



Co-incidence of BA.1 and BA.2 at the start of Singapore's Omicron wave revealed by Community and University Campus wastewater surveillance



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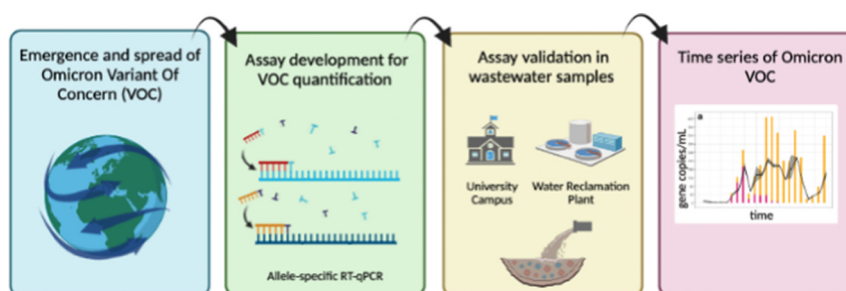
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HIGHLIGHTS

- Co-incidence of Omicron BA.1 and BA.2 in Singapore's wastewater
- This comes after strategic relaxation of policies and reopening of borders.
- AS RT-qPCR can be an accurate and reliable way of tracking VOCs in wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater surveillance (WWS) has been globally recognised to be a useful tool in quantifying SARS-CoV-2 RNA at the community and residential levels without biases associated with case-reporting. The emergence of variants of concern (VOCs) have given rise to an unprecedented number of infections even though populations are increasingly vaccinated. This is because VOCs have been reported to possess higher transmissibility and can evade host immune responses. The B.1.1.529 lineage (Omicron) has severely disrupted global plans to return to normalcy. In this study, we developed an allele-specific (AS) RT-qPCR assay which simultaneously targets the stretch of deletions and mutations in the spike protein from position 24–27 for quantitative detection of Omicron BA.2. Together with previous assays that detect mutations associated with Omicron BA.1 (deletion at position 69 and 70) and all Omicron (mutation at position 493 and 498), we report the validation and time series of these assays from September 2021 to May 2022 using influent samples from two wastewater treatment plants and across four University campus sites in Singapore. Viral RNA concentrations at the treatment plants corroborate with locally reported clinical cases, AS RT-qPCR assays revealed co-incidence of Omicron BA.1 and BA.2 on 12 January 2022, almost two months after initial BA.1 detection in South Africa and Botswana. BA.2 became the dominant variant by the end of January 2022 and completely

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displaced BA.1 by mid-March 2022. University campus sites were similarly positive for BA.1 and/or BA.2 in the same week as first detection at the treatment plants, where BA.2 became rapidly established as the dominant lineage within three weeks. These results corroborate clinical incidence of the Omicron lineages in Singapore and indicate minimal silent circulation prior to January 2022. The subsequent simultaneous spread of both variant lineages followed strategic relaxation of safe management measures upon meeting nationwide vaccination goals.

1. Introduction

A novel coronavirus outbreak was first reported in Wuhan, China in December 2019 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Zu et al., 2020). In February 2020, this outbreak was termed as the Coronavirus Disease 2019 or COVID-19 (WHO, 2020a) and in March 2020 it was declared as a global pandemic (WHO, 2020b). Since then (August 2022), it has infected over 604 million people and caused more than 6.49 million deaths worldwide (Ritchie et al., 2022). Due to the widespread nature of COVID-19, variants with mutation in its viral genome have been found and some variants are known to pose a higher risk due to increased transmissibility and reduced vaccination effectiveness (Harvey et al., 2021). To date, the WHO has declared five variants of concern (VOC) which include Alpha, Beta, Gamma, Delta, and Omicron lineages (WHO, 2022). By September 2021, Alpha, Beta and Gamma had been downgraded to variants being monitored by the US Centers for Disease Control and Prevention (CDC) as they were circulating at very low levels (CDC, 2022). Delta followed suit in April 2022, leaving Omicron as the only VOC in circulation and the dominant strain worldwide since early 2022 according to the Nextstrain database (accessed 1 August 2022, Hadfield et al., 2018).

Wastewater surveillance (WWS) has emerged during this COVID-19 pandemic as an effective tool in providing situational awareness of COVID-19 trends, allowing policy makers to make better informed decisions (Betancourt et al., 2021; Wong et al., 2021). This is because symptomatic and asymptomatic people infected with SARS-CoV-2 can shed the viral RNA in urine and feces (Peccia et al., 2020; Peng et al., 2020; Jones et al., 2020; Murata et al., 2021). WWS offers many advantages over clinical surveillance such as being non-invasive, unbiased, able to cover a broad spectrum, and its low cost in providing real-time data (Daughton, 2020; Hart and Halden, 2020; Thompson et al., 2020). WWS has also been proven to be a useful early warning tool as it can detect silent circulation of the virus before clinical cases are confirmed (Mao et al., 2020; Medema et al., 2020; Randazzo et al., 2020; Albastaki et al., 2021; Saguti et al., 2021; Wong et al., 2021). At the residential level, such as on a University campus, positive SARS-CoV-2 signal detected through WWS can lead to actionable information, such as ad-hoc testing of all residents and subsequent isolation to prevent the spread of the virus (Corchis-Scott et al., 2021; Gibas et al., 2021). This is especially important when vaccinations are not widely available, or when VOCs emerge that are better able to evade the host immune response, thus posing greater risk (Hu et al., 2022; Nasreen et al., 2022).

The need for timely situational awareness of COVID-19 trends, especially when new VOCs emerge, has motivated multiple variant tracking studies (Yaniv et al., 2021; Lee et al., 2022a). There has been increasing use of next generation sequencing (NGS) methods such as shotgun metagenomics and amplicon sequencing to determine VOCs circulating in the community (Chiara et al., 2020; Karthikeyan et al., 2022). However, due to complex sample and library preparation, cost, and long turnaround time, molecular diagnostic tools such as RT-qPCR remain a gold standard for rapid detection of SARS-CoV-2 (Jayamohan et al., 2020). We have previously developed and validated allele-specific (AS) RT-qPCR methods for VOCs Alpha, Delta and total Omicron (Lee et al., 2021a, 2021b, 2022a).

Omicron BA.1 and BA.2 possess unique mutations which allow them to be distinguished (Ayadi et al., 2022; Majumdar and Sarkar, 2022). In this study, we develop and deploy an assay targeting mutations associated with Omicron BA.2, which has been a dominant variant in many countries including the United States, South Africa, and Singapore (Buchholz, 2022; Sguazzin, 2022; Ooi, 2022). This assay targets deletions at loci 24–27

which the Omicron BA.1 variant does not possess. The objective of this study was to understand the dynamics of VOCs in Singapore's wastewater from September 2021 to May 2022 during a time where Singapore progressively eased restrictions and reopened borders, after achieving vaccination targets. We compared wastewater VOC signals on a University campus to those obtained at water reclamation plants (WRP), which treat and reclaim wastewater in Singapore, to determine the timing for initiation and displacement of prevailing variants at both residential and community scales. We ask whether circulation of either or both Omicron variants occurred in Singapore prior to the first case reports and following initial detection in South Africa and Botswana (Viana et al., 2022). We also ask whether BA.1 was displaced by BA.2 similar to dynamics observed in other countries, or if simultaneous detection signified co-incidence of both variant lineages in Singapore. These findings highlight that WWS of VOCs at residential and community scales enable insights into disease dynamics and the efficacy of policies to control disease spread.

2. Materials and methods

2.1. Assay design

AS RT-qPCR assays were designed based on the principles following our previous work (Lee et al., 2021a, 2021b, 2022a, 2022b) and targeting the stretch of nine-nucleotide deletions at loci 24–27 in the spike protein gene that was associated with the BA.2 VOC over the study period (September 2021–May 2022) and is now associated with all non-BA.1 Omicron variants. Briefly, this was done using the Integrated DNA Technologies (IDT) PrimerQuest Tool. Target mutations were placed near the 3' end of the forward primer. All primers have a melting temperature range of 59–65 °C while probes are in the range of 64–72 °C. Probes were designed to anneal to the same strand as the AS primer, with the probe as close as possible to the 3'-end of the AS primers. The primer F-WT-24-27 is designed with mixed bases at one position to enable binding to more WT (non-BA.2) sequences. All primers and probes used were purchased from IDT (Table S1). A summary table is included to clarify the purpose of each assay (Table S2).

2.2. Wastewater collection

Wastewater collection was done as part of a University surveillance program. Briefly, collection was scheduled twice a week using an Aquacell P2 Multiform autosampler (Aquatic, United Kingdom). The autosampler collected wastewater every 10 min forming one composite sample every 6 h in a 24 h period. Composite samples were subsequently delivered to the lab and processed immediately. From 10 January to 15 February 2022, 45 campus sampling points with service populations from 200 to 1200 persons were monitored twice weekly for the N gene and the 69–70 mutation in the S gene associated with the alpha and BA.1 (Omicron) variants. Additional retrospective analyses were carried out on pooled 24 h composite samples from four campus sampling locations using the Omicron-specific assays developed in this study and in Lee et al. (2022a, 2022b). WRP samples were collected weekly from two treatment plants in Singapore serving a total of more than 50 % of the country's population. These samples were then transported on ice to the lab and processed immediately.

2.3. Sample concentration and RNA extraction

Campus residential samples were processed by ultrafiltration using protein concentrators excluding a three-week period from 11 October to 2

November 2021 where the PEG method of viral particle concentration was used due to a global supply chain shortage of concentrators. Efficiencies of both methods for recovering SARS-CoV-2 were comparable (not statistically different) during internal evaluation (unpublished data; Chandra et al., 2023). Wastewater samples were first centrifuged at 4000g at 4 °C for 30 min to remove large debris. When using the 30 kDa Amicon® Ultra 15 mL Centrifugal Filters or Sartorius Vivaspin® Turbo 15 RC 30kDA, samples were centrifuged for 4000g at 4 °C for 20 min for further concentration. The second method was using polyethylene glycol (PEG). Briefly, the clarified supernatant was added to 3.6 g of PEG (8 % w/v, PEG 8000) and 0.875 g of sodium chloride (Sigma Aldrich, USA). The mixture was left overnight at 4 °C on a rotating shaker at 130 rpm and subsequently centrifuged at 6000g for 60 min at 4 °C. The supernatant was removed, and the pellet was resuspended in 300 µL of phosphate buffered saline (PBS). A negative process control was included in all processing runs to ensure the absence of contamination for all concentration methods. 140 µL of retentate from the centrifugal columns or resuspended PEG pellets in PBS was used for RNA extraction using QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The final elution was done in 60 µL of AVE buffer (Qiagen, Germany). Retrospective analysis was done by pooling equal volumes of each available 6 h composite, to form a 24 h composite, for downstream RT-qPCR.

For WRP samples, retrospective analysis was done on PEG pellets that had been stored between one to eight months at −80 °C followed by resuspension, extraction, and analysis. Concentrations of the N gene were confirmed to be within 50 % concentration (approximately 1 CT) of samples analysed in near-real-time, indicating no significant sample degradation during storage. Upstream sample concentration was carried out using only the PEG method described above. RNA extraction was done using 140 µL of the resuspended PEG pellets using the KingFisher Apex System and MagMax Viral Pathogen II Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

2.4. RNA standards and their quantification by RT-ddPCR

Synthetic SARS-Cov-2 RNA controls were used for Delta (B.1.617.2), Omicron BA.1 and BA.2 (B.1.1.529) (Twist Bioscience, USA). RNA standards were prepared as single-use aliquots. The respective controls were quantified by digital droplet RT-PCR by using the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad Laboratories, USA) according to the manufacturer's instructions. AS-RT-qPCR was carried out using TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, USA) with technical duplicates, at a final volume of 20 µL according to manufacturer's recommendations. The final concentration of the AS RT-qPCR primers were 500 nM, probe at 200 nM, with 2 µL of template. No template controls were included for each assay and none of them amplified. The reactions were performed on a Bio-Rad CFX96 or Bio-Rad Opus instrument under the following conditions, 5 min at 50 °C and 20 s at 95 °C, followed by 45 cycles of 3 s at 95 °C and 30 s at 60 °C. N1 assay amplification was performed at 50 °C for 5 min and 2 min at 95 °C, followed by 45 cycles of 3 s at 95 °C and 45 s at 55 °C. Standard curves were applied across the Ct values obtained

from the samples, with technical variations across runs monitored and normalised with the use of inter-plate calibrators.

2.5. Data analysis

Frequency of targeted mutations was analysed using R. qPCR data and graphs were analysed using Microsoft Excel and Graphpad Prism.

3. Results

3.1. Assay validation

In this study we developed and validated an AS RT-qPCR assay for non-BA.1 Omicron VOCs (corresponding to BA.2 during the study period) targeting the unique stretch of mutations at the loci 24–27. We also validated previously established assays targeting mutations at the loci 69–70 (BA.1) and 493–498 (total Omicron) at our sampling sites (WRP and University Campus). Validation was carried out against synthetic RNA showing that all mutant (MT) specific assays do not cross-react with wild type (WT) RNA at and below 10^3 copies, conferring good specificity for determining wastewater concentrations of the Omicron VOCs (Fig. S1). Table S3 shows the efficiencies, slopes and intercepts of the assays used in this study. The efficiency of the assays was within the range of 91.3 %–102.7 % with similar slopes and intercepts. Results obtained from the WRPs and University campus sites using the AS RT-qPCR assays were plotted against the U.S. CDC N1 assay results (Fig. S2). For each sample that was positive, the sum of viral RNA concentrations for the WT and MT specific assays correlated well with the N1 assay and with a Pearson correlation coefficient (R) greater than 0.82 (Fig. 1).

3.2. WRP time series

To determine the prevalence of SARS-CoV-2 variant signals in Singapore's wastewater and to detect if transmission occurred before case reports, we applied our AS RT-qPCR and the US CDC N1 assay on wastewater influent samples from two WRPs. WRP 1 has a treatment capacity of 900,000 m³ per day and aggregates flow from more than 50 % of Singapore's land mass, while WRP 2, which is smaller, has a treatment capacity of 361,000m³ per day (PUB, 2022). Weekly samples were collected as part of an ongoing monitoring program from September 2021 to May 2022 and analysed using the US CDC N1 and the 493–498 assays, where the MT 493–498 detects all Omicron, and the WT 493–498 detects all non-Omicron target sequences. Both WRPs showed a similar trend in which both the N1 and the WT 493–498 assay yielded positive signals in September 2021 before subsiding by early December 2021. This coincided with the Delta wave in Singapore (Nextstrain, accessed 1 August 2022, Hadfield et al., 2018). Following the delta wave, SARS-CoV-2 viral signals were detected again in early January 2022 by the N1 and MT 493–498 assays, while the WT locus at 493–498 remained near or below the limit of detection (LOD) and continued until the end of the study period which represented the Omicron BA.1 and BA.2 wave (Fig. 2).

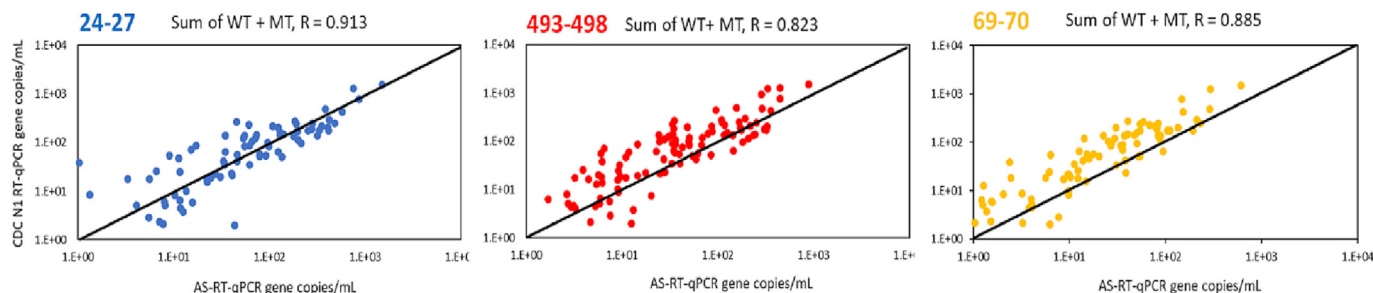


Fig. 1. Comparison of AS RT-qPCR assays against US CDC N1 assay. The panels show the comparison between the concentration of SARS-CoV-2 RNA obtained using the CDC N1 assay and sum of concentrations measured by each AS RT-qPCR assay obtained from wastewater samples in this study.

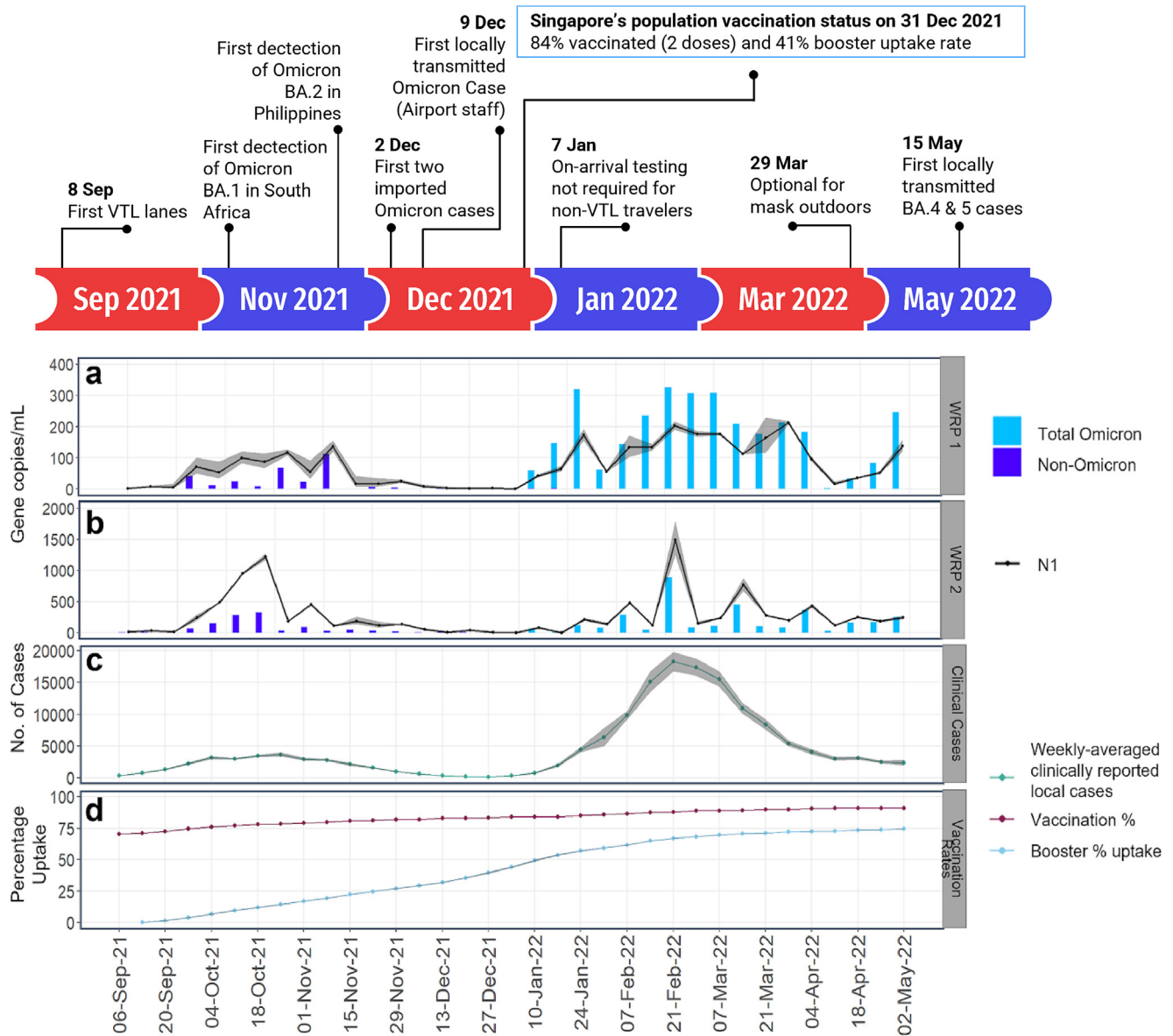


Fig. 2. Timeline of events from September 2021 to May 2022 and VOC associated mutations compared at loci 493–498 against the US CDC N1 assay at 95 % confidence interval at a) WRP 1 b) WRP 2. Dark blue bars represent WT 493–498 which corresponds to non-omicron signal while light blue bars represent MT 493–498 which corresponds to total omicron signal and c) weekly-averaged clinically reported local cases d) COVID-19 vaccination and booster uptake percentage in Singapore. VTL: Vaccinated Travel Lane, which allowed air travel without mandatory quarantine for vaccinated travellers testing negative for SARS-CoV-2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To further differentiate the Omicron variants circulating in Singapore, we employed two additional assays. Firstly, the 24–27 assay (Fig. 3a and b) which quantifies the WT and MT sequences at a stretch of mutations specific to Omicron BA.2, BA.4 and BA.5 (Hodcroft, 2022) and secondly, the 69–70 assay (Fig. 3c and d), which determines the relative abundance of the WT and MT sequences at the designated locus, reflecting a shared mutation in Alpha, Eta, and Omicron BA.1. During our study period these two assays were indicative of Omicron BA.1 and BA.2, respectively since Alpha and Eta variants were no longer in global circulation and Singapore only started to observe Omicron BA.2.12.1 cases on 28 April 2022 (Begum, 2022) while BA.4 & 5 were only clinically detected in three persons on 15 May 2022 (MOH, 2022b), which is outside the study period. Silent circulation of BA.2.12.1, BA.4 and BA.5 prior to clinical detection would increase values reported by the MT 24–27 assay relative to the WT assay.

The 69–70 and 24–27 assays were used on wastewater samples from December 2021 to early May 2022, covering the period before Omicron

was detected in the wastewater to the time it became the dominant variant. In December 2021, clinical cases indicated the delta VOC wave had largely subsided, and RT-qPCR of WRPs indicated N gene levels near the LOD, with no detection of MT sequences by all AS assays. The WT of all AS assays had sporadic detection near its LOD which in Singapore's context likely corresponded to residual traces of the Delta VOC. On 5 January 2022, there was a low abundance (3.44 gene copies/mL) of MT 24–27 (BA.2) picked up from WRP 1. However, the overall N gene levels were near the LOD. The following week, we observed the co-incidence of Omicron BA.1 and BA.2 in Singapore's wastewater on samples collected on 12 Jan 2022 from both WRP 1 and WRP 2 (Fig. 3). In the subsequent weeks for both WRPs, BA.2 levels rose relative to BA.1 and was the dominant variant by 31 January 2022. BA.2 completely displaced BA.1 by the end of February 2022 at WRP 2 and by mid-March 2022 at WRP 1. From December 2021 to early May 2022 the 24–27 assay indicated that the sum of MT (BA.2) and WT (non-BA.2) sequences at the locus mirrored the dynamics of the N1 gene which is universally detected in all variants (Fig. 3).

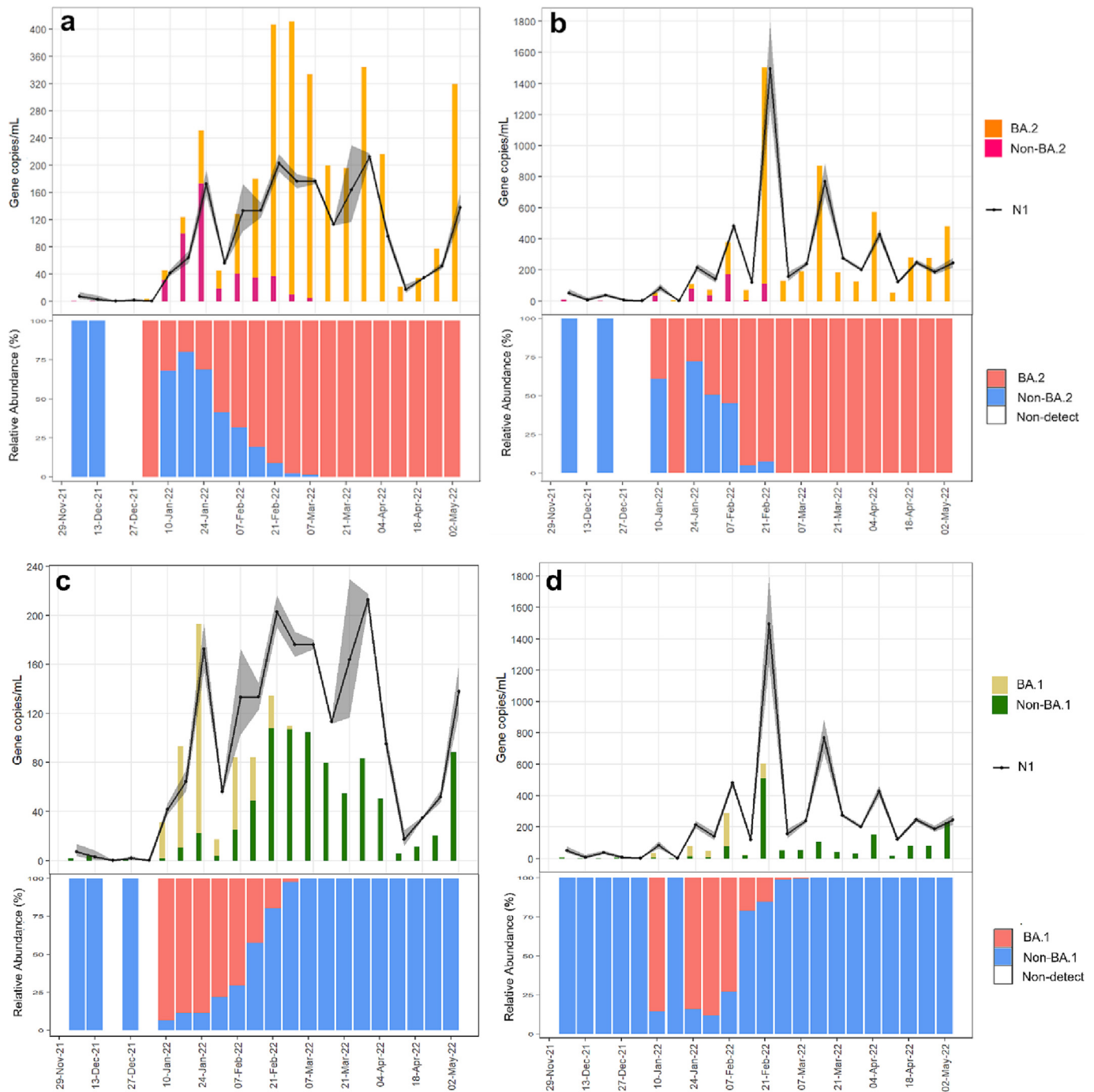


Fig. 3. Displacement dynamics of WT and MT sequences at locus 24–27 a) WRP 1 b) WRP 2 using our AS RT-qPCR assay on community wastewater = samples collected in Singapore. Assay MT 24–27 corresponds to the BA.2 lineage, while WT 24–27 corresponds to non-BA.2 lineages, inclusive of BA.1 over the study period. For assay 69–70 c) WRP 1 d) WRP 2, MT corresponds to BA.1, while WT corresponds to non-BA.1 lineages, inclusive of BA.2. N1 is represented by a black line with 95 % confidence interval shaded in grey.

3.3. University campus time series

To examine the SARS-CoV-2 VOC wastewater trends at a residential scale, we applied the Omicron AS RT-qPCR assays to sites across a University campus. These sites were part of a campus monitoring project aggregating 24-hour flow from 45 sites around undergraduate housing. All sites were subjected to real-time surveillance for the N gene and the AS RT-qPCR 69–70 assay associated with BA.1 from 10 January to 15 February 2022 (corresponding to the first four weeks of the semester). Four sites (Hall A-D) were selected for longitudinal retrospective analysis of total Omicron, BA.1 and BA.2 WT and MT sequences from December 2021 to early

May 2022. Sites A, B, C and D accommodated a population of approximately 250, 550, 1200 and 400 students, respectively. As the catchment size was much smaller compared to the WRPs, we hypothesised that trends would be more variable, corresponding to local infection patterns.

N1 and WT 493–498 (non-Omicron) signals were detected at relatively low levels and frequency at campus sites during the Delta wave while social distancing measures and border restrictions were implemented in Singapore (Fig. 4). At the start of the semester (10 January 2022) real-time surveillance indicated that 11 of the 45 sites were positive for the N gene, with 8 of these sites also positive for MT 69–70 (indicative of BA.1) corresponding with the first detection of BA.1 and BA.2 at the WRPs on 5

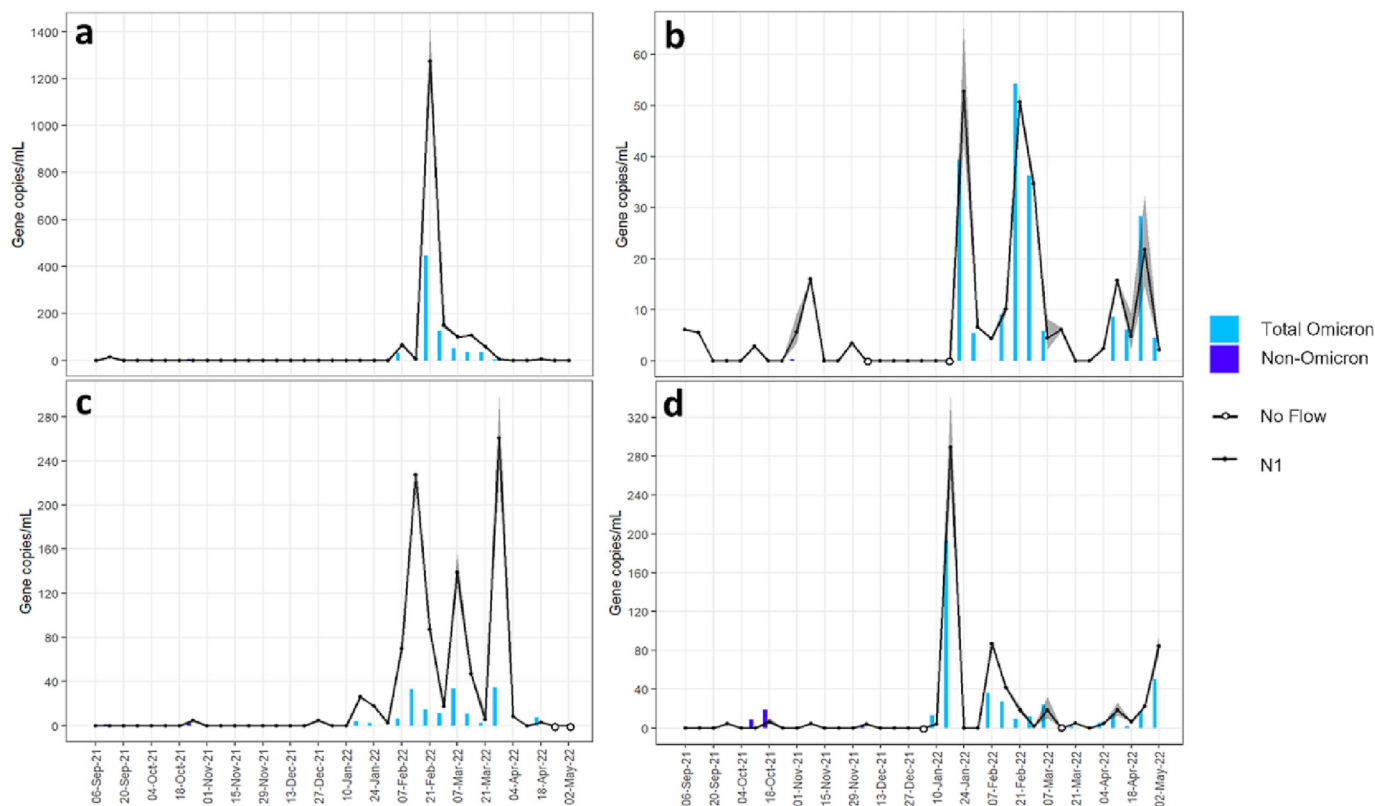


Fig. 4. Timeline of VOC associated mutations using our 493–498 assays compared against U.S. CDC N1 assay at 95 % confidence interval from University campus sites from Halls A to D, a) to d), respectively. Assay 493–498 MT corresponds to all known Omicron lineages inclusive of BA.1 and BA.2 while WT corresponds to non-Omicron lineages, indicative of the delta VOC in Oct. 2021.

January and 12 January 2022, respectively (Fig. 3). Within the first four weeks of the term, there was positive detection of BA.1 at 26 sites (Fig. S3). Detailed retrospective analysis was carried out to shed light on the dynamics of both BA.1 and BA.2 variants prior to the semester's start, and through the term-end.

We observed a variable order of arrival for Omicron BA.1 and BA.2 at the four University campus sites (Fig. 5). The first detection of Omicron in Halls C and D were of WT 24–27, corresponding to Omicron BA.1. In contrast, MT 24–27 was detected in Hall A, which corresponded to BA.2. Only in Hall B was co-occurrence of BA.1 and BA.2 detected, albeit with a relatively low abundance of BA.1. Our 69–70 assay showed similar results with the only difference being the initial detection of MT 69–70 which represents BA.1 from Hall A on 10 February 2022 (Fig. S4). Dynamics of Omicron BA.1 and BA.2 at campus hall sites also differed from the WRPs. Complete displacement by BA.2 occurred about two to three weeks earlier than the WRPs for Halls B and D. Halls A and C only detected a mixture of BA.1 and BA.2 on two occasions from February to March 2022. MT 24–27 which corresponds to BA.2 detection dominated these sites for the majority of the study period. Based on our assay, BA.2 was the only VOC circulating on the 4 sites from 17 March 2022 onwards, similar to dynamics observed at the WRPs.

3.4. Wastewater surveillance and its link to clinical reporting of local cases

We compared our wastewater variant data from WRP 1 against daily clinically reported local case in Singapore, obtained from the Ministry of Health, Singapore (MOH) (<https://www.moh.gov.sg/covid-19/statistics>) and found that our wastewater VOC analysis agree with the clinically reported local cases in Singapore (Fig. 2e). VOC concentrations and clinical cases started to experience a sharp rise within the first two weeks of January 2022 and started to decline towards the end of April 2022. It is noted that wastewater concentrations started to rise again in the first week of

May 2022 as the initial Omicron BA.1 and BA.2 wave was coming to an end. By 15 May 2022, there were 3 reported local cases of BA.4 and BA.5 and whether silent circulation of these variants preceded clinical identification, will be examined in follow-up work.

4. Discussion

The Omicron wave has had an unprecedented level of impact on the global scale due to its higher transmissibility and possibility of reinfection compared to Delta (Pulliam et al., 2022; Sato et al., 2022). Its first lineage was detected in South Africa and Botswana in early November 2021 (Omicron B.1.1529 or BA.1) and in less than three weeks had been found in 87 countries (Viana et al., 2022). Its second lineage BA.2 was first detected in the Philippines in late November 2021 (Li et al., 2022) and has displaced BA.1 to be the dominant variant in numerous countries such as the United States, South Africa, Belgium, Denmark, Italy, and many more (Buchholz, 2022; Boehm et al., 2022; Sguazzin, 2022; Bloemen et al., 2022; Lyngse et al., 2022; La Rosa et al., 2022). Here, we validated an additional AS RT-qPCR assay to quantify the relative abundance of viral mutations and deletions at the spike protein locus 24–27 in Singapore's wastewater, which in this study period from September 2021 to May 2022 mainly detected BA.2. Together with our previously validated assays which targets BA.1 and all Omicron, respectively (Lee et al., 2021a, 2021b; Lee et al., 2022a; Lee et al., 2022b) we compared the wastewater trends obtained from two WRPs serving more than half of Singapore's population, as well as four sites located on a University campus. We report co-occurrence of Omicron BA.1 and BA.2 by 12 January 2022 in the WRPs, months after its first detection of BA.1 in South Africa and BA.2 in the Philippines. Real-time surveillance of 45 University campus sites for BA.1 showed a staggered pattern of detection with 8 sites positive for the MT 69–70 assay the first week of classes (10 Jan 2022) increasing to 26 positive sites by mid-February 2022. Based on our longitudinal analysis of four

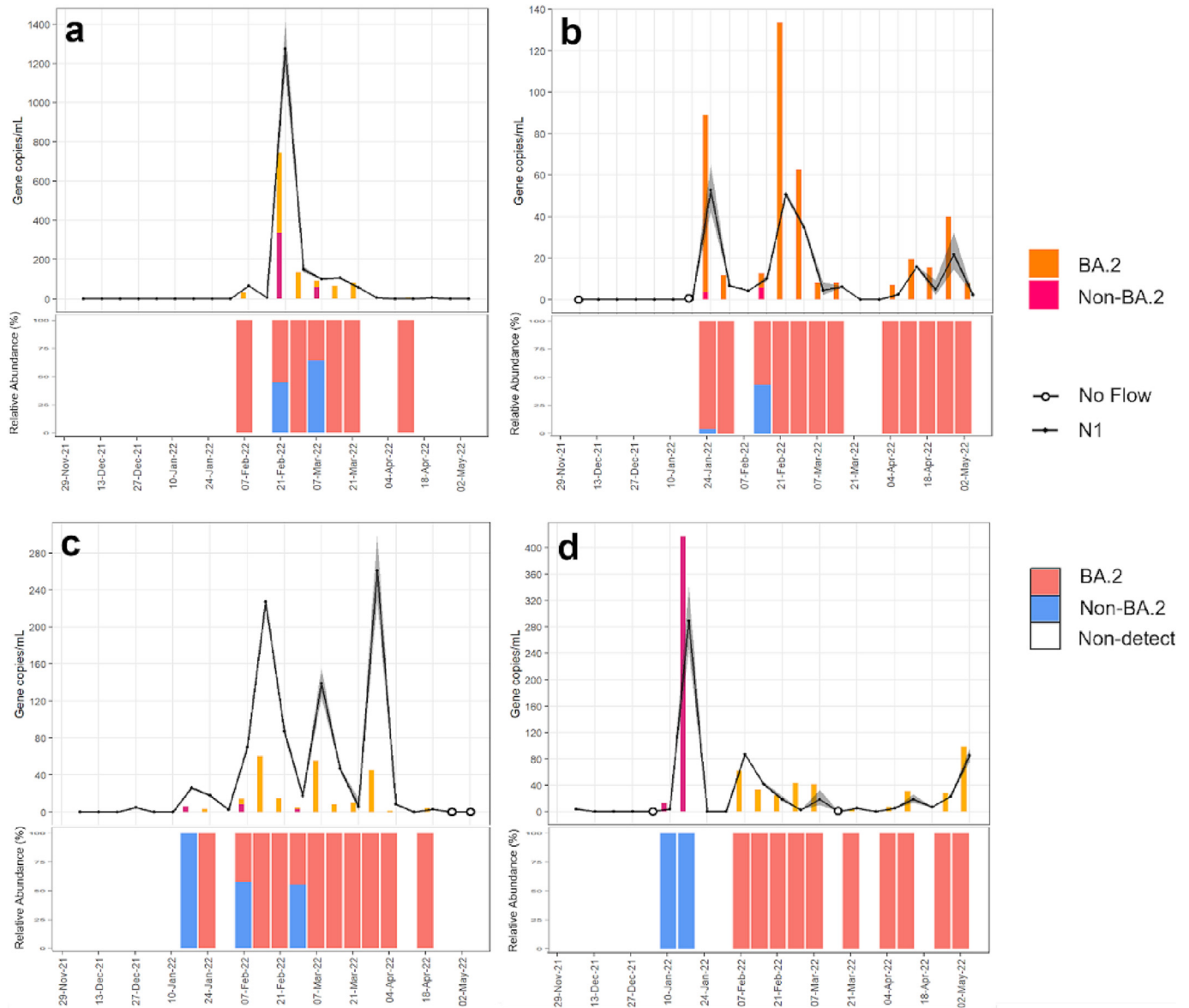


Fig. 5. Dynamics of BA.1 and BA.2 using our 24–27 AS RT-qPCR assay on University campus samples collected in Singapore from Halls A to D, a) to d), respectively. Assay MT 24–27 corresponds to the BA.2 lineage, while WT 24–27 corresponds to non-BA.2 lineages, inclusive of BA.1 over the study period. N1 is represented by a black line with 95 % confidence interval shaded in grey.

campus sites, BA.2 became the dominant variant by the end of January 2022 and completely displaced BA.1 by mid-March 2022.

WWS has been shown to complement clinical reporting, serve as an early warning tool in the case where silent circulation is occurring (Mao et al., 2020; Medema et al., 2020; Randazzo et al., 2020; Albastaki et al., 2021; Saguti et al., 2021) and is recognised as a valuable public health tool to track VOCs (Lee et al., 2022a; Smyth et al., 2022).

The co-occurrence of BA.1 and BA.2 is an interesting finding as many countries reported initial BA.1 arrival before BA.2 displacement. Furthermore, there was no crossover or displacement of Delta by Omicron as seen in other studies (Bloemen et al., 2022; Lee et al., 2022a). This can be credited to the policies put in place by the Singapore government to limit the spread of SARS-CoV-2 as well as tightening border controls to delay the arrival of new variants. Local policies such as mandatory wearing of masks in public areas, contact tracing and safe entry check-ins to venues such as shopping malls, restaurants and workplaces were among the regulations that were implemented during the first part of the study period. Relaxation of regulations for mask wearing outdoors occurred on 29 March

2022 while masks were still required in indoor settings (MOH, 2022a; MOH, 2022c).

Singapore is a global travel hub and popular transit location. Prior to COVID-19, air travel contributed to 11.8 % of Singapore's gross domestic product (IATA, 2018). During COVID-19 travellers entering Singapore were subjected to a mandatory fourteen day stay home notice (SHN) period accompanied by COVID-19 testing. As part of a strategic plan to reopen the country and the economy following a nation-wide vaccination campaign, the SHN was reduced to seven days for countries with a lower number of cases and vaccinated travel lanes (VTL) were first launched on 8 Sept 2021 to Brunei and Germany (Fig. 2) whereby fully vaccinated travellers were exempted from a seven-day SHN if an on-arrival COVID-19 test was negative (Ang, 2021). By the end of November 2021, there were up to 27 countries with VTLs with Singapore (CAAS, 2021).

When the WHO declared Omicron BA.1 a VOC on 26 November 2021 (WHO, 2021), Singapore responded with enhanced testing regimes for arriving travellers and announced on 22 December 2021 that it had detected 65 Omicron imported cases (MOH, 2021a) excluding the first case of local

transmission originating from an airport worker on 9 December 2021 (Fig. 2). Omicron cases increased to 685 by 31 December 2021 in which 75 % were detected through the enhanced testing regime for VTL travellers (MOH, 2021b). By 31st December 2021, Singapore's vaccination rate was at 84 % of the total population while 41 % had already taken their booster shot (Fig. 2f). Coinciding with reports indicating the Omicron VOC elicited less severe outcomes than previous lineages (Sigal et al., 2022) and in an effort to return to normalcy, Singapore eased travel restrictions on 7 January 2021 where on-arrival testing was not required for non-VTL travellers (MOH, 2021b). Prior to 11 January 2022, clinically reported COVID-19 cases were low and imported case numbers were always higher than locally transmitted ones (Lai, 2022). However, soon after travel restrictions were eased in Singapore, a rise in cases due to the Omicron variant, initially comprising both lineages BA.1 and BA.2 was observed, heralding a transition to "endemic COVID" (MOH, 2022a). Hence, it is likely due to local government policies which delayed the mass community spread of BA.1 from imported cases in December, coupled with the arrival of travellers carrying a mixture of BA.1 and BA.2 upon re-opening of travel bubbles that contributed to the co-occurrence of Omicron BA.1 and BA.2 as evidenced by Singapore's wastewater.

At the University campus wastewater network, overall trends largely agreed with those observed at the community level WRPs however, variability in individual displacement dynamics can be attributed to the smaller catchment size of these sites. For the WRPs, there was gradual decrease in WT 24–27 (BA.1) and the corresponding increase in MT 24–27 (BA.2) from 12 January 2022 to early March 2022 (Fig. 3), however this trend was absent in the University campus sites. Even though BA.1 was first detected in Halls C and D, BA.2 rapidly became the dominant variant and subsequently the only variant across all sites. Omicron BA.2 is found to be more transmissible than BA.1 (Lyngse et al., 2022) and in a University setting where social interactions between individuals tend to happen more often compared to a typical residential setting, this is not surprising.

As more countries transit towards endemicity and living with COVID-19, wastewater surveillance can continue to be a non-invasive, unbiased and accurate way of obtaining an overall community outlook of circulating viruses and enable policy makers to make informed public health responses and to evaluate the efficacy of measures adopted. Indeed, this work reveals that arrival of Omicron variant in wastewater was delayed in Singapore relative to many reports from other countries, with arrival of the Omicron variant lineages occurring soon after a relaxation of governing policies when nation-wide vaccination targets were met and spread of the Omicron variant was deemed an acceptable risk. Thus, WWS data supports clinical observations that Omicron variant circulation coincided with the first case reports and was not preceded by a significant period of silent circulation in Singapore.

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CRediT authorship contribution statement

JT, SW, WLL and FJDC conceptualised the project. WJN, WCGK, MT, CCJT, SW, JT managed the campus network for sample collection. FJDC and JT designed the experiments. WLL, XQ, FA, FC, HJC, EA, JT designed the BA.2 assay. FJDC, SYK and SAAA performed experiments. FJDC, EH, XQG, JWC, JT analysed the data. FJDC wrote the manuscript. All authors contributed to editing the manuscript. JT supervised the project. All authors read and approved the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

EA is a Biobot Analytics advisor and holds shares in the company. All the other authors have no competing interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162611>.

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