Tracking SARS-CoV-2 Variants in Georgia Wastewater Using dPCR: A One-Year Study and Comparison with Clinical Sample Genotyping and GISAID Sequencing

Sayed Mosavi [1], Patrick Andersen [1], Robbie Barbero [1], Ben Lepene [1], Christine L. Moe [2], Marlene Wolfe [2], Orlando Sablon [2], Stephen Hilton [2], Pengbo Liu [2], Jean Lozach [3], Tim Wesselman [3]

1. Ceres Nanosciences, Manassas, VA | 2. Emory University, Atlanta, GA | 3. Rosalind Bio, San Diego, CA

Introduction

Wastewater surveillance has become a vital tool for monitoring infectious diseases like COVID-19 at the community level.[1] As microbes continue to mutate, giving rise to variants with differing transmissibility and virulence, tracking the emergence and prevalence of these variants has become a critical component of wastewater-based monitoring. Although PCR-based methods are the gold standard for pathogen detection and quantification, they are generally not designed to distinguish among a broad range of variants. Meanwhile, genomic sequencing—while comprehensive—is often too complex, time-consuming, and resource-intensive for many wastewater testing laboratories.[2]

In this study, we developed a customizable digital PCR (dPCR)-based genotyping approach to detect SARS-CoV-2 variants in wastewater.[3] This method provides a rapid and cost-effective way to screen for specific variants, enabling detection of both known and emerging variants beyond predefined markers. We also built a streamlined data analysis pipeline and integrated the results into a public-facing dashboard to deliver real-time insights alongside clinical and GISAID sequencing data. Results from a year-long surveillance effort across Georgia (April 2023–April 2024) highlight the potential of wastewater-based dPCR genotyping as a scalable, timely, and community-representative approach for tracking SARS-CoV-2 variants.

Methods

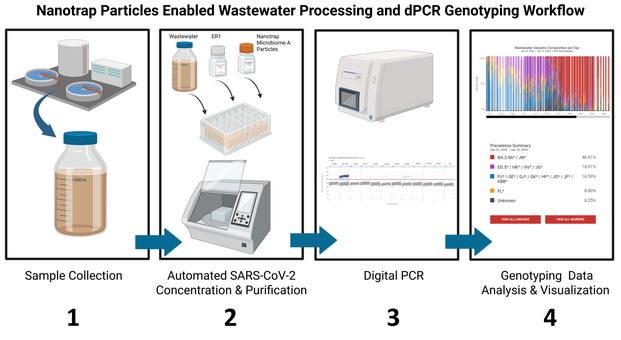

Figure 1: Workflow for wastewater processing and dPCR detection, quantification, and genotyping.[4] (1) Sample Collection: 528 untreated wastewater samples are collected from 17 wastewater treatment facilities across the state of Georgia, including metro Atlanta and rural regions. (2) Automated SARS-CoV-2 Concentration & RNA Extraction: Using an automated system, viruses are concentrated from the wastewater using Nanotrap® Microbiome A Particles, and nucleic acids are purified using a nucleic acid extraction kit. (3) dPCR: Samples are analyzed on the QIAGEN QIAcuity Digital PCR system using mutation-specific genotyping assays to detect SARS-CoV-2 variants deployed in three panels. (4) Genotyping Data Analysis and Visualization: The composition and prevalence of SARS-CoV-2 variants are analyzed and visualized over time.

Results

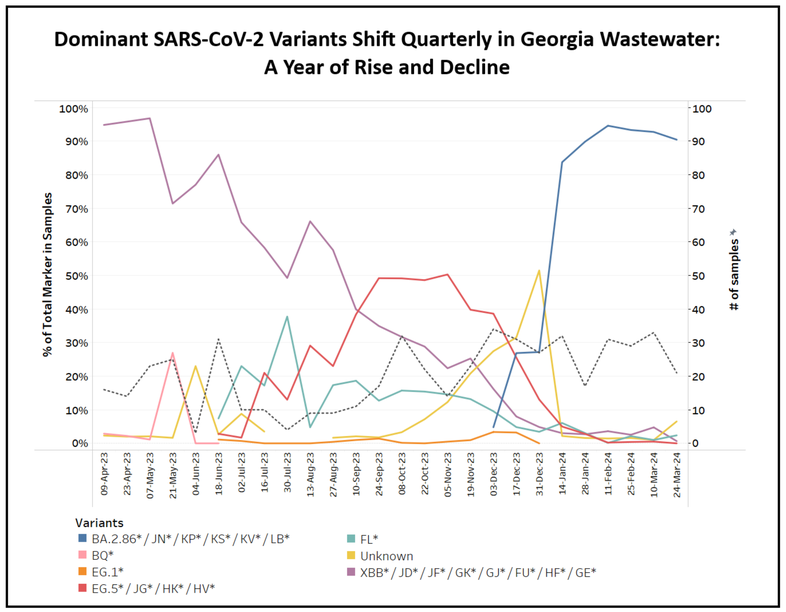

Figure 2: Temporal Distribution of SARS-CoV-2 Genotypes in Georgia Wastewater Samples (Two-Week Averages, April 2023 - April 2024). Initially, XBB* (purple) dominated but declined by December 2023. BA.2.86* / JN* (blue) surged to 90% around February 2024. EG.5* (red) peaked at 50% in October 2023. FL* (cyan) showed sporadic peaks, while BQ* (pink) briefly peaked in May 2023. The Unknown marker (yellow) demonstrates an upward trend from October 2023 to January 2024, indicating the introduction of a new variant in the wastewater. The black dotted line represents the number of samples collected, which remained approximately 20 samples every two weeks. *The asterisk (*) denotes the parent lineage and all of its descendant sublineages.*

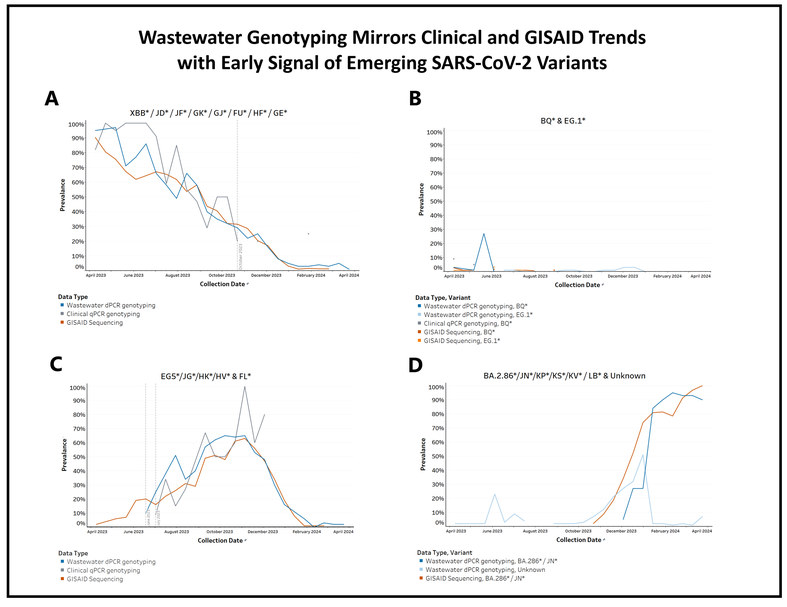

Figure 3. Prevalence of SARS-CoV-2 Variants in Georgia: Clinical, GISAID, and Wastewater Data (Two-Week Averages, April 2023–April 2024). A total of 528 wastewater samples were processed and analyzed, alongside 330 clinical genotyping samples and 2,855 clinical sequencing datasets from GISAID. (A) XBB* prevalence declined across all data; by Oct 2023, it persisted in wastewater and GISAID (30%) but was absent from clinical data. (B) BQ* peaked at 27% in wastewater in May 2023, undetected in clinical/GISAID; EG.1* appeared only in wastewater (1–3% in fall/winter). (C) EG.5*/FL* were detected in wastewater 22 days before clinical reports. (D) BA.2.86*/JN* and an unknown marker rose in wastewater and GISAID; the unknown marker's Oct–Jan peak aligned with BA.2.86*/JN*. Clinical data were limited during this period and were excluded.

Key Takeaways

- **dPCR-based genotyping is a cost-effective, scalable alternative to whole-genome sequencing for wastewater surveillance.** Singleplex dPCR reduced reagent costs by 36% and processing time by 68% compared to Illumina sequencing; multiplexing further reduced costs by 84% and time by 90%.[3]
- **One variant typically dominated each quarter,** with no more than three variants prevalent at a time. This indicates that dPCR genotyping of wastewater provides sufficient resolution for community-level surveillance and enables early detection of major shifts, without requiring high-resolution sequencing (Figure 2).
- **Wastewater trends closely mirrored clinical and GISAID data,** supporting dPCR genotyping as a reliable and complementary tool for population-level viral surveillance (Figure 3).

Scan to learn more about the dPCR-based genotyping study protocol and Rosalind Tracker.

Study protocol for SARS-CoV-2 variant detection in wastewater (https://www.ceresnano.com).

Public-facing dashboard for rapid genotyping program for SARS-CoV-2 in the United States (https://tracker.rosalind.bio).

Acknowledgments

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4. Figure 1 Created in BioRender. Patnaik, A. (2025) https://BioRender.com/030bkg0.