Changing Tides: West Michigan Based Genomic Analysis of SARS-CoV-2 Variants in Wastewater

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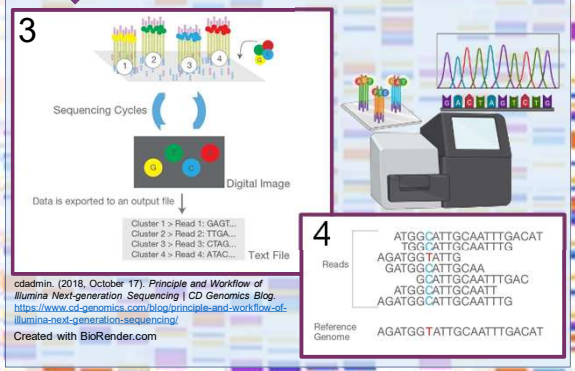
Introduction

SARS-CoV-2 wastewater surveillance provides a complimentary approach to public health surveillance and quantification of viral presence within a population. However, robust quantification of variants and de novo detection of emerging variants remains challenging and is ever evolving for existing strategies. Evaluation of SARS-CoV-2 RNA in wastewater within the west Michigan population was conducted from 11/2020-ongoing. Following initial quantification of SARS-CoV-2 utilizing gene targets N1 and N2, samples that exceeded the laboratory threshold-1 of 10,000 gene copies/100 mL underwent variant analysis utilizing discriminatory assays provided by GT-Molecular. Regional genomic targets were determined based on circulating variants of concern as noted by the World Health Organization.

Data Strategy

316 sample extracts were analyzed for variant status from 03/2021-03/2023. A subset of 8 samples that exceeded the secondary threshold-2 value of 20,000 gene copies/100 mL were evaluated using WGS via Illumina's MiSeq System to cross reference with the previously analyzed subset that noted ambiguous signal on ddPCR.

0. RNA Extraction from wastewater
   Translation to cDNA
   Quality Control
1. Library preparation
2. Cluster generation
3. Sequencing
4. Alignment and Data analysis



cdadmin. (2018, October 17). *Principle and Workflow of Illumina Next-generation Sequencing | CD Genomics Blog.* https://www.cd-genomics.com/blog/principle-and-workflow-of-illumina-next-generation-sequencing/

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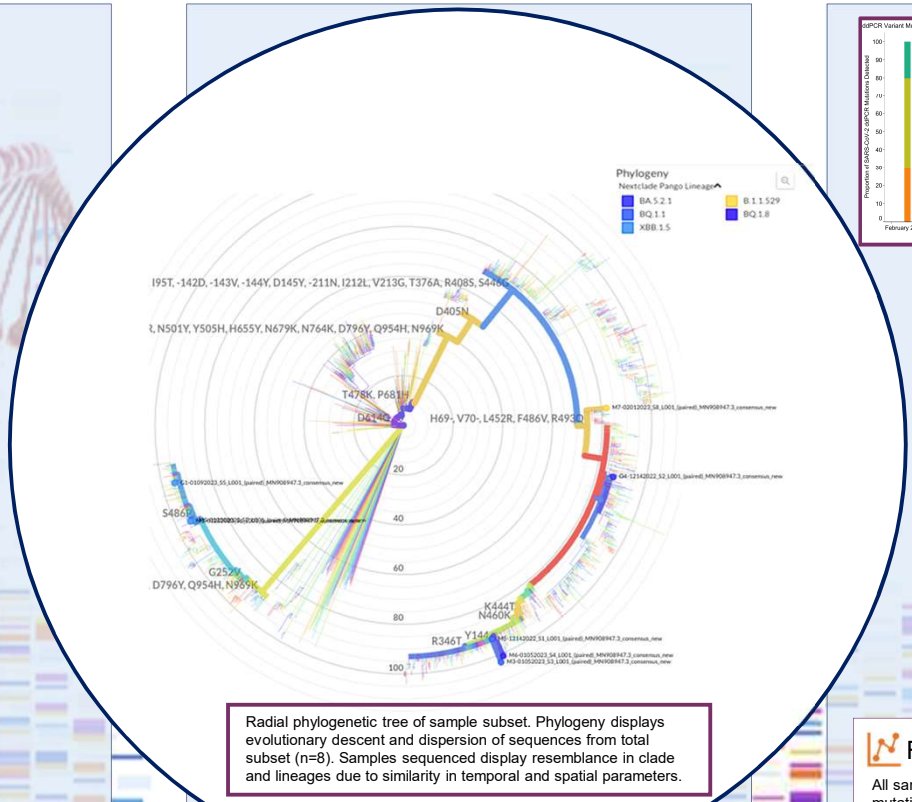
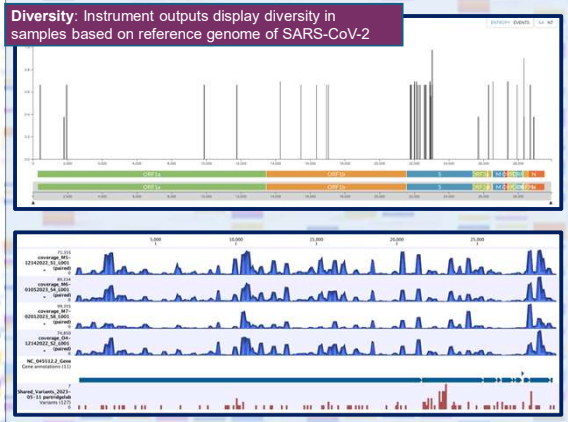
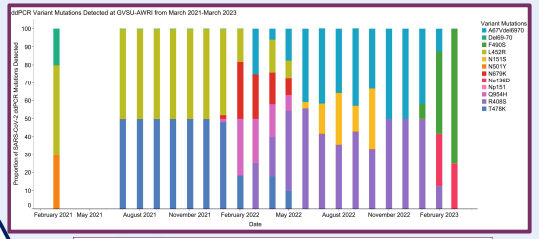


Radial phylogenetic tree of sample subset. Phylogeny displays evolutionary descent and dispersion of sequences from total subset (n=8). Samples sequenced display resemblance in clade and lineages due to similarity in temporal and spatial parameters.

Diversity: Instrument outputs display diversity in samples based on reference genome of SARS-CoV-2





Mutations that were detected using ddPCR for specific gene targets as prioritized by variant of concern status. Testing was limited to circulating variant of concern and kit manufacturing.

Sample ID	ddPCR Variant ID	ddPCR Reported Detections	Sequenced Variant ID	Clade	# Sequenced Mutations Detected
M5-12/14/2022	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron BQ.1.1	22E	64
O4-12/14/2022	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron BA.5.2.1	22B	57
M3-01/05/2023	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron BQ.1.1	22E	67
M6-01/05/2023	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron BQ.1.8	22E	81
G1-01/09/2023	Omicron XBB/XBB.1.5	R408S, F490S	Omicron XBB.1.5	23A	85
M3-01/25/2023	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron XBB.1.5	23A	62
M5-01/25/2023	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron XBB.1.5	23A	77
M7-02/01/2023	Omicron BA.5/BQ.1	R408S, 67V;del69-70	Omicron BA.1.1.529	21L	63

Results and Discussion

All samples evaluated came back with detection of targeted gene mutations when using ddPCR. A subset of 8 samples selected from 2022-2023 that met threshold-2 were investigated for sequencing potential. Results revealed detections of new variants of concern entering the population during peaks in wastewater signal. These variants included Alpha, Delta, Epsilon, Omicron, Omicron sub-variants BA.1, BA.1.1529, BA.2, BA.2.1.2, BA.4, BA.5, BA.5.2.1, BQ.1/BQ.1.1, BQ.1.8, and XBB/XBB.1.5. Based on clinically available timelines and ddPCR kit manufacture and distribution, variation was seen for variant point of entry and detection in known clinical cases. All 8 samples sequenced came back with 92-100% genomic coverage. Mutation profiles and phylogeny status revealed samples had anywhere from 57-85 mutations that deviated from the SARS-CoV-2 reference genome. The sample subset was collected during a transitional period of BQ.1 to XBB lineages and revealed co-dominance in variants during this time within our sample geographical region.

Despite challenges in variant quantification for SARS-CoV-2, genomic analysis of SARS-CoV-2 in wastewater can inform epidemiological work and public health officials as a complement to the established infrastructure for viral analysis during periods of high variant diversity.

Acknowledgments

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