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Uncovering DENV, CHIKV, and ZIKV in Urban Wastewater in Brazil Through Genomic and Molecular Screening

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Abstract

This study evaluated and compared molecular methods for the detection of arboviruses Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV) in wastewaters collected from July 2022 to May 2023 in the region of Belo Horizonte, Brazil. Detection rates varied substantially across the methods. DENV was identified in 24% (15/63) of samples using hybrid capture method of whole-genome sequencing (WGS) and MinION sequencing, in 66.6% (20/30) using only WGS, but was not detected using the CDC Trioplex RT-PCR Assay Kit and ZDC (IBMP). CHIKV was detected in 19.0% (12/63) of the samples by WGS and MinION, in 85.7% (12/14) using only MinION sequencing, and in 4.7% (3/63) by RT-PCR. ZIKV was found in only one sample (1/63) by WGS, while RT-qPCR yielded a high false-positive rate (65.1%, 41/63). The potential of genomic wastewater surveillance for arboviruses was demonstrated and that diagnostic RT-qPCR kits used for clinical samples were not directly suitable for environmental surveillance.

Keywords: Arbovirus; genomic surveillance; molecular screening; RT-qPCR diagnostic kit; public health laboratories; wastewater surveillance

1. Introduction

Arboviruses such as Dengue virus (DENV), Chikungunya virus (CHIKV), and Zika virus (ZIKV) continue to pose serious public health threats across tropical and subtropical regions, particularly in Brazil. In recent years, the country has experienced increasingly severe outbreaks of arboviral diseases, with significant impacts on healthcare infrastructure and population health. Between

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October 2023 and June 2024, Brazil reported an unprecedented surge in Dengue and Chikungunya cases, underscoring the urgent need for enhanced, real-time surveillance tools. By May 2024, Brazil had recorded over 4.2 million probable cases of Dengue—the highest ever reported in a single year—with more than 2,900 confirmed deaths. During the same period, Chikungunya accounted for over 250,000 probable cases, with a marked increase in co-circulation of arboviruses in several regions, particularly in the Southeast and Central-West [1].

Traditional surveillance systems—primarily based on clinical and laboratory-confirmed cases—are essential for outbreak response but often suffer from reporting delays, underdiagnosis, and limited coverage, particularly in areas with poor healthcare access. In this context, Wastewater Environmental Surveillance (WES) has emerged as a promising tool for early detection and community-level monitoring of pathogens. WES involves monitoring the presence and concentration of viral RNA or DNA in wastewater and environmental samples to infer the circulation of infectious agents within the population [2]. This approach is especially relevant for diseases with asymptomatic or subclinical presentations, such as Zika and mild Dengue cases, which frequently go unreported in clinical systems.

However, the application of WES for arboviruses remains limited by several technical and methodological challenges. One of the main limitations is the typically low concentration of viral RNA in sewage, necessitating the use of highly sensitive detection methods such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) [3]. Although RT-qPCR is widely regarded for its sensitivity, performance can be compromised by inhibitors present in sewage [4], as well as by RNA degradation and genetic variability [5]. Additionally, detection efficiency is influenced by the viral shedding rate, which varies across individuals and viruses. For instance, Chen and Bibby [6] emphasized that successful detection of ZIKV depends not only on method sensitivity but also on a sufficiently high rate of viral shedding in excreta.

While WES has proven successful for fecal-shed viruses like SARS-CoV-2 [7,8], human adenovirus, norovirus, rotavirus and hepatitis A virus [9], evidence for arboviruses remains scarce. Some viruses, such as the polyomaviruses BKV and JCV, which are shed in urine, are consistently detected in wastewater [10,11], suggesting a potential route for arbovirus detection. Diamond et al. [12] reported the potential of wastewater surveillance, a cost-effective and scalable approach for generating high-resolution health data and highlighted the medium to high feasibility of detecting climate-sensitive pathogens such as malaria, Dengue, Zika, West Nile viruses in wastewater. Nonetheless, studies specific to DENV, CHIKV, and ZIKV monitoring in sewage remain limited. For example, Zhu et al. [5], attempted to detect ZIKV in archived wastewater samples from the 2015–2016 outbreak in Bahia, Brazil, but found no detectable RNA, although the study provided valuable insights into RNA stability and recovery. Chandra et al. [13] investigated the persistence of Dengue (Serotypes 2 and 3), Zika, Yellow Fever and Murine Hepatitis virus RNA in wastewater matrices and reported that the RNA of these viruses persisted in wastewater over a range of temperature (6, 25 and 37°C), supporting the potential for WES of arboviral outbreaks. Thakali et al. [14] reported that conducting DENV wastewater surveillance remains challenging due to the lack of established protocols and standardized wastewater processing methods. On the other hand, Monteiro et al. [15] reported the detection of DENV in 25% and CHIKV in 11% of 273 wastewater samples collected in Portugal (during May 2022 to April 2023) using RT-qPCR, demonstrating feasibility under optimized conditions. Additionally, Wolfe et al. [16] reported DENV RNA detection in wastewater solids samples from three WWTP in Miami, Florida, USA. They showed that wastewater detection of DENV was possible with as few as 4.23 confirmed dengue cases per 1 million people. Yet, these examples remain isolated, and further investigation into effective, scalable methodologies is urgently needed—particularly in high-burden countries like Brazil.

Building on our previous findings, which detected multiple human viruses, including Mpox in sewage samples [17], this study investigates the detection of arboviruses in wastewaters using three molecular approaches: RT-qPCR, hybrid capture method for whole genome sequencing, and Oxford Nanopore's MinION sequencing. We conducted an 11-month surveillance campaign in Belo Horizonte (during 2022 and 2023), the third-largest metropolitan area in Brazil, during a period of active arboviral circulation. In fact, the city of Belo Horizonte, a densely populated urban center in

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southeastern Brazil, experiences recurring outbreaks of arboviral diseases, making it a critical location for improving and validating surveillance techniques. This comparative analysis aims to evaluate the sensitivity and practicality of each method, and to advance the use of WES as an early warning tool for arbovirus outbreaks in urban settings. We aim to inform the development of robust surveillance frameworks capable of supporting public health interventions and guiding timely responses to arboviral threats.

While molecular detection methods such as RT-qPCR are widely used for virus identification in environmental samples, recent advances in genomic technologies—including nanopore-based whole genome sequencing (e.g., Oxford Nanopore's MinION)—offer complementary approaches for detecting and characterizing viral genomes directly from complex matrices like wastewater. The aim of this study was to evaluate and compare the effectiveness of different molecular methods (hybrid capture for WGS, MinION sequencing, and RT-qPCR diagnostic kit used for clinical samples) for detecting arboviruses in wastewater. This is the first pilot study that has applied and compared these methods to successfully detect and genetically characterize DENV, CHIKV, and ZIKV in wastewater during a dengue outbreak in Brazil. Our study used wastewater samples from hospitals and community wastewaters and we made agreements with the responsible authorities of the hospitals and the Sanitation company to collect these samples.

2. Materials and Methods

Belo Horizonte is a largest Brazilian city from the state of Minas Gerais, Southeast of Brazil, with a population around 2.7 million people and a metropolitan area encompassing around 6 million inhabitants [18]. Sewage samples were collected from five sites in the metropolitan region: two hospitals (designed Hospital A and Hospital B, with Hospital A serving as a reference center for infectious disease treatment in the state), and two municipal Wastewater Treatment Plants (WWTP) that both treat sewage from approximately 2.3 million people, with a mean influent load of 2,300 $L.s^{-1}$ for WWTP-A (WWA) and 2,089 $L.s^{-1}$ for WWTP-B (WWB). The third WWTP (WWC) receives sewage from an international airport (influent flow of 5.0 $L.s^{-1}$, and population equivalent of 2,560 inhabitants). The geographic coordinates for these locations are: WWTP-A: 19°54′20.2″S 43°53′15.5″W, WWTP-B: 19°49′22.5″S 43°53`44.2″W, WWTP-C: 19°63′57.1″S 43°96`69.3″W Hospital A: 19°59′13.5″S 43°59′12.1″W, and hospital B: 19°49′03.0″S 43°56′53.4″W.

Sewage samples were collected twice monthly from July 23, 2022, to May 2023. In total 63 samples were collected and sequenced: 28 from the hospitals (14 samples each), 18 from WWTP-A and 15 from WWTP-B. Additionally, 2 samples were obtained from WWTP-C at the international airport on March 15, 2023. Hospital sewage sampling followed previously established protocols [8]. For the WWTPs, 24h-composite samples were collected at the plant inlets and filtered using electronegative membranes (30 to 50 mL), as previously described [7,8], with minor modifications: $MgCl_2$ (2.5 M) was omitted, and pH adjustment to 3.5 was not performed. Viral genetic material was extracted from the membranes using the AllPrep PowerViral DNA/RNA Kit (Qiagen®, Hilden, Germany), following the manufacturer's instructions. Extracted nucleic acids were resuspended in 100 µL of RNase-free ultrapure water and stored at –80 °C.

All 63 samples underwent whole-genome sequencing through target enrichment using a hybrid capture method. This approach employs biotinylated probes that hybridize specifically to viral target sequences, which are then isolated via magnetic pulldown to enrich the libraries. Target enrichment was performed using the Illumina VSP panel, which targets 66 DNA and RNA viruses—including Polyomavirus, HPV, Mpox, Poliovirus, Influenza, Dengue, Chikungunya, Zika, and SARS-CoV-2—following the protocol described previously [17]. Sequencing libraries were prepared by synthesizing cDNA from the concentrated wastewater samples using the Illumina RNA Prep with Enrichment Indexes Set A (96-sample format; Catalog No. 20026121) and the Illumina VSP panel (Catalog No. 20088154). Libraries were sequenced on the NextSeq™ 2000 System (2 × 150 bp), generating 8–10 million reads per sample. FASTQ files were analyzed using the Illumina DRAGEN™ Microbial Enrichment pipeline, available via BaseSpace™ Sequence Hub, with default settings for viral detection. Additionally, raw data were analyzed using the Genome Detective software [19]. The sequences were deposited in GenBank under the sample codes SAMN42174356 to SAMN42174437.

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For some samples (15 selected samples, results shown in Table 2) MiniION sequencing was used to confirm the presence of DENV1 and CHIKV. Multiplex PCR was conducted using Q5 Hot Start high-fidelity DNA polymerase (New England Biolabs) and a CHIKV whole-genome sequencing primer scheme (the primers are divided into two separate pools, A and B) [20,21]. For Dengue, samples were processed using CADDE primers and Oxford Nanopore Technologies, following protocols validated in previous studies [22].

For DENV, CHIKV and ZIKV, we used the GoTaq Probe 1-Step RT-qPCR kit (Promega) with primers and probes of Trioplex Real-time RT-PCR Assay kit (CDC (https://www.cdc.gov/zika/pdfs/trioplex-real-time-rt-pcr-assay-instructions-for-use.pdf), according to the manufacture instructions. Primers in house were also applied (for CHIKV according to Lanciotti et al. [23], for ZIKV according to Lanciotti et al. [24], and for DENV according to Johson et al. [25]), and kit ZDC (IBMP), which detects ZIKV, CHIKV, and DENV1–4 through RT-qPCR using hydrolysis probes for specific molecular targets defined by the manufacturer, though the exact genomic regions are not disclose (https://www.ibmp.org.br/wp-content/uploads/2025/01/INF-047-15-Kit-IBMP-Biomol-ZDC.pdf). We sequenced 3 CHIKV and 4 ZIKV positive amplified fragments of RT-PCR to try to confirm the detection of the targets. The amplicons were purified using magnetic beads and subsequently sequenced on the Ion PGM platform (Thermo Fisher Scientific), following the manufacturer's recommendations.

3. Results and Discussion

As shown in Table 1, the detection frequencies of DENV, CHIKV and ZIKV varied depending on the method used target enrichment for WGS, RT-qPCR and MinION sequencing, applied to 63 wastewaters samples collected from five locations (3 WWTPs and 2 hospitals) over an 11-month period (July 2022 to May 2023). DENV-1 was detected in 15 out of 63 samples (23.8%), ZIKV in only one sample (1.6%) and CHIKV in 12 (19%) (Table 1) using WGS and MinION sequencing. The highest detection rates were observed in samples from Hospital A (Table 1). Notably, the only ZIKV-positive sample originated from Hospital A and was collected on February 8, 2023. This finding is interesting, though should be interpreted with caution, especially considering that the official data for Belo Horizonte showed that there were no confirmed cases of Zika in the city in 2022, 2023, and 2024 [26,27]. It may reflect a background signal or artifact, and does not, on its own, provide sufficient evidence to suggest cryptic or subclinical circulation of ZIKV in the population. In contrast, dengue cases were substantially higher, with 776 and 2,874 confirmed cases reported in February and March 2023, respectively [27]. For Chikungunya, 404 and 1,314 confirmed cases were recorded during the same period [27]. RT-qPCR testing using the Trioplex- CDC diagnostic kit —routinely applied to clinical samples—yielded negative results for DENV across all 63 wastewater samples (Table 1). Even when using an alternative diagnostic assay (ZDC kit from IBMP), no DENV-positive samples were identified (Table 2). For CHIKV, 95.2% of the samples (60/63) yielded negative results, while 4.7% (3/63) were false positives (Tables 1 and 2). For ZIKV, non-specific amplification occurred in 65.1% of the samples (41/63), resulting in a high rate of false positives (Tables 1 and 4).

Table 1. Arbovirus Detection in Wastewater Samples Using WGS (NextSeq 2000), MinION, and RT-PCR.

Sample/ location	DENV WGS+ MinION	DENV RT-PCR*	CHIKV WGS+ MinION	CHIKV RT-PCR *	ZIKV WGS	ZIKV RT-PCR*
WWTP A	3/18 = 16.7%	ND	4/18=22.2%	1/18=5.5%	0/17	10/18=55.5%
WWTP B	3/15= 20%	ND	2/15=13.3%	ND	0/15	9/15=60
WWTP C	1/2= 50%	ND	1 /2=50%	ND	0/2	1 / 2=50%
Hospital A	5/14= 35.5%	ND	3/14=21.4%	1/14=7.1%	1/14=7.1%	12/14=85.7%

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Hospital B	3/14= 21.4%	ND	2/14=14.3%	1/14=7.1%	0/14	9/14=64.3%
Total	15/63=23.8%	ND	12/63=19%	3/63=4.7%**	1/63=1.6%	41/63= 65.1%***

*RT-qPCR using kit Trioplex (CDC) for DENV, CHIKV and ZIKV, kit ZDC (IBMP) and in house primers; Results presented are from all 3 assays (kits) combined. ND not detected; **the amplified fragments were sequenced and no CHIKV sequences were retrieved.*** amplified fragments were sequenced and no ZIKV sequences were retrieved. MinION sequencing was not performed for ZIKV.

Table 2 presents a comparison between two sequencing methods, hybrid capture WGS and MinIOn sequencing, for the detection DENV-1 and CHIKV detection. Detection frequencies differed notably between methods. For DENV-1, the hybrid capture WGS method identified 20 positive samples out of 30 (66.6%) whereas MinION sequencing detected only 2 out of 15 samples (13.3%). Conversely, for CHIKV MinION sequencing yielded a much higher rate 12 out of 14 samples (85.7%) compared to only 2 out of 30 samples (6.7%) using WGS. These differences might be associated with the primers used and the amplification of different regions of the virus genomes, DENV1 and CHIKV. For MinION sequencing, the regions amplified and sequencing for DENV and CHIKV use overlapping primers technique to capture the entire genome. So, if some primer fails, we would still sequence some fragments. For Illumina, the hybrid capture WGS method using a viral surveillance panel, we do not know the regions amplified because it is a commercial panel, and the oligonucleotides region were not mentioned by the company.

Table 2. Comparison of DENV1 and CHIKV detection by WGS, MinION, and RT-qPCR (Number of reads / genome coverage %)

Sample ID/Date	**DENV1-WGS**	**DENV1-MinION**	**DENV RT-PCR***	**CHIKV-WGS**	**CHIKV-MinION**	**CHIKV RT-PCR****
47-HA (07/23/22)	42 / 9.02%	ND	ND	ND	32123 /7.2%	ND
49-HA (08/12/22)	ND	ND	ND	ND	477 / 25.5%	ND
50-HB (08/12/22)	30 / 4.27%	ND	ND	ND	ND	ND
51-HA (09/01/22)	14 /3.2 %	NS	ND	ND	NS	ND
52-HB (09/01/22)	ND	ND	ND	ND	2853 / 17.7%	ND
54-WWA (02/08/23)	ND	ND	ND	ND	84 / 61.8%	ND
55- HA (02/08/23)	8 / 3.90%	NS	ND	ND	NS	ND
56- WWB (02/08/23)	50 /9.53%	NS	ND	ND	NS	ND
59- WWA (02/23/23)	ND	NS	ND	ND	NS	ND
61-HA (02/23/22)	10 / 3.3%	NS	ND	ND	NS	ND
65- WWA (03/01/23)	68 /9.44%	5863/ 80.1%	ND	ND	1781 / 12.8%	ND
66- WWA (03/01/23)	10 / 3.35%	ND	ND	ND	22640 / 33.4%	ND
67- HA(03/01/23)	12 / 4.52%	ND	ND	ND	19788 /6.5%	ND
68-WWB-I (03/01/23)	82 /7.22%	ND	ND	ND	NS	ND
69-WWB-E (03/01/23)	61 /5.5%	ND	ND	61 /2.8%	84 /40.4%	ND

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70- HB (03/01/23)	97 /13.1%	ND	ND	ND	5423 /26.4%	ND
71-WWA (03/15/23)	12 / 5.05%	NS	ND	ND	NS	ND
72-WWA (03/15/23)	28 / 1.3%	NS	ND	ND	NS	ND
74/75-WWB(03/15/23)	ND	ND	ND	ND	ND	ND
77-WWC-I(03/15/23)	34 /10.9%	NS	ND	ND	NS	ND
78-WWC-E(03/15/23)	ND	ND	ND	ND	63 / 19.6%	ND
79-WWA (02/23/23)[1]	2 / 1.36%	3 /79.3%	ND	76/ 6.8%	70 / 7.4%	ND
81-HA (02/23/22)[2]	8 / 1.22%	NS	ND	ND	NS	ND
82-WWA (02/23/23)[1]	12 / 4.6%	NS	ND	ND	NS	ND
62/83-WWB(02/23/23)	70 / 3.99%	ND	ND	ND	26495/ 15.4%	ND
84-HA (02/24/23)[2]	ND	NS	ND	ND	NS	ND
87/34-HB (12/01/22)	2 / 1.33%	NS	ND	ND	NS	ND
320-HB (03/14/23)	ND	NS	ND	ND	NS	+(27)***
323-HA (04/11/23)	ND	NS	ND	ND	NS	+(29)***
329-WWA (05/2023)	ND	NS	ND	ND	NS	+(28)***
Detecion	**20/30=66.6%**	**2/15=13.3%**	**0**	**2/30= 6.7%**	**12/14=85.7%**	**3/30=10%**

ND:not detected. NS:not sequenced; [1] 59/79/82:same sample. [2] 84/81/61: same sample; *for DENV RT-qPCR was performed using Trioplex CDC kit, ZDC (IBMP kit), and with in house primers for (subtypes 1, 2 3 and 4).**for CHIKV RT-qPCR was performed using Trioplex CDC kit and in house primers.***CHIKV positive amplification with Trioplex CDC kit (Cq number); the amplified fragments were sequenced and no CHIKV sequences were retrieved. HA, and HB, hospital wastewater from hospital A and B, respectively. WWA-I: influent sample from Wastewater Treatment Plant A, B and C, WWA-E: effluent sample from WWTP.

Importantly, DENV and CHIKV RNA were first detected in hospital wastewater samples as early as July and August 2022, when the reported number of new dengue and chikungunya cases per epidemiological week in Belo Horizonte were relatively low (Table 3): 31 and 3 cases (July 23, 2022), and 23 and 6 cases (August 12, 2022), respectively [28,29]. In contrast, detection in community wastewater (WWTP-A) occurred later, in February and March 2023, coinciding with a substantial increase in reported cases: 267 and 148 cases for dengue and chikungunya, respectively (February 23, 2023), and 430 and 253 cases, respectively (March 1, 2023) [28,29].

These findings suggest that hospital wastewater can serve as an effective sentinel system for early arbovirus surveillance in urban settings—even during periods of low community case numbers (under 20 cases per week)—by mitigating the dilution effects observed in large-scale municipal sewage systems, where each WWTP services around 1.3 million people. In the case of ZIKV, the only positive sample was from the sewage of Hospital A (in 02/08/2023), which is the reference hospital for infectious diseases in the state of Minas Gerais, and Zika cases were not reported in the municipality in 2022 and 2023, despite having reported cases of Zika in the state of Minas Gerais (20 and 54 cases, respectively, in 2022 and 2023 [26]. Thus, cryptic circulation of ZIKV could be occurring

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in hospital sewage. The comparison between the hybrid capture WGS method and RT-qPCR for ZIKV is shown in Table 4. ZIKV was detected in only one out of 30 samples (3.33%) using WGS, while RT-qPCR resulted in a high rate of false positives 22 out of 28 samples (78.6%). Sequencing of the amplified fragments from both diagnostic kits (Trioplex CDC and ZDC-IBMP) confirmed the absence of ZIKV sequences. In one case, sequencing of the amplicons generated by RT-qPCR—short fragments under 100 base pairs, which constrain more robust analyses—suggested that the amplified products may correspond to bacterial sequences, including species from the *Sulfurospirillum* genus. Thus, indicating that the positive RT-PCR results may have resulted from non-specific amplification.

Our results suggest that the clinical kits evaluated for arboviruses detection, Trioplex CDC and ZDC-IBMP, are not directly applicable to genetic material extracted from environmental matrices and may generate false positives or negatives. Nonetheless, in a previous study [17], we showed that qPCR diagnostic kit used on clinical samples for the detection of Mpox (five PLEX assays diagnostic kit from Bio-Manguinhos), and RT-qPCR assays for Influenza A, Influenza B and SARS-CoV-2 (developed by Bio-Manguinhos) performed well for monitoring wastewater samples.

It is also important to note that the detection of viral RNA via WGS and MinION sequencing in this study was not accompanied by absolute quantification (e.g., genome copies per liter) or threshold cycle (Cq) values derived from calibrated RT-qPCR assays.Without standard curves and quantification controls, the sequencing data reflect presence/absence only, and the number of reads or genome coverage percentage does not necessarily correlate with meaningful viral loads.

Nevertheless, the prevalence of DENV in wastewaters samples observed in this study using genomic sequencing, 23.8% (15 of 63 samples) were similar to those reported in previous studies, 21% (24/112 samples) and 25% (68/273), respectively conducted in Portugal [15] and the United States [16] using RT-PCR. Future studies should include quantitative RT-qPCR with external RNA standards to determine viral load levels in environmental samples and assess their public health significance.

Table 3. Detection of DENV1 and CHIKV in Wastewater (Number of reads / genome coverage %) vs. Weekly Reported Cases in Belo Horizonte*

Sample ID	DENV1-WGS	DENV1-MinION	Dengue cases	CHIKV-WGS	CHIKV-MinION	Chikungunya cases
47-HA (07/23/22)	42 / 9.02%	ND	31	ND	32123 /7.2%	3
49-HA (08/12/22)	ND	ND	23	ND	477 / 25.5%	6
50- HB (08/12/22)	30 / 4.27%	ND	23	ND	ND	6
51- HA (09/01/22)	14 /3.2 %	NS	19	ND	NS	5
52- HB (09/01/22)	ND	ND	19	ND	2853/17.7%	5
54-WWA(02/08/23)	ND	ND	161	ND	84 /61.8%	117
55- HA (02/08/23)	8 / 3.90%	NS	161	ND	NS	117
56-WWB(02/08/23)	50 /9.53%	NS	161	ND	NS	117
59- WWA (02/23/23)[1]	ND	NS	267	ND	NS	148
61-HA (02/23/22)[2]	10 / 3.3%	NS	267	ND	NS	148
65- WWA(03/01/23)	68 /9.44%	5863/80.1%	430	ND	1781 / 12.8%	253

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66- WWA(03/01/23)	10 / 3.35%	ND	430	ND	22640/33.4%	253
67- HA (03/01/23)	12 /4.52%	ND	430	ND	19788/6.5%	253
68-WWB (03/01/23)	82 /7.22%	ND	430	ND	NS	253
69-WWB (03/01/23)	61 /5.5%	ND	430	61/2.8%	84 /40.4%	253
70- HB (03/01/23)	97 /13.1%	ND	430	ND	5423 /26.4%	253
71-WWA (03/15/23)	12 / 5.05%	NS	604	ND	NS	414
72-WWA (03/15/23)	28 / 1.3%	NS	604	ND	NS	414
74/75-WWB (03/15/23)	ND	ND	604	ND	ND	414
77-WWC-I(03/15/23)[3]	34 /10.9%	NS	604	ND	NS	414
78-WWC-E(03/15/23)[3]	ND	ND	604	ND	63 / 19.6%	414
79-WWA (02/23/23)[1]	2 / 1.36%	3 /79.3%	267	76/6.8%	70 / 7.4%	148
81-HA (02/23/23)[2]	8 / 1.22%	NS	267	ND	NS	148
82-WWA (02/23/23)[1]	12 / 4.6%	NS	267	ND	NS	148
62/83-WWB (02/23/23)	70 / 3.99%	ND	267	ND	26495/ 15.4%	148
84-HA (02/24/23)[2]	ND	NS	267	ND	NS	148
87/34-HB (12/01/22)	2 / 1.33%	NS	22	ND	NS	1
320-HB (03/14/23)	ND	NS	604	ND	NS	414
323-HA (04/11/23)	ND	NS	1415	ND	NS	719
329-WWA(05/10/23)	ND	NS	883	ND	NS	479

*Number of cases according to BRASIL. Ministério da Saúde. Dengue - Minas Gerais: Cases per municipality Belo Horizonte. DATASUS. Available at: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinannet/cnv/denguebmg.def. Acessed on 9 June 2025. ND: not detected (Genome not present); NS: not sequenced; [1] 59/79/82: same sample; [2] 61/81/84:same sample; [3] Sewage sample from the WWTP (WWC) at the international airport in Confins-Influent and Effluent.

Table 4. Comparison of ZIKV Detection in Wastewater Using WGS (NextSeq 2000) and RT-qPCR.

Sample ID/date	ZIKV-WGS	ZIKV - RT-qPCR
47-HA (07/23/22)	ND	+ (29.02)
49-HA (08/12/22)	ND	+ (26.63)
50- HB (08/12/22)	ND	-

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51- HA (09/01/22)	ND	+ (34.06)*
52- HB (09/01/22)	ND	+ (26.9)
54- WWTPA (02/08/23)	ND	+ (26.81)
55- HA (02/08/23)	215 reads /1.40 coverage%	+ (34.73)*
56- WWB (02/08/23)	ND	+ (35.83)*
59- WWA (02/23/23)[1]	ND	+ (37.63)*
61-HA (02/24/23)[2]	ND	- * ZDC
65- WWA (03/01/23)	ND	+ (24.67)
66- WWA (03/01/23)	ND	+ (25.94)**
67- HA (03/01/23)	ND	+ (27.46)
68- WWB (03/01/23)	ND	+ (21.38)
69- WWB (03/01/23)	ND	+ (25.48)
70- HB (03/01/23)	ND	+ (26.83)
71-WWA (03/15/23)	ND	NT
72-WWA (03/15/23)	ND	- *
74/75-WWB (03/15/23)	ND	+ (28.02)
77- WWC-I (03/15/23)[3]	ND	- *
78-WWC-E (03/15/23)[3]	ND	+ (21.03)
79-WWA (02/23/23)[1]	ND	+ (19.83)
81-HA (02/24/23)[2]	ND	-
82-WWA (02/23/23)[1]	ND	NT
62/83-WWB (02/23/23)	ND	+ (20.74)
84-HA (02/24/23)[2]	ND	+ (31.34)*
87/34-HB (12/01/22)	ND	- *
320-HB(03/14/23)	ND	+ (23)***
323-HA (04/11/23)	ND	+ (26)***
329-WWA (05/2023)	ND	+ (23)***
Detection	1/30=3.33%	22/28=78.6%(false positive)

ND: not detected (Genome not present). NT:not tested. [1] 59/79/82 are the same sample; [2] 61/81/84, same sample; [3] sewage from WWC, WWTP from the international airport in Confins-Influent and Effluent. MinION sequencing was not performed for ZIKV. *RT-qPCR was performed using Kit ZDC (IBMP) (Cq number). For other samples RT-qPCR was performed using Kit Trioplex CDC (Cq number) and RT-PCR with in house primers; ** fragment was sequenced and retrieved sequences of *Sulfurospirillum* bacteria. ***fragments were sequenced and no ZIKV sequences were retrieved.

4. Conclusions

This study demonstrates that genomic approaches, specifically hybrid-capture whole-genome sequencing (WGS) and MinION nanopore sequencing, can be effective for detecting arboviruses such DENV, CHIKV, and ZIKV in wastewater from both hospital and community settings. However, their apparent superiority over RT-qPCR in this context likely reflects methodological differences, including the use of probe-based enrichment and high nucleic acid input volumes, rather than inherent analytical robustness. Furthermore, despite their sensitivity, sequencing-based approaches

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remain limited by cost, infrastructure requirements, and turnaround time, which may constrain their routine applicability in environmental surveillance systems.

Our study demonstrated that RT-qPCR assays designed for clinical diagnostics, such as the Trioplex (CDC) and ZDC (IBMP) kits, which are standard across Brazilian public health laboratories during Dengue and Chikungunya outbreaks, are not directly suitable for wastewater samples, likely due to issues with matrix interference, primer specificity, and inadequate validation for environmental conditions. Rather than discarding RT-qPCR entirely, these findings underscore the need for tailored protocols, including optimized primers and controls, for environmental matrices.

Overall, our findings emphasize that a hybrid environmental surveillance strategy using adapted RT-qPCR assays for routine, cost-effective screening and sequencing-based methods as confirmatory tools for pathogen characterization and thus establishing early outbreak signals of arboviruses.

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Abbreviations

The following abbreviations are used in this manuscript:

RT-qPCR	reverse transcription quantitative polymerase chain reaction
WGS	Whole Genome Sequencing
WWTP	Waste Water Treatment Plant

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