Rapid Reviews COVID-19

Review 1: "Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

Review:

This preprint titled "Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants" is a very timely addition to the pool of literature related to SARS-CoV-2 and wastewater-based epidemiology. This study provides a successful demonstration of genome sequencing and single nucleotide variant (SNV) detection of SARS-CoV-2 present in wastewater, in turn allowing one to obtain ecological and evolutionary insights of SARS-CoV-2 through WBE.

Despite the merits of this study, there are certain aspects that need to be further clarified, namely the authors need to provide more information on their approach to allow one (i) to determine how feasible the approach can be used in other geographical regions, where SARS-CoV-2 abundance is generally on the low end (with Cq value > 33) and with the outbreak possibly at the non-peak phase; (ii) to perform the needed enrichment steps that would help in genome sequencing.

To further illustrate, it seems that most of the samples sequenced in this study have low Cq values of < 33. However, a search in most published WBE papers show that the detected Cq values are usually higher, ranging from 36 to 39. Hence questions remain on whether samples with high Cq values (and therefore with low abundance of SARS-CoV-2 in wastewater) can achieve such successful demonstrations of SARS-CoV-2 genome and SNV sequencing.

Although the authors have also mentioned that one way to circumvent the abovementioned problem is to perform rRNA depletion or viral enrichment, not much information is given on both oligo probe sets (Gut microbiome, which does not seem to be commercially available; Respiratory virus oligo panel, which is commercially available at Illumina). Information on what oligos were used and their associated targets would be useful. A balanced discussion on the pros and cons of performing such enrichment steps would also be informative.

Considering that the Illumina sequencing approach can be very sensitive (with the multiple rounds of emulsion-based PCR) and that the authors also performed additional enrichment steps, the authors should also include negative controls to determine if they are still recovering SARS-CoV-2 genomes from their approach.

Although it is out of the scope of this study, the authors used three different extraction methods without really explaining why. The sampling point and nature of the wastewater matrix is also not well described. The authors mentioned "Wastewater interceptors" but it is not clear what that means.

Despite these technical comments, it is in my opinion that this preprint still provides potentially informative data that would help decision makers utilize WBE data to manage the COVID-19 pandemic.