

Control Number: 2022-A-3-NGS
Topic 1: Microbial Chatter: Microbial ecology in health and disease
Publishing Title: Enabling Earlier Detection of Recently Emerged SARS-CoV-2 Variants of Concern in Wastewater
Author Block: **N. Sapoval** (1), Y. Liu (1), E. G. Lou (2), L. Hopkins (3), K. Ensor (4), R. Schneider (3), L. Stadler (2), T. Treangen (1)
Rice University, Houston, TX.

Abstract Body: As clinical testing declines, wastewater monitoring can provide crucial surveillance on the emergence of SARS-CoV-2 variant of concerns (VoCs) in communities. Multiple recent studies support that wastewater based SARS-CoV-2 detection can precede clinical cases by up to two weeks. Furthermore, wastewater based epidemiology enables wide population based screening and study of the viral evolutionary dynamics. However, highly sensitive detection of emerging variants remains a complex task due to the pooled nature of environmental samples and genetic material degradation. In this paper we present quasi-unique mutation based identification (QualD), a novel bioinformatics tool for VoC detection based on quasi-unique mutations. The benefits of QualD are three-fold: (i) provides up to 3 week earlier VoC detection, (ii) more sensitive VoC detection (tolerant of >50% mutation drop-out), and (iii) leverages all mutational signatures (including insertions & deletions). We validate our tool on simulated data, and evaluate it on more than 2,600 real wastewater samples collected in Houston, TX. Our results support early detection hypotheses for the Delta and Omicron VoCs. Furthermore, due to the inclusion of indels in our pipeline we show higher sensitivity than other wastewater VoC detection pipelines. Additionally our data indicates higher stability of the indel based markers in the wastewater data. We note that QualD is optimized for sensitivity and detection of emerging VoCs, and hence should ideally be used together with other SARS-CoV-2 screening tools, especially when tracking quantitative changes in well established circulating variants. This tool is currently being used for routine surveillance in Houston, TX. In the future we plan on extending QualD for monitoring additional pathogens in wastewater

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Control Number: 2022-A-100-NGS
Topic 1: Epidemiological Cues: NGS in Clinical and Public Health Microbiology
Publishing Title: Wastewater Surveillance Using Short Read and Long Read Sequencing Detects Emerging SARS CoV-2 Variants
Author Block: **P. Ramachandran**, T. Kayikcioglu, T. Walsky, J. Woods, A. Windsor, H. Chen, C. Boerner, J. Amirzadegan, C. H. Bias, M. Balkey, H. Rand, J. Pettengill, R. Timme, C. Grim, M. Hoffmann; FDA, College Park, MD.

Abstract Body: Wastewater surveillance has become a routine public health monitoring tool for detecting and tracking SARS-CoV-2 (SC2) variants. Several waves of SC2 variants spread across the country over the past several months; understanding their prevalence and trajectory is essential. This study assesses detection performance, variant calling accuracy, and time from sample collection to public data release across 3 sites, Alabama (AL), Mississippi (MS), and Maryland (MD), using 2 SC2 target enrichment panels on Illumina MiSeq and Oxford Nanopore Technologies (ONT) GridIon sequencing platforms. Routine wastewater sample collection began in November 2021 in AL and MS and mid-January 2022 in MD. Viral RNA was extracted using the Zymo Environ Water RNA kit on wastewater concentrates from AL

and MS. Promega Enviro TNA extraction kit was used on raw grabs and composites from MD. cDNA was prepared using Invitrogen Superscript IV First Strand Synthesis kit. Target enrichment was performed with QIAseq DIRECT or NEBNext VarSkip Short amplicon kit and sequenced on MiSeq or ONT Gridion, respectively. Sequences were analyzed with our custom CFSAN Wastewater Analysis Pipeline. Performance was assessed with 4 metrics: percent reads aligned to the SC2 genome, mean genome coverage depth, completeness of coverage, and precision of variant detection. Raw sequence data and detailed metadata were submitted to NCBI under the umbrella BioProject [PRJNA757291](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA757291). Estimated variant proportions are updated as records become available in NCBI on the FDA-CFSAN “Wastewater Surveillance for SARS-CoV-2 Variants” website. We detected the spike in Omicron sub-lineages in wastewater about a week ahead of the clinical data publicly available at ZIP-level for MD. Our wastewater data also revealed that the onset of BA.2, BA.2.12 and BA.5 waves occurred about 2 weeks earlier in AL than in MS. Quality metrics of our output varied noticeably with the rise and fall of BA waves affecting the population serviced by the respective sewer sheds. Our lead time from sample collection to public release of raw sequence data via NCBI was as short as 57 hours. Continually evaluating target enrichment panels is crucial to accurately detect emerging sub-lineages. By using updated target enrichment panels to overcome S-gene dropout, continually updating protocols, and releasing our data publicly, we supported data-driven public health efforts. Our methods for processing, sequencing, and analysis allows for rapid detection of SC2 trends in a community.

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Control Number: 2022-A-136-NGS
Topic 1: Microbial Chatter: Microbial ecology in health and disease
Publishing Title: Identifying Bias in Metagenomics for Wastewater Surveillance
Author Block: I. Keenum, S. Jackson, N. Lin;
NIST, Silver Spring, MD.

Abstract Body: As the One Health framework for considering antibiotic resistance (AR) gains traction, routine surveillance for AR genes (ARGs) and pathogens is increasing. A necessary element of widespread monitoring is standardized methodology to generate comparable results. Wastewater surveillance has emerged as an increasing area of interest for public health monitoring for disease prevalence (most prominently SARS-COV-2) and has the potential for targeting additional elements such as AR prevalence in a community. Efforts has been taken by for culturable organisms through the World Health Organization’s Tricycle program and the U.S. CDC’s National Wastewater Surveillance System has presented plans for targeting ARGs in wastewater influents via digital PCR. However, choosing specific ARGs for widespread monitoring presents challenges in assay selection and emergent gene tracking. Shotgun metagenomic sequencing has been identified as a promising method to monitoring all ARGs present in an environment. It has the potential to allow for simultaneous untargeted gene monitoring, however, due to the variation present in metagenomics workflows, standardization is still needed to identify biases present that could influence the results. In this meta-analysis, 1000 wastewater influent metagenomes were collected via a systematic literature review from publicly available datasets. Metadata was extracted from the relevant publications and samples were processed with identical ARG annotation and

taxonomic annotation bioinformatic workflows. Ideally, the differences seen in microbiome and resistome, will be primarily driven by sample location and timing and less effected by sample processing and authors affiliated with the work. The results show that while overall the sampling location was the primary driver in the annotation of the resistomes and microbiomes (ANOSIM, Resistome: $p = 0.001$, $R = 0.25$; Microbiome $p = 0.001$, $R = 0.25$), DNA extraction and (DNA extraction: ANOSIM, Resistome: $p = 0.001$, $R = 0.18$; Microbiome $p = 0.001$, $R = 0.21$). Additionally, the sequencing depth also was seen to affect the total annotations of ARGs and the effect of the bibliographic network is still being assessed (how do the co-authors of a paper directly impact the results of a resistome). Understanding the bias' present in existing sample data is critical to identifying points where standardization should be focused for metagenomics sampling workflows for widespread AR monitoring.

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Late Breaking Abstract Submission

Sam Sherchan

Title: Environmental surveillance of SARS-CoV-2 variants in Nepal

Abstract

Genomic surveillance of wastewater sampling sites are increasingly exploited to map the prevalence and spread of SARS CoV-2 variants in Nepal. For effective public health measures, the understanding of the circulating variants of the virus is very important. Environmental genomic surveillance is expected to predict upcoming variant(s) with the possible next wave of COVID-19. The main aim of the genomic surveillance project in Nepal is to inform public health authorities and relevant stakeholders regarding the circulating SARS CoV-2 variants. The project also aims to understand the feasibility of utilizing wastewater based epidemiology for minimizing the infectious diseases in lower income countries like Nepal. So far, key variant assignment is based on mutations in spike protein of the virus. Therefore we approached spike gene amplicon sequencing with NGS strategy on Illumina sequencing instrument. Out of 72 sewage and hospital wastewater attempts, a significant number of reads were obtained, only 49 samples with higher reads in hospital wastewater samples. We assigned variants in the sequences using the "kallisto" bioinformatics pipeline. Most frequent mutations were SNPs - T477K, D614 and L452, indicating mutations in ACE-2 receptors. Among assigned eleven variants the dominant one was Delta variant until mid-December 2021, then replaced by Omicron which correlates with clinical scenario. Nepal had an Omicron wave with massive positivity rates in January 2022. Though further analysis with the whole genome is continued, our results of genomic surveillance provide usefulness of wastewater testing to predict genomic variants of SARS CoV-2 in poor resource setting countries.