Short Communication

Non-intrusive wastewater surveillance for monitoring of a residential building for COVID-19 cases

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HIGHLIGHTS

- Non-intrusive wastewater surveillance was implemented in a high-risk apartment building.
- SARS-CoV-2 signals in wastewaters prompted identification of a COVID-19 case.
- Detection of SARS-CoV-2 in wastewaters preceded symptom onset of a case.
- Wastewater surveillance is useful for non-intrusive monitoring of premises with relatively small population.

GRAPHICAL ABSTRACT
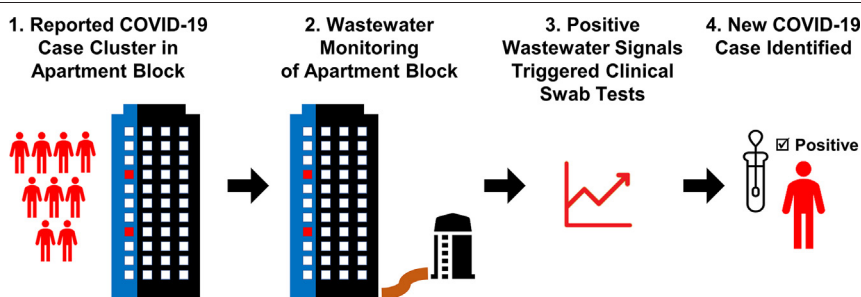
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ABSTRACT

Wastewater-based surveillance for SARS-CoV-2 has been used for the early warning of transmission or objective trending of the population-level disease prevalence. Here, we describe a new use-case of conducting targeted wastewater surveillance to complement clinical testing for case identification in a small community at risk of COVID-19 transmission. On 2 July 2020, a cluster of COVID-19 cases in two unrelated households residing on different floors in the same stack of an apartment building was reported in Singapore. After cases were conveyed to healthcare facilities and six healthy household contacts were quarantined in their respective apartments, wastewater surveillance was implemented for the entire residential block. SARS-CoV-2 was subsequently detected in wastewaters in an increasing frequency and concentration, despite the absence of confirmed COVID-19 cases, suggesting the presence of fresh case/s in the building. Phone interviews of six residents in quarantine revealed that no one was symptomatic (fever/respiratory illness). However, when nasopharyngeal swabs from six quarantined residents were tested by PCR tests, one was positive for SARS-CoV-2. The positive case reported episodes of diarrhea and the case's stool sample was also positive for SARS-CoV-2, explaining the SARS-CoV-2 spikes observed in wastewaters. After the case was conveyed to a healthcare facility, wastewaters continued to yield positive signals for five days, though with a decreasing intensity. This was attributed to the return of recovered cases, who had continued to shed the virus. Our findings demonstrate the utility of wastewater surveillance as a non-intrusive tool to monitor high-risk COVID-19 premises, which is able to trigger individual tests for case detection, highlighting a new use-case for wastewater testing.

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1. Introduction

Wastewater-based testing for SARS-CoV-2 has been shown to be a useful COVID-19 surveillance tool (Daughton, 2020). At wastewater treatment plants, it could provide early warning of transmission or objective trending of the population-level disease prevalence (Thompson et al., 2020). In countries such as the Netherlands (Medema et al., 2020), Spain (Randazzo et al., 2020), France (Wurtzer et al., 2020) and the United States (Nemudryi et al., 2020), the detection of SARS-CoV-2 RNA at wastewater treatment plants preceded symptomatic cases. Wastewater surveillance has also been used as an additional indicator to provide prevalence trends of SARS-CoV-2 in the sampled communities (National Institute for Public Health and the Environment, 2020; Peccia et al., 2020). More recently, surveillance at high-density living premises types, such as student hostels, has also been carried out (Chelvan, 2021; Peiser, 2020).

In Singapore, more than 58,000 COVID-19 cases have been reported from January to December 2020, mainly in community clusters and migrant worker dormitories. Similar to other countries, active wastewater monitoring is also being conducted in Singapore since February 2020 at water reclamation plants for wide area surveillance, and at high density living premises types, such as worker dormitories to provide timely assessment of the COVID-19 situation (Mohan, 2020; National Environment Agency, 2020).

On 2 July 2020, the Ministry of Health in Singapore reported a cluster of COVID-19 cases in two unrelated households on different floors of the same apartment building (Goh, 2020). Six apartment units on each floor of this building share lifts, lift lobbies and stairwell. The first case (index case) was reported on 23 Jun 2020, followed by eight additional cases identified through active clinical surveillance. Six of these were among seven family members who reside with the index case. Two remaining cases were among seven residents in another unit neighboring the index case (Fig. 1). Residents of the two affected units had no known interactions.

All cases were conveyed to healthcare facilities for clinical management and isolation, and household members in the two affected units were placed under home quarantine for 14 days. Active clinical surveillance that comprised a one-time offer of PCR testing and phone interview for symptoms was conducted among other residents and visitors of the affected building. To complement clinical surveillance, wastewater surveillance was implemented from 4 to 20 July 2020 for continuous monitoring of the building.

Here, we describe a new use-case of wastewater surveillance that facilitated the detection of a new COVID-19 case prior to onset of symptoms in the affected apartment building, demonstrating the utility of wastewater surveillance as a non-intrusive tool to monitor high-risk COVID-19 premises.

2. Materials and methods

2.1. Clinical surveillance

Persons under quarantine (PUQs) who resided in the two affected units (one and five PUQs, respectively) were placed on active phone surveillance. Nasopharyngeal swab PCR tests were conducted at the start and end of their quarantine period. Another 152 residents and 25 visitors of the affected section of the building were offered PCR tests and were placed on active phone surveillance and monitored for fever or respiratory symptoms until 12 July 2020. All epidemiological investigations and outbreak containment measures were implemented under the Infectious Diseases Act (Singapore Statutes Online, 2003).

2.2. Wastewater sampling

Wastewater samples were collected from a manhole serving the residential building using an autosampler (Aquacell P2 Multiform,

Aquamatic, United Kingdom). Hourly wastewater composites were collected during the diurnal sewage peaks (0500–1500 h and 1700–2200 h) from 4 to 20 July 2020. Each hourly composite sample collected ($n = 159$) included four 200 mL wastewater samples drawn from the manhole every 15 min. Nevertheless, samples could not be collected due to low sewage flow during certain hours. Details of sample collection and the list of samples successfully collected are provided in the Supplementary Data (Tables S1 and S2). All samples were transported daily to the laboratory on ice at 4–8 °C.

2.3. Virus concentration and RNA extraction

An aliquot of 45 mL of each hourly composite sample was concentrated using an adapted polyethylene glycol (PEG) precipitation method (Ahmed et al., 2020; World Health Organization, 2003). Briefly, each sample aliquot was clarified through centrifugation at 4000 ×g for 30 min at 4 °C. The supernatant was added to 3.6 g of PEG (8% w/v, polyethylene glycol 8000, Sigma Aldrich) and 0.875 g of sodium chloride (Sigma Aldrich). The supernatant-PEG mixture was incubated overnight at 4 °C on a rotating shaker at 200 rpm and was subsequently centrifuged at 4000 ×g for 180 min at 4 °C. The supernatant was removed, and the virus pellet was suspended in 300 μL of phosphate buffered saline.

This adapted method had a SARS-CoV-2 recovery rate of 59.5 ± 10.4% and was chosen because of its higher recovery rate in our laboratory settings when compared with other reported virus concentration methods (Ahmed et al., 2020). The recovery rate for the virus concentration method was obtained using SARS-CoV-2 positive wastewater samples with known SARS-CoV-2 virus concentration, where virus concentrations of the samples had been previously determined.

The recovery rate was obtained using the following formula, and tests were conducted in duplicate:

$$\text{Recovery } (\%) = \frac{\text{Number of virus copies per μL of virus concentrate}}{\text{Number of virus copies per μL of raw sewage used}} \times 100$$

Virus RNA was extracted from 200 μL of the suspended virus pellet using the KingFisher Flex System and MagMAX Viral Pathogen II Kit (ThermoFisher, USA) according to the manufacturer's recommendations.

2.4. Molecular testing

SARS-CoV-2 ORF1ab gene (World Health Organization, 2020) and the PMMoV faecal indicator (Gu et al., 2018) were detected in RNA extracted from wastewater samples by using quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) assays described elsewhere (Niu et al., 2020; Zhang et al., 2006). PMMoV, which is widespread and abundant in human faeces (Kitajima et al., 2018; Rosario et al., 2009), was primarily used as an indicator of human faecal contamination in wastewater samples and as an indirect indicator of PCR inhibition. A negative PMMoV result was assumed to indicate either the absence of faecal matter in tested samples or inhibition of PCR assay due to various inhibitors present in wastewater.

The original assays were optimized to perform a one-step RT-PCR, by using Luna Universal Probe One-Step RT-qPCR Kit (NEB, USA). Details on assay optimisation are provided in the Supplementary Data. Each PCR reaction was carried out in a 20 μL final reaction volume containing 1× Luna Universal Probe One-Step Reaction Mix, 1× Luna WarmStart RT Enzyme Mix, either 0.5 μM (ORF1ab) or 0.9 μM (PMMoV) of each primer, either 0.25 μM (ORF1ab) or 0.2 μM (PMMoV) of the probe and 2.5 μL of RNA template. The reaction protocol consisted of reverse transcription for 10 min at 55 °C, initial denaturation at 95 °C for 1 min, and 45 amplification cycles of denaturation at 95 °C for 10 s and extension at 55 °C for 30 s. The copy numbers of each target were calculated from
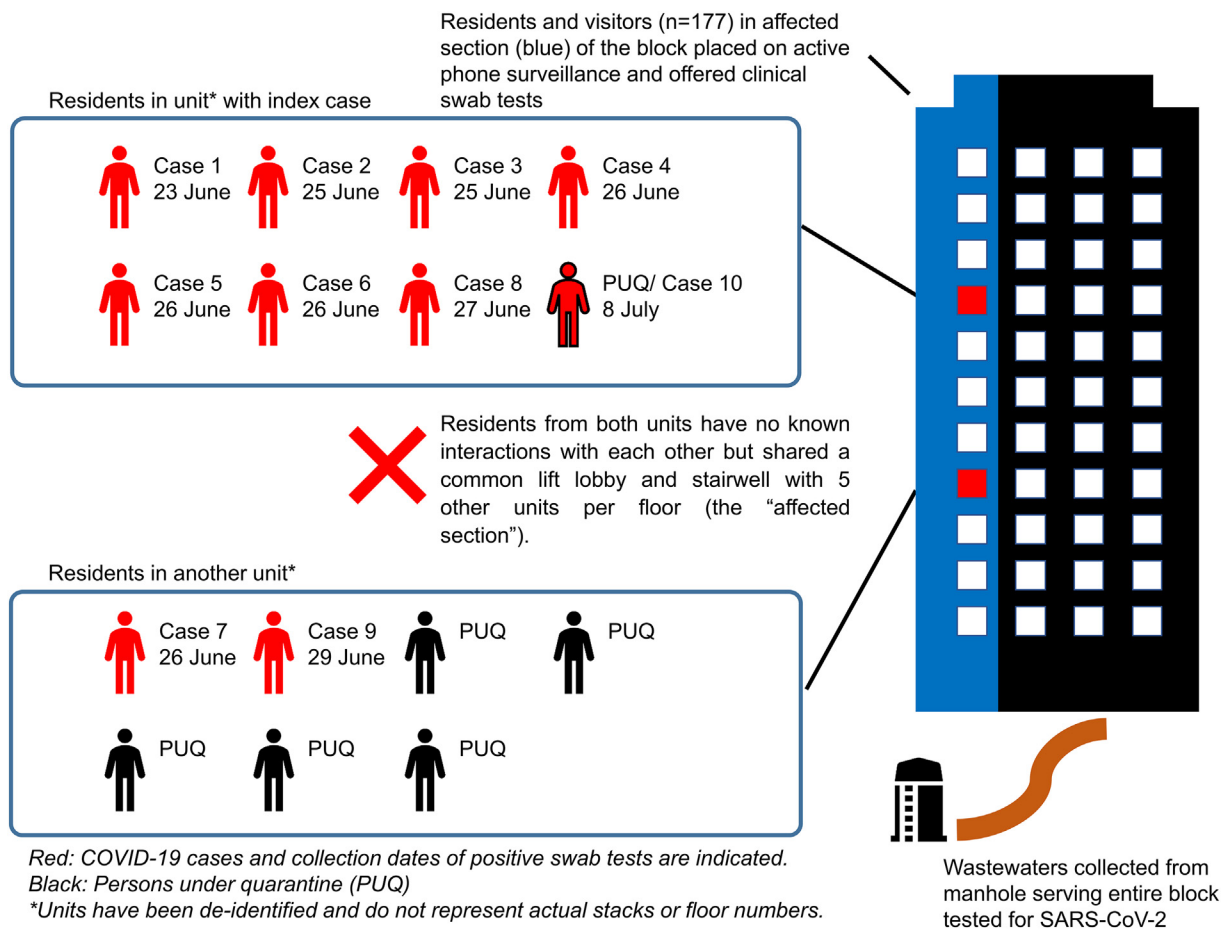
Fig. 1. Schematic representation of the cluster of cases in the residential building. The cluster comprised of nine cases residing in two units located on different floors of the same apartment building. Residents from both units had no known interactions, but shared common lift lobby and stairwell with five other units in each floor (the "affected section").

quantification cycle (Cq) values by using standard curves generated from serial dilutions of known copy numbers of a SARS-CoV-2 RNA synthetic control (complete genome based, Twist Synthetic SARS-CoV-2 RNA Control 2, MN908947.3; Twist Bioscience, USA) and PMMoV RNA synthetic control (110 bp; Integrated DNA Technologies, Singapore). Both standard curves were generated by using triplicates of 5-log serial dilutions. The thresholds of detection were determined based on the dynamic range of amplification for ORF1ab and PMMoV targets. The lowest level of detection for ORF1ab was 10 copies of synthetic SARS-CoV-2 RNA per reaction at an average Cq of 39.36. The respective value for PMMoV target was 150 copies of synthetic RNA per reaction at an average Cq of 39.96. Therefore, evidence of amplification at Cq <40 was considered as a "positive signal" for each target. All tests were performed in triplicates and an average concentration is reported.

3. Results

The one-time swab tests were done on 110 residents and 13 visitors of the affected building from 1 to 3 July 2020. All tests yielded negative SARS-CoV-2 results. However, SARS-CoV-2 was detected in one of eight wastewater samples collected on the first day of deployment on 4 July 2020 (7000 RNA copies/L sewage) (Fig. 2A), suggesting active virus shedding in the building. SARS-CoV-2 was detected in wastewaters daily from 4 to 9 July 2020, with frequent spikes ranging from 600 to 246,700 RNA copies/L sewage (Fig. 2A).

Despite positive wastewater test results, none of the persons under quarantine ($n = 6$) in the two affected apartment units reported COVID-19 symptoms. Nasopharyngeal swab PCR tests were carried out on all persons under quarantine on 8 July 2020, and one was positive

for SARS-CoV-2 (Cq value 22.64, 22.16). The new case (Case 10) was subsequently conveyed to a healthcare facility. Case 10 reported gastrointestinal symptoms, with episodes of diarrhea since 7 July 2020. The stools of case 10 were positive for SARS-CoV-2 on 11 July 2020.

The continuous screening of wastewater facilitated the identification of a COVID-19 case in the affected building. Additionally, the diarrheal episodes and positive stool of this new case explained the spikes of virus RNA levels observed from 4 to 9 July 2020. Notably, SARS-CoV-2 RNA was detected in wastewaters more than three days before the case recalled diarrheal, fever and respiratory symptoms, suggesting that viral shedding in faecal material could have started in the pre-symptomatic period.

The index case (Case 1) returned from the healthcare facility on 8 July 2020 after clinical recovery. Therefore, wastewater signals detected on 9 July 2020 could be either due to viral shedding from the newly identified case (Case 10) or from the returned index case. However, SARS-CoV-2 levels declined after the newly identified case was conveyed to a healthcare facility (1100–39,400 to 600–1700 RNA copies/L sewage from 10 to 13 July 2020) (Fig. 2B), suggesting that subsequent viral shedding was from the recovered index case, rather than from another additional new case/s. This contrasts to the frequent spikes in virus RNA levels observed from 4 to 9 July 2020, before the new case was identified.

Interestingly, SARS-CoV-2 levels increased again during 15–16 July 2020 (3900–28,300 RNA copies/L sewage) and was attributed to two recovered cases who returned on 14 July 2020 (Fig. 2B). SARS-CoV-2 levels were not detected from the evening of 19 July 2020, corroborating the viral shedding pattern of recovered cases and the cessation of active virus transmission in the building.
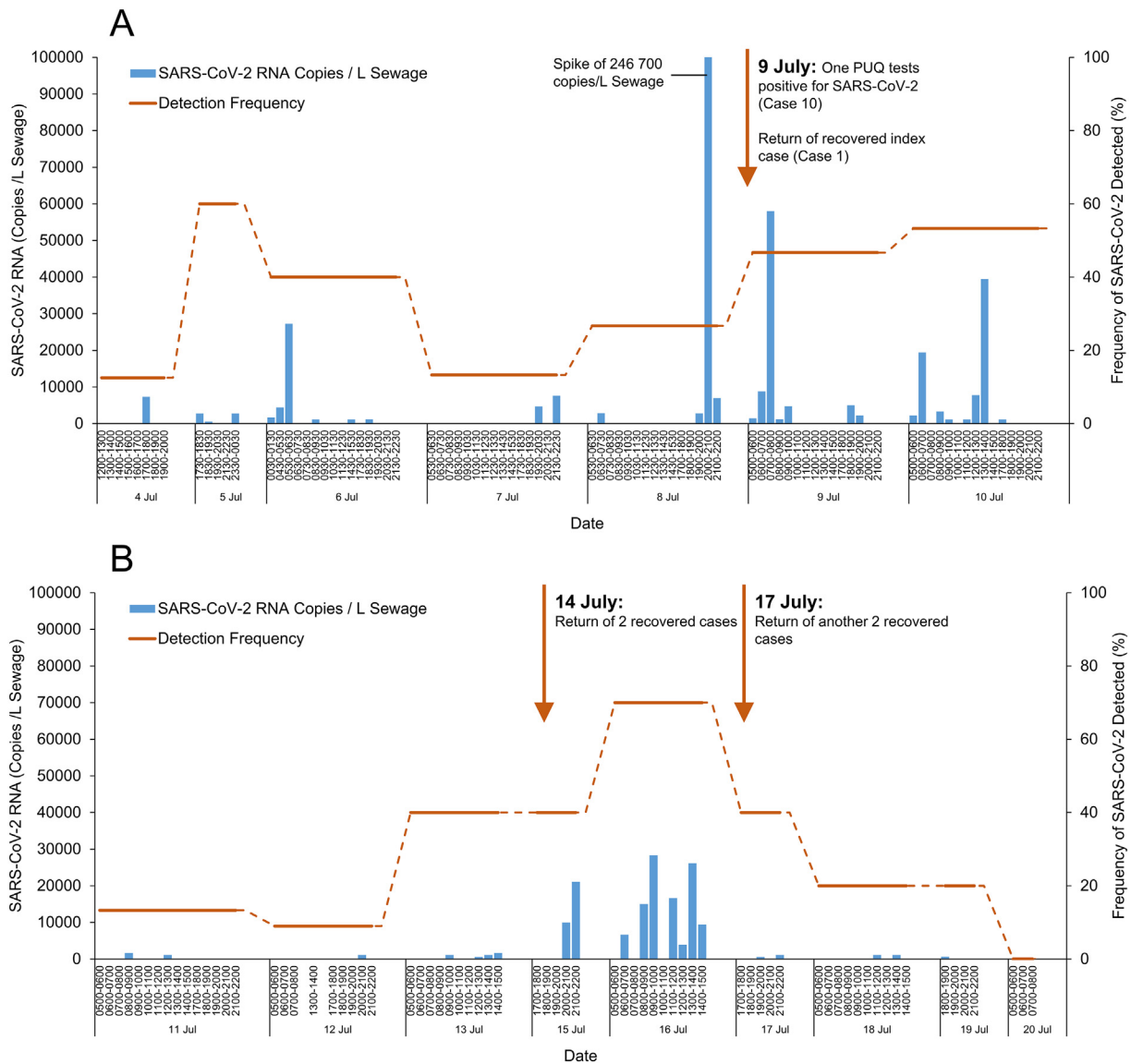
Fig. 2. SARS-CoV-2 virus RNA in wastewater samples collected from the apartment building. Panel A – SARS-CoV-2 RNA levels from 4 to 10 July 2020; frequent spikes in virus RNA levels corroborated the new case reported on 9 July 2020. Panel B – SARS-CoV-2 RNA levels from 11 to 20 July 2020; detection of SARS-CoV-2 RNA levels during 15–16 July 2020 and subsequent decline in virus RNA signals corroborated the return of two recovered cases on 14 July 2020. Return of another two recovered cases on 17 July 2020 did not result in spikes of SARS-CoV-2 RNA levels.

4. Discussion

This report describes a new use-case for wastewater surveillance, in which monitoring of a relatively small community at high risk of COVID-19 provided actionable data that informed operational decisions. Positive and intensifying SARS-CoV-2 signals in wastewater triggered intrusive individual tests for case identification, while negative or weakening signals assured the gradual cessation of transmission. In contrast to the widely reported, regional-based surveillance at wastewater treatment plants implemented for situational assessment (La Rosa et al., 2020; Prado et al., 2021), this targeted approach of wastewater surveillance complemented the cost- and logistics- intensive individual testing to facilitate early identification of a COVID-19 case.

Detection of virus RNA in wastewaters before the onset of symptoms in the present study supports the notion that faecal material can be positive for SARS-CoV-2, even when cases are asymptomatic (Ahmad et al., 2020; Tang et al., 2020). It is now known that SARS-CoV-2 shedding can persist longer in stool samples than in nasopharyngeal swabs (Gupta et al., 2020). Taken together, this suggests that stool samples have a

longer detection window for SARS-CoV-2 than other commonly-tested biological samples, underscoring the utility of wastewater testing as a COVID-19 surveillance strategy.

The present study also revealed the importance of longitudinal wastewater surveillance for the continuous monitoring of SARS-CoV-2 in the community. Single time point detection of SARS-CoV-2 in wastewaters provided limited information on whether a positive signal originates due to viral shedding by newly-emerging cases or from a recovered case. In contrast, continued monitoring and trend analyses could reveal whether positive signals occur due to viral shedding either by a new or a recovered case. Our findings also emphasized that the contribution of residual viral shedding from recovered cases must be accounted for when modelling wastewater surveillance-based estimates of COVID-19 prevalence in affected communities.

4.1. Limitations

It was a challenge to discern whether viral signals were due to new or recovered cases, although the increasing or decreasing trends

of viral RNA levels could be ascribed to new and recovered cases respectively. Another limitation was that wastewater samples could not be collected in every hour for each sampling day due to low sewage flow. Although 24-h daily composites have been recommended for other surveillance studies (Centres for Disease Control and Prevention, 2020), hourly composites provided improved sensitivity for case detection presumably because samples were not diluted by negative samples collected at different hours. However, the hourly approach requires a large number of wastewater samples to be tested, and could thus be a limitation for laboratories with limited resources.

Despite demonstrating a new use-case for wastewater surveillance, the approach described in the present study might be resource-intensive and/or limited by the access to suitable sampling sites to capture the targeted population.

5. Conclusions

- This study highlights a new use-case for non-intrusive wastewater testing at high-risk sites that facilitates case identification with minimal inconvenience to the community.
- The study demonstrated that viral shedding by even a single case could be detected in wastewater from a small community.
- The findings also emphasized limitations when interpreting single time point wastewater test results, as positive signals might be either from active infections or recovered cases. Longitudinal monitoring of wastewater could thus be appropriate in wastewater-based COVID-19 surveillance programmes.

CRediT authorship contribution statement

Judith Chui Ching Wong: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Joanna Tan:** Formal analysis, Investigation, Writing – original draft. **Ying Xian Lim:** Formal analysis, Investigation, Writing – original draft. **Sathish Arivalan:** Methodology, Investigation. **Hapuarachchige Chanditha Hapuarachchi:** Methodology, Investigation, Writing – review & editing. **Diyar Mailepessov:** Investigation. **Jane Griffiths:** Investigation. **Praveena Jayarajah:** Investigation. **Setoh Yin Xiang:** Investigation. **Wei Ping Tien:** Investigation. **Swee Ling Low:** Investigation. **Carmen Koo:** Investigation. **Surya Pavan Yenamandra:** Investigation. **Marcella Kong:** Investigation. **Vernon Jian Ming Lee:** Supervision, Writing – review & editing. **Ng Lee Ching:** Supervision, Conceptualization, Methodology, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147419.

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