

Evaluation of Wastewater Surveillance as an Early Warning Tool for COVID-19 in a Conflict-Affected Setting, Maiduguri, Northeastern Nigeria

Bamaiyi Joseph Musa^{1*}; Ibrahim Yusuf Ngoshe²; Abubakar Shettima²; Maryceline Mandu Baba^{1,3}; Mohammed Aja¹; Leonard Adamu Hamatu¹; Ishaq Haruna¹

¹WHO National Polio/ITD Laboratory, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria

²Department of Microbiology, Faculty of Life Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria

³Department of Medical Laboratory Science, College of Medical Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria

*Corresponding author: Bamaiyi Joseph Musa. Email: bjmusa@gmail.com; Telephone: +2348030601033

ARTICLE INFORMATION	ABSTRACT
Article history: Published: August 2026 Keywords: SARS-CoV-2 COVID-19 Wastewater Surveillance Wastewater-Based Epidemiology Early Warning	Background: Wastewater-based epidemiology (WBE) can provide population-level information on SARS-CoV-2 circulation and may complement clinical surveillance, but performance depends on wastewater infrastructure, sampling, environmental conditions and laboratory recovery. Objective: This study evaluated the potential utility of wastewater surveillance as an early warning tool for COVID-19 in Maiduguri, northeastern Nigeria, by comparing wastewater detection with clinical specimens and characterizing SARS-CoV-2 genomes from clinical samples. Methods: Five hundred nasopharyngeal specimens and 192 wastewater samples were collected during a six-month longitudinal sentinel surveillance programme covering eight catchments. Wastewater was concentrated using a polyethylene glycol-based method, followed by RNA extraction and RT-qPCR. Clinical positives were sequenced on the Oxford Nanopore MinION using an ARTIC workflow, with Pangolin lineage assignment and phylogenetic analysis in MEGA 12. Results: SARS-CoV-2 RNA was detected in 119/500 clinical specimens (23.8%), whereas 0/192 wastewater samples were positive. Of 54 clinical positives subjected to sequencing, 10 were assigned lineages and 44 remained unassigned because of incomplete coverage or ambiguous regions. B.1.462 was the most frequent classified lineage. Mean SNP count was 6.30 per genome, with transitions predominating. Conclusion: The study did not demonstrate a detectable wastewater early-warning signal during the study period. The negative environmental result should not be interpreted as absence of transmission; rather, it highlights the need for locally adapted sampling, concentration, quality control and integration of environmental, clinical and genomic surveillance in conflict-affected settings.

1. Introduction

The COVID-19 pandemic demonstrated the importance of surveillance systems capable of identifying community transmission rapidly and reliably. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, spread globally following its emergence in late 2019. Clinical surveillance based primarily on individual diagnostic testing became the principal method for identifying infections; however, this approach is influenced by healthcare access, testing availability, symptom status, willingness to seek care and surveillance capacity. These limitations are particularly important in low-resource and humanitarian settings, where healthcare systems may be disrupted or inaccessible.

The present study therefore aimed to evaluate wastewater surveillance as a potential early warning tool for SARS-CoV-2 in a conflict-affected setting by comparing environmental and clinical detection and by characterizing SARS-CoV-2 genomes detected in clinical samples.

2. Literature Review

2.1 Wastewater-based epidemiology as complementary surveillance

Wastewater-based epidemiology (WBE) provides an alternative population-level surveillance approach. SARS-CoV-2 RNA can be shed by infected individuals and subsequently enter wastewater systems. Consequently, measurement of viral RNA in wastewater can provide an aggregate indication of viral circulation within a defined catchment without requiring identification and testing of individual infected persons. Early studies demonstrated the feasibility of detecting SARS-CoV-2 RNA in untreated wastewater in Australia and Europe, while subsequent studies demonstrated relationships between wastewater viral concentrations and clinical COVID-19 indicators. Ahmed et al. (2020) provided an early proof of concept for SARS-CoV-2 detection in untreated wastewater in Australia, while Medema et al. (2020) demonstrated the presence of SARS-CoV-2 RNA in sewage and its relationship with reported COVID-19 prevalence in the Netherlands.

The potential value of wastewater surveillance lies not only in its ability to detect the presence of SARS-CoV-2 but also in its potential to identify changes in community transmission before increases become apparent through clinical surveillance. The World Health Organization recognizes environmental surveillance as a complementary source of population-level information that may

provide early warning of increasing or decreasing SARS-CoV-2 circulation and information about variants of concern. Importantly, WHO recommends that wastewater surveillance should be integrated with other surveillance systems rather than replacing clinical surveillance. The evidence supporting WBE is nevertheless context dependent. The relationship between wastewater viral concentrations and clinically confirmed infections varies substantially according to sampling design, catchment characteristics, environmental conditions, viral shedding, sewer connectivity and analytical methodology. A systematic review and meta-analysis found substantial variation in correlations between wastewater SARS-CoV-2 concentrations and clinical indicators, emphasizing that environmental and epidemiological factors can influence the performance of WBE.

2.2 WBE in conflict-affected and Nigerian settings

These challenges may be amplified in conflict-affected settings. Maiduguri, the capital of Borno State in northeastern Nigeria, has experienced prolonged humanitarian and security challenges associated with insurgency, population displacement and pressure on health and sanitation infrastructure. The study area includes densely populated residential communities, major markets, healthcare facilities and internally displaced population settings. The project document describes extensive open drainage systems and heterogeneous wastewater pathways within the metropolis, conditions that may complicate conventional wastewater surveillance. Maiduguri therefore represents an important setting in which to evaluate whether WBE can provide useful supplementary surveillance information. The study specifically incorporated healthcare-associated, residential, commercial and vulnerable-population catchments. The sampling framework included the University of Maiduguri Teaching Hospital (UMTH), Mairi, Gwange, Bolori, Shehuri, Bulabulin Market, Gamboru Market and an IDP camp outflow.

In Nigeria, evidence supporting the feasibility of wastewater surveillance has emerged from other urban settings. A study conducted at the University College Hospital in Ibadan detected SARS-CoV-2 RNA in untreated wastewater, demonstrating the feasibility of WBE in a Nigerian healthcare environment. Similarly, recent surveillance of wastewater canals in Lagos demonstrated the potential of environmental surveillance for monitoring public-health pathogens in a densely populated Nigerian urban environment.

Despite these advances, evidence from northeastern Nigeria remains limited. The project identifies a particular gap in state-level genomic surveillance and the absence of paired clinical-wastewater genomic analyses from Maiduguri or Borno State.

3. Methodology

3.1 Study design and setting

A longitudinal sentinel environmental surveillance design was implemented in Maiduguri metropolitan area, Borno State, northeastern Nigeria. The study included Maiduguri Metropolitan Council and Jere Local Government Area. Maiduguri is a major urban and commercial centre with high population density, heterogeneous settlement patterns and significant population mobility. The city also hosts internally displaced populations resulting from the prolonged humanitarian crisis in northeastern Nigeria. Laboratory analyses were conducted at the WHO National/Intra-typic Differentiation (ITD) Polio Laboratory located at the University of Maiduguri Teaching Hospital (UMTH), Maiduguri.

3.2 Wastewater surveillance sites

Eight sentinel wastewater sites were selected to represent different epidemiological and environmental contexts:

UMTH sewer outflow; Mairi Ward drainage; Bulabulin Market drainage; Gamboru Market drainage; Gwange drainage; Bolori drainage; Shehuri drainage; and IDP camp outflow.

The sites represented healthcare-associated, residential, commercial and vulnerable-population catchments. The original study design specified weekly sampling over six months, generating an expected total of 192 wastewater samples.

This site selection was particularly relevant to a conflict-affected setting because it sought to capture areas where conventional clinical surveillance could be constrained by population displacement, healthcare access, high mobility and heterogeneous sanitation systems.

3.3 Clinical sampling

A total of 500 nasopharyngeal specimens were collected from individuals presenting in healthcare and community settings. Participants were enrolled following informed consent and specimens were collected using standard nasopharyngeal swab procedures. Samples were transported in viral transport medium and maintained at appropriate refrigerated or frozen temperatures before laboratory processing.

3.4 Wastewater sampling and processing

Wastewater sampling used repeated sentinel collection. Composite sampling was incorporated into the surveillance framework to improve temporal representation and reduce variability associated with intermittent viral shedding.

Samples were subjected to clarification and concentration before RNA extraction. The study employed a PEG-based concentration strategy, with PEG 6000, dextran and sodium chloride used to facilitate viral concentration. Concentrated material was subsequently subjected to chloroform treatment and RNA extraction. The project protocol describes processing of approximately 500 mL of wastewater, clarification by centrifugation, PEG-dextran two-phase separation, overnight incubation at 4°C and subsequent recovery of the concentrated fraction. RNA was extracted using a QIAamp Viral RNA Mini-based approach, followed by RT-qPCR for SARS-CoV-2 detection.

3.5 SARS-CoV-2 detection

SARS-CoV-2 RNA in clinical and wastewater samples was assessed using real-time reverse transcription polymerase chain reaction (RT-qPCR). The assay included reverse transcription followed by amplification of SARS-CoV-2 targets. Clinical samples were considered positive according to the assay's established cycle-threshold criteria.

For clinical specimens with valid Ct values, Ct values were used as a relative proxy for viral RNA abundance. The study appropriately recognized that Ct values are influenced by specimen quality, assay characteristics and amplification efficiency and therefore should not be interpreted as direct quantitative viral-load measurements without calibration.

3.6 Whole-genome sequencing

SARS-CoV-2-positive clinical samples were selected for whole-genome sequencing using the Oxford Nanopore Technologies MinION platform and ARTIC amplicon sequencing strategy. Sequencing data were processed using the EPI2ME workflow. Lineage classification was undertaken using Pangolin, while multiple sequence alignment and phylogenetic analysis were performed using MEGA version 12. Neighbour-Joining phylogenetic analysis was conducted with 1,000 bootstrap replicates.

3.7 Statistical analysis

Descriptive statistics were used to summarize SARS-CoV-2 positivity, Ct values and genomic characteristics. Because all wastewater samples were negative, inferential statistical comparison between clinical and wastewater viral loads could not be undertaken.

3.8 Ethical considerations

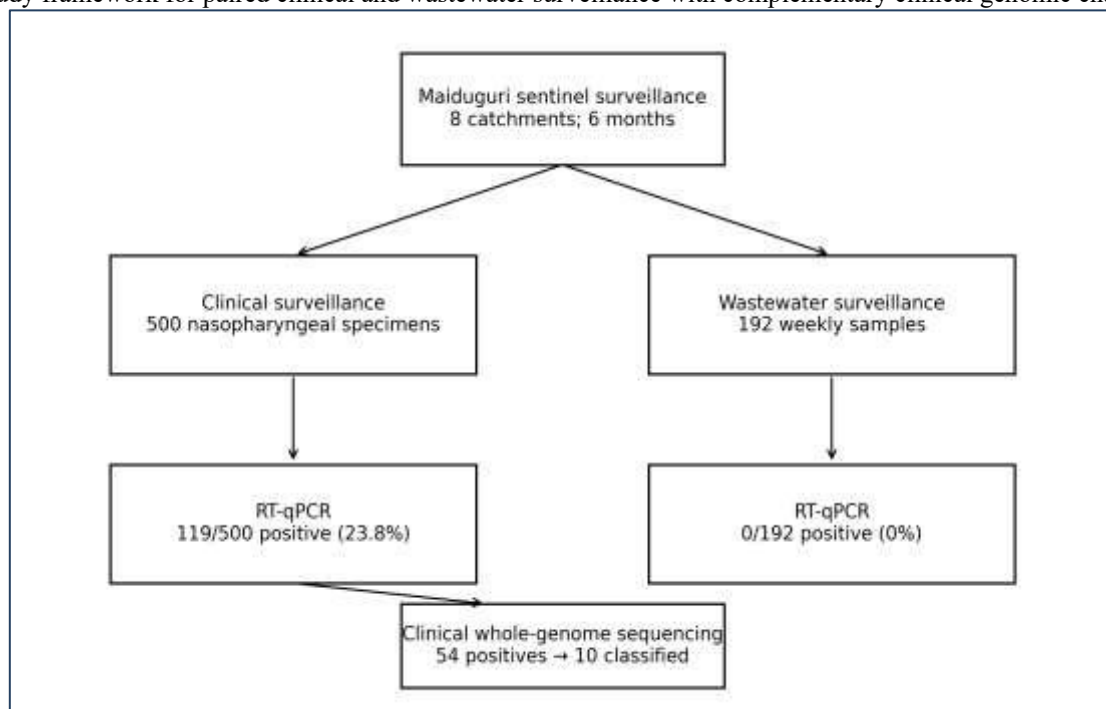
Ethical approval was obtained from the Borno State Ministry of Health Ethical Clearance Committee. Informed consent was obtained from participants providing clinical specimens. The environmental surveillance component did not involve identification of individual persons.

Table 1: Sentinel wastewater surveillance sites and catchment contexts

Sentinel site	Catchment context	Sampling framework
UMTH sewer outflow	Healthcare-associated	Weekly sentinel sampling
Mairi Ward drainage	Residential	Weekly sentinel sampling
Bulabulin Market drainage	Commercial market	Weekly sentinel sampling
Gambaru Market drainage	Commercial market	Weekly sentinel sampling
Gwange drainage	Residential	Weekly sentinel sampling
Bolori drainage	Residential	Weekly sentinel sampling
Shehuri drainage	Residential	Weekly sentinel sampling
IDP camp outflow	Vulnerable/displaced population	Weekly sentinel sampling

Source: Study protocol and surveillance framework reported in the manuscript.

Figure 1: Study framework for paired clinical and wastewater surveillance with complementary clinical genomic characterization.



Source: Study data.

4. Findings

4.1 SARS-CoV-2 detection in clinical and wastewater samples

A total of 500 nasopharyngeal specimens were analysed. SARS-CoV-2 RNA was detected in 119 samples, corresponding to an overall positivity rate of 23.8%. In contrast, none of the wastewater samples tested positive for SARS-CoV-2 RNA, resulting in a wastewater detection rate of 0%.

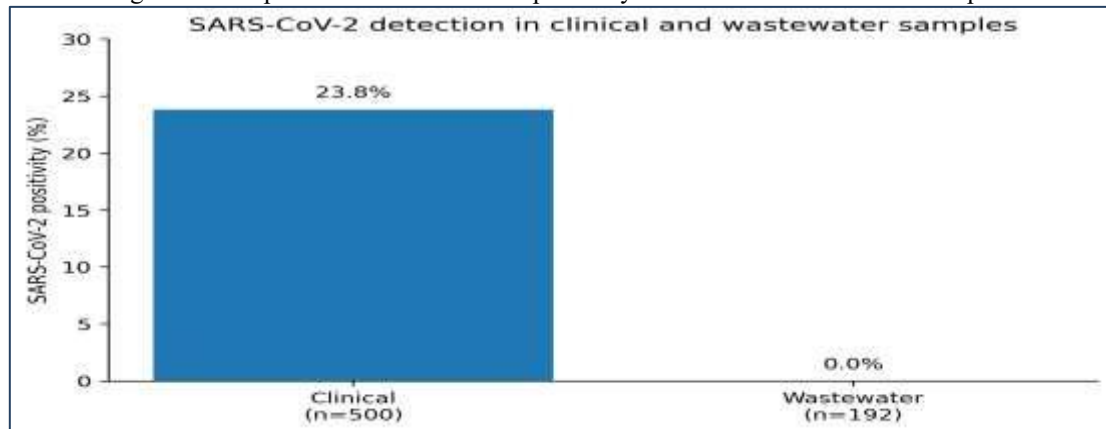
The comparative detection results are summarized in Table 2 and Figure 2.

Table 2: SARS-CoV-2 detection in clinical and wastewater samples

Sample type	Total tested	Positive	Negative	Positivity (%)
Nasopharyngeal specimens	500	119	381	23.8
Wastewater samples	192	0	192	0.0

Source: Study data.

Figure 2: Comparison of SARS-CoV-2 positivity in clinical and wastewater samples.



Source: Study data.

4.2 Clinical viral-load distribution

Among SARS-CoV-2-positive clinical samples with valid Ct measurements, 54 samples were available for viral-load-related analysis. The mean Ct value was 26.75 ± 2.70 , with a median of 27.32 and a range of 19.19–33.57.

The relatively low mean Ct value indicates that a substantial proportion of the sequenced clinical specimens contained appreciable quantities of viral RNA. However, Ct values should be interpreted as assay-dependent proxies rather than absolute measures of viral load.

Table 3: Summary of Ct values among SARS-CoV-2-positive clinical specimens with valid measurements

Measure	Value	Interpretation
Number with valid Ct values	54	Clinical positive specimens available for viral-load-related analysis
Mean Ct $\pm$ SD	26.75 ± 2.70	Assay-dependent proxy for viral RNA abundance
Median Ct	27.32	Assay-dependent proxy
Range	19.19–33.57	Observed Ct range

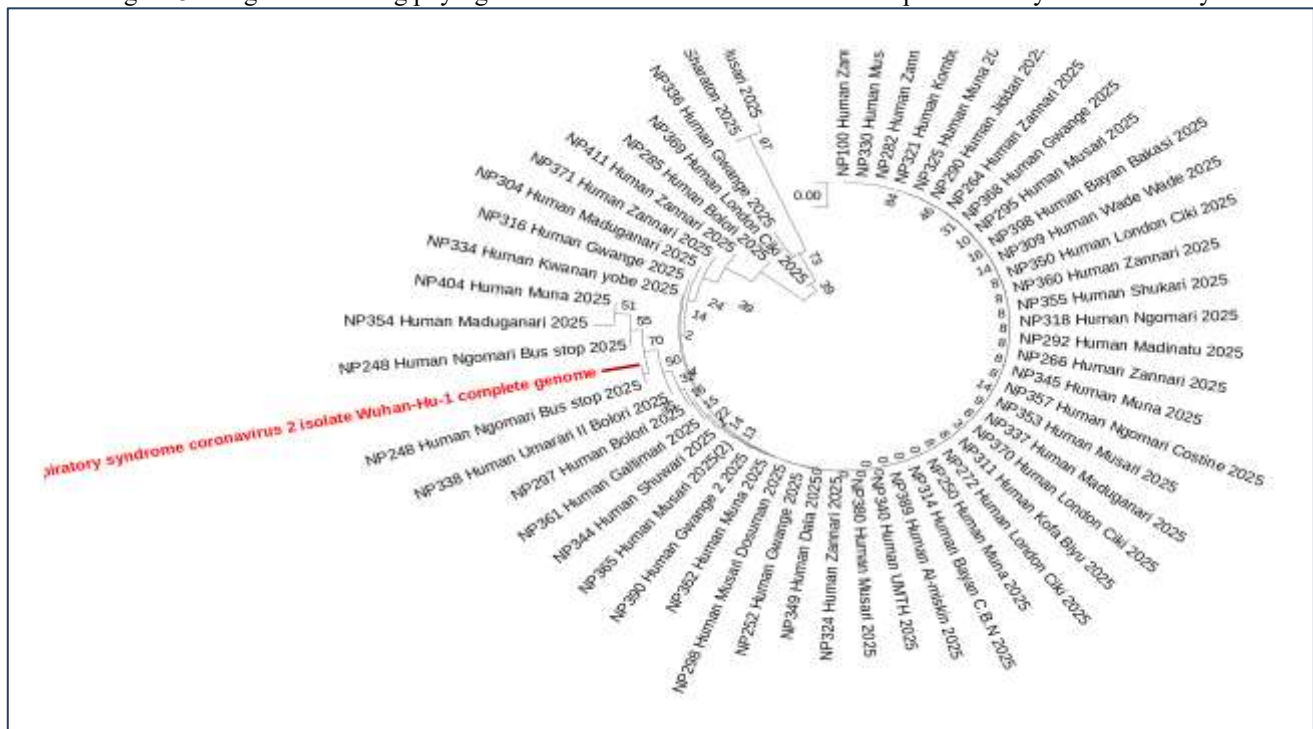
Source: Study data.

4.3 Clinical genomic characterization

Mutation analysis showed a mean of 6.30 SNPs per genome, with values ranging from one to ten. The mean number of transitions was 5.89 compared with 0.41 transversions, indicating a strong predominance of transition substitutions.

Phylogenetic analysis demonstrated clustering of several genomes with relatively short branch lengths and limited nucleotide divergence. This pattern suggests that the viruses represented in the clinical specimens were closely related and may have resulted from relatively recent transmission chains within the study population.

Figure 3: Neighbour-Joining phylogenetic tree of SARS-CoV-2 clinical sequences analysed in the study.



Source: Study data.

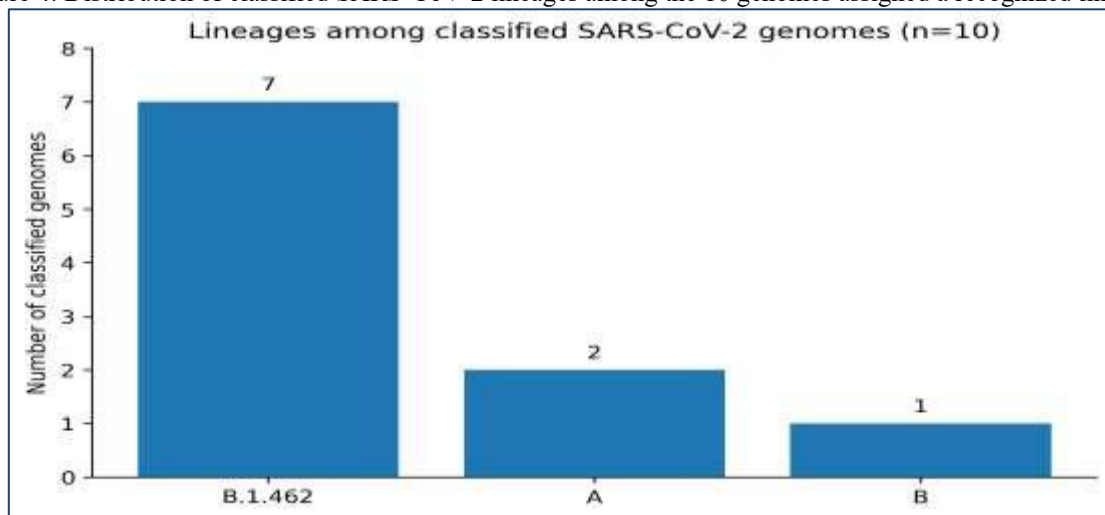
Sequencing yield and lineage assignment are summarized in Table 4 and Figures 4 and 5.

Table 4: Whole-genome sequencing outcome and lineage assignment

Category	Number	Percentage (%)	Key finding
Clinical positives subjected to sequencing	54	100.0	SARS-CoV-2-positive clinical samples
Recognized lineages assigned	10	18.5	Complete/adequate sequence information for lineage assignment
Unassigned genomes	44	81.5	Incomplete genome coverage or ambiguous sequence regions
Most frequent classified lineage	7	70.0 of classified	B.1.462

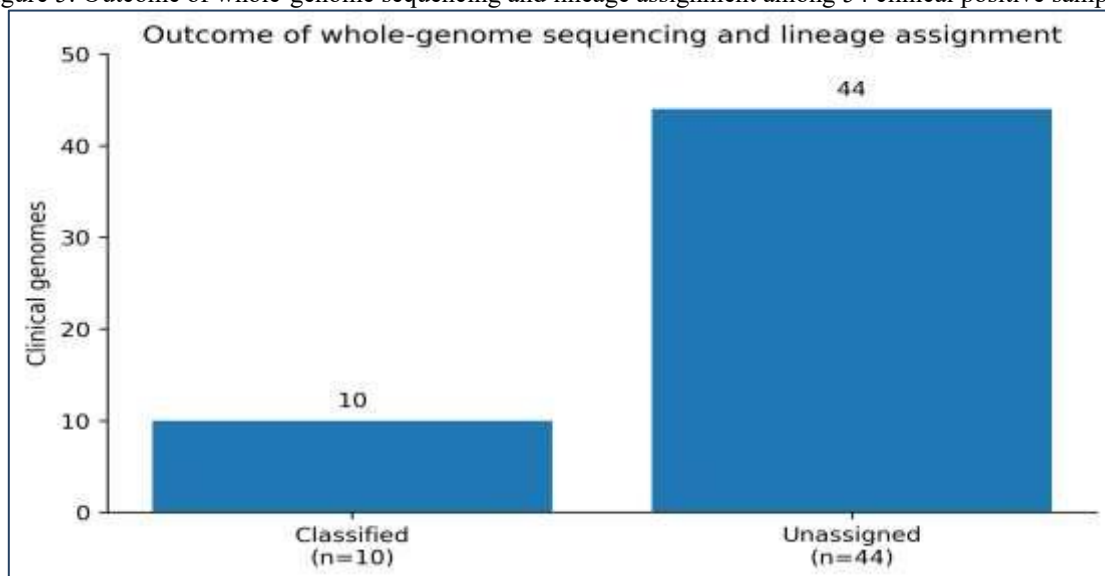
Source: Study data.

Figure 4: Distribution of classified SARS-CoV-2 lineages among the 10 genomes assigned a recognized lineage.



Source: Study data.

Figure 5: Outcome of whole-genome sequencing and lineage assignment among 54 clinical positive samples.



Source: Study data.

Table 5: Mutation summary among characterized clinical SARS-CoV-2 genomes

Genomic measure	Mean / range	Summary
Single nucleotide polymorphisms (SNPs)	6.30 per genome; range 1–10	Low overall nucleotide divergence among characterized genomes
Transitions	5.89 per genome	Strong predominance of transition substitutions
Transversions	0.41 per genome	Substantially fewer than transitions

Source: Study data.

4.4 Phylogenetic relationships

However, because SARS-CoV-2 RNA was not detected in wastewater, phylogenetic comparison between wastewater and clinical genomes was not possible.

4.5 Discussion

4.5.1 Principal finding

The principal finding of this study was the discordance between clinical and environmental surveillance. SARS-CoV-2 was detected in 23.8% of clinical specimens, while all wastewater samples were negative. This result indicates that the wastewater surveillance system, as implemented during the study period, did not generate a detectable early-warning signal despite clear evidence of SARS-CoV-2 circulation in the population represented by the clinical samples.

This finding differs from studies in which wastewater SARS-CoV-2 concentrations correlated with clinical incidence. Medema et al. (2020) demonstrated that SARS-CoV-2 RNA could be detected in sewage and correlated with reported COVID-19 prevalence, while Ahmed et al. (2020) demonstrated detection of SARS-CoV-2 RNA in untreated wastewater in Australia.

The difference does not necessarily imply that WBE is ineffective. Rather, it emphasizes that wastewater surveillance is a system whose performance depends on biological, environmental, infrastructural and analytical conditions.

4.5.2 Wastewater negativity does not establish absence of transmission

An important interpretation of the present findings is that a negative wastewater result should not be equated with absence of SARS-CoV-2 transmission. WHO has emphasized that environmental surveillance is a complementary surveillance method and that interpretation requires consideration of the characteristics of the wastewater system and the sensitivity of the environmental sampling approach. In the present study, the 23.8% clinical positivity rate demonstrates that SARS-CoV-2 was circulating even though environmental detection was unsuccessful. Consequently, the negative wastewater findings represent a surveillance limitation rather than evidence that the community was free of infection.

This distinction is particularly important in humanitarian settings, where an environmental surveillance programme could otherwise generate false reassurance.

4.5.3 Environmental and infrastructural factors

Maiduguri presents several environmental characteristics that may reduce the sensitivity of conventional wastewater surveillance. The study area contains interconnected open drainage systems rather than a uniformly reticulated sewer network. These drainage channels receive variable contributions from households, markets, healthcare facilities and other sources.

Such systems differ fundamentally from large centralized wastewater treatment plants. In a centralized sewer catchment, wastewater is relatively well defined in terms of population contributing to the flow. In open drainage systems, however, flow, population contribution, dilution, environmental exposure and retention times can vary substantially.

WHO guidance recognizes that wastewater surveillance is more challenging in locations with limited sewerage and that environmental sampling may need to be adapted to local conditions. In settings without conventional sewer networks, human-impacted environmental waters can potentially be considered as part of broader wastewater and environmental surveillance approaches.

4.5.4 Dilution and environmental degradation

The concentration of SARS-CoV-2 RNA reaching an environmental sampling point depends on the number of infected individuals shedding viral RNA, faecal shedding intensity, wastewater volume, retention time, environmental degradation and sampling location.

Peccia et al. and other early studies demonstrated relationships between wastewater viral concentrations and COVID-19 incidence, but subsequent work has shown that environmental and sampling factors can substantially influence the strength of these relationships. A systematic review and meta-analysis reported wide variation in correlations between wastewater SARS-CoV-2 RNA concentrations and clinical indicators.

In Maiduguri, open drainage systems may be exposed to environmental conditions that promote dilution or degradation of viral RNA before sampling. The project itself identifies dilution, environmental degradation and infrastructural limitations as possible explanations for the absence of detectable SARS-CoV-2 RNA.

Therefore, the negative results may reflect a failure to recover sufficient target RNA rather than true absence of SARS-CoV-2 from wastewater.

4.5.5 Sampling design and repeated environmental surveillance

The study used eight sentinel sites and weekly sampling over six months. This design provided broad geographic representation across healthcare, residential, commercial and vulnerable-population settings.

Nevertheless, a wastewater early-warning system requires sufficient temporal resolution and appropriately selected sampling points. WHO notes that frequent sampling is important when wastewater data are intended to provide actionable early warning.

In a city such as Maiduguri, the most appropriate sampling points may not necessarily be conventional drains. Future studies should compare multiple matrices and locations, including raw wastewater immediately downstream of high-density settlements, healthcare catchments, septic-tank discharge points, sludge and other human-impacted waters.

The use of human faecal normalization markers could also help determine whether a negative SARS-CoV-2 result reflects low population contribution or poor viral recovery.

4.5.6 Implications for conflict-affected settings

The most important contribution of this study is its focus on a conflict-affected environment. In settings affected by insecurity, displacement and humanitarian pressures, clinical surveillance may be compromised by interrupted healthcare access, movement restrictions, shortages of diagnostic supplies and reduced reporting.

Under such circumstances, a successful wastewater surveillance system could provide a population-level surveillance mechanism that does not require individuals to present at healthcare facilities. This is particularly valuable for detecting asymptomatic or undiagnosed infections.

However, conflict can simultaneously undermine the environmental surveillance system itself. Population displacement may change the population contributing to a catchment; temporary settlements may lack formal sewerage; sanitation systems may change rapidly; and insecurity may restrict access to sampling sites.

Thus, the value of WBE in conflict-affected settings is potentially high, but its implementation requires a surveillance design that is specifically adapted to humanitarian and environmental realities.

4.5.7 Comparison with evidence from Nigeria

The negative wastewater findings in Maiduguri contrast with evidence from other Nigerian settings. Olatunji et al. demonstrated SARS-CoV-2 RNA detection in wastewater associated with the University College Hospital in Ibadan, supporting the feasibility of environmental surveillance in a healthcare-associated catchment.

Similarly, surveillance of wastewater canals in Lagos has demonstrated the value of environmental sampling for monitoring public-health pathogens in a densely populated urban environment.

The contrast between these findings and those from Maiduguri illustrates an important principle: WBE performance cannot necessarily be transferred from one city to another without local validation. Differences in sewerage, drainage, rainfall, wastewater composition, population density, sanitation practices and sampling access may materially affect detection.

4.5.8 Genomic surveillance provides complementary evidence

Although environmental surveillance was negative, clinical genomic surveillance provided evidence of circulating SARS-CoV-2 diversity. Of the 54 clinical genomes analysed, 10 were assigned to recognized lineages, with B.1.462 the most frequently identified lineage. The high proportion of unassigned genomes demonstrates the technical challenge of generating complete SARS-CoV-2 genomes from clinical specimens, particularly where viral concentration and genome coverage are suboptimal.

The relatively low genomic divergence among sequences suggests circulation of closely related viruses within the sampled population.

These findings reinforce the importance of integrating environmental surveillance with genomic surveillance. A future wastewater programme should not focus solely on RT-qPCR positivity but should also aim, where viral concentration permits, to recover genomic information about circulating lineages and variants.

4.5.9 Implications for early-warning surveillance

An early-warning system must satisfy at least three conditions: it must detect the pathogen reliably, it must detect meaningful changes in transmission early enough to influence decision-making, and the resulting signal must be sufficiently specific to trigger an appropriate public-health response.

The present study fulfilled neither the first nor the second criterion for SARS-CoV-2 wastewater surveillance because all wastewater samples were negative and no environmental trend could be generated. Consequently, it would be scientifically inappropriate to conclude that wastewater surveillance provided an early warning of COVID-19 in Maiduguri.

Instead, the study demonstrates the importance of validating WBE locally before it is incorporated into routine outbreak decision-making. WHO's current guidance emphasizes that environmental surveillance should be integrated with other surveillance systems and used where it provides actionable information, is technically feasible and can be sustainably implemented.

4.6 Strengths of the study

This study has several important strengths.

First, it represents one of the few investigations specifically examining SARS-CoV-2 environmental surveillance in Maiduguri and northeastern Nigeria. Second, the study used repeated wastewater sampling across multiple epidemiologically distinct catchments rather than relying on a single sampling location. Third, the environmental surveillance framework incorporated healthcare, residential, commercial and vulnerable-population settings. Fourth, clinical surveillance was integrated with molecular and genomic characterization, allowing the environmental findings to be interpreted in the context of confirmed community transmission. Finally, the study provides valuable evidence that can guide future wastewater surveillance programmes in conflict-affected and resource-constrained settings.

4.7 Limitations

Several limitations should be considered.

First, the absence of detectable SARS-CoV-2 RNA in wastewater prevented direct evaluation of the temporal lead-lag relationship between wastewater signals and clinical cases. Second, no environmental genomes were obtained; therefore, genomic concordance between wastewater and clinical samples could not be evaluated. Third, the large proportion of unassigned clinical genomes limits interpretation of lineage diversity. Fourth, open drainage systems and heterogeneous wastewater infrastructure may have reduced the representativeness of some sampling locations. Fifth, the study did not establish quantitative recovery efficiency for SARS-CoV-2 RNA from every wastewater matrix, making it difficult to determine whether negative results resulted from true absence or analytical loss. Finally, the study was not designed to calculate the sensitivity, specificity, positive predictive value or negative predictive value of wastewater surveillance because the wastewater dataset contained no positive samples.

4.8 Public health and policy implications

4.8.1 Wastewater surveillance should complement rather than replace clinical surveillance

Clinical testing remains essential for individual diagnosis and immediate patient management. Wastewater surveillance should provide an additional population-level signal rather than substitute for clinical testing. This approach is consistent with current WHO guidance.

4.8.2 *Environmental surveillance must be locally validated*

A surveillance strategy that performs well in a centralized sewer system may perform poorly in an open-drainage environment. Local pilot studies should therefore be undertaken before routine implementation.

4.8.3 *Sampling should target epidemiologically meaningful catchments*

Future programmes should prioritize high-density communities, healthcare facilities, markets, IDP settlements and other locations where population movement or vulnerability may increase outbreak risk.

4.8.4 *Wastewater surveillance should incorporate quality-control markers*

Future studies should incorporate human faecal markers and process controls to distinguish true absence of viral RNA from poor recovery, dilution or insufficient human wastewater contribution.

4.8.5 *Environmental and genomic surveillance should be integrated*

Where sufficient viral RNA can be recovered, sequencing of wastewater samples should be incorporated into surveillance programmes to enable detection of circulating lineages and potentially emerging variants.

5. Conclusion and Recommendations

5.1 *Conclusion*

This study evaluated the potential of wastewater surveillance as an early-warning tool for COVID-19 in Maiduguri, a conflict-affected and resource-constrained setting in northeastern Nigeria. Clinical surveillance demonstrated substantial SARS-CoV-2 circulation, with 119 of 500 nasopharyngeal samples testing positive, corresponding to a positivity rate of 23.8%. However, none of the 192 wastewater samples tested positive for SARS-CoV-2 RNA.

The absence of a wastewater signal meant that the study could not demonstrate a predictive or early-warning relationship between wastewater SARS-CoV-2 detection and clinical infection. Rather than demonstrating that WBE is unsuitable for Maiduguri, the findings highlight the importance of environmental context, drainage infrastructure, sampling strategy, viral concentration efficiency and quality control in determining surveillance sensitivity.

The study therefore supports a cautious but continued role for wastewater surveillance as a complementary tool, particularly in conflict-affected settings where conventional clinical surveillance may be incomplete. Future programmes should employ locally optimized sampling and concentration methods, population-normalization controls, longitudinal sampling and integrated clinical-genomic analysis. In conclusion, wastewater surveillance should not be interpreted as a stand-alone replacement for clinical surveillance in Maiduguri. Its greatest potential lies in becoming part of a multimodal surveillance system combining clinical diagnostics, genomic surveillance and environmental monitoring. Such integration could strengthen preparedness for future COVID-19 waves and other emerging infectious disease threats in northeastern Nigeria and comparable humanitarian settings.

5.2 *Recommendations*

Based on the findings of this study, the following recommendations are proposed:

- Optimize wastewater concentration methods for the specific wastewater matrices encountered in Maiduguri, including open drainage water and sewage-associated environmental waters.
- Evaluate alternative sampling matrices, including settled solids, sludge and other human-impacted waters where conventional wastewater sampling is unreliable.
- Increase temporal sampling frequency during periods of suspected increased transmission to improve the likelihood of detecting early changes.
- Use population-normalization markers such as human faecal indicators to account for changes in population contribution and wastewater dilution.
- Establish recovery and inhibition controls for every wastewater batch to improve interpretation of negative RT-qPCR results.
- Integrate environmental and clinical surveillance data within a common epidemiological platform.
- Strengthen clinical genomic surveillance in northeastern Nigeria to address the under-representation of Borno State in national genomic datasets.
- Develop a sentinel wastewater network incorporating UMTH, densely populated residential communities, major markets and IDP settlements.
- Conduct prospective longitudinal studies specifically designed to evaluate the lead time between wastewater SARS-CoV-2 signals and clinical case increases.
- Develop locally appropriate action thresholds for wastewater increases before environmental surveillance is used to trigger public-health interventions.

Declarations

Ethics approval and consent to participate: Ethical approval was obtained from the Borno State Ministry of Health Ethical Clearance Committee. Informed consent was obtained from participants contributing clinical specimens.

Consent for publication: Not applicable.

Availability of data: Data availability should be stated according to the institutional and ethical requirements governing the underlying study. De-identified genomic data may be made available through an appropriate public sequence repository where applicable.

Competing interests: The authors declare no competing interest.

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Authors' contributions: Bamaïyi Joseph Musa: conceptualization, study design, laboratory investigation, sample processing, RT-qPCR testing, data curation, formal analysis, interpretation of findings, writing - original draft, and revision of the manuscript. Ibrahim Yusuf Ngoshe: supervision, methodological guidance, data interpretation, critical review, and editing of the manuscript. Abubakar Shettima: supervision, study coordination, validation of methods, critical review, and editing of the manuscript. Marycelin

M. Baba: scientific supervision, laboratory quality oversight, interpretation of virological findings, critical review, and editing of the manuscript. Mohammed Aja: laboratory support, sample handling, data verification, and manuscript review. Leonard Adamu Hamatu: laboratory support, data management, interpretation support, and manuscript review. Ishaq Haruna: laboratory support, data checking, technical assistance, and manuscript review. All authors read and approved the final version of the manuscript and agree to be accountable for the integrity and accuracy of the work.

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