Scientific Research Publishing

American Journal of Molecular Biology, 2022, 12, 109-121
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

A Retrospective Analysis of Wastewater Confirms Dominant Circulation of SARS-CoV-2 Delta Variant in Nairobi, Kenya, between April 2021 and August 2021

Patson Sichamba[1*], Fred Wamunyokoli[2*], Peter Borus[3], Rosemary Nzunza[3], Opanda Silvanos[3], Samwel Symekher[3], Samoel Ashimosi Khamadi[3], Janet Majanja[3], Meshack Wadegu[3], Esther Chitechi[3], Benlick Mwangi[3], Evans Komen[3], Stephen Ombija[3], Wallace Dimbuson Bulimo[3*]

[1]Pan African University Institute for Basic Sciences Technology and Innovation (PAUSTI), Nairobi, Kenya
[2]Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya
[3]Kenya Medical Research Institute Centre for Virus Research, Nairobi, Kenya
Email: *sichambapatd@gmail.com

How to cite this paper: Sichamba, P., Wamunyokoli, F., Borus, P., Nzunza, R., Silvanos, O., Symekher, S., Khamadi, S.A., Majanja, J., Wadegu, M., Chitechi, E., Mwangi, B., Komen, E., Ombija, S. and Bulimo, W.D. (2022) A Retrospective Analysis of Wastewater Confirms Dominant Circulation of SARS-CoV-2 Delta Variant in Nairobi, Kenya, between April 2021 and August 2021. *American Journal of Molecular Biology*, **12**, 109-121.
https://doi.org/10.4236/ajmb.2022.123010

Received: May 8, 2022
Accepted: June 26, 2022
Published: June 29, 2022

Abstract

Wastewater surveillance has been applied in various parts of the world to monitor the introduction and transmissions of SARS-CoV-2 variants in a population. The knowledge of SARS-CoV-2 variants circulating in a population is critical to COVID-19 management and timing of the application of public health countermeasures. Contrary to the routine clinical surveillance of SARS-CoV-2 where cases from asymptomatic patients are often underreported, wastewater surveillance offers an unbiased tool for monitoring the extent of SARS-CoV-2 transmissions in a community. The present study aimed to characterize SARS-CoV-2 variants that circulated in Nairobi County, Kenya, between April 2021 and August 2021 utilizing wastewater samples. Viral RNA was extracted from wastewater samples, followed by SARS-CoV-2 screening by real-time RT-qPCR before targeted sequencing of the Spike gene. Forty samples were analyzed, of which 50% (n = 20) tested positive for SARS-CoV-2 by real-time RT-qPCR. Of these, 45% (n = 9) were successfully amplified by RT-PCR and sequenced. The majority (78%, 7/9) of the viruses belonged to the Delta (B.1.617.2) lineage of SARS-CoV-2, while a minority (22%) belonged to the Alpha (B.1.1.7) and Alpha-Delta lineages. Phylogenetic analysis of the SARS-COV-2 delta lineage strains revealed scattered clustering of the Kenyan viruses among the global strains included in the analysis, suggesting different introductory routes into the country. On the whole, our re-

sults confirm previous clinical findings that SARS-CoV-2 variants belonging to the Alpha (B.1.1.7) and Delta (B.1.617.2) lineages circulated in Nairobi County, Kenya during the study period, with the latter predominating. This is the first study to describe the diversity of SARS-CoV-2 variants circulating in Kenya, through wastewater analysis.

Keywords

Wastewater Surveillance, SARS-CoV-2, Delta Variants, Spike Gene, COVID-19, Kenya

1. Introduction

The Coronavirus disease 2019 (COVID-19) is caused by a human coronavirus strain known as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [1]. The Virus is transmitted through respiratory droplets and direct contact with both asymptomatic and symptomatic carriers [2]. The main symptoms of the disease include fever, coughing, sneezing, diarrhoea, dyspnoea, headache, sore throat, and rhinorrhoea [3]. COVID-19 was declared a global pandemic by the World Health Organization (WHO) on 11th March 2020 [4]. The first case of COVID-19 was confirmed in Kenya on 12th March 2020 [5], and as of December 31, 2021, more than 295,028 cases and 5378 deaths had been reported in the country [6].

Wastewater-based epidemiology is a surveillance tool for the collection, extraction, and detection of infectious agents present in a population using wastewater as a sample source [7]. The technique has been employed for the detection of illicit drugs [8], antimicrobial resistance (AMR) [9], viruses such as poliovirus [10], hepatitis A virus, norovirus outbreaks [11] and SARS-CoV-2 [12] [13] [14]. COVID-19-infected individuals usually shed the virus in the nasal, oropharyngeal, and gastrointestinal tract [15] [16] [17] [18]. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) has shown that viral RNA shedding continues after symptoms have subsided, and that viruses shed into the wastewater environment can be detected for an extended period [19].

The major benefits of the wastewater-based epidemiology approach are that it is non-invasive, unbiased, in-expensive, and can serve as an early-warning system for SARS-CoV-2 emergence due to its inherent inclusiveness, and the ability to capture both asymptomatic and symptomatic cases [14] [20] [21]. Additionally, wastewater represents a pooled sample from a population for its ability to blend the viruses coming from different sources such as hand washing, sputum, feaces, and fomites contributed by both symptomatic and asymptomatic cases [22].

The SARS-CoV-2 nucleic RNA material has previously been detected in wastewater in various countries, globally [23] [24] [25] [26]. Case in point, countries

such as Australia, New Zealand, Brazil, the Netherlands, and the United States have adopted wastewater surveillance as a routine COVID-19 monitoring method [27]. Despite its convenience and the various advantages that come with it, data on SARS-CoV-2 from wastewater surveillance in Kenya is lacking.

In an attempt to bridge the gap, the present study aimed to describe the genetic diversity of SARS-CoV-2 variants that circulated in Nairobi County, Kenya between April 2021 and August 2021 through real-time RT-qPCR screening of the virus nucleic RNA in wastewater, coupled with nucleotide sequencing of the complete Spike protein-encoding gene. The SARS-CoV-2 spike protein (S) is the major surface virus antigenic protein, responsible for host Angiotensin-converting enzyme 2 (ACE2) receptor recognition and attachment [28]. Furthermore, the spike protein is a major determinant of SARS-CoV-2 variant designation [29] which is caused by genetic mutations in the gene that codes for this protein [30].

2. Materials and Methods

2.1. Study Sites and Sampling

The study utilized grab samples (n = 40) that were previously collected as part of routine poliovirus environment surveillance (ES) by the Centre for virus research of Kenya Medical Research Institute (KEMRI). The sampling period was between April 2021 and August 2021. The samples were collected from eight sampling sites, namely, Kibera, Dandora treatment plant, Mukuru Kware river, Eastleigh A, Eastleigh B, Majengo river, Kaverera bridge, and Mathare within the Nairobi County, as previously mapped and validated by WHO (Figure 1).

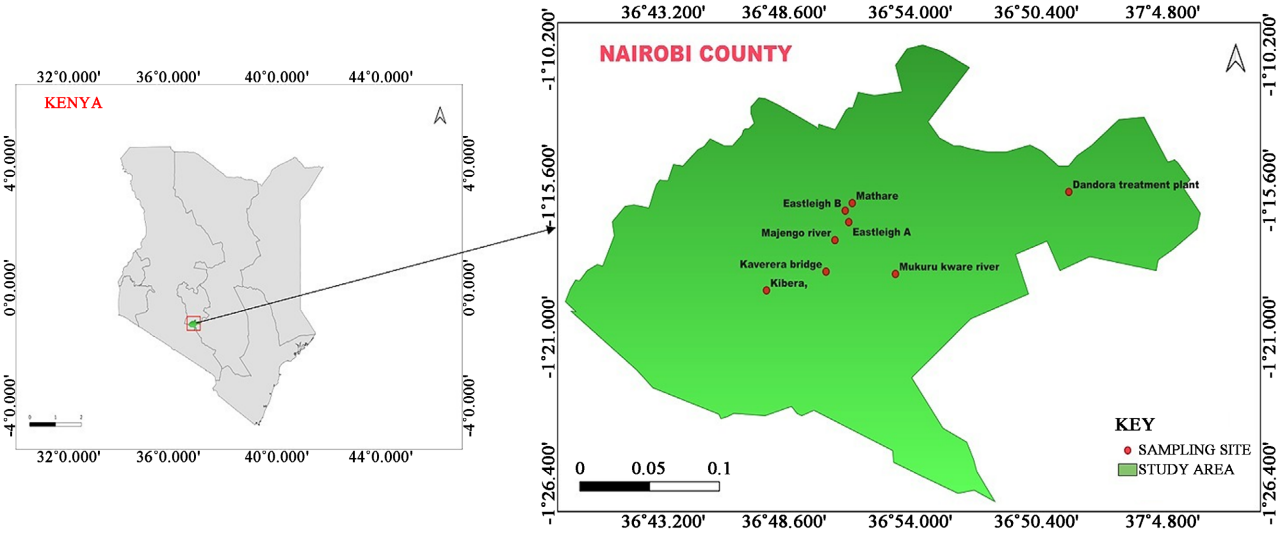

Figure 1. A sketch diagram of the map of Kenya depicting study-sampling sites. The map was generated using QGIS version 3.10 [31].

2.2. Viral RNA Extraction and Real-Time RT-qPCR Screening

Prior to viral RNA extraction, the wastewater samples were concentrated fol-

lowing the two-phase separation method of the World Health Organization [32] protocol for environmental surveillance of Poliovirus. Briefly, 500 ml wastewater was centrifuged, at 1500 g for 20 minutes. The pellet was stored at 4˚C for later use and the pH of the supernatant was adjusted to neutral (pH 7 - 7.5) using NaOH and HCl. The supernatant was mixed with defined amounts of dextran and polyethylene glycol (PEG). The Mixture was kept in constant agitation for 1 hour at room temperature using a magnetic stir plate then poured into the separating funnels which were left to stand overnight at 4˚C. The concentrate was harvested by collecting the lower layer and the interphase slowly drop-wise, and this concentrate was mixed with the pellet from the initial centrifugation. The viral RNA was extracted using the RADI PREP swab and stool DNA/RNA kit following the manufacturer's instructions (KH Medical Co. Ltd. Republic of Korea). The RADI COVID-19 Detection kit was used to screen the extracted RNA for SARS-CoV-2 using a real-time RT-qPCR assay targeting the virus S and RdRp genes as directed by the manufacturer (KH Medical Co., ltd. Korea).

2.3. Complementary DNA (cDNA) Synthesis and Conventional RT-PCR

The cDNA synthesis was performed using the Accuris qMax™ First Strand cDNA Synthesis Kit (USA), based on the manufacturer's instructions. PCR amplification of the SARS-CoV-2 surface spike protein-encoding gene was performed using a set of five pairs of primers described elsewhere [33]. The PCR master mix was prepared using the FIREPol® Master Mix, based on the manufacturer's specifications (Solis Biodyne, Tartu, Estonia).

The PCR amplicons generated were electrophoresed on a 1% agarose gel (Sigma-Aldrich Co, USA) stained in 2 µg/ml ethidium bromide (Sigma-Aldrich Co, USA) solution and viewed using the E-box gel imaging system (Vilber Lourmat, France). The amplicons were next purified using Exonuclease I/Shrimp Alkaline Phosphatase (ExoSap-IT) enzyme (Affymetrix, USA), based on the manufacturer's instructions. Subsequently, the purified products were sequenced on both strands on an automated 3500×L Genetic Analyzer (Applied Biosystems, USA). Cycle sequencing was done using the Big Dye Terminator Cycle sequencing kit v3.1 (Applied Biosystems, USA), based on the manufacturer's instructions. The SARS-CoV-2 Spike protein-encoding gene sequences reported in this work are available in GenBank under the accession numbers: ON063425 - ON063433.

2.4. Sequence Analysis

The generated DNA nucleotide sequence fragments were assessed for quality and assembled into contig using finchTV v1.4.1 [34] and DNA Baser v4 [35] software respectively. Mutation calling and genetic lineage grouping were performed using Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) [36] and NextStrain [37] online tools. The sequences were compared to NC_045512.2-Wuhan-Hu-1 as well as those obtained from other regions during the study period, which were drawn from the Global Initiative for Sharing All In-

fluenza Data (GISAID) [38]. Multiple sequence alignment was performed using Muscle version v3.8 software [39]. A phylogenetic tree was generated using MrBayes software v3.2 [40] under the best fit TN93 + G substitution model as predicted by the jModelTest software [41]. The tree was visualized and annotated using Fig Tree v1.4.0 software [42].

3. Results

Overall, 20 (50%) of the extracted grab samples tested positive for SARS-CoV-2 by real-time RT-PCR, with Ct values ranging from 27.1 to 40 for both the target S and RdRp virus genes. The frequency of SARS-CoV-2 detections among the study sites is summarized in Figure 2.

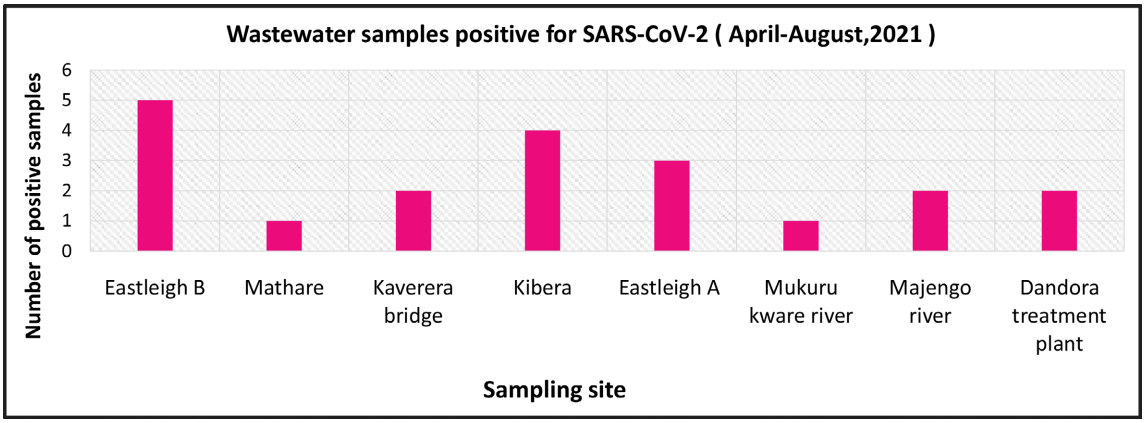

Figure 2. Summary of the number of SARS-CoV-2 detections by real-time RT-PCR assay among the study sampling sites.

Nine (45%) of the positives were successfully amplified and sequenced using conventional RT-PCR, and yielded usable sequences. Genetic analysis revealed that the majority (n = 7) of the sequences belonged to the SARS-CoV-2 Delta (B.1.617.2) lineage, while a minority (n = 2) belonged to the Alpha (B.1.1.7) and a recombinant (Alpha-Delta) lineages (Table 1). All the Kenya viruses exhibited various amino acid substitutions in the Spike protein relative to the prototype NC_045512.2-Wuhan-Hu-1 strain as summarized in Table 1.

Table 1. SARS-CoV-2 lineage classification and amino acid variations in the spike protein of the Kenyan viruses relative to the prototype NC_045512.2-Wuhan-Hu-1 strain.

Sample ID	Date of collection	WHO Lineage	Spike protein mutation
KENYA_104_19042021	6/4/21	Delta	T19R, P681R, D614G, L452R, F157del, R158del, D228H, E156G, G142D, T478K
KENYA_125_19052021	19/5/21	Delta	T19R, P681R, D614G, D950N, Q613H, T478K, L452R, F157del, R158del, E156G, G142D
KENYA_138_10062021	10/6/21	Delta	T19R, P681R, D614G, D950N, T478K, L452R, F157del, R158del, E156G, T95I, G142D

Continued

KENYA_139_10062021	10/6/21	Delta	P681R, D614G, L452R, F157del, R158del, E156G, T478K, D142D, D950N
KENYA_141_10062021	10/6/21	Delta	T19R, P681R, D614G, D950N, T478K, L452R, F157del, R158del, E156G, S112L, G142D
KENYA_143_10062021	10/6/21	(Alpha,V1)	A570D.S982A, P681H, D614G, T716I, Y144del, N501Y, D1118H, V70del, H69del
KENYA_164_15072021	15/7/21	recombinant (Delta and Alpha)	P681R, D614G, D950N, T478K, L452R, A520S
KENYA_203_12082021	12/8/21	Delta	T19R, P681R, D614G, L452R, F157del, R158del, D228H, E156G, D142D, T478K, D950N
KENYA_205_12082021	12/8/21	Delta	T19R, V90I, P681R, D614G, D950N, T478K, L452R, F157del, R158del, E156G, T95I, G142D

These changes were however not unique to the Kenyan viruses as they were common among sequences of the global strains included in the analysis. Phylogenetic comparisons revealed general interspersion of the Kenyan SARS-CoV-2 Delta lineage viruses among the foreign reference strains included in the analysis (**Figure 2**). The Kenyan virus KENYA 205 12082021 clustered closely with the Indian and USA strains, KENYA 138 10062021 with Botswana and Australian strains, KENYA 139 10062021 with a strain from the United States of America, KENYA 203 12082021 and KENYA 104 19042021 with strains from Denmark and Cameroon, KENYA 125 19052021 with strains from Vietnam and India, KENYA 141 10062021 with strains from Tunisia & Congo, and KENYA 164 15072021 with strains from southern Sudan and Japan (**Figure 3**).

4. Discussion

Wastewater surveillance for SARS-CoV-2 offers an alternative approach for epidemiological tracking of the extend of virus spread in a community [43]. SARS-CoV-2 nucleic RNA has previously been detected in wastewaters [43] [44] [45]. This approach is mostly utilized in situations of low virus prevalence or during early periods of a pandemic [43] [46] [47]. Due to its low cost in comparison to clinical epidemiology, which requires individual patients to be swabbed, the approach may be used in low resource settings (where it will quickly indicate circulating strains in the general population) and may also be used routinely once the pandemic has established in the population [46]. The present study used real-time RT-qPCR assay coupled with targeted sequencing to detect and characterize the diversity of SARS-CoV-2 in Nairobi County, Kenya, between April 2021 and August 2021. Overall, nine SARS-CoV-2 variants were identified. The majority (78%, n = 7) of the variants belonged to the Delta SARS-CoV-2 lineage while a minority (22%) belonged to the Alpha (B.1.1.7) and Alpha-Delta recombinant lineages. This result is consistent with previous findings in Kenya using clinical epidemiologic approach [48] [49] as well as related studies elsewhere, where increased cases of COVID-19 caused by the Delta SARS-CoV-2

Figure 3. Bayesian phylogenetic tree based on nucleotide sequences of the Spike gene of SARS-CoV-2 Delta lineage viruses. The tree was generated using MrBayes v3.2. The numbers at the nodes denote posterior probability values. The scale bar indicates the number of nucleotide substitutions per site. The study strains are shown in blue. The prototype NC_045512.2-Wuhan-Hu-1 reference strain is shown in red.

variants were reported during the same period [50] [51]. The increased global circulation of the Alpha (B.1.1.7) SARS-CoV-2 lineage was reported beginning in 2021, before being replaced by the Delta variant [51].

Notably, only 45% (9/20) of the SARS-CoV-2 viral RNA positive samples were detected by real-time RT-qPCR subsequently amplified by conventional RT-PCR prior to sequencing. The failure of 55% of the samples to amplify by conventional RT-PCR may be attributed to low copy numbers of template SARS-CoV-2 RNA (high Ct value samples) in the samples. Real-time PCR has statistically higher sensitivity compared to conventional RT-PCR [52] [53]. Cumulatively, the Eastleigh (Eastleigh A/B) and Kibera sampling sites had the highest detection levels, accounting for nearly 60% of the total extractions. This could be attributed to the high population density in these two slum areas, as well as the proximity of the sampling sites to very fresh and raw stool, implying that the viruses had not been exposed to the environment for too long since they were shed in the stool. The observed differences in SARS-CoV-2 detection levels by real-time RT-qPCR could be attributed to varying degrees of virus damage in the wastewaters. Findings from a previous study have shown that the period for the survival of SARS-CoV-2 RNA in wastewater is hinged on various factors including the presence of other microorganisms and physicochemical properties such as pH, solids, and disinfectant compounds, known to destroy viruses and their nucleic acids, rendering viral detection cumbersome [54]. Furthermore, the higher SARS-CoV-2 concentrations in in sewage of the two sites could be attributed to a failure to comply with government COVID-19 public health countermeasures such as masking and social distancing, resulting in increased community transmissions and concomitant increased virus shedding in stool.

Phylogenetic analysis of the SARS-CoV-2 Delta (B.1.617.2) lineage strains based on the complete surface glycoprotein spike gene sequence data revealed interspersion of the Kenyan viruses among the global strains included in the analysis. This result suggests seeding of the Delta SARS-CoV-2 variant strains into Kenya from different source countries.

5. Conclusion

This study had some pitfalls. The limited number of wastewater samples analyzed may not provide a true representation of the frequency of SARS-CoV-2 variants that circulated in Nairobi County, Kenya, during the studied period, thus introducing bias in the reported data. Secondly, the targeted sequencing of solely the virus Spike gene is limiting as evolutionary patterns exhibited by the other genes comprising the SARS-CoV-2 genome are missed. Despite the limitations, we have demonstrated that SARS-CoV-2 variants belonging to the Alpha (B.1.1.7) and Delta (B.1.617.2) lineages circulated in Nairobi County, Kenya between April 2021 and August 2021, with the latter predominating. This is the first study to describe the diversity of SARS-CoV-2 variants circulating in Kenya, through wastewater analysis.

Acknowledgments

We thank the Kenya Medical Research Institute (KEMRI), Centre for Virus Research (CVR), for unwavering support during the implementation of this study. We also thank the Pan African University Institute for Basic Sciences, Technology, and Innovation (PAUSTI) for finding the project.

Ethical Approval

The KEMRI Scientific and Ethics Review Unit (SERU) approved this study under protocol approval number SERU4323.

Authors' Contributions

Conceptualization: Bulimo Dimbuson Wallace, Nzunza Rosemary, Wamunyokoli Fred.

Data curation: Sichamba Patson, Opanda Silvanos, Bulimo Dimbuson Wallace.

Formal analysis: Sichamba Patson, Opanda Silvanos, Symekher Samwel, Bulimo Dimbuson Wallace.

Investigation: Sichamba Patson.

Methodology: Sichamba Patson, Nzunza Rosemary, Symekher Samwel, Majanja Janet, Wadegu Meshack, Wanguche Esther, Mwangi Benlick, Ombija Stephen, Borus Peter, Evans Komen.

Supervision: Bulimo Dimbuson Wallace, Wamunyokoli Fred, Borus Peter, Nzunza Rosemary.

Writing—original draft: Sichamba Patson, Nzunza Rosemary, Symekher Samwel, Majanja Janet, Wadegu Meshack, Wanguche Esther, Ombija Stephen.

Writing—review & editing: Bulimo Dimbuson Wallace, Opanda Silvanos, Samoel Ashimosi Khamadi, Wamunyokoli Fred.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hu, B., Guo, H., Zhou, P. and Shi, Z.L. (2020) Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, **19**, 141-154. https://doi.org/10.1038/s41579-020-00459-7

[2] Wiersinga, W.J., Rhodes, A., Cheng, A.C., Peacock, S.J. and Prescott, H.C. (2020) Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA—Journal of the American Medical Association*, **324**, 782-793. https://doi.org/10.1001/jama.2020.12839

[3] Mehta, O.P., Bhandari, P., Raut, A., Kacimi, S.E.O. and Huy, N.T. (2021) Coronavirus Disease (COVID-19): Comprehensive Review of Clinical Presentation. *Frontiers in Public Health*, **8**, Article No. 582932. https://doi.org/10.3389/fpubh.2020.582932

[4] Di Gennaro, F., Pizzol, D., Marotta, C., Antunes, M., Racalbuto, V., Veronese, N. and

Smith, L. (2019) Coronavirus Diseases (COVID-19) Current Status and Future Perspectives: A Narrative Review.

[5] Adetifa, I.M.O., Uyoga, S., Gitonga, J.N., Mugo, D., Otiende, M., Nyagwange, J., Karanja, H.K., Tuju, J., Wanjiku, P., Aman, R., Mwangangi, M., Amoth, P., Kasera, K., Ng'ang'a, W., Rombo, C., Yegon, C., Kithi, K., Odhiambo, E., Rotich, T., *et al.* (2021) Temporal Trends of SARS-CoV-2 Seroprevalence during the First Wave of the COVID-19 Epidemic in Kenya. *Nature Communications*, **12**, Article No. 3966. https://doi.org/10.1038/s41467-021-24062-3

[6] Ministry of Health, Kenya (2021) Press Statement on COVID-19. https://www.health.go.ke/press-releases

[7] Sims, N. and Kasprzyk-Hordern, B. (2020) Future Perspectives of Wastewater-Based Epidemiology: Monitoring Infectious Disease Spread and Resistance to the Community Level. *Environment International*, **139**, Article ID: 105689. https://doi.org/10.1016/j.envint.2020.105689

[8] Huizer, M., ter Laak, T.L., de Voogt, P. and van Wezel, A.P. (2021) Wastewater-Based Epidemiology for Illicit Drugs: A Critical Review on Global Data. *Water Research*, **207**, Article ID: 117789. https://doi.org/10.1016/j.watres.2021.117789

[9] Mtetwa, H.N., Amoah, I.D., Kumari, S., Bux, F. and Reddy, P. (2021) Wastewater-Based Surveillance of Antibiotic Resistance Genes Associated with Tuberculosis Treatment Regimen in Kwazulu Natal, South Africa. *Antibiotics*, **10**, 1362. https://doi.org/10.3390/antibiotics10111362

[10] Ndiaye, A.K., Diop, P.A.M. and Diop, O.M. (2014) Eirus and Non-Polio Enterovirus Nvironmental Surveillance of Poliovin Urban Sewage in Dakar, Senegal (2007-2013). *Pan African Medical Journal*, **19**, Article No. 243. https://doi.org/10.11604/pamj.2014.19.243.3538

[11] Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T. and Norder, H. (2014) Detection of Pathogenic Viruses in Sewage Provided Early Warnings of Hepatitis A Virus and Norovirus Outbreaks. *Applied and Environmental Microbiology*, **80**, 6771-6781. https://doi.org/10.1128/AEM.01981-14

[12] McClary-Gutierrez, J.S., Mattioli, M.C., Marcenac, P., Silverman, A.I., Boehm, A.B., Bibby, K., Balliet, M., de los R.F.L., Gerrity, D., Griffith, J.F., Holden, P.A., Katehis, D., Kester, G., LaCross, N., Lipp, E.K., Meiman, J., Noble, R.T., Brossard, D. and McLellan, S.L. (2021) SARS-CoV-2 Wastewater Surveillance for Public Health Action: Connecting Perspectives from Wastewater Researchers and Public Health Officials during a Global Pandemic. *Emerging Infectious Diseases*, **27**, 1-8. https://doi.org/10.20944/preprints202104.0167.v1

[13] Mousazadeh, M., Ashoori, R., Paital, B., Kabdaslı, I., Frontistis, Z., Hashemi, M., Sandoval, M.A., Sherchan, S., Das, K. and Emamjomeh, M.M. (2021) Wastewater Based Epidemiology Perspective as a Faster Protocol for Detecting Coronavirus RNA in Human Populations: A Review with Specific Reference to Sars-Cov-2 Virus. *Pathogens*, **10**, 1008. https://doi.org/10.3390/pathogens10081008

[14] Jacobsen, H., Pediatrics, B.M.C., Niemann, J., Jacobsen, H., Vonasek, J.H., Hagstrøm, S., Donneborg, M.L. and Sørensen, S. (2021) Prolonged Rectal Shedding of SARS-CoV-2 in a 22-Day-Old-Neonate: A Case Report. *BMC Pediatrics*, **21**, Article No. 506.

[15] Vaselli, N.M., Setiabudi, W., Subramaniam, K., Adams, E.R., Turtle, L., Iturriza-Gómara, M., Solomon, T., Cunliffe, N.A., French, N., Hungerford, D. and Group, C.-L.S. (2021) Investigation of SARS-CoV-2 Faecal Shedding in the Community: A Prospective Household Cohort Study (COVID-LIV) in the UK. *BMC Infectious Diseas-*

es, **21**, Article No. 784. https://doi.org/10.2139/ssrn.3820521

[16] Lewis, N. and Pray, W. (2021) COVID-19 Response Team and Epidemic Intelligence Service.

[17] Yan, D., Zhang, X., Chen, C., Jiang, D., Liu, X., Zhou, Y., Huang, C., Zhou, Y., Guan, Z., Ding, C., Chen, L., Lan, L., Fu, X., Wu, J., Li, L. and Yang, S. (2021) Characteristics of Viral Shedding Time in SARS-CoV-2 Infections: A Systematic Review and Meta-Analysis. *Frontiers in Public Health*, **9**, Article ID: 652842. https://doi.org/10.3389/fpubh.2021.652842

[18] Cevik, M., Tate, M., Lloyd, O., Maraolo, A.E., Schafers, J. and Ho, A. (2021) SARS-CoV-2, SARS-CoV, and MERS-CoV Viral Load Dynamics, Duration of Viral Shedding, and Infectiousness: A Systematic Review and Meta-Analysis. *The Lancet Microbe*, **2**, e13-e22. https://doi.org/10.1016/S2666-5247(20)30172-5

[19] Owusu, D., Pomeroy, M.A., Lewis, N.M., Wadhwa, A., Yousaf, A.R., Whitaker, B., Dietrich, E., Hall, A.J., Chu, V., Thornburg, N., Christensen, K., Kiphibane, T., Willardson, S., Westergaard, R., Dasu, T., Pray, I.W., Bhattacharyya, S., Dunn, A., Tate, J.E., Buono, S., *et al.* (2021) Persistent SARS-CoV-2 RNA Shedding without Evidence of Infectiousness: A Cohort Study of Individuals with COVID-19. *Journal of Infectious Diseases*, **224**, 1362-1371. https://doi.org/10.1093/infdis/jiab107

[20] Hamouda, M., Mustafa, F., Maraqa, M., Rizvi, T. and Aly, A. (2021) Science of the Total Environment Wastewater Surveillance for SARS-CoV-2: Lessons Learnt from Recent Studies to Define Future Applications. *Science of the Total Environment*, **759**, Article ID: 143493. https://doi.org/10.1016/j.scitotenv.2020.143493

[21] Hart, O.E. and Halden, R.U. (2020) Science of the Total Environment Computational Analysis of SARS-CoV-2/COVID-19 Surveillance by Wastewater-Based Epidemiology Locally and Globally: Feasibility, Economy, Opportunities and Challenges. *Science of the Total Environment*, **730**, Article ID: 138875. https://doi.org/10.1016/j.scitotenv.2020.138875

[22] Giacobbo, A., Rodrigues, M.A.S., Zoppas Ferreira, J., Bernardes, A.M. and de Pinho, M.N. (2021) A Critical Review on SARS-CoV-2 Infectivity in Water and Wastewater. What Do We Know? *Science of the Total Environment*, **774**, Article ID: 145721. https://doi.org/10.1016/j.scitotenv.2021.145721

[23] Agrawal, S., Orschler, L. and Lackner, S. (2021) Long-Term Monitoring of SARS-CoV-2 RNA in Wastewater of the Frankfurt Metropolitan Area in Southern Germany. *Scientific Reports*, **11**, Article No. 5372. https://doi.org/10.1038/s41598-021-84914-2

[24] Peccia, J., Zulli, A., Brackney, D.E., Grubaugh, N.D., Kaplan, E.H., Casanovas-Massana, A., Ko, A.I., Malik, A.A., Wang, D., Wang, M., Weinberger, D.M. and Omer, S.B. (2020) SARS-CoV-2 RNA Concentrations in Primary Municipal Sewage Sludge as a Leading Indicator of COVID-19 Outbreak Dynamics. https://doi.org/10.1101/2020.05.19.20105999

[25] Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., Haramoto, E. and Rose, J. B. (2020). SARS-CoV-2 in Wastewater: State of the Knowledge and Research Needs. *Science of the Total Environment*, **739**, Article ID: 139076. https://doi.org/10.1016/j.scitotenv.2020.139076

[26] Wurtzer, S., Marechal, V., Mouchel, J.M., Maday, Y., Teyssou, R., Richard, E., Almayrac, J.L. and Moulin, L. (2020) Evaluation of Lockdown Effect on SARS-CoV-2 Dynamics through Viral Genome Quantification in Waste Water, Greater Paris, France, 5 March to 23 April 2020. *Eurosurveillance*, **25**, Article ID: 2000776. https://doi.org/10.2807/1560-7917.ES.2020.25.50.2000776

[27] Aguiar-Oliveira, M.L., Campos, A., Matos, A.R., Rigotto, C., Sotero-Martins, A.,

Teixeira, P.F.P. and Siqueira, M.M. (2020) Wastewater-Based Epidemiology (Wbe) and Viral Detection in Polluted Surface Water: A Valuable Tool for Covid-19 Surveillance—A Brief Review. *International Journal of Environmental Research and Public Health*, **17**, 9251. https://doi.org/10.3390/ijerph17249251

[28] Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T. and Veesler, D. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, **181**, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

[29] O'Toole, Á., Pybus, O.G., Abram, M.E., Kelly, E.J. and Rambaut, A. (2022) Pango Lineage Designation and Assignment Using SARS-CoV-2 Spike Gene Nucleotide Sequences. *BMC Genomics*, **23**, Article No. 121. https://doi.org/10.1186/s12864-022-08358-2

[30] Magazine, N., Zhang, T., Wu, Y., McGee, M.C., Veggiani, G. and Huang, W. (2022) Mutations and Evolution of the SARS-CoV-2 Spike Protein. *Viruses*, **14**, 640. https://doi.org/10.3390/v14030640

[31] QGIS Development Team (2020) QGIS Geographic Information System (Version 3.10) Software. https://qgis.org/en/site

[32] World Health Organization (WHO) (2003) Guidelines for Environmental Surveillance of Poliovirus Circulation Guidelines for Environmental. *Vaccine and Biologicals*, **3**, 28. https://apps.who.int/iris/bitstream/handle/10665/67854/WHO_V-B_03.03_eng.pdf?sequence=1

[33] Freed, N.E., Vlková, M., Faisal, M.B. and Silander, O.K. (2021) Rapid and Inexpensive Whole-Genome Sequencing of SARS-CoV-2 Using 1200 bp Tiled Amplicons and Oxford Nanopore Rapid Barcoding. *Biology Methods and Protocols*, **5**, bpaa014. https://doi.org/10.1093/biomethods/bpaa014

[34] Geospiza, L. (2008) FinchTV 1.4.0. https://digitalworldbiology.com/FinchTV

[35] BioSoft H (2014) DNA Sequence Assembler. http://www.dnabaser.com/download/DNA-Baser-sequence-assembler/

[36] Rambaut, A., Holmes, E.C., O'Toole, Á., Hill, V., McCrone, J.T., Ruis, C., du Plessis, L. and Pybus, O.G. (2020) A Dynamic Nomenclature Proposal for SARS-CoV-2 Lineages to Assist Genomic Epidemiology. *Nature Microbiology*, **5**, 1403-1407. https://doi.org/10.1038/s41564-020-0770-5

[37] Hadfield, J., Megill, C., Bell, S.M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T. and Neher, R.A. (2018) NextStrain: Real-Time Tracking of Pathogen Evolution. *Bioinformatics*, **34**, 4121-4123. https://doi.org/10.1093/bioinformatics/bty407

[38] Shu, Y. and McCauley, J. (2017) GISAID: Global Initiative on Sharing All Influenza Data—From Vision to Reality. *Eurosurveillance*, **22**, 30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

[39] Edgar, R.C. (2004) MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Research*, **32**, 1792-1797. https://doi.org/10.1093/nar/gkh340

[40] Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. (2012) Mrbayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology*, **61**, 539-542. https://doi.org/10.1093/sysbio/sys029

[41] Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, **25**, 1253-1256. https://doi.org/10.1093/molbev/msn083

[42] Rambaut, A. (2010) FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree

[43] Gregory, D.A., Wieberg, C.G., Wenzel, J., Lin, C.H. and Johnson, M.C. (2021) Monitoring Sars-Cov-2 Populations in Wastewater by Amplicon Sequencing and Using the Novel Program Sam Refiner. *Viruses*, **13**, Article No. 1647. https://doi.org/10.3390/v13081647

[44] Pandemic, C., Martin, J., Klapsa, D., Wilton, T., Zambon, M., Bentley, E., Bujaki, E., Fritzsche, M., Mate, R. and Majumdar, M. (2020) Tracking SARS-CoV-2 in Sewage.

[45] Dzinamarira, T., Murewanhema, G., Iradukunda, P.G., Madziva, R., Herrera, H., Cuadros, D.F., Tungwarara, N., Chitungo, I. and Musuka, G. (2022) Utilization of SARS-CoV-2 Wastewater Surveillance in Africa—A Rapid Review. *International Journal of Environmental Research and Public Health*, **19**, Article No. 969. https://doi.org/10.3390/ijerph19020969

[46] Sinclair, R.G., Choi, C.Y., Riley, M.R. and Gerba, C.P. (2008) Pathogen Surveillance through Monitoring of Sewer Systems. *Advances in Applied Microbiology*, **65**, 249-269. https://doi.org/10.1016/S0065-2164(08)00609-6

[47] Thongpradit, S., Prasongtanakij, S., Srisala, S., Kumsang, Y., Chanprasertyothin, S., Boonkongchuen, P., Pitidhammabhorn, D., Manomaipiboon, P., Somchaiyanon, P., Chandanachulaka, S., Hirunrueng, T. and Ongphiphadhanakul, B. (2022) A Simple Method to Detect SARS-CoV-2 in Wastewater at Low Virus Concentration. *Journal of Environmental and Public Health*, **2022**, Article ID: 4867626. https://doi.org/10.1155/2022/4867626

[48] Kilifi, K.W.T.R.P. (2021) Explaining the Three Waves of the COVID-19 Transmission in Kenya Using a Mathematical Model/Policy Brief Key Message.

[49] Brand, S.P.C., Ojal, J., Aziza, R., Were, V., Emelda, A., Kombe, I.K., Mburu, C., Ogero, M., Agweyu, A., Warimwe, M., Nyagwange, J., Karanja, H., Gitonga, J.N., Mugo, D., Uyoga, S., Adetifa, I.M.O., Scott, J.A.G., Otieno, E., Otiende, M., Nokes, D.J., *et al.* (2022) COVID-19 Transmission Dynamics Underlying Epidemic Waves in Kenya. *Science*, **374**, 989-994. https://doi.org/10.1126/science.abk0414

[50] Herlihy, R., Bamberg, W., Burakoff, A., Alden, N., Severson, R., Bush, E., Kawasaki, B., Berger, B., Austin, E., Shea, M., Gabrieloff, E., Matzinger, S., Burdorf, A., Nichols, J., Goode, K., Cilwick, A., Stacy, C., Staples, E. and Stringer, G. (2021) Rapid Increase in Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant—Mesa County, Colorado, April-June 2021. *MMWR Recommendations and Reports*, **70**, 1084-1087. https://doi.org/10.15585/mmwr.mm7032e2

[51] Chen, Z., Azman, A.S., Chen, X., Zou, J., Tian, Y., Sun, R., Xu, X., Wu, Y., Lu, W., Ge, S., Zhao, Z., Yang, J., Leung, D.T., Domman, D.B. and Yu, H. (2022) Global Landscape of SARS-CoV-2 Genomic Surveillance and Data Sharing. *Nature Genetics*, **54**, 499-507. https://doi.org/10.1038/s41588-022-01033-y

[52] Ferreira, M.B., de-Paris, F., Paiva, R.M. and Nunes, L.S. (2018) Assessment of Conventional PCR and Real-Time PCR Compared to the Gold Standard Method for Screening *Streptococcus agalactiae* in Pregnant Women. *Brazilian Journal of Infectious Diseases*, **22**, 449-454. https://doi.org/10.1016/j.bjid.2018.09.005

[53] Zemtsova, G.E., Montgomery, M. and Levin, M.L. (2015) Relative Sensitivity of Conventional and Real-Time PCR Assays for Detection of SFG Rickettsia in Blood and Tissue Samples from Laboratory Animals. *PLOS ONE*, **10**, e0116658. https://doi.org/10.1371/journal.pone.0116658

[54] Pino, N.J., Rodriguez, D.C., Cano, L.C. and Rodriguez, A. (2021) Detection of SARS-CoV-2 in Wastewater Is Influenced by Sampling Time, Concentration Method, and Target Analyzed. *Journal of Water and Health*, **19**, 775-784. https://doi.org/10.2166/wh.2021.133