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RBM-targeted amplicon sequencing coupled with the WasteCov pipeline for rapid surveillance of SARS-CoV-2 lineage groups in municipal wastewater

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Wastewater-based epidemiology is a tool for population-level surveillance when clinical testing capacity is limited. We developed a rapid amplicon sequencing workflow targeting the spike RBM, together with WasteCov, our custom bioinformatic pipeline for SARS-CoV-2 lineage surveillance in wastewater. This method combines one-step RT-PCR amplification with targeted sequencing. Candidate assignments are constrained using contemporaneous epidemiological priors derived from clinical reports of globally circulating variants. WasteCov performs mapping, variant calling and lineage estimation using an updated UShER matrix operating through two modes: a Freyja-adapted mutation-centric approach and "sign_mol", a haplotype-aware mode that detects RBM molecular signatures, estimates their frequencies and assigns them to lineage groups. We applied the framework to 76 weekly composite wastewater samples from Thessaloniki, Greece, covering the Delta-to-Omicron transition (W47-2021 to W18-2022) and subsequent Omicron diversification (W48-2022 to W48-2023). Our approach captured major replacements, including the BA.1-to-BA.2 shift, the transition from BA.5/BA.2.75 to XBB descendants, and the rise of BA.2.86, while remaining informative at low prevalence. Wastewater-derived profiles were concordant with 28803 genomes from clinical surveillance and, in some cases, detected variant emergence earlier. This approach supports timely, cost-efficient variant surveillance, extendable to other viruses.

Keywords

SARS-CoV-2, Wastewater-based epidemiology, Receptor-binding motif targeted NGS, Variant quantification, Lineage dynamics, Public health surveillance

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Professional Status of the Speaker

PhD Student

Junior Scientist Status

Yes, I am a Junior Scientist.

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