Assessing the presence of SARS-CoV-2 in wastewater and implications for worker's health at three WWTP in Gauteng, South Africa

Don Jambo[1,2], **Noncy Gomba**[1], **Tanusha S Singh**[1,3,4]

[1] National Institute for Occupational Health, National Health Laboratory Services, Johannesburg, South Africa
[2] School of Microbiology, North-West University, Potchefstroom, South Africa
[3] Department of Clinical Microbiology and Infectious Diseases, University of Witwatersrand, Johannesburg, South Africa
[4] Department of Environmental Health, University of Johannesburg, South Africa

INTRODUCTION

Despite the continuously changing developments observed during the course of the COVID-19 pandemic (corona virus disease 2019), the high death toll and socio-economic consequences resulting from the spread of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infections has catapulted intense research into the virus' modes of transmission with significant focus also being placed on faecal-oral transmission as it has been previously linked to SARS-CoV outbreaks in the past. Uncertainties arise on the persistence of SARS-CoV-2 in sewage wastewater raising great concern over the potential of infection risk for highly exposed wastewater treatment plant (WWTP) personnel as well as the health risk from water reuse.

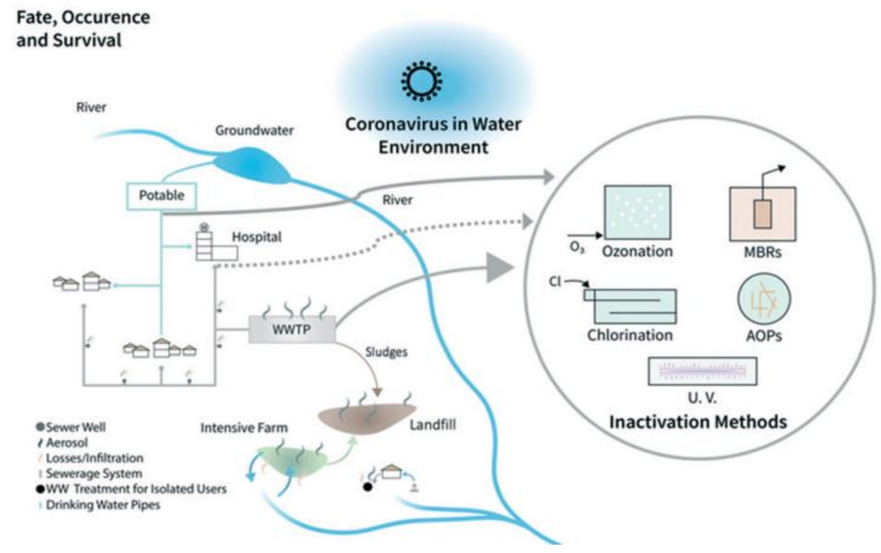

Figure 1 Coronavirus in water and sanitation systems: fate, occurrence, inactivation methods (Naddeo and Liu 2020)

AIMS

- To assess the occurrence of SARS-CoV-2 in wastewater and aerosols at aeration basins
- To determine genetic diversity of detected genetic fragments of SARS-CoV-2 using metagenomics analysis
- To evaluate treatment process efficacy in removal of SARS-CoV-2
- To evaluate potential health risks to wastewater workers and implications for water reuse

METHODS

Water (influent; primary sludge; activated sludge; secondary settling tank (SST) effluent; final effluent), contact surface swab and bioaerosol samples were collected weekly at three WWTPs (referred to as WWTP A, B and C) between July and September, 2020. No activated sludge, swab and bioaerosol samples were collected from WWTP A as these were only associated with WWTP B and C with surface aeration bioreactors. Samples were processed according to type as shown in Figure 2 and thereafter subjected to RNA extraction and real time reverse transcription-quantitative polymerase chain reaction (rRT-qPCR).

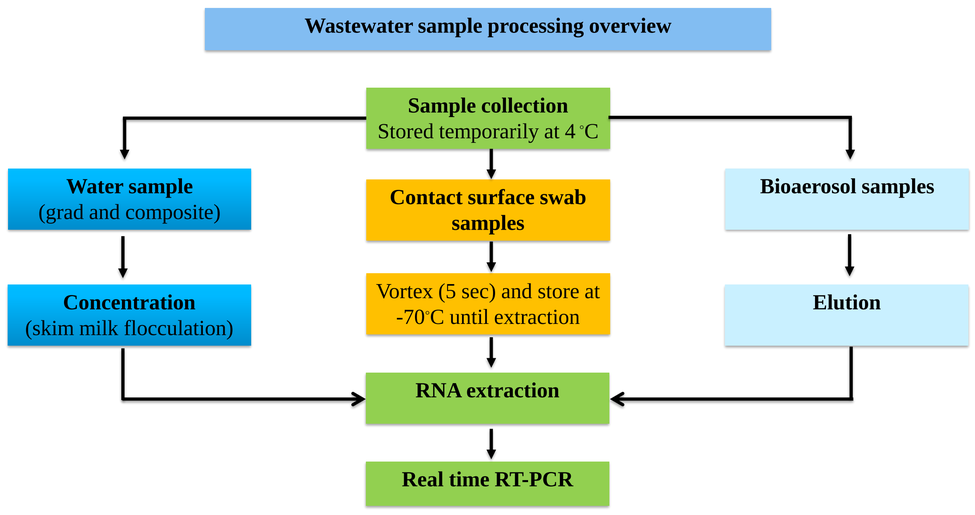

Figure 2 Schematic overview of the work flow adopted for water, swab and bioaerosol sample processing

RESULTS

- 85.3% (58/68) samples collected over a five-week period showed a positive detection for SARS-CoV-2 (Ct values <40 were regarded as positive)
- The influent and primary sludge samples from all three WWTPs were consistently positive
- SARS-CoV-2 RNA was also detected in the final effluents across all three WWTPs
- However WWTP C and B had no detection at week 3 and week 2 & 5, respectively
- Three swabs from WWTP B and 1 from WWTP C had a positive detection result
- SARS-CoV-2 was detected in 93.3% (14/15) of samples collected in week 5 (Figure 3)
- All targeted gene fragments (N, ORF1ab, and S) were detected in all WWTPs A and C samples of week 5
- However WWTP B was negative for the final effluent sample and only S gene amplification was detected for the contact swab sample
- Only WWTP B's contact swab samples was processed for week 5

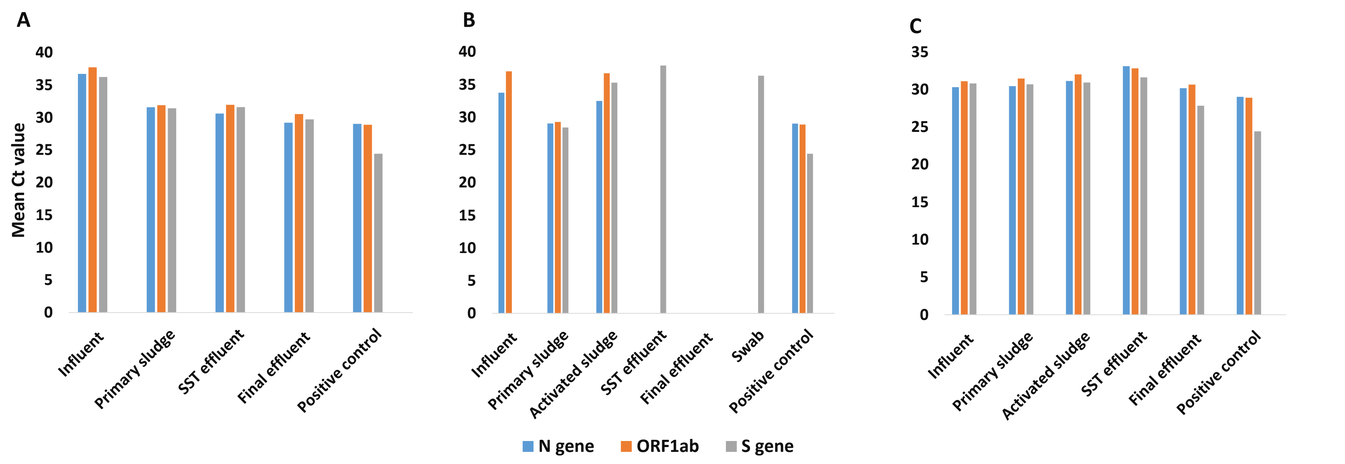

Figure Mean Ct values (*n*=2) for week 5 samples collected from WWTP A, B and C

Observed gene copy equivalents/ml (GCEs/ml) ranged between 1.8 x 10^3 and 4.8 x 10^5 GCEs/ml (Figure 4). Primary sludge samples had the highest GCEs/ml except for WWTP C week 5, where final effluent GCEs/ml exceeded other samples. There was a decline in Week 5 GCEs/ml for samples leading up to the SST effluent while an increase in CGEs was observed for final effluent samples. SST effluent sample GCEs/ml for WWTP A and B decreased between week 1 and week 5 while the opposite was true for primary sludge and final effluent samples. Low GCEs/ml were observed for WWTP B's swab sample (1.83x10^3 GCEs/ml for the S gene). The concurrent presence of SARS-CoV-2 gene fragments in the activated sludge and swab samples from WWTP B is significant.

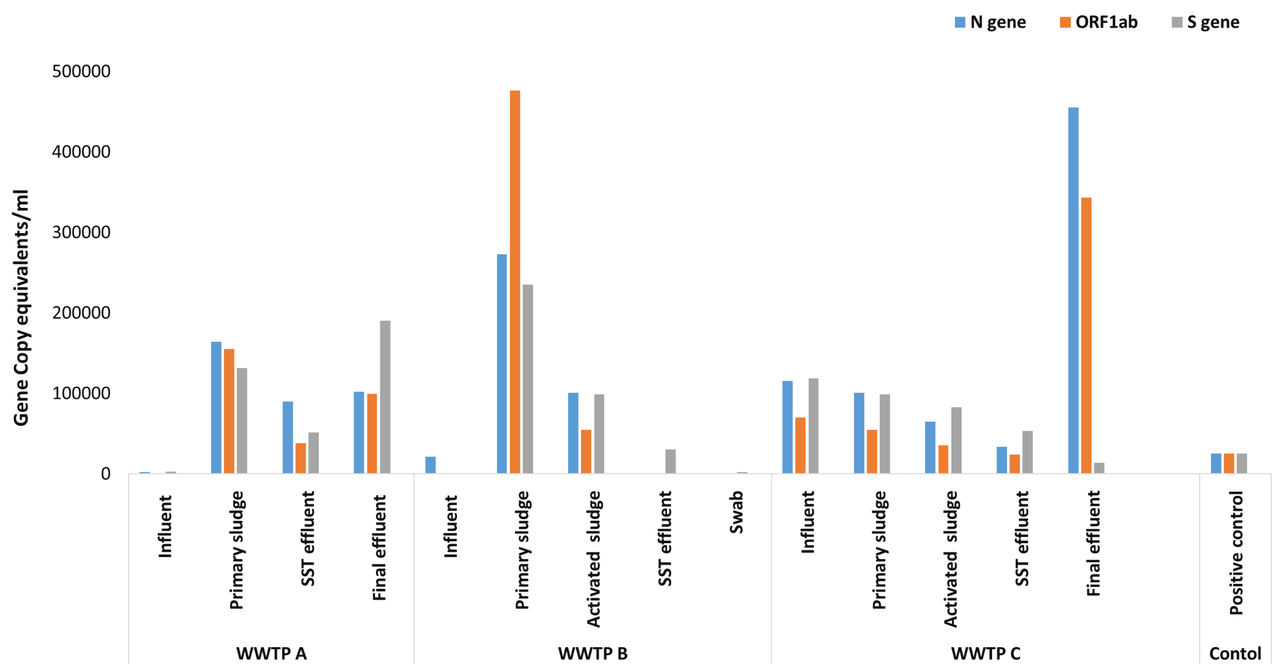

Figure 4 Gene copy equivalents/ ml for SARS-CoV-2 of week 5 samples collected from WWTP A, B and C

DISCUSSION

- The S gene fragment was detected in 85.3% samples and appears to be more readily detected compared to the N gene and ORF1ab. This demonstrates that detection sensitivity varies for different target genes
- SARS-CoV-2 gene fragments was successfully detected in the final effluent contrary to reports from previous studies of high-income countries
- The results suggest possible viral degradation along the WWTP treatment chain although this observation is not consistent with the spikes in the final effluent GCEs/ml for WWTP A and B in week 5
- The positive viral RNA results for swab and activated sludge samples suggest the likelihood of exposure to workers although this does not indicate a health risk in wastewater workers a viable SARS-CoV-2 was not detected
- Some samples showed no detection of the internal control (MS2 phage) even though there was a positive PCR result for the gene targets suggesting inhibition either from high RNA concentration or impurities in the sample matrix
- Repeats with lower extracted RNA concentrations showed improved results for rRT-PCR

CONCLUSION

SARS-CoV-2 gene fragments were detected in over 80 % of samples including effluent samples raising concerns of potentially negative implications for water reuse. More importantly, the presence of detectable SARS-CoV-2 gene fragments in samples associated with key WWTP process points suggests that WWTP workers could be at risk of exposure to the virus. Further investigations on viral infectivity will be necessary in order to elucidate the health risks posed to WWTP personnel.

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