5.1.2.21                                  | Durham           | 44,590,041,57, -79,413,22,97              | 2,917,708          | 96.19          | 96.47                 | Delta sublineage AY.25.3     | None                                    | SRR18680488       |
| 116       | 6-Dec-21                                    | Windsor WWTP-1          | Windsor Lou Romano WRP 173                           | Windsor          | 42,283,84, -83,085,27                     | 2,649,640          | 54.05          | 90.05                 | Delta sublineage AY.4        | None                                    | SRR18680485       |
| 118       | 6-Dec-21                                    | Windsor WWTP-2          | Leamington PCC 149                                   | Windsor          | 42,034,43, -82,588,50                     | 3,013,594          | 44.39          | 97.40                 | Delta sublineage AY.103      | None                                    | SRR18680463       |
| 150       | 8-Dec-21                                    | Northern Ontario WWTP-1 | Sudbury/TPKL221208                                   | Northern Ontario | 46,465,566,027,240, -81,032,855,716,62,95 | 2,145,830          | 30.57          | 98.75                 | Delta sublineage AY.74       | None                                    | SRR18680452       |
| 155       | 9-Dec-21                                    | Toronto WWTP-1-Site 1   | S2A-177  | Toronto          | 43,735,84, -79,495,83,2                   | 2,767,236          | 52.66          | 96.52                 | Delta B.1.617.2              | Traces of Omicron BA.1                  | SRR18680491       |
| 176       | 13-Dec-21                                   | Windsor WWTP-2          | Leamington PCC 152                                   | Windsor          | 42,034,43, -82,588,50                     | 2,837,338          | 96.14          | 97.52                 | Delta sublineage AY.103      | None                                    | SRR18680487       |
| 284       | 15-Dec-21                                   | Halton WWTP-1           | Maplehurst Correctional Complex                      | Halton           | 43,520,000, -79,900,000                   | 3,446,194          | 99.64          | 99.14                 | Delta sublineage AY.25.1     | None                                    | SRR18680462       |
| 210       | 17-Dec-21                                   | Guelph WWTP-1           | Guelph   | Wellington       | 43,520,000, -80,270,000                   | 2,458,380          | 75.02          | 96.74                 | Delta sublineage AY.39       | Omicron BA.1                            | SRR18680480       |
| 208       | 20-Dec-21                                   | Guelph WWTP-1           | Guelph   | Wellington       | 43,520,000, -80,270,000                   | 3,406,436          | 67.05          | 98.86                 | Omicron BA.1                 | Delta B.1.617.2                         | SRR18680481       |
| 191       | 21-Dec-21                                   | Frontenac WWTP-2        | Loyalist/Amherstview WPCP                            | Frontenac        | 44,231,127, -76,660,988                   | 3,231,114          | 49.13          | 98.79                 | Omicron BA.1                 | Delta B.1.617.2                         | SRR18680484       |
| 200       | 21-Dec-21                                   | Niagara WWTP-1          | Crystal Beach Wastewater Treatment Plant (Fort Erie) | Niagara          | 42,860,000, -79,060,000                   | 3,075,436          | 35.10          | 99.69                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680482       |
| 211       | 22-Dec-21                                   | Guelph WWTP-1           | Guelph   | Wellington       | 43,520,000, -80,270,000                   | 3,053,446          | 80.99          | 98.71                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680479       |
| 192       | 23-Dec-21                                   | Frontenac WWTP-3        | Eastern Ontario/Hawkesbury                           | Frontenac        | 45,611,676, -74,596,091                   | 4,867,030          | 96.39          | 98.12                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680483       |
| 287       | 29-Dec-21                                   | Halton WWTP-1           | Maplehurst Correctional Complex                      | Halton           | 43,520,000, -79,900,000                   | 3,058,088          | 91.51          | 99.08                 | Delta sublineage AY.25.1     | Traces of Omicron BA.1                  | SRR18680461       |
| 250       | 3-Jan-22                                    | Frontenac WWTP-4        | Kingston/Catarauqui Bay WWTP                         | Frontenac        | 44,214,819, -76,550,722                   | 3,616,518          | 82.89          | 98.34                 | Omicron BA.1.1               | Delta B.1.617.2, Traces of Omicron BA.2 | SRR18680467       |
| 304       | 3-Jan-22                                    | Niagara WWTP-2          | Queenston Wastewater Treatment Plant                 | Niagara          | 43,160,000, -79,050,000                   | 3,891,574          | 76.68          | 98.88                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680460       |
| 222       | 4-Jan-22                                    | Durham WWTP-4           | Courtice WWTP/C04.4.1.22                             | Durham           | 43,871,319,28, -78,755,977,8              | 3,276,026          | 68.05          | 98.29                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680478       |
| 227       | 5-Jan-22                                    | Toronto WWTP-1-Site 2   | S2B-167  | Toronto          | 43,761,87, -79,508,388                    | 3,884,772          | 98.55          | 97.02                 | Omicron BA.1.1               | None                                    | SRR18680477       |
| 229       | 5-Jan-22                                    | Toronto WWTP-1-Site 3   | S1B-190  | Toronto          | 43,730,131, -79,549,578                   | 3,068,436          | 73.87          | 97.54                 | Omicron BA.1.1               | None                                    | SRR18680476       |
| 251       | 5-Jan-22                                    | Frontenac WWTP-5        | Eastern Ontario/Hawkesbury WWTP                      | Frontenac        | 45,611,676, -74,596,091                   | 3,373,878          | 67.69          | 98.40                 | Omicron BA.1                 | Delta B.1.617.2                         | SRR18680466       |
| 230       | 6-Jan-22                                    | Waterloo WWTP-1         | Waterloo 845R-I-B 1/6                                | Waterloo         | 43,485,09, -80,504,12                     | 2,655,472          | 97.84          | 99.68                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680475       |
| 231       | 6-Jan-22                                    | Waterloo WWTP-2         | Galt 847R-I-B 1/6                                    | Waterloo         | 43,340,827, -80,313,357                   | 3,138,934          | 98.75          | 99.59                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680473       |
| 232       | 6-Jan-22                                    | Waterloo WWTP-3         | Kitchener 846R-I-B 1/6                               | Waterloo         | 43,396,587, -80,421,080                   | 3,486,778          | 88.36          | 99.71                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680472       |
| 233       | 7-Jan-22                                    | Waterloo WWTP-4         | Peel 830F-I-B 1/7                                    | Waterloo         | 43,754,622, -79,626,407                   | 2,987,086          | 53.29          | 99.57                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680471       |
| 235       | 7-Jan-22                                    | Waterloo WWTP-5         | York 831F-I-B 1/7                                    | Waterloo         | 43,845,555, -79,335,031,5                 | 2,720,060          | 76.96          | 98.40                 | Omicron BA.1.1               | None                                    | SRR18680470       |
| 254       | 10-Jan-22                                   | Northern Ontario WWTP-1 | Sudbury/TPKL220110                                   | Northern Ontario | 46,465,566,027,240, -81,032,855,716,62,95 | 3,003,880          | 15.27          | 99.69                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680465       |
| 307       | 10-Jan-22                                   | Guelph WWTP-1           | Guelph   | Wellington       | 43,520,000, -80,270,000                   | 3,532,702          | 99.75          | 97.55                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680459       |
| 245       | 11-Jan-22                                   | Toronto WWTP-1-Site 1   | S2A-187  | Toronto          | 43,735,84, -79,495,83,2                   | 2,095,676          | 98.73          | 99.77                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680469       |
| 368       | 12-Jan-22                                   | Niagara WWTP-3          | Port Weller Sewer Treatment Plant                    | Niagara          | 43,230,000, -79,220,000                   | 3,412,742          | 94.39          | 100.00                | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680450       |
| 248       | 13-Jan-22                                   | Toronto WWTP-1-Site 1   | S2A-189  | Toronto          | 43,735,84, -79,495,83,2                   | 3,149,968          | 91.69          | 98.63                 | Omicron BA.1.1               | Delta B.1.617.2                         | SRR18680468       |

(Continued on next page)

**TABLE 1 (Continued)**

| Sample ID | Date of collection (day-mo-yr) <sup>a</sup> | Sampling point          | Wastewater sample location ID                     | Region           | GPS coordinates                     | No. of input reads | % mapped reads | % breadth of coverage | Variant detected (consensus) | Variant detected (subconsensus) | SRA accession no.           |
|-----------|---|-------------------------|---|------------------|-------------------------------------|--------------------|----------------|-----------------------|------------------------------|---------------------------------|-----------------------------|
| 365       | 13-Jan-22                                   | Niagara WWTP-4          | Baker Road Wastewater Treatment Plant (Grimsby)   | Niagara          | 43.190000, -79.540000               | 2,374,706          | 98.36          | 99.00                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680451</a> |
| 330       | 14-Jan-22                                   | Halton WWTP-1           | Maplehurst Correctional Complex                   | Halton           | 43.520000, -79.900000               | 3,637,030          | 97.02          | 99.69                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680458</a> |
| 341       | 14-Jan-22                                   | Guelph WWTP-1           | Guelph  | Wellington       | 43.520000, -80.270000               | 3,529,346          | 74.85          | 97.64                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680454</a> |
| 257       | 17-Jan-22                                   | Northern Ontario WWTP-1 | Sudbury/TPKL220117                                | Northern Ontario | 46.4655660272402, -81.0328557166295 | 3,175,686          | 91.77          | 99.70                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680464</a> |
| 373       | 17-Jan-22                                   | Northern Ontario WWTP-2 | Sault Ste. Marie/TPSSM220117                      | Northern Ontario | 46.5057224005555, -84.25739500029   | 3,313,904          | 89.77          | 99.00                 | Omicron BA.1.1               | Delta B.1.617.2, Omicron BA.2   | <a href="#">SRR18680448</a> |
| 338       | 18-Jan-22                                   | Niagara WWTP-5          | Niagara Falls-Stamford Wastewater Treatment Plant | Niagara          | 43.120000, -79.090000               | 2,917,972          | 96.83          | 99.71                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680455</a> |
| 359       | 18-Jan-22                                   | Durham WWTP-5           | Corbett WWTP/OUT/A02.18.1.22                      | Durham           | 43.85542682, -78.89248301           | 2,366,054          | 85.02          | 99.00                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680453</a> |
| 332       | 19-Jan-22                                   | Halton WWTP-1           | Maplehurst Correctional Complex                   | Halton           | 43.520000, -79.900000               | 3,260,302          | 40.88          | 99.66                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680457</a> |
| 333       | 21-Jan-22                                   | Halton WWTP-1           | Maplehurst Correctional Complex                   | Halton           | 43.520000, -79.900000               | 3,834,210          | 99.53          | 99.21                 | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680456</a> |
| 372       | 26-Jan-22                                   | Northern Ontario WWTP-1 | Sudbury/TPKL220126                                | Northern Ontario | 46.4655660272402, -81.0328557166295 | 2,985,948          | 99.15          | 100.00                | Omicron BA.1.1               | Delta B.1.617.2                 | <a href="#">SRR18680449</a> |
| 421       | 5-Feb-22                                    | Ottawa WWTP-1           | Ottawa influent                                   | Ottawa           | 45.461111, -75.589167               | 2,634,062          | 93.99          | 96.00                 | Omicron BA.1.1               | None                            | <a href="#">SRR18680447</a> |
| 422       | 7-Feb-22                                    | Ottawa WWTP-1           | Ottawa influent                                   | Ottawa           | 45.461111, -75.589167               | 2,482,608          | 99.95          | 96.00                 | Omicron BA.1.1               | None                            | <a href="#">SRR18680446</a> |
| 423       | 9-Feb-22                                    | Ottawa WWTP-1           | Ottawa influent                                   | Ottawa           | 45.461111, -75.589167               | 2,668,098          | 50.47          | 95.00                 | Omicron BA.1.1               | None                            | <a href="#">SRR18680492</a> |

<sup>a</sup> Data in the table are sorted by date of collection. WWTP, wastewater treatment plant.



**FIG 1** Heatmap showing estimated frequencies of Omicron (BA.1) mutations in composite samples of wastewater influent from 10 regions across Ontario. Each column represents a sample, and they are ordered by date of collection. Each row represents the genomic locus of a BA.1-defining mutation (<https://github.com/cov-lineages/constellations/tree/main/constellations/definitions>). Colors depict the percentage frequency of mutations in the samples; the deeper the color, the higher the mutation frequency. The heatmap was generated using VCFparser v1.0.0 (<https://github.com/kbessonov1984/VCFParser>).

synthesis system (Thermo Fisher Scientific). SARS-CoV-2 amplicons were generated as previously described (15) but with ARTIC V4 primers ([https://github.com/artic-network/artic-ncov2019/tree/master/primer\\_schemes/nCoV-2019](https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019)). DNA libraries were generated using the Nextera XT DNA library prep kit (Illumina). Paired-end ( $2 \times 150$  bp) sequencing was performed using the MiniSeq system (Illumina). Raw sequence reads were analyzed using ViralRecon v2.4.1 (16). Variants were called with iVar v1.3.1 (17) using minimum quality and depth of 15 and 10, respectively. Consensus and subconsensus sequences were defined using mutation frequency thresholds of  $>50\%$  and 10 to 50%, respectively. Variant lineages were inferred using Pangolin v3.1.20 (18). Default parameters were used for all tools unless otherwise specified.

We received 148,603,298 total reads across all 48 samples (mean, 3,095,902; range, 2,095,676 to 4,867,030). In most of the samples (85%;  $n = 41$ ),  $\geq 50\%$  of reads mapped to the SARS-CoV-2 Wuhan strain. The average breadth of coverage of the consensus sequences generated was 98%. In consensus sequences, the Delta variants, including 8 sublineages, were detected in 14 samples from 7 regions in December 2021 (Table 1). Omicron BA.1/BA.1.1 was detected in 34 samples collected from all the regions studied except in Windsor, located in the extreme southwest of the province (Fig. 1). In subconsensus sequences, 26 samples contained the Delta VOC, 2 samples contained both Delta and Omicron BA.2 mutations, while traces ( $\leq 6$  mutations) of Omicron BA.1-defining mutations were detected in 4 samples. Overall, multiple SARS-CoV-2 VOCs were detected in 32 samples (Table 1). Collectively, continuous genomic surveillance of wastewater provides sufficient specificity to infer individual VOC lineages in mixed samples and is effective for monitoring SARS-CoV-2 VOCs in the community.

**Data availability.** The metagenomic sequences are available in the NCBI Sequence Read Archive under BioProject accession number [PRJNA824537](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA824537).

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