Detection of SARS-CoV-2 Variants in Wastewater in Two Metropolitan Areas of Arkansas

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Abstract

Wastewater surveillance has been successfully used to detect outbreaks of poliovirus, hepatitis A virus, norovirus, and other pathogens. It has also been implemented as an effective approach for the monitoring of SARS-CoV-2 and variants at the community level during the COVID-19 pandemic. More recently, the detection of other pathogens in wastewater, such as poliovirus and monkeypox, has shown promise beyond COVID-19 pandemic and emphasizes its impact as an early warning of disease outbreaks. Since April 2020, we have been investigating the presence of SARS-CoV-2 and its genomic changes in wastewater influent sampled from two metropolitan areas in Arkansas. The levels of viral RNA were quantified by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) targeting three different viral genes (encoding ORF1ab polyprotein, ORF1ab; surface glycoprotein, S-protein; and nucleocapsid phosphoprotein, N-protein). The identity and genetic diversity of the virus were investigated using allele-specific RT-qPCR and RNA sequencing. SARS-CoV-2 variants of concern (e.g. Delta, Omicron) were detected in wastewater samples throughout the duration of the study and matched those found in COVID-19 patients from Arkansas during the same period. Changes observed in the detection pattern of S- and N-genes due to viral mutations suggest that this type of data could serve as an early warning signal to investigate for new variants in the population. This study supports the use of wastewater surveillance as a reliable complementary tool for the monitoring of SARS-CoV-2 variants at the community level.

Introduction

The emergence of new SARS-CoV-2 genetic variants has raised concerns due to their potential impact on viral pathogenicity, vaccine effectiveness, and the sensitivity of available detection tests. While sequencing of the viral genome from individual patients can provide information about the genetic diversity of the virus, RNA sequencing of wastewater samples has the potential to detect circulating SARS-CoV-2 variants at the community level. Here, we monitored the presence of SARS-CoV-2 and its genomic changes in wastewater sampled from two metropolitan areas in Arkansas during major surges of COVID-19 cases. The levels of three different viral genes (encoding ORF1ab polyprotein, ORF1ab; surface glycoprotein, S-protein; and nucleocapsid phosphoprotein, N-protein) were quantified between April 2020 and January 2023 by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). An allele-specific RT-qPCR approach was used to screen these samples for SARS-CoV-2-specific mutations in the S-gene, particularly those featured in the Delta and Omicron variants. The identity and genetic diversity of the virus were further investigated through amplicon-based RNA sequencing.

Materials and Methods

- 24-hour composite samples of untreated wastewater influent
  - Little Rock, AR (Adams Field, Fourche Creek, and Little Maumelle) and Pine Bluff, AR water treatment facilities
- Viral heat-inactivation: 56 °C for 30 min

Materials and Methods

- **Process/Recovery control**
  - Bovine coronavirus (BCoV, *Betacoronavirus*); spiked into wastewater samples prior to further processing and quantified by RT-qPCR
- Polyethylene glycol (viral concentration)
- Viral RNA was isolated through a magnetic bead–based purification method using MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Applied Biosystems)
- **RT-qPCR**
  - TaqMan™ 2019-nCoV Assay Kit v1 (Applied Biosystems): assays specific to three SARS-CoV-2 genes (ORF1ab, S- and N-proteins)
  - A subset of wastewater samples collected between October 2020 and January 2022 and that tested positive for SARS-CoV-2 was screened for the presence of selected mutations on the S-gene using TaqMan™ SARS-CoV-2 mutation panel assays (Applied Biosystems)
  - Positive control: synthetic DNA target sequences for each of the assays
  - Negative control: no template added on RNA isolation and RT-qPCR steps
  - Standard curve using known amounts of heat-inactivated SARS-CoV-2 isolate USA-WA1/2020 (BEI Resources)
- **Human fecal control**
  - Pepper mild mottle virus (PMMoV); RNA content in wastewater samples quantified by RT-qPCR
- **SARS-CoV-2 Genome Sequencing**
  - Amplicon-based RNA sequencing libraries were generated using AmpliSeq Library PLUS for Illumina library preparation kits and sequenced on an Illumina NextSeq 500 system. The Illumina DRAGEN COVID Lineage application and Explify Respiratory Pathogen ID/AMR Panel (RPIP) software within BaseSpace Sequence Hub were used for sequencing data analysis
  - The relative abundance of SARS-CoV-2 lineages was estimated in wastewater samples by using the bioinformatic package Freyja (https://github.com/andersen-lab/Freyja)

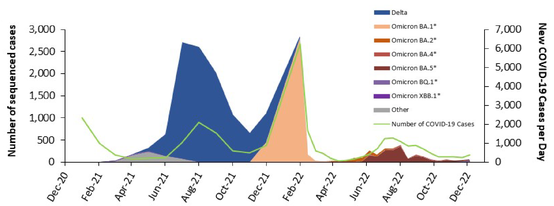

Figure 1. Distribution of SARS-CoV-2 variants among selected COVID-19 cases in the state of Arkansas between December 2020 and December 2022, https://www.gisaid.org/hcov19-variants.

Results and Discussion

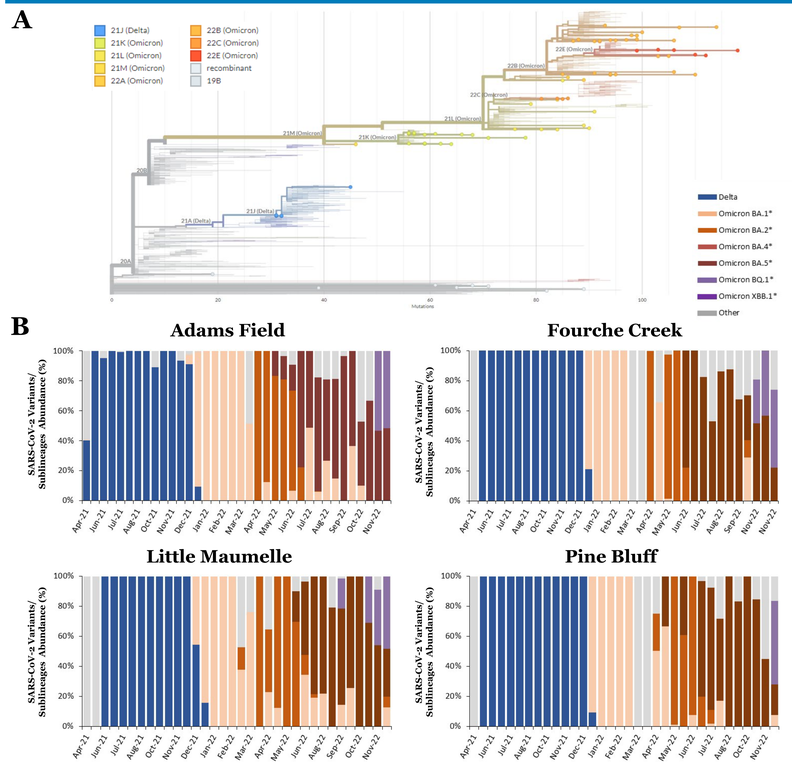

Figure 2. SARS-CoV-2 variants as assessed by RNA sequencing of wastewater samples. **A.** Phylogenetic analysis of SARS-CoV-2 viral genome consensus sequences detected in wastewater samples collected in Little Rock, AR and Pine Bluff, AR wastewater treatment facilities. Lines with dots corresponding to the legend color indicate samples sequenced in the study. Clades were assigned by using the NextClade tool (https://clades.nextstrain.org). **B.** Diversity of SARS-CoV-2 lineages based on amino acid changes in wastewater samples in Little Rock and Pine Bluff, AR overtime. The relative abundance of SARS-CoV-2 lineages in wastewater samples was estimated by using the bioinformatic package Freyja. These data are now available at the NCBI public repository, https://www.ncbi.nlm.nih.gov/bioproject/865728 and can be followed through the CFSAN dashboard - GenomeTrakr Network, https://www.fda.gov/food/whole-genome-sequencing-wgs-program/wastewater-surveillance-sars-cov-2-variants.

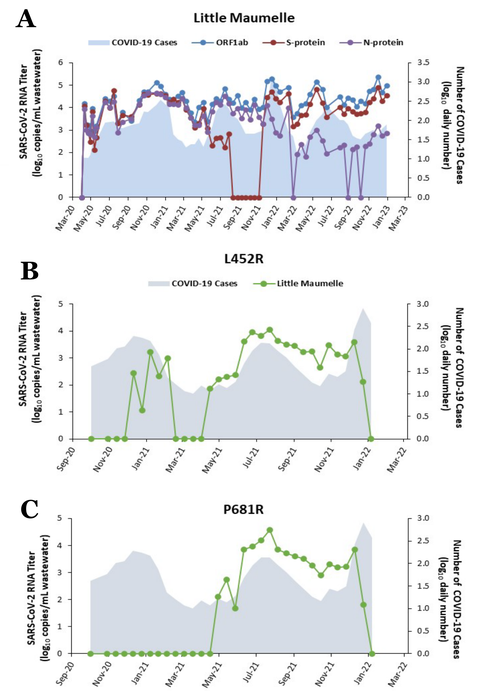

Figure 3. Temporal dynamics of SARS-CoV-2 genes in wastewater and COVID-19 case count in Little Rock, AR. **A.** The primary y-axis presents the levels of the viral RNA by gene in wastewater samples. The secondary y-axis shows the daily number of COVID-19 cases in Little Rock, AR, as reported by the Arkansas Department of Health during the same period. Rise of mutations in SARS-CoV-2 S-gene L452R **(B)** and P681R **(C)**, featured in the Delta variant, as detected by RT-qPCR in wastewater samples. The SARS-CoV-2 titers were normalized to PMMoV and are presented as viral RNA $\log_{10}$ copy number/mL of wastewater. Silva et al. Sci Total Environ. 2022 Nov 25;849:157546.doi: 10.1016/j.scitotenv.2022.157546.

Conclusion

- ✓ Our data show how changes in the SARS-CoV-2 virus genome can affect the sensitivity of specific RT-qPCR assays used in COVID-19 testing.
- ✓ SARS-CoV-2 variants (Alpha, Delta, and Omicron) responsible for the epidemic outbreaks in the area were identified in wastewater in the study locations. These same variants were found in COVID-19 patients from Arkansas during the same period and the viral titers in wastewater correlated with the number of COVID-19 cases in the areas studied.
- ✓ These findings support the use of wastewater surveillance as a reliable complementary tool for monitoring SARS-CoV-2 and its genetic variants at the community level.

Disclaimer: The views expressed in this poster do not necessarily reflect those of the US Food and Drug Administration or the Arkansas Department of Health. **Acknowledgements:** This study is being supported with COVID Supplemental funds.