

Abstract citation ID: ckae144.2136

Viral pathogen surveillance in wastewater using metagenomic approaches: obstacles and prospects

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Background: Wastewater monitoring offers an important tool for pathogen detection and tracking within large populations at a minimized cost and labor. Untargeted metagenomic approaches for the detection and sequencing of viruses still face limitations. In this study we tested the application of the sequence-independent single-primer amplification (SISPA) protocol in conjunction with nanopore sequencing to the study of viromes in wastewater samples.

Methods: Influent wastewater was collected from four Wastewater Treatment Plants located in the Lisbon region and Alentejo, Portugal in 2019 and 2023. Samples were processed with different methods including filtration, flocculation, Nanotraps or absorbent tampons. We applied the SISPA protocol, that includes a random amplification step, followed by nanopore sequencing on flow cells R9.4.1. We used the CZ ID (<https://czid.org>) pipeline for metagenomic analysis and taxonomic reporting.

Results: A large heterogeneity was found between samples in sequencing output (ranging from 17 thousand to 12 million reads) and the proportion of sequence reads classified as viral (ranging from 0.06% to 4% per sample). Viral taxa with higher number of reads were Picornavirales, dsDNA and ssDNA phages, Mimiviridae and Circoviridae. Known viral pathogens included Rotavirus A, Orthopoxvirus, Human betaherpesvirus and Avian orthoavulavirus.

Conclusions: Metagenomic studies of environmental samples generally exhibit a low proportion of viral reads, even after viral enrichment. A substantial sequencing effort is thus required to achieve a comprehensive virome characterization. As alternative, a more targeted approach, e.g. to target families of viruses of interest, may be considered. Funding: Fundação para a Ciência e Tecnologia (FCT): GHTM - UID/04413/2020; LA-REAL - LA/P/0117/2020; <https://doi.org/10.54499/CEECINST/00102/2018/CP1567/CT0040> to SGS. Research council of the Vrije Universiteit Brussel (OZR-VUB): OZR3863BOF to PL.

Key messages:

- Enrichment for viruses in metagenomic approaches remain challenging in very complex samples as are influent wastewaters.
- Virome characterization still necessitates high sequencing efforts but may be used as a complementary environmental survey tool integrated with more targeted approaches.