Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Use of sewage surveillance for COVID-19 to guide public health response: A case study in Hong Kong

Yu Deng a,1, Xiaoqing Xu a,1, Xiawan Zheng a, Jiahui Ding a, Shuxian Li a, Ho-kwong Chui b, Tsz-kin Wong c, Leo L.M. Poon d, Tong Zhang a,⁎

a Environmental Microbiome Engineering and Biotechnology Lab, Center for Environmental Engineering Research, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China
b Environmental Protection Department, The Government of the Hong Kong SAR, Tamar, Hong Kong SAR, China
c Drainage Services Department, The Government of the Hong Kong SAR, Wanchai, Hong Kong SAR, China
d School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong SAR, China

HIGHLIGHTS
• Standardized testing procedure and reporting results for routine sewage samples
• Sewage surveillance helped to determine the area for a compulsory testing order.
• A new workflow expedited the Delta variant identification in sewage.
• Sewage surveillance helped thwart a looming Delta outbreak in the community.

GRAPHICAL ABSTRACT

ABSTRACT

Sewage surveillance could help develop proactive response to the Coronavirus Disease 2019 (COVID-19) pandemic, but currently there are limited reports about examples in practical exercises. Here, we report a use case of intensified sewage surveillance to initiate public health action to thwart a looming Delta variant outbreak in Hong Kong. On 21 June 2021, albeit under basically contained COVID-19 situation in Hong Kong, routine sewage surveillance identified a high viral load of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a sewage sample from one site covering over 33,000 population, suggesting infected cases living in the respective sewershed. The use of a newly developed method based on allele-specific real-time quantitative polymerase chain reaction (AS RT-qPCR) served to alert the first documentation of the Delta variant in local community sewage three days before the case was confirmed to be a Delta variant carrier. Intensified sewage surveillance was triggered. Targeted upstream sampling at sub-sewershed areas pinpointed the source of positive viral signal across spatial scales from sewershed to building level, and assisted in determining the specific area for issuing a compulsory testing order for individuals on 23 June 2021. A person who lived in a building with the positive result of sewage testing was confirmed to be infected with COVID-19 on 24 June 2021. Viral genome sequences determined from the sewage sample were compared to those from the clinic specimens of the matched patient, and confirmed that the person was the source of the positive SARS-CoV-2 signal in the sewage sample. This study could help build confidences for public health agencies in using the sewage surveillance in their own communities.

Keywords: Sewage surveillance, Public health intervention, Allele-specific RT-qPCR, Delta variant, Targeted upstream sampling

⁎ Corresponding author.
E-mail address: zhangt@hku.hk (T. Zhang).
1 These authors contributed equally.
1. Introduction

During the COVID-19 pandemic, accurate use of diagnostic tests in high volumes and quantities as well as the rapid response to the testing results help implement proactive public health response (Vandenbergen et al., 2021). Most frequently used diagnostic tests for individuals are based on reverse transcriptase-polymerase chain reaction (RT-PCR) and immunoassays (Mercer and Salit, 2021). However, the use of individual diagnostics for mass screening to track disease transmission would generate very high demands on laboratory test resources, affecting the implementation feasibility. Sewage surveillance detects the presence of SARS-CoV-2 and its variants at the community-level, facilitating adaptive allocation of clinical diagnostic resources for the targeted population (Polo et al., 2020). Long-lasting shedding of SARS-CoV-2 in the human excreta was identified regardless of disease severity (Zheng et al., 2020; Guo et al., 2021). The value of integrating sewage surveillance into the management system of COVID-19 outbreaks is high, especially for early identification of asymptomatic or presymptomatic individuals at the community-level in a scalable, cost-effective and non-invasive way (Keshaviah et al., 2021).

Sewage surveillance has been demonstrated to be a useful tool in combating infectious diseases such as Hepatitis E in UK (Smith et al., 2016), Hepatitis A in Sweden (Hellmér et al., 2014), and the paralytic polio in Israel (Manor et al., 2014). The COVID-19 pandemic has catalysed the research development of using sewage surveillance to measure population-level infections (Thompson et al., 2020; Daughton, 2020). As of now, more than 50 countries around the globe are monitoring the sewage for SARS-CoV-2 virus (Naughton et al., 2021). The European Union had encouraged its member states to establish nation- and territory-wide sewage monitoring systems by 1 October 2021 (EU Commission Recommendation (EU), 2021). A few countries, including Netherlands (Kitajima et al., 2020), Australia (Ahmed et al., 2020), Israel (Bogler et al., 2020), and U.S.A (Keshaviah et al., 2021), are incorporating sewage surveillance data into its established routine COVID-19 monitoring.

A number of research groups around the world have gathered evidences on the feasibility of employing the sewage surveillance as a tool to address public health needs, such as providing early warning signals of an outbreak (Medema et al., 2020; Xu et al., 2021), revealing community infection trends (Wu et al., 2022), and monitoring effectiveness of lockdown measures (Hillary et al., 2021). However, most of the reported studies are under the context of research studies, and barriers still remain regarding directly using sewage surveillance data to guide public health decisions. Only a few examples on the practical use of sewage surveillance were reported, such as the use of sewage surveillance at university campuses to guide public health actions (Harris-Lovett et al., 2021). More practical examples are needed to transform sewage surveillance from academic researches to a public health management tool (McClary-Gutierrez et al., 2021).

A territory-wide sewage surveillance system has been well established in Hong Kong through close collaboration between an interdisciplinary research team of environmental microbiology and public health in the University of Hong Kong and the Government of the Hong Kong SAR, including the Environment Bureau, the Food and Health Bureau, the Drainage Services Department, the Environmental Protection Department, etc. This system was built on top of a well-established public sewerage network covering the majority of the total population of Hong Kong, which provided a readily available platform for monitoring SARS-CoV-2 in sewage. The laboratory analytical method for SARS-CoV-2 in sewage was developed and validated locally at residential buildings and city blocks (Xu et al., 2021). In addition, to facilitate the planning of strategic sampling in the territory and to contextualize the sewage testing data for assisting the decision making process, an interactive online dashboard was built to integrate data from multiple sources based on Geographic Information Systems (GIS), including sewage testing data, geographical data of the sampling site sewershed, population survey data of individual sewersheds, georeferenced clinic case record, COVID-19 hospitals, and designated quarantine hotels and centres.

The use of sewage surveillance in Hong Kong started from pilot studies for target vulnerable populations at residential care homes when the fourth COVID-19 wave hit Hong Kong in November 2020, geared to scale implementation at ad hoc sites of residential buildings and city blocks with clusters of infections since December 2020, to sentinel monitoring at stationary sites across for early warning after the fourth wave decelerated in March 2021. Total sampling points of both stationary sites and ad hoc sites as of August 2021 exceeded over 1500 (Fig. 1).

The objectives of this study were to exemplify how systematic sewage surveillance identified a single building with only one COVID-19 patient as the source of viral signal in sewage from a sewershed of 33,000 people and the way in using a new workflow to help rapidly identify the Delta variant in sewage samples, with the aim to provide near real-time results for decision making in assisting the successful containment of a silent transmission event of SARS-CoV-2 in the local community in Hong Kong.

2. Materials and methods

2.1. Sewage sample collection

All sewage samples were collected from manholes and taken at 15-min interval during 3 h in the morning peak (8 am to 11 am). The adopted sampling strategy using 3-h composite sampling spanning the morning peak hours 8–11 am could allow for timely processing of the sewage samples within 12 h after the sampling event as well as minimisation of dilution effect of the viral signals caused by longer sampling time for the composite sample, though it may miss signals from other time slots. Sewage samples were regularly collected from 112 stationary sampling sites across various districts in Hong Kong. The site TP site 9A was one of the stationary sites located in the Tai Po (TP) district, which covered a serving population of 33,000.

Samples were delivered to the laboratory on ice. 50 mL samples were inactivated at 60 °C for 30 min and were firstly centrifuged at 4750 g at 4 °C for 30 min, and then 30 mL supernatant was used for further ultracentrifugation at 150,000 g at 4 °C for 1 h (Beckman Coulter, Indianapolis, IN). Finally, the concentrated pellet from the last step was re-suspended with ~200 µL PBS for RNA extraction. RNA was extracted using QIAamp Viral RNA Kits (Qiagen) according to the manufacturer’s instruction with a final elution volume of 50 µL.

2.2. Primer and probe design principle

The primers and probes were designed using Clone Manager 8.0 (Sci-Ed Software), following the principle of allele-specific RT-qPCR to place the mutation at the 3’ end of forward or reverse primer. We designed two forward primer sets respectively targeting wild type and mutation type for each target site. In addition, we introduced an artificial mismatch close to the 3’ end of the forward primer to boost the discrimination between the wild type and the mutant. The parameters including hairpin, self-dimerization, and heterodimerization of all primer and probe sequences were analyzed by Ghether (Sequenom, USA). Following the principle of allele-specific PCR, we designed an artifical mismatch close to the 3’ end of the forward primer to boost the discrimination between the wild type and the mutant (McClary-Gutierrez et al., 2021).

A territory-wide sewage surveillance system has been well established in Hong Kong through close collaboration between an interdisciplinary research team of environmental microbiology and public health in the University of Hong Kong and the Government of the Hong Kong SAR, including the Environment Bureau, the Food and Health Bureau, the Drainage Services Department, the Environmental Protection Department, etc. This system was built on top of a well-established public sewerage network covering the majority of the total population of Hong Kong, which provided a readily available platform for monitoring SARS-CoV-2 in sewage. The laboratory analytical method for SARS-CoV-2 in sewage was developed and validated locally at residential buildings and city blocks (Xu et al., 2021). In addition, to facilitate the planning of strategic sampling in the territory and to contextualize the sewage testing data for assisting the decision making process, an interactive online dashboard was built to integrate data from multiple sources based on Geographic Information Systems (GIS), including sewage testing data, geographical data of the sampling site sewershed, population survey data of individual sewersheds, georeferenced clinic case record, COVID-19 hospitals, and designated quarantine hotels and centres.

The use of sewage surveillance in Hong Kong started from pilot studies for target vulnerable populations at residential care homes when the fourth COVID-19 wave hit Hong Kong in November 2020, geared to scale implementation at ad hoc sites of residential buildings and city blocks with clusters of infections since December 2020, to sentinel monitoring at stationary sites across for early warning after the fourth wave decelerated in March 2021. Total sampling points of both stationary sites and ad hoc sites as of August 2021 exceeded over 1500 (Fig. 1).

The objectives of this study were to exemplify how systematic sewage surveillance identified a single building with only one COVID-19 patient as the source of viral signal in sewage from a sewershed of 33,000 people and the way in using a new workflow to help rapidly identify the Delta variant in sewage samples, with the aim to provide near real-time results for decision making in assisting the successful containment of a silent transmission event of SARS-CoV-2 in the local community in Hong Kong.

2. Materials and methods

2.1. Sewage sample collection

All sewage samples were collected from manholes and taken at 15-min interval during 3 h in the morning peak (8 am to 11 am). The adopted sampling strategy using 3-h composite sampling spanning the morning peak hours 8–11 am could allow for timely processing of the sewage samples within 12 h after the sampling event as well as minimisation of dilution effect of the viral signals caused by longer sampling time for the composite sample, though it may miss signals from other time slots. Sewage samples were regularly collected from 112 stationary sampling sites across various districts in Hong Kong. The site TP site 9A was one of the stationary sites located in the Tai Po (TP) district, which covered a serving population of 33,000.

Samples were delivered to the laboratory on ice. 50 mL samples were inactivated at 60 °C for 30 min and were firstly centrifuged at 4750 g at 4 °C for 30 min, and then 30 mL supernatant was used for further ultracentrifugation at 150,000 g at 4 °C for 1 h (Beckman Coulter, Indianapolis, IN). Finally, the concentrated pellet from the last step was re-suspended with ~200 µL PBS for RNA extraction. RNA was extracted using QIAamp Viral RNA Kits (Qiagen) according to the manufacturer’s instruction with a final elution volume of 50 µL.

2.2. Primer and probe design principle

The primers and probes were designed using Clone Manager 8.0 (Sci-Ed Software), following the principle of allele-specific RT-qPCR to place the mutation at the 3’ end of forward or reverse primer. We designed two forward primer sets respectively targeting wild type and mutation type for each target site. In addition, we introduced an artificial mismatch close to the 3’ end of the forward primer to boost the discrimination between the wild type and the mutant. The parameters including hairpin, self-dimerization, and heterodimerization of all primer and probe sequences were analyzed by Ghether (Sequenom, USA). Detailed information of the primers and probes designed were included in Table S1. All primers and probes were synthesized by BGI.

2.3. Analytical performance of AS RT-qPCR primers and probes

To verify the performance of designed primers and probes for sensitivity and specificity, we synthesized three plasmids from BGI respectively carrying the wild type of the spike gene, the mutant type with L452R, N501Y and P681R mutation sites, and the mutant type with T478K. They were referred to as the wild type (WT) and the mutant type (MT). All fragments were cloned to a commercial PMV vector. All synthesized plasmids were dissolved into DEPC-treated water and quantified using Qubit dsDNA HS assay kit (ThermoFisher, USA). The copy number was obtained based on
the length and Avogadro's number. The standard curves were generated by tenfold dilution of the plasmids from $10^5$ to $10^0$ copy $\mu$L$^{-1}$.

### 2.4. Quantification of positive samples in the routine detection

The final concentration of SARS-CoV-2 in the raw sewage, as measured in copy per L, was derived by dividing the viral concentration in one RNA extraction by the amount of the tested raw sewage sample (30 mL). One RNA extraction was the total RNA volume (i.e., 50 $\mu$L) extracted from the concentrated sample obtained from the 30 mL raw sewage sample. And the viral concentration in one RNA extraction was calculated by multiplying the measured concentration from RT-qPCR reaction by the ratio of the RNA amount for one RNA extraction (i.e., 50 $\mu$L) to that used for one RT-qPCR reaction (4 $\mu$L).

For routine analysis, we used N1 (US Centers for Disease Control) and E (Charité-Universitätsmedizin Berlin Institute of Virology, Germany) primer-probe sets. A positive sewage sample means both N1 and E having signals under the cutoff cycle threshold (Ct) value of 40, and the final viral concentration in the positive sewage sample was reported using the primer-probe set that has a higher average viral concentration for duplicated RT-qPCR reactions calculated using the standard curves of N1- or E-carrying plasmids. The plasmid carrying N1 target or E target was synthesized commercially by BGI. The concentration of plasmid was obtained using Qubit dsDNA HS assay kit (ThermoFisher, USA) and the copy number was calculated based on the length and Avogadro's number. In each batch of RT-qPCR, the standard curves for N1 and E were freshly made by serial tenfold dilution of the plasmid ranging from $10^6$ to 1 copy $\mu$L$^{-1}$ to quantify the copy number of SARS-CoV-2 in positive sewage samples.

#### 2.5. One-step RT-qPCR reaction conditions

For the variant detection, we used specifically designed primer sets. The One-step RT-qPCR reactions were set up in a final volume of 20 $\mu$L, using 4 $\mu$L template, 5 $\mu$L TaqMan Fast Virus 1-step Master Mix (ThermoFisher, USA) and the primers and probes with the final concentration of 500 nM and 250 nM, respectively. The One-step RT-qPCR was performed on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR system (ThermoFisher, USA) following the PCR cycler conditions, reverse transcribed for 5 min at 50 °C and initial denaturation for 20 s at 95 °C, followed by 45 cycles of 5 s at 95 °C and 30 s at 55 °C (AS primer-sets and N1 set) or 58 °C (E set). The samples were determined as positive when the Ct was below 40. For every batch of RT-qPCR detection assay, a non-template control (NTC) was included.

#### 2.6. Library preparation and sequencing

We applied targeted sequencing using Nanopore and NovaSeq platforms in this study. ARTIC amplicons (~400 bp) of TP site 9A sample were prepared according to the ARTIC V3 protocol for sequencing with some modifications, specifically, we added 10 $\mu$L of cDNA template reverse instead of 2.5 $\mu$L. The purified amplicons were subjected to Next-Generation sequencing (NGS, NovaSeq 6000, Novogene) and Nanopore sequencing. For nanopore sequencing, we used ONT ligation Kit (SQK-LSK109) following the manufacturer's protocol. The library was run into a flowcell (FLO-MIN106) and sequenced on a GridION X5 device. The amplicon-based sequencing for the patient was performed via Illumina platform using method as previously reported (Sit et al., 2020).

#### 2.7. Raw data analysis

The resulting reads for NGS were aligned to the reference genome sequence of a SARS-CoV-2 virus isolate strain (GenBank Accession Number MN908947.3) using bwa mem. And then, we did primer trimming, quality trimming and consensus calling using iVar (1.3.1), samtools mpileup (v1.13) following the iVar pipeline. The raw reads of nanopore were basecalled using Guppy (4.3.4) under the high-accuracy basecalling
model, and then the consensus-level variant candidates were identified using Medaka developed by ARTIC workflow (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). The called consensus was identified to the specific lineage by Nextclade (https://clades.nextstrain.org/). We blasted the consensus sequences from sewage samples and patient’s specimen using Geneious Prime 2021 which generated the mapping chart. The phylogenetic tree was created by Nextclade (https://clades.nextstrain.org/).

2.8. Collection of demographic, clinical and epidemiological data

The confirmed patient was reported by the Centre for Health Protection of the Government of the Hong Kong SAR. Geographic data, including individual sewersheds, and the population size, were provided by the Environmental Protection Department, the Government of the Hong Kong SAR. Besides, the sampling site diagram was plotted by ArcGIS 10.6. The clinical data related to the confirmed case were supplied by the Hospital Authority of Hong Kong.

2.9. Statistical analysis

We used GraphPad Prism (GraphPad Software, San Diego, CA, USA) and Microsoft Excel for linear regression and other statistical analyses.

3. Results

3.1. The territory-wide sewage surveillance system in Hong Kong

The sewage surveillance system in Hong Kong now serves as a management tool for proactive preparedness and responses to the COVID-19 pandemic, with adaptive sampling strategy for providing an early warning signal, monitoring different phases of pandemic progression to meet the public health needs, as well as real-time reporting to assist the decision making process. Once a sewage sample is tested as “positive” for SARS-CoV-2, the health authority will consider relevant clinical factors to decide public health interventions on the need, such as a compulsory testing notice or a restriction-testing declaration, to be conducted for the concerned population to identify the hidden cases.

For the ongoing routine sewage surveillance in Hong Kong, sewage samples are daily collected and analyzed for the presence of SARS-CoV-2. As of June 2021, around 220 sewage samples were collected and tested from nearly 5.3 million people (70% of the total population). This provides broad monitoring on the overall COVID-19 condition across the territory in a proactive manner.

3.2. Detection of SARS-CoV-2 in sewage and intensified sewage surveillance

As of 21 June 2021, Hong Kong achieved a 14-d streak of zero local infections. In line with the epidemiological data, routine sewage surveillance did not detect SARS-CoV-2 circulation in the local community sewage during that time. Since the last documentation of positive sewage sample on 11 May 2021, no positive signal of SARS-CoV-2 had been detected in sewage samples during the routine daily monitoring until 21 June 2021. On 21 June 2021, a sewage sample collected from one of the stationary sampling sites, the Tai Po site 9A (TP site 9A) with a sewershed serving over 33,000 people, returned positive sewage test results (high viral load of 568,000 RNA copies per L) for SARS-CoV-2, indicating a possible re-introduction of COVID-19 to the local community (Fig. 1).

The detection was considered alarming. Fig. 2 illustrated the timeline of key events in response to detection of SARS-CoV-2 in the community sewage during the routine sewage surveillance. As soon as the positive signal of SARS-CoV-2 was detected, intensification of sewage surveillance in that particular sewershed was initiated. This involved adapting sewage sampling strategy to accommodate the expanded sewage surveillance at the targeted sewershed of TP site 9A, and applying a newly developed allele-specific RT-qPCR workflow for a rapid analysis of the variant type in the sewage sample (Fig. 3).

The sampling strategy included continued sewage sample collection at the TP site 9A, and simultaneous daily sampling at upstream sewage manholes. Besides, a new workflow was implemented for identifying the variant type in sewage. The upstream sampling sites comprised 9 second-tier and 17 third-tier sites located within the sewershed of the first-tier site of TP site 9A, with population coverage ranging from approximately 1000 to 30,000. The 9 second-tier sites provided a complete spatial coverage for the entire sewershed of TP site 9A, and the third-tier sites were manholes of individual buildings located within the sewershed of a few second-tier sites to ease the tracing of hidden cases.

Following the first detection of positive SARS-CoV-2 signal from the stationary site of TP site 9A on 21 June 2021, next came two positive samples on 22 June 2021, including an additional sample consecutively taken at TP site 9A and the other sample from a second-tier site TP-008 which covers 15,000 people. The detected viral RNA concentration were 2322 and 1807 copies per L of sewage for these two sites, respectively. Of the 9 second-tier sites sampled on that day (22 June 2021), only the site TP-008 was tested positive for SARS-CoV-2 RNA. On 23 June 2021, the site TP-008 was found repeatedly positive, with a low viral concentration of 680 copies per L of sewage, while the site TP site 9A was negative. On the same day, SARS-CoV-2 was detected in a sewage sample from the third-tier site WTTE2 representing a single residential building (Wan Hang House) having over 4700 population living in a single residential building (Wan Hang House) (Fig. 4). After that, no positive viral signal was detected across all 27 sampling sites during the continued monitoring period from 24 June to 28 June 2021 (Fig. 5).

Fig. 2. The timeline of key events in response to detection of SARS-CoV-2 and the Delta variant in the community sewage during routine sewage surveillance.
3.3. A new workflow expedited the Delta variant identification

We implemented a rapid testing workflow for detecting SARS-CoV-2 Delta variant. This new genotyping workflow was based on AS RT-qPCR comprising four parallel reactions that target four signature mutations (N501Y, L452R, T478K, and P681R) situated in the gene of spike protein of SARS-CoV-2. Each assay involved a pair of primer-probe set, which included a common reverse primer and two allele-specific forward primers that enable single-nucleotide discrimination of genome targets between the mutation type and its opposite genotype (i.e., the wild type). Four pairs of primer-probe sets were designed for four mutation sites and their opposite genotypes (Details in Materials and Methods).

In-silico tests showed that the two characteristic mutations, including the N501Y and L452R, can classify the current four variant of concerns (VOCs, including Alpha, Beta, Gamma, Delta) and the Mu variant of interest (VOI) as designated by the WHO as of September 2021 into two groups: presence of N501Y in the absence of L452R could indicate a group consisting the Alpha, Beta, Gamma, Lambda, and Mu variants, respectively. Additionally, two signature mutations of the Delta variant, i.e. the T478K, and P681R, were also detected in this sewage sample, ascertaining the presence of the Delta variant. A very low amount of the L452 and T478 wild types were also detected with respective concentrations of 2 and 3 viral DNA copy per reaction, which could possibly due to cross-reactivity in the presence of high amount of the mutation types as observed in the evaluation results of analytical specificity. The implementation of the newly developed AS RT-qPCR based workflow identified the SARS-CoV-2 in the TP site 9A sewage as the Delta variant within one day after the sample collection.

To confirm the Delta variant obtained from the AS RT-qPCR workflow, we determined the viral genome sequence in the same sewage sample using both short-read (Illumina) and long-read (Nanopore) sequencing platforms, and compared the obtained sequences to the whole genome sequences derived from the matched patient’s specimen. By comparing to the reference genome sequence of a SARS-CoV-2 virus isolate strain (GenBank MN908947.3), we determined the genome coverage and consensus
variants (Fig. 6). Both Illumina and Nanopore sequencing resulted in near-complete (99.6%) genome sequences with an average coverage of 5500 and 421, respectively. In total, we found 42 consensus variants (39 substitutions and 3 deletions) with Illumina data and 39 consensus variants (36 substitutions and 3 deletions) with Nanopore data (Table S2). The resulting consensus sequence from the matched patient exhibited a lower genome coverage of 96%, comprising 41 substitutions and 3 deletions. The consensus variants were detected by Illumina data with high accuracy, and there were only two undetected substitutions (G21987A and A23994G) when compared with the consensus variants from the matched patient, while for the Nanopore data, there were six undetected substitutions (G21987A, A23994G, C6402T, C19220T, G29402T, and C29555T) as well as one erroneous substitution (C15925T), compared with the virus sequence from the matched patient (Fig. S2).

The consensus-level phylogeny was resolved by placing these three consensus genome sequences on a high-quality SARS-CoV-2 reference tree constructed based on clade-defining mutations (https://clades.nextstrain.org/). The resulting phylogenetic tree (Fig. S3) indicated that the Delta variant was detected from the sewage samples, and this matched with the patient’s specimen being as the Delta variant, which was concordant with the results obtained by the AS RT-qPCR workflow developed in the present study. Nevertheless, the SARS-CoV-2 genome from the patient was more closely related to the genome obtained from Illumina data instead of that from Nanopore data, probably due to the absence of one of five clade-defining mutations of Delta variant (T22917G, C22995A, T27638C, G28881T, and G29402T) in the Nanopore data.

3.4. Mandatory individual PCR testing triggered by the sewage surveillance

In Hong Kong, community spread of fourth wave of COVID-19 has been largely contained since the end of April 2021. As of 21 June 2021, the last reported local case was on 7 June 2021, when a resident who contracted the SARS-CoV-2 Alpha variant locally was confirmed. No other local COVID-19 cases had been recorded since then, achieving 14-d streak of zero local infections until 21 June 2021. Furthermore, there had been no single case with the Delta variant in local communities by then. The sewage sample collected from the site TP site 9A on 21 June 2021 was tested positive for SARS-CoV-2 during Hong Kong’s routine sewage surveillance. On 22 June 2021, the intensified sewage surveillance tracked the source of the positive viral signal to an upstream sampling site TP-008. On the same day, evidences from genotypic analyses indicated that the SARS-CoV-2 Delta variant was involved. As an emergency response to the first detection of SARS-CoV-2 Delta variant in the sewage of the local communities in Hong Kong, on 23 June 2021, the health authority issued a compulsory testing notice for the sampled population covered by the sampling site TP-008 (https://www.info.gov.hk/gia/general/202106/23/P2021062300471.htm). Over 15,000 residents living in the respective area were required to undergo individual testing via RT-qPCR. A case who lived in Wan Hang House...
Fig. 5. (a) Hierarchy structure of the first-tier, and its second-tier and third-tier sampling sites. (b) The detection results of these sampling sites.

| Site   | Tier | Population | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
|--------|------|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| TP005  | 1st  | 3,000      | —     | —     | —     | —     | —     | —     | —     | —     |
| TP006  | 1st  | 1,724      | X     | —     | —     | —     | —     | —     | —     | —     |
| TP007  | 1st  | 1,785      | X     | —     | —     | —     | —     | —     | —     | —     |
| TP008  | 1st  | 1,540      | X     | —     | —     | —     | —     | —     | —     | —     |
| TP009  | 1st  | NA         | X     | —     | —     | —     | —     | —     | —     | —     |
| TP010  | 1st  | 5,490      | X     | —     | —     | —     | —     | —     | —     | —     |
| TP012  | 1st  | 884        | X     | —     | —     | —     | —     | —     | —     | —     |
| TP013  | 1st  | 31,537     | X     | —     | —     | —     | —     | —     | —     | —     |
| WTTK1  | 2nd  | 5,465      | X     | X     | —     | —     | —     | —     | —     | —     |
| WTTK2  | 2nd  | 778        | X     | X     | —     | —     | —     | —     | —     | —     |
| WTTK3  | 2nd  | 2,400      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK4  | 2nd  | 2,400      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK5  | 2nd  | 1,026      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK6  | 2nd  | 2,050      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK7  | 2nd  | 1,790      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK8  | 2nd  | 1,790      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK9  | 2nd  | 3,602      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK10 | 2nd  | 6,745      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK11 | 2nd  | 1,162      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK12 | 2nd  | 4,388      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK13 | 2nd  | 2,694      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK14 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK15 | 2nd  | 3,884      | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK16 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK17 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK18 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK19 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK20 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK21 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |
| WTTK22 | 2nd  | NA         | X     | X     | X     | —     | —     | —     | —     | —     |

Fig. 6. Alignments of consensus sequences obtained from the sewage sample and the matched patient's specimen to the reference genome sequence of a SARS-CoV-2 virus isolate strain (GenBank MN908947.3). Genetic disagreements were highlighted in black in the query sequences. Query 1 and 2 refer to the consensus sequences of SARS-CoV-2 in sewage samples obtained using the Nanopore and Illumina sequencing platforms, respectively. Query 3 refers to the consensus sequences of SARS-CoV-2 in the matched patient's specimen using the Illumina sequencing platform.
(located within the TP-008 sewershed) was reported as preliminary positive on 23 June and confirmed on 24 June by the local authorities. This patient began to have symptoms including fever, cough and sore throat on 21 June 2021 (the same day when the first positive sewage sample was taken in the morning). On 25 June 2021, the case was confirmed to be infected with the Delta variant via whole-genome sequencing, three days after AS RT-qPCR test of sewage gave the alert about the Delta variant.

4. Discussion

In this study, we showed an example on the use of sewage surveillance for COVID-19 to address the operationalization needs and the decision making timeline for a public health intervention to control a looming Delta variant outbreak. The used sewage testing method for SARS-CoV-2 was shown to be able to alert the presence of a single COVID-19 patient in a community with 33,000 residents. Routine sewage surveillance served to alert the local authorities on 21 June 2021 about the re-introduction of SARS-CoV-2 in local communities when the COVID-19 was largely contained in Hong Kong. Subsequent intensified sewage surveillance pinpointed the specific sewershed area of the individual for the intervention planning. Genotyping of the SARS-CoV-2 RNA in the sewage sample via a rapid workflow informed the local authorities about suspected local infections involving the Delta variant within one day after the sample collection, enabling evidence-based decision making to the first documentation of the Delta variant in local community sewage, which predated the Delta variant confirmation in the matched patient by three days. Since 24 June 2021 after the matched case was hospitalized, continued sewage surveillance at target areas did not detect any positive signal of the SARS-CoV-2 for five consecutive days, suggesting the Delta variant was not circulating in the local communities. As of the end of September 2021, no single local case involving the Delta variant was reported according to the clinical epidemiological data of local public health agencies, which verified the stop of a potential transmission of the Delta variant in local communities in Hong Kong.

The intensified sewage surveillance activities were essential for managing the looming outbreak in communities, which compromised the implementation of targeted upstream sampling and the adoption of a newly developed AS RT-qPCR based workflow. Unlike previously reported work on a silent polio outbreak in Israel (Manor et al., 2014; Kaliner et al., 2015), in which the targeted upstream sampling was used to assess the intensity of the poliovirus circulation across different communities, this study provided evidences in identifying buildings and places for statutory public health intervention to discover infected individuals. This strategy allowed the tracking of the viral source from the site covering a large area where the first positive signal was picked up (with over 33,000 sewered population) on 21 June 2021, and on the next day to a second-tier site TP-008 representing over 15,000 people, and finally on the third day to the single building (Wan Hang House) where the matched COVID-19 case lived. As a precautionary measure, a compulsory testing notice was implemented on 22 June 2021 for all residents living in the sewershed of the site TP-008. This decision mainly considered the population risk due to the highly transmissible Delta variant despite the challenges in the implementation for a large population. Experience gained from the case described in this study showed that proactive planning of upstream sites at building or sub-building level for target upstream sampling and strategic prioritization of the targeted upstream sampling sites can help identify the precise locations of the possible patient for the follow-up public health intervention to capture the infected individuals. Besides, the truth value of sewage surveillance could be manifested when complemented with follow-up public health interventions such as compulsory testing.

Adoption of the newly developed AS RT-qPCR based workflow improved sewage surveillance efforts by providing results of the variant type within one day after sewage sample collection, enabling the real-time risk evaluation based on the genotypic analyses of the detected SARS-CoV-2 RNA. Though whole-genome sequencing (WGS) approach can provide rich surveillance data such as the determination of transmission networks and the evolution by whole genome analysis, and a few pioneering studies (Cris-Cristoph et al., 2021; Martin et al., 2020) have demonstrated the feasibility in applying this approach to sewage samples, it remains challenging considering the low viral titers and complex matrix in sewage samples (Chiara et al., 2021). To inform the follow-up public health action, the rapid assessment is of prominent importance, especially when the dominant circulating variant type in a community could be obtained from the epideimology studies based on clinic epidemiologic surveillance. AS RT-qPCR based approach has its unique strength: the shorter turnaround time (usually a few hours) and higher sensitivity when compared with the sequencing-based approach make it a more suitable assessment tool to address the timelines and align with the information needs of policymakers. It should be noted that, one limitation for the AS-qPCR method used in this study was the cross-reactivity observed for the primer-probe sets designed for the mutation sites of T478K and L452R. Future work is needed to improve the specificity by optimizing the design of the primer sequence and the RT-qPCR conditions. In addition to detect the Alpha variant in sewage in previous studies (La Rosa et al., 2021; Lee et al., 2021) and the Delta variant in sewage in this study, the AS RT-qPCR based approach could also be flexible deployed for other fast-spreading SARS-CoV-2 variants, such as the Omicron, by selecting and validating unique sets of characteristic mutations that have a high predictive value of the variant type.

5. Conclusion

Hong Kong established routine city-wide sewage surveillance for COVID-19 since end of December 2020 to provide daily sewage test results for statutory public health interventions. In light of the emergence of SARS-CoV-2 variants worldwide, the sewage surveillance system now incorporates new testing workflow for rapid assessment of threat levels. This study clearly demonstrated how public health response and intervention were implemented following the detection of the Delta variant through routine sewage surveillance and the newly developed AS RT-qPCR based variant testing workflow. Standardized procedures of testing and reporting, fast variant detection method based on AS RT-qPCR, and proactive planning of upstream sites were the major factors for the rapid turnaround needed for public health intervention against the spread of the SARS-CoV-2 variants in a community.

CRediT authorship contribution statement

Yu Deng: Conceptualization, Methodology, Data analysis, Writing.
Xiaojing Xu: Conceptualization, Methodology, Experiments, Data analysis and Writing.
Xiaowan Zheng: Methodology, Experiments, Reviewing and Editing.
Jiahui Ding: Methodology, Experiments, Data analysis.
Shuxian Li: Experiments, Data analysis.
Ho-kwong Chui: Methodology, Reviewing and Editing.
Tsz-kin Wong: Methodology.
Leo LM Poon: Methodology, Reviewing and Editing.
Tong Zhang: Conceptualization, Methodology, Data analysis, Writing, and Supervision.

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.

Acknowledgements

This study was financially supported by Health and Medical Research Fund (HMRF) (COVID190209 and COVID1903015) from the Food and Health Bureau, the Government of the Hong Kong Special Administrative Region (SAR), China. We thank both the Centre for Health Protection of Food and Health Bureau of Hong Kong and the Hospital Authority of Hong Kong for sharing clinical data and epidemiological details. We thank HKU-Pasteur Research Centre for the technical support on lab facilities. Xiaojing Xu, Xiaowan Zheng, Jiahui Ding and Shuxian Li would like to thank for the University of Hong Kong for the Postgraduate Studentship (PGS).
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153250.

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., et al., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 138764.

Bogler, A., Packman, A., Furman, A., Gross, A., Kushmaro, A., Ronen, A., et al., 2020. Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic. Nat. Sustain. 3 (12), 981–990.

Chiara, M., De'Erchia, A.M., Giunti, C., Manzari, C., Parisi, A., Resta, N., et al., 2021. Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. Brief. Bioinform. 22 (2), 616–630.

Cris-Christoph, A., Kantor, R.S., Olm, M.R., Whitney, O.N., Al-Shayeb, B., Lou, Y.C., et al., 2021. Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants. MBio 12 (1), e02703–e02720.

Daughton, C.G., 2020. Wastewater surveillance for population-wide Covid-19: the present and future. Sci. Total Environ. 736, 139631.

EU Commission Recommendation (EU), 2021. 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU.

Guo, M., Tao, W., Flavell, R.A., Zhu, S., 2021. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. Nat. Rev. Gastroenterol. Hepatol. 18 (4), 269–283.

Harris-Lovett, S., Nelson, K.L., Beamer, P., Bischel, H.N., Bivins, A., Bruder, A., et al., 2021. Wastewater surveillance for SARS-CoV-2 on college campuses: initial efforts, lessons learned and research needs. Int. J. Environ. Res. Public Health 18 (9), 4455.

Hellmér, M., Paxius, N., Magnus, L., Eanche, L., Arnholm, B., Johansson, A., et al., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80 (21), 6771–6781.

Hillary, L.S., Farkas, K., Mahor, K.H., Locaci, A., Thorpe, J., Distaso, M.A., et al., 2021. Monitoring SARS-CoV-2 in municipal wastewater to evaluate the success of lockdown measures for controlling COVID-19 in the UK. Water Res. 200, 117214.

Kaliner, E., Kopel, E., Anis, E., Mendelson, E., Moran-Gilad, J., Shulman, L.M., et al., 2015. The Israeli public health response to wild poliovirus importation. Lancet Infect. Dis. 15 (10), 1236–1242.

Keshaviah, A., Hu, X.C., Henry, M., 2021. Developing a flexible national wastewater surveillance system for COVID-19 and beyond. Environ. Health Perspect. 129 (4), 045002.

Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., et al., 2020. SARS-CoV-2 in wastewater: state of the knowledge and research needs. Sci. Total Environ. 739, 139076.

La Rosa, G., Mancini, P., Ferraro, G.B., Veneti, C., Iaconelli, M., Lucentini, L., et al., 2021. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein. Water Res. 197, 117104.

Lee, W.L., Imkaeve, M., Armas, F., McElroy, K.A., Gu, X., Duvall, C., et al., 2021. Quantitative SARS-CoV-2 alpha variant B.1.7 tracking in wastewater by allele-specific RT-qPCR. Environ. Sci. Technol. Lett. 8 (8), 675–682.

Manor, Y., Shulman, L., Kaliner, E., Hindiyeh, M., Ram, D., Sofer, D., et al., 2014. Intensified environmental surveillance supporting the response to wild poliovirus type 1 silent circulation in Israel, 2013. Eurosurveillance 19 (7), 20708.

Martin, J., Klapsa, D., Wilton, T., Zambron, M., Bentley, E., Bujski, E., et al., 2020. Tracking SARS-CoV-2 in sewage: evidence of changes in virus variant predominance during COVID-19 pandemic. Viruses 12 (10), 1144.

McClary-Gutierrez, J.S., Mantoli, M.C., Marcenac, P., Silverman, A.L., Boehm, A.B., Bibby, K., et al., 2021. SARS-CoV-2 wastewater surveillance for public health action. Emerg. Infect. Dis. 27.

Medema, G., Heijnen, L., Elkinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-CoV-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7 (7), 511–516.

Mecer, T.R., Salit, M., 2021. Testing at scale during the COVID-19 pandemic. Nat. Rev. Genet. 22 (7), 415–426.

Naughton, C.C., Roman, F.A., Alvarado, A.G.F., Tariqi, A.Q., Deeming, M.A., Bibby, K., et al., 2021. Show us the data: Global COVID-19 wastewater monitoring efforts, equity, and gaps. medRxiv https://doi.org/10.1101/2021.03.14.21253564.

Polo, D., Quintela-Baluja, M., Corbíbí, A., Jones, D.L., Singer, A.C., Graham, D.W., et al., 2020. Making waves: wastewater-based epidemiology for COVID-19-approaches and challenges for surveillance and prediction. Water Res. 186, 116404.

Sit, T.H., Brackman, C.J., Ip, S.M., Tam, K.W., Law, P.Y., Yo, E.M., et al., 2020. Infection of dogs with SARS-CoV-2. Nature 586 (7831), 776–779.

Smith, D.B., Paddy, J.O., Simmonds, P., 2016. The use of human sewage screening for community surveillance of hepatitis A virus in the UK. J. Med. Virol. 88 (5), 915–918.

Thompson, J.R., Naucharlicia, V.Y., Gu, X., Lee, W.L., Rajal, V.B., Haines, M.B., et al., 2021. Making waves: wastewater surveillance of SARS-CoV-2 for population-based health management. Water Res. 184, 116181.

Vandenberg, D., Martini, D., Rochas, O., van Belkum, A., Kozlakides, Z., 2021. Considerations for diagnostic COVID-19 tests. Nat. Rev. Microbiol. 19 (3), 171–183.

Wu, F., Xiao, A., Zhang, J., Moniz, K., Endo, N., Armas, F., et al., 2022. SARS-CoV-2 RNA concentrations in wastewater foreshadow dynamic and clinical presentation of new COVID-19 cases. Sci. Total Environ. 805, 150121.

Xu, X., Zheng, X., Li, S., Lam, N.S., Wang, Y., Chu, D.K., et al., 2021. The first case study of wastewater-based epidemiology of COVID-19 in Hong Kong. Sci. Total Environ. 148000.

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., et al., 2020. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. BMJ 369.