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Wastewater monitoring in tourist cities as potential sentinel sites for near-real-time dynamics of imported SARS-CoV-2 variants

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HIGHLIGHTS

• Alpha, Delta, Omicron BA.1 and BA.2 WBE reflected clinical variant distribution.
• Omicron BA.1 and BA.2 viral loads predominantly drove SARS-CoV-2 resurgence.
• Flow-rate normalization of SARS-CoV-2 increased the correlations with clinical data.
• Bangkok WBE shifted from 22-d early warning to near-real-time pattern during 2021.
• Tourist cities’ WBE suggested for surveillance of new variants or infectious agents.

ABSTRACT

Wastewater-based epidemiology (WBE) complements the clinical surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants’ distribution in populations. Many developed nations have established national and regional WBE systems; however, governance and budget constraints could be obstacles for low- and middle-income countries. An urgent need thus exists to identify hotspots to serve as sentinel sites for WBE. We hypothesized that representative wastewater treatment plants (WWTPs) in two international gateway cities, Bangkok and Phuket, Thailand, could be sentinel for SARS-CoV-2 and its variants to reflect the clinical distribution patterns at city level and serve as early indicators of new variants entering the country. Municipal wastewater samples (n = 132) were collected from eight representative municipal WWTPs in Bangkok and Phuket during 19 sampling events from October 2021 to March 2022, which were tested by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using the US CDC N1 and N2 multiplex and variant (Alpha, Delta, and Omicron BA.1 and BA.2) singleplex assays.

Keywords:
Gateway cities
COVID-19
Omicron
Lineage
Normalizer
Wastewater surveillance

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Wastewater-based epidemiology (WBE) complements the clinical surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants’ distribution in populations. Many developed nations have established national and regional WBE systems; however, governance and budget constraints could be obstacles for low- and middle-income countries. An urgent need thus exists to identify hotspots to serve as sentinel sites for WBE. We hypothesized that representative wastewater treatment plants (WWTPs) in two international gateway cities, Bangkok and Phuket, Thailand, could be sentinel for SARS-CoV-2 and its variants to reflect the clinical distribution patterns at city level and serve as early indicators of new variants entering the country. Municipal wastewater samples (n = 132) were collected from eight representative municipal WWTPs in Bangkok and Phuket during 19 sampling events from October 2021 to March 2022, which were tested by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using the US CDC N1 and N2 multiplex and variant (Alpha, Delta, and Omicron BA.1 and BA.2) singleplex assays.
The variant detection ratios from Bangkok and Phuket followed similar trends to the national clinical testing data, and each variant’s viral loads agreed with the daily new cases (3-4 moving average). Omicron BA.1 was detected in Phuket wastewater prior to Bangkok, possibly due to Phuket’s WWTPs serving tourist communities. We found that the Omicron BA.1 and BA.2 viral loads predominantly drove the SARS-CoV-2 resurgence. We also noted a shifting pattern in the Bangkok WBE from a 22-d early warning in early 2021 to a near real-time pattern in late 2021. The potential application of tourist hotspots for WBE to indicate the arrival of new variants and re-emerging or unprecedented infectious agents could support tourism-dependent economies by complementing the reduced clinical regulations while maintaining public health protection via wastewater surveillance.

1. Introduction

Since its first application for coronavirus disease 2019 (COVID-19) surveillance in early 2020, wastewater-based epidemiology (WBE) has been applied continually to monitor severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, and to foreshadow clinical case reports of COVID-19 in communities (de Araujo et al., 2022; Medema et al., 2020; Morvan et al., 2022). SARS-CoV-2 variants, which are defined as related viruses with one or more mutations in their genomes (Centers for Disease Control and Prevention, 2022), have emerged and threatened public health globally since the beginning of the COVID-19 pandemic. WBE has been used successfully to monitor the SARS-CoV-2 variants of concern, namely, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), using various analytical methods, such as next generation sequencing (Amman et al., 2022; Wang et al., 2022), reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (La Rosa et al., 2022; Xu et al., 2022; Yaniv et al., 2022), and digital PCR (Boehm et al., 2022; Heijnen et al., 2021).

National and continental levels of WBE networks have been established in developed nations such as the United States (CDC, 2021), countries in the European Union (European Commission, 2021), and Canada (Canadian Water Network, 2022). However, maintaining sustainable networks for national WBE requires cooperation between the governmental, academic, and private sectors, a sound economic status, and sufficient budget, as well as public knowledge and acceptance (Canadian Water Network, 2020; CDC, 2021). For low- and middle-income countries (LMICs), WBE offers an economical and effective method to support clinical surveillance at a population level (Adelodun et al., 2020; Gwenzl, 2021; Sangsanont et al., 2022). However, constraints on governance and legislative frameworks, as well as available budgets, may present challenges to the establishment of national WBE systems in LMICs (Takeda et al., 2021). It is therefore imperative to identify WBE activity in sentinel cities to represent disease dynamics and/or offer early warnings of new variants arriving in a country.

Because COVID-19 initially spread quickly from region to region across the world, international air travel is considered the main global transmission route. We hypothesized that tourist destinations and gateway cities could be sentinelized for SARS-CoV-2 and its variants to reflect the clinical distribution patterns and serve as early indicators of new variants arriving in the country. In this study, we selected Phuket, which represents a tourist city, and Bangkok, a capital and gateway city. Phuket, located in the southern part of Thailand, is the third most popular tourist city in the country. In 2021, it hosted over 294,078 airplane international visitors, accounting for 70% of the city population (Ministry of Tourism and Sports, 2022; National Statistical Office, 2022a). Phuket is also the first city with the “no-quarantine Phuket sandbox” for fully vaccinated travelers to promote the reopening of Thailand for international travelers (CCSA, 2021). Bangkok is a capital city, government center and business hub of Thailand, with a population of over 5.5 million as of December 2021 (Bangkok Metropolitan Administration, 2022). Bangkok also accommodates non-registered populations who move from their hometown to work or study, accounted for 42% of Bangkok population (National Statistical Office, 2022b), and over 1.29 million international visitors entered the country through its airports in 2021 (Ministry of Tourism and Sports, 2022). Clinical records show that the first patients infected with the Alpha, Delta, and Omicron BA.1 variants in Thailand were in Bangkok in March, May, and December 2021, respectively, emphasizing the importance for Bangkok to serve as a sentinel city (Department of Disease Control, 2022a).

The specific objectives of this study were (1) to assess the applicability of WBE targeting SARS-CoV-2 and its variants (Alpha, Delta, and Omicron BA.1 and BA.2) in the municipal wastewater in Bangkok and Phuket to represent the clinical circulation of the virus, (2) to compare the dynamics of SARS-CoV-2 and its variants between the capital city Bangkok and the tourist city Phuket using WBE, and (3) to investigate the leading indicative patterns of SARS-CoV-2 via WBE in comparison to clinical testing dynamics. The implications of this study are that WBE in tourist and gateway cities could be a leading indicator of the arrival of new variants or emerging infectious agents. Tourist destinations could also benefit from WBE disease surveillance as it could help protect public health, complement the reduced clinical regulations and thus help promote tourism-reliant economies.

2. Materials and methods

2.1. Study sites and wastewater sample collection

A total of 132 wastewater samples comprising 124 wastewater influent (after the bar screening process) and eight effluent (after the secondary sedimentation) samples were collected from eight municipal WWTPs in Bangkok and Phuket, Thailand (Fig. 1), during 19 sampling events from October 2021 to March 2022 (Supplemental Table S1). The four large WWTPs in Bangkok (L1–L4) were selected from the city’s 19 facilities due to their effective representation of COVID-19 outbreaks on a city scale, as suggested in our previous work (Sangsanont et al., 2022). The L1–L4 WWTPs each serve 432,000–580,000 people and together cover around 36% of the population of Bangkok (Supplemental Table S1) (Bangkok Metropolitan Administration, 2022). We further selected a WWTP at an international airport (site A1) and three WWTPs in downtown Phuket (sites P1–P3) because their service areas are located in tourist and downtown areas. These four WWTPs serve approximately 25% of the Phuket population. One WWTP (site P1) also receives a portion of the wastewater from the airport.

The samples from Bangkok WWTPs were collected using the 24 h composite autosamplers available at each facility, while the samples from the Phuket WWTPs and the Phuket airport were collected by grab sampling between 8:30 a.m. and 10:30 a.m. The two-liter samples were collected in sterile plastic containers and transported on ice to the respective laboratories within 8 h. The samples were stored at 4 °C for no longer than 24 h before further analysis.

2.2. Water processing, nucleic acid extraction, and bacteriophage phi6 recovery analysis

To maintain the required safety protocol, 45–100 mL of the samples were incubated at 60 °C in a water bath for 1 h. The samples were subsequently centrifuged at 4750 × g for 10 min at 4 °C using an Amicon® Ultra-15 centrifugal filter device (Merck Millipore Ltd, Burlington, MA, USA) with a 30 kDa molecular weight cut-off. However, to reduce the inactivating effect on the viral RNA, the incubating step was disregarded for the Bangkok samples collected from December 7, 2021, onward and for the Phuket samples collected from January 12, 2022, onward, while all other safety protocols were adhered to. Comparisons of the data sets prior to and following the changed protocol were achievable because our
preliminary results indicated a very slight change in the cycle threshold (Ct) values of 1.39 due to the effect of the 60 °C incubation (Supplemental Table S2). The 140 μL concentrated samples were extracted using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Nucleic acid concentrations were measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The 60 μL eluted extracts were stored at −30 to −20 °C until further analysis within 2–3 months. The recovery efficiency analysis was performed by spiking the enveloped bacteriophage phi6 (NBRC 105899, Biological Research Center, NITE, Japan), which was grown in accordance with the protocol provided by Sangsanont et al. (2022). Phi6 has been used as an enveloped virus surrogate for human viruses, such as Ebola virus, and coronaviruses (e.g., SARS, SARS-CoV-2, and MERS) (Gallandat et al., 2017; Sherchan et al., 2020; Ye et al., 2016). Our preliminary data showed that phi6 was undetected in eight representative unspiked samples. Triplicate serial 10-fold dilutions of the phi6 RNA was measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to determine the end-point dilutions. The RT-qPCR qualitative results (i.e., positive or negative) were converted to a concentration unit of PCR-detection unit (PDU) using the statistical most probable number table (Haramoto et al., 2007; Huang et al., 2000; Sangsanont et al., 2020). The recovery was conducted by spiking phi6 into the water samples to achieve an approximately 1 × 10⁶ PDU/mL, and then calculated by dividing the bacteriophage phi6 after the concentration by that before the concentration.

2.3. RT-qPCR assays

The US CDC N1 and N2 multiplex, variant (Alpha, Delta, and Omicron BA.1 and BA.2) singleplex, and bacteriophage phi6 RT-qPCR assays were performed with the QuantiStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using the primers, probes, and PCR amplification conditions listed in Supplemental Table S3 (CDC, 2020; Gendron et al., 2010; Puenpa et al., 2022; Sherchan et al., 2020). The RT-qPCR variant assays were originally designed for variant identification in 155 positive sequences from clinical samples of Thai patients by targeting the TaqMan probes at the deletion regions of the gene encoding the spike protein (Puenpa et al., 2022). The specific targets of the spike genes were Alpha (69–70 deletion), Delta (157–158 deletion), Omicron BA.1 (3-residue insertion: ins214EPE) and Omicron BA.2 (24–26 deletion). The methods showed 100 % sensitivity and 100 % specificity in tested clinical specimens (n = 1200) for all four variant assays (N = 1200) (Saengdao, 2022). No cross-reactions were observed with other RNA viruses examined (influenza A and B, human parainfluenza virus, respiratory syncytial virus, human coronavirus NL63, human metapneumovirus, human rhinovirus, adenovirus and bocavirus) (Saengdao, 2022). The nucleic acid archived at −80 °C from January to April 2021 were also analyzed for the Alpha variant for comparison (Sangsanont et al., 2022). The 10 μL RT-qPCR reaction mixture for the N1 and N2 multiplex assay comprised 2.5 μL of TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific), 0.3 μL each of the 10 μM forward and reverse primers, 0.2 μL each of the 10 μM hydrolysis probe, 2.5

Fig. 1. Sampling locations in Bangkok’s municipal wastewater treatment facilities (sites L1–L4), and an international airport (site A1) and municipal wastewater treatment facilities (sites P1–P3) in Phuket.
μL of the extracted template, and 3.4 μL of sterile water. The 10 μL RT-qPCR reaction mixture for each of the variants and bacteriophage phi6 assays comprised 2.5 μL of TaqMan Fast Virus 1-Step Master Mix, 1 μL each of the 10 μM forward and reverse primers, 0.3 μL of 10 μM hydrolysis probe, 2.5 μL of the extracted template, and 2.7 μL of sterile water. The crAssphage qPCR assay was conducted on DNA-copurified nucleic acid samples using the QuantStudio3 Real-Time PCR System (Applied Biosystems), with the 20 μL qPCR mixture comprising 0.8 μL of each 10 μM forward and reverse primer, 0.4 μL of 10 μM hydrolysis probe, 2 μL of the extracted template, 6 μL of 1 μg/μL BSA, and 10 μL of the Luna Universal Probe qPCR Master Mix (New England BioLabs, Inc., Ipswich, MA, USA). The RT-qPCR and qPCR results were analyzed using QuantStudio Design & Analysis software (Applied Biosystems) with automatic baseline and manual adjustments of the threshold value to 0.115, 0.047, 0.040, and 0.036 for N1, BA.1, BA.2, and crAssphage, respectively. Each sample was run in duplicate, and the positive and negative controls were included in every instrumental run. The RT-qPCR and qPCR protocols were performed according to the MIQE guideline, with recommended information shown in Supplemental Table S4 (Bustin et al., 2009). The N1 standard curve was as previously published (Sangsanont et al., 2022). The Delta and Omicron BA.1 and BA.2 standard curves were constructed using linearized synthetic plasmid standards (GeneArt®, Invitrogen, Thermo Fisher Scientific) and quantified using the Qubit 3.0 fluorometer (Thermo Fisher Scientific). The Delta standard curves were calculated to PDU/mL through the end-point dilution assay. The standard curve characteristics and assay limits are shown in Supplemental Table S5. The gene copies calculation from the N1 and variant standard curves for the data below limit of quantification (LOQ) were conducted using LOQ/2. A sample below the limit of detection (LOD) was defined as negative.

2.4. SARS-CoV-2 calculations and COVID-19 case data

The flow-rate normalization of SARS-CoV-2 (gene copies [GC]/d) was calculated by multiplying the N1 concentrations (GC/L) with the average monthly flow rate (cu.m./d) of each facility and a constant factor of 1000 L/cu.m. Due to the unavailability of the daily flow rates, the flow-normalized loads were used to calculate the combined viral loads from more than one WWTP. The crAssphage normalization was calculated by incorporating the crAssphage concentration from each sample in the form of the population equivalent, as previously described (Sangsanont et al., 2022). The daily new COVID-19 cases that were reported per district for Bangkok were accessed through the public website of the Department of Disease Control, Ministry of Public Health, and for Phuket, these were requested from the Phuket Provincial Public Health Office. The variant-specific COVID-19 cases in Thailand were retrieved from the GISAID database (https://www.gisaid.org/).

2.5. Statistical analysis

The time-series cross-correlation function (CCF) between the 3-d moving averages of the daily new cases at different lag times from 0 to 15 days and the SARS-CoV-2 viral loads were analyzed using IBM Statistical Package of Social Science Statistics, version 23.0 (SPSS Inc., USA). Lag time was defined as the number of days of wastewater monitoring data preceding the average of the 3-d moving average of daily new cases. The illustrations were prepared using GraphPad Prism version 9.4.1 (GraphPad Software, USA).

3. Results and discussion

3.1. SARS-CoV-2 variant WBE reflected variant circulation in the population

The Alpha, Delta, and Omicron BA.1 and BA.2 variants were analyzed in the wastewater influent from four Bangkok and four Phuket locations (Fig. 2). The reported variant detection based on clinical data from Thailand nationally (https://www.gisaid.org/) was plotted from January 2021 to March 2022 (Fig. 2a). The Alpha variant was detected in the Bangkok wastewater in our April 2021 nucleic acid archives, and although the RNA quantity could be affected by longer storage, the results corresponded with the rising phase of the Alpha variant among Thai COVID-19 patients (Fig. 2b). The occurrence of the Delta variant in the initial Bangkok samples in October 2021 coincided with its predominance in Thai patients during the same period. However, the December 2021 period showed low SARS-CoV-2 N1 loads in the wastewater (Fig. 3a), which was in agreement with the decreasing number of COVID-19 cases, and may thus have caused the low positive rates of the Delta variant during this time. The Phuket samples demonstrated the presence of the Delta variant from four Bangkok wastewater treatment facilities (B), in the wastewater influent from three Phuket wastewater treatment facilities and one international airport (C) from January 2021 to March 2022. The dates are shown as day/month/year.
3.2 Omicron BA.1 and BA.2 detected in the tourist city Phuket wastewater earlier than in the capital city Bangkok

Interestingly, Omicron BA.1 was first detected in the Phuket wastewater (site P2) on December 29, 2021, which was one week before its detection in the Bangkok wastewater (site L1) on January 4, 2022 (Fig. 4). Omicron BA.2 appeared first in the Phuket wastewater on January 12, 2022, which closely followed its first appearance in the Thai COVID-19 patient data from the GISAID database on December 26, 2021 (Fig. 2a). The BA.2 variant subsequently co-circulated consistently in the Phuket wastewater together with BA.1 and predominated after early February 2022 (Fig. 4b). In Bangkok, BA.2 emerged on February 22, 2022, and co-circulated with BA.1 (Fig. 4a). Variant dynamics with the rise of Omicron and its replacement of or co-circulation with Delta have been demonstrated via wastewater surveillance (Lee et al., 2022a; Wolfe et al., 2022c; Yaniv et al., 2022).

Our results may have occurred because, during the initial reopening of Thailand for international travelers and as the first city with the “no-quarantine Phuket sandbox” for fully vaccinated travelers (CCSA, 2021), Phuket welcomed seven times more visitors in December 2021 than the average number of visitors in earlier months (Ministry of Tourism and Sports, 2022). Moreover, the Bangkok wastewater may have had lower sensitivity in detecting viral circulation due to over two million people being served by the combination of the four WWTPs. The Phuket wastewater samples, on the other hand, were from three WWTPs serving approximately 100,000 people in downtown and tourist areas, as well as one WWTP serving a Phuket international airport. The imported new variants may therefore have had a higher chance of being detected in the study areas in Phuket than those in Bangkok. Furthermore, the first imported case of monkeypox, which was very recently declared a public health emergency of international concern by the World Health Organization (Taylor, 2022), was...
identified in Phuket on July 21, 2021 (Department of Disease Control, 2022b). Consequently, urban and tourist communities and the corresponding airports could act as hotspots for WBE of not only COVID-19, but also emerging or re-emerging infectious agents such as influenza viruses, respiratory syncytial virus, poliovirus, arboviruses, and monkeypox virus (Hughes et al., 2022; Lanese, 2022; Lee et al., 2022b; Wolfe et al., 2022a, 2022b).

3.3. Omicron BA.1 and BA.2 viral loads predominantly drove SARS-CoV-2 resurgence

The flow-normalized viral loads of the Delta and Omicron BA.1 and BA.2 variants in wastewater presented similar dynamics to the total SARS-CoV-2 loads and clinical data in Bangkok (Fig. 5a) and Phuket (Fig. 5b). The detectable concentrations ranged from $1.21 \times 10^5$ GC/L to $2.46 \times 10^4$ GC/L for BA.1, and at one quantifiable concentration of $3.65 \times 10^4$ GC/L for BA.2. The replacement of the Delta variant by BA.1 viral loads escalated the surge of the new COVID-19 wave observed in this study. Omicron BA.1 was first detected near the lowest point of the daily reported clinical cases during the time the Delta variant was dominant in early January 2022 in Bangkok (Fig. 5a) and late December 2021 in Phuket (Fig. 5b), which was prior to the subsequent COVID-19 resurgence. The infection rates between Bangkok and Phuket could have differed due to differences in the public health interventions as well as the background immunity and vaccination rates of the local residents and international travelers in the exposed population (Department of Disease Control, 2022c). Additionally, the surge of the COVID-19 sub-wave occurred with the dominance of the BA.2 variant in February 2022 in both Bangkok and Phuket (Fig. 5a and b). The higher transmissibility of BA.1 and BA.2 compared to the Delta variant may have explained such COVID-19 waves. The effective reproductive numbers of Omicron BA.1 were reported to be $1.99 \pm 0.38$ times higher than those of Delta (Ito et al., 2022; Liu and Rocklöv, 2022), while those of BA.2 were $2.51 \pm 0.47$ times higher those of Delta (Ito et al., 2022).

3.4. Flow-rate, but not crAssphage, SARS-CoV-2 normalization increased correlations with clinical data

SARS-CoV-2 RNA (combined N1 and N2) was detected in 66 of 92 (71.7 %) wastewater influent samples in Bangkok and 37 of 48 (77.1 %) influent samples in Phuket. Of the eight effluent samples from Bangkok, five were negative, and three were detectable but not quantifiable (DNQ). The effluent samples mostly showed higher Ct values than their corresponding influent samples, which implied certain levels of removal through wastewater treatment (Supplemental Table S6). The average phi6 recovery rates were 4.18 % and 5.95 % for the Bangkok and Phuket wastewater samples, respectively. The N1 concentrations at each WWTP ranged from $9.43 \times 10^3$ to $1.04 \times 10^5$ GC/L in Bangkok and from $4.91 \times 10^3$ to $1.01 \times 10^5$ GC/L in Phuket. The SARS-CoV-2 (combined N1 and N2) positive rates and flow-normalized and crAssphage-normalized N1 loadings were plotted with the 3-d moving average of the daily new cases for Bangkok (Fig. 3a) and Phuket (Fig. 3b). The combined N1 loadings from the four Bangkok WWTPs from January to April 2021, as previously reported (Sangsanont et al., 2022), were also plotted for comparison with the Bangkok 3-d moving average of daily new cases (Supplemental Fig. S1). Interestingly, the CCFs indicated a higher correlation with the clinical data (3-d moving average) when flow-normalized SARS-CoV-2 rather than crAssphage-normalized SARS-CoV-2 was used (Fig. 6). Similarly, flow-normalization provided a higher correlation with the clinical data for each WWTP in Bangkok and Phuket (Supplemental Table S7).

As observed in previous studies, normalized SARS-CoV-2 with biomarkers, such as crAssphage, pepper mild mottle virus, or other fecal indicators, did not improve correlations with clinical data (Ai et al., 2021; Greenwald et al., 2021). Different decay rates between SARS-CoV-2 and each of the normalizers could be affected by environmental conditions and wastewater compositions (e.g., chemicals released by industrial wastewaters in sewersheds), which could have different inactivation mechanisms toward enveloped and non-enveloped viruses (Ahmed et al., 2020a; Bayati et al., 2022; Zhan et al., 2022). It is therefore suggested that flow- and biomarker-normalized viral loads be used to report SARS-CoV-2 WBE.

3.5. Shifting patterns from leading indications to near real-time manifestations of SARS-CoV-2 WBE

The lag time analyses of SARS-CoV-2 demonstrated no lag times, as shown by the cross-correlation analyses from the combined Bangkok WWTPs and combined Phuket WWTPs (Fig. 6), as well as from each WWTP (Supplemental Fig. S2). Notably, the 22-d lag time for the four same WWTPs in Bangkok (sites L1–L4) from January to April 2021 with a 5-d moving average of clinical data (Sangsanont et al., 2022) was not evident in the wastewater data during the period October 2021–March 2022 in this study. A reduction in the early warning lag time of WBE over time when monitored at the same locations has also been observed in the United States (Zhao et al., 2022). The availability of COVID-19 antigen test kits and increased awareness, clinical testing access, and reporting
System efficiencies could have enhanced the clinical case reporting rates and thus shortened or eliminated the lag times (Xiao et al., 2022; Zhao et al., 2022). Intrinsic properties, such as different asymptomatic portions and fecal shedding rates and durations, as well as vaccinations, could also play an important role (Bertels et al., 2022; Zhao et al., 2022). The Omicron variants shortened the period to symptom manifestation and shedding duration (Baker et al., 2022; Lamers et al., 2022; Zhao et al., 2022). Moreover, community-specific shedding rates, in which fecal shedding rates could be affected by demographics, especially age, ethnicity, and socioeconomic factors, have been suggested (Prasek et al., 2022). Furthermore, wastewater characteristics, sampling, and analysis methods, as well as quality control, for WBE research could also affect the detectability of the viruses (Ahmed et al., 2020b, 2022b; Bertels et al., 2022; Zhao et al., 2022).

Consequently, the shifting patterns of WBE lag times preceding clinical data emphasizes the need for WBE in future infectious disease outbreaks. In particular, many clinical surveillance limitations, such as asymptomatic or late clinical symptomatic disease manifestation, the insufficient availability of related supplies, public health personnel, and service facilities, and the ignorance or resistance of patients to seeking medical testing may be encountered in the early stages of unprecedented pandemics (Lee et al., 2022c; Lieberman-Cribbin et al., 2020; Schmitz et al., 2021).

3.6. WBE implementation in tourist cities as potential sentinel sites

This study demonstrated that cities visited by international travelers could provide a sentinel site for the arrival of the newly emerging infectious agents or variants, with Phuket as having higher proportion of visitors showing earlier emergence of the COVID-19 variants in wastewater. The results of our study provided evidence for LMICs that support trends observed in high-income countries, e.g., wastewater from the respective airplanes and airports captured the first instances of the import of Omicron via patients arriving in Australia and Germany (Agrawal et al., 2021; Ahmed et al., 2022c); Lieberman-Cribbin et al., 2020; Schmitz et al., 2021).

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Notwithstanding, the higher detection rates of SARS-CoV-2 in wastewater in major cities that serve as transportation gateways from international destinations have been shown to reflect national clinical data trends as well as the early emergence of imported variants (Oloye et al., 2022; Tiwari et al., 2022; Weidhaas et al., 2021). Such cities thus offer an intriguing opportunity to serve as WBE sentinel sites for countrywide SARS-CoV-2 monitoring. Furthermore, the gateway cities in LMICs likely have higher capability than other cities in the country to maintain the wastewater monitoring efforts in terms of budget and governance, which will benefit the populations of the cities as well as the entire country according to the research results. With the worldwide relaxation of COVID-19 entry requirements, and indoor and outdoor measures (https://covid19.who.int/measures), WBE in gateway cities could facilitate the tourism-dependent economies while maintaining public health protection via wastewater surveillance to reduce the outbreak impact that could be less resilient for LMICs (United Nation Thailand, 2020).

4. Conclusions

WBE is useful for determining the circulation of COVID-19 and its variants in populations. The Alpha, Delta, and Omicron BA.1 and BA.2 variants were successfully detected in wastewater from four large WWTPs in Bangkok, three WWTPs serving tourist communities in Phuket, and one WWTP from a Phuket international airport. The variant detection rates over time demonstrated similar patterns to the variant circulation in clinical patients, which underlines the benefit of WBE at tourist and gateway sites to determine national circulation patterns. We found that Omicron BA.1 and BA.2 emerged earlier in Phuket than Bangkok wastewater, potentially due to Phuket’s status as a tourist destination. The Omicron BA.1 and BA.2 viral loads predominantly drove the SARS-CoV-2 resurgence, possibly due to their intrinsic transmission characteristics. Moreover, the combined SARS-CoV-2 viral loads in each city, as normalized by the flow rate, exhibited real-time dynamics with the 3-d moving average of the cities’ daily new cases during the study periods October 2021–March 2022 for Bangkok and December 2021–March 2022 for Phuket. Our results underline the significance of WBE in supporting the clinical surveillance of COVID-19 and its variants, and we suggest the use of WWTPs of tourist cities as sentinel sites for monitoring the import of new variants or other infectious agents during unprecedented pandemics.

CRediT authorship contribution statement

Jatuwat Sangsanont: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. Surapong Rattanakul: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. Prasert Makkaew: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Nopadol Precha: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Pratchaya Rukthanapitak: Investigation. Montakarn Sresung: Investigation. Yadpironin Siri: Investigation. Masaaki Kitajima: Conceptualization, Writing – review & editing. Tomoko Takeda: Conceptualization, Writing – review & editing. Eiji Haramoto: Conceptualization, Writing – review & editing. Nasamon Wanlapakorn: Resources, Writing – review &


**Appendix A. Supplementary data**

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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