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WATER RESEARCH COMMISSION

ENVIRONMENTAL SURVEILLANCE FOR NON SEWERED COMMUNITIES: A TOOL FOR DISEASE MITIGATION IN DEVELOPING COUNTRIES

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Environmental surveillance and Wastewater-Based Epidemiology (WBE) has proven to a useful tool in fighting a pandemic. Recent application of techniques for SARS-CoV-2 detection in sewage has provided vital information on the emergence of disease, circulating variants of interest and variants of concern, and possibility of predicting hospital admissions. Developing countries do not have extensive sewer networks and therefore cannot use WBE for the same population scale. A South African study has successfully demonstrated the application of non-sewered surveillance across multiple sites and provinces in South Africa. To the authors' knowledge, this has been the largest non sewered surveillance programme undertaken globally.

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INTRODUCTION

1.1 Background

Environmental Surveillance has been shown to be a useful tool for monitoring pathogens of concern and disease outbreaks (WHO, 2020). Techniques developed have been used for Polio and Typhoid detection. Recently, the virus – *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) – that causes *Coronavirus Disease 2019* (COVID-19) has been monitored at *Wastewater Treatment Works* (WWTWs) around the world. Infected patients shed the virus in their faeces and to a limited extent, in their urine (Jones, et al., 2020), that ultimately ends up at WWTWs, if the toilets are connected to a sewer system. A sample taken at the head of the WWTWs can be analysed for understanding and estimating the prevalence of pathogens including SARS-CoV-2. This approach is known as *Wastewater Based Epidemiology* (WBE), fits under the umbrella of *Environmental Surveillance* and can provide population-level health data for those connected to the sewer system. This data can be used to guide public health responses and strategies during a pandemic. Using molecular techniques like *Reverse Transcription Polymerase Chain Reaction* (RT-qPCR), fragments of SARS-CoV-2 RNA can be detected in samples from the head of the WWTWs. The benefit of the approach is that it provides a snapshot of the community health of a population and includes asymptomatic and symptomatic and possibly some pre-symptomatic patients as SARS-CoV-2 RNA can be detected in faeces of these individuals (Jones, et al., 2020) and by assumption, a sample taken at the head of a WWTWs. It is important to note that the molecular techniques traditionally used in this approach provide information whether the virus RNA is present in sample and does not distinguish whether the virus is viable, i.e. infectious.

Researchers have demonstrated many useful findings based on WBE. In early 2020, during the early stages of the pandemic, Medema and coworkers tested for and detected the SARS-CoV-2 virus in wastewater samples in the six cities and an airport in the Netherlands (Medema, Heijnen, Elsinga, Italiaander, & Brouwer, 2020). The researchers made several significant findings. First, they detected signal of SARS-CoV-2 RNA before the first clinical diagnosis. And second, they found that the surveillance of wastewater samples was sensitive enough to pick up SARS-CoV-2 RNA when COVID-19 cases were low and that a significantly correlated increase in RNA signal was observed as clinical cases increased (Medema, Heijnen, Elsinga, Italiaander, & Brouwer, 2020).

In Italy, La Rosa and co-workers also showed detection of the virus before the first clinical diagnosis (La Rosa, et al., 2020). In the USA, Peccia and colleagues were able to show a clear correlation between hospital admissions and the increase in SARS-CoV-2 in the sludge at the WWTWs (Peccia, et al., 2020). They noted that positive test results for SARS-CoV-2 in the municipal sludge were several days ahead of date of patient specimen collection, local hospital admissions and positive tests results from patients by reporting date. Similar findings have been made in France and Greece (Galani, et al., 2022; Wurtzer, et al., 2020). These results highlight the usefulness of WBE in providing a non-invasive warning tool conferring advanced notice of infection rates at population-level and the possible effect on medical infrastructure, including requirement for hospitalisations.

Since 2020, there have been numerous countries that have adopted WBE for SAR-CoV-2 early detection and disease mitigation (Medema, Been, Heijnen, & Petterson, 2020; Tlhagale, et al., 2022). Within South Africa, the S*outh African Collaborative COVID-19 Environmental Surveillance System* (SACCESS) network was established and led by the South Africa *National Institute of Communicable Diseases* (NICD). The network consists of the NCID and SACCESS partners monitoring 85 WWTWs across the country.

1.2 Targeted WBE

As an off-shoot, targeted WBE has emerged and provides community-level data rather than population level data. This monitoring may be potentially a more convenient and cheaper means of monitoring for viruses (than individual patients) during pandemics. Examples of targeted WBE include monitoring of the wastewater from residences, aeroplanes, and clinics. In Australia, the toilet wastewater from aeroplanes and cruise ships were monitored and the data compared to traveller screening data (Ahmed, et al., 2020). Other successful applications include university campuses and institutions (Betancourt, et al., 2021; Gibas, et al., 2021; Wang, et al., 2022).

1.3 The Challenge for Implementing WBE in Developing Countries

In developing countries, the sewer coverage is generally low. WBE can therefore not be applied in these countries for large proportions of the population. Multiple sanitation systems can be used within a developing country city. These include sewered and non-sewered technologies. For the latter, it refers to a sanitation system that is not connected to a sewer network and includes septic tanks, latrines (wet or dry), conservancy tanks, aqua privies, and the next generation of off-the-grid systems which are yet to reach scale.

In South Africa, around two-thirds of the population are connected to the sewer system. Coverage is not uniform across the country, with the provinces of the Western Cape and Gauteng having relatively higher access than less developed provinces like Limpopo and the Eastern Cape. Within a province, coverage can also be uneven. Each province can consist of metropolitan municipalities which are the highly-urbanised areas and district and local municipalities. There is no metropolitan municipality in South Africa that has 100% sewer coverage. A district municipality can consist of several local municipalities with varying levels of servitude and possibly large rural components where there has been historic under-development.

WBE provides unique cost-effectiveness and efficiencies that would suit developing countries during pandemics. The challenge for developing countries is how to gain the benefits of WBE in areas in cities which include varying proportions of sewered and non-sewered sanitation coverage. If WBE can be adapted to developing countries and their contexts, many of issues faced during pandemics can be mitigated. These include cost of individual testing, supply chain issues related to availability of kits, laboratories with specialised equipment and trained personnel to undertake diagnostic testing (Giri & Rana, 2020; International Finance Corporation World Bank Group, 2020; Nkengasong, 2020; Peplow, 2020).

Mushrooming informal settlements is also a unique developing country challenge. High urbanisation trends make it challenging to provide formalised servitudes in informal settlements within cities. Conventional disease mitigation practices, like social distancing, is challenging in these areas. Shared use of ablution facilities is common, and the disposal of human faecal waste and human activity wastes is challenging. Informal settlements are usually inhabited by the most vulnerable population which have limited income and limited access to private healthcare. It is assumed that disease outbreaks in these areas would be likely due to the congested nature of settlements and lack of servitudes. A WBE tool adapted for these areas would be most beneficial in these areas and provide a cost-effective measure to monitor disease outbreaks and monitor flare-ups during a pandemic.

In 2020, the Water Research Commission (WRC) set a challenge to the sector to develop a nonsewered surveillance framework for SARS-CoV-2.

The strategy of the research was to:

- Develop the appropriate methodology for detection of SARS-CoV-2 in non-sewered communities.
- Develop the business case for non-sewered SARS-CoV-2 surveillance to be incorporated as part of city-wide WBE surveillance. The inclusion of non-sewered surveillance within city-wide surveillance programmes ensures that everyone benefits from surveillance programmes and health outcomes.

The challenge that we faced at the onset was the variety in sampling points in non-sewered areas. Potential sampling points include individual toilets, collected faecal sludge, greywater and other runoff from communities which do not have a system to dispose of wastewaters and use nearby stream or rivers (Figure 1).

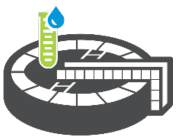
Sampling	**Successful business case**
	WWTWs
Targeted WBE	
	Planes, cruise ships
	Hospitals, clinics
	Campuses and other residential buildings
Non-sewered sampling	**Can non-sewered surveillance be effective and efficient as WBE?**
	Do we sample the environment near settlements?
	Do we sample a single toilet, the faecal sludge from one household or many, a communal system?

Figure 1. Could Non-Sewered Surveillance be cost-effective and efficient as shown for WBE for WWTWs and targeted WBE?

The programme initiated by the WRC aimed to resolve these issues and provide the business case for inclusion of non-sewered surveillance with the sewered WBE as part of City-Wide Environmental Surveillance. This paper presents the major findings from the case study in South Africa. Other developing countries would need to adapt the findings to their local context and conditions.

ESTABLISHING THE METHOD

2.1 Local Partnerships and Sites Sampled

One of the key aspects to achieving successful outcomes for this project was establishing sampling partnerships and collaborations. At WWTWs, the sample can be taken at the head of works by staff who are accustomed to taking wastewater samples. For the non-sewered settlements, Waterlab had to provide the necessary training and facilitation to ensure proper sample collection.

Waterlab PTY Ltd made collaborative partnerships with various river action groups, community leaders, universities and research facilitators to enable the collection of samples from identified sites. To determine the trends within a river or other contaminated water source, a sampling point both up - and downstream of a selected informal settlement was identified to ensure that the impact of the community can be assessed based on the background upstream contamination. As part of the study standing pools of water have also been identified as sampling points especially in the KwaZulu-Natal (KZN) area where these are semi-permanent fixtures within the community.

Samples were either collected by Waterlab staff or couriered to Waterlab in Pretoria when sampled by others for sample preparation and analysis of water quality parameters.

Samples from identified sample sites were collected from March 2021 to September 2021, as presented in **Table 1**. In September 2021, the sites and results were reviewed and based on preliminary study findings, some sites were retained for expansion of longitudinal data for historical trend analysis, sampling at some sites was stopped, and additional sites were added, to be sampled for an additional 12 weeks. Through experience, it was decided that the run-off, streams and lakes were ideal sampling sites for SARS-CoV-2 detection. Sampling faecal sludge collected from Urine Diversion Dehydrating Toilets (UDDTs) were not fruitful and proved to be impractical. Further, no SARSCoV-2 signals could be detected from UDDT faecal sludge analysed. This aspect was removed from the non-sewered surveillance programme.

Table 1. List of initial sample locations

No.	Pro-vince	City/Town	District	Site name	Type	Co-ordinates	Sample frequency	Sample duration
1	Gauteng	City of Johannesburg	Johannesburg MM	Jukskei upstream Alex	River	-26.109573, 28.113083	Monthly	20 weeks
2*		City of Johannesburg	Johannesburg MM	Jukskei Source	River	-26.19338, 28.07110	bi-weekly	20 weeks
2		City of Johannesburg	Johannesburg MM	Jukskei downstream Alex	River	-26.079089, 28.108555	bi-weekly	20 weeks
3		City of Johannesburg	Johannesburg MM	Silvertown, Alexandra standpipe/wetland	Surface	-26.087994, 28.107073	bi-weekly	20 weeks
4		Kliptown, Soweto	Johannesburg MM	Klipspruit (K5) at Kliptown	River	-26.290033, 27.885617	bi-weekly	20 weeks
5		Sebokeng, Emfuleni LM	Sedibeng	Rietspruit (RV1) at Sebokeng	River	-26.728650, 27.717950	Monthly	20 weeks
6		City of Tshwane	Tshwane MM	Rietspruit (Hennops tributary) at Thatchfield	River	-25.895948, 28.119746	bi-weekly	20 weeks
7		City of Tshwane	Tshwane MM	Kaalspruit (Hennops tributary) downstream Tembisa	River	-25.957542, 28.206773	bi-weekly	20 weeks
8		City of Ekurhuleni	Ekurhuleni MM	Glenshaft Pan (DS Benoni WWTW at informal settlement)	Surface	-26.215597, 28.314323	bi-weekly	20 weeks
9		City of Ekurhuleni	Ekurhuleni MM	Klip River (Upstream Waterval WWTW)	River	-26.435323, 28.092954	bi-weekly	20 weeks

No.	Pro-vince	City/Town	District	Site name	Type	Co-ordinates	Sample frequency	Sample duration
10	Western Cape	Stellenbosch	Cape Winelands	Plankenbrug River upstream informal settlement	River	-33.891337, 18.828423	bi-weekly	20 weeks
11		Stellenbosch	Cape Winelands	Plankenbrug/Krom River at runoff	River	-33.934110, 18.851063	bi-weekly	20 weeks
12		Franschoek/ Langrug	Cape Winelands	Franschoek River upstream Langrug settlement	River	-33.905828, 19.101960	bi-weekly	20 weeks
13		Franschoek/ Langrug	Cape Winelands	Langrug settlement runoff into Stielbeuelrivier	Surface	-33.896159, 19.100807	bi-weekly	20 weeks
14	KZN	eThekwini	eThekwini	Palmiet river upstream Quarry Road West informal settlement	River	-29.804962, 30.965609	bi-weekly	20 weeks
15		eThekwini	eThekwini	Palmiet river downstream Quarry Road West informal settlement	River	-29.802616, 30.970598	bi-weekly	20 weeks
16		eThekwini	eThekwini	Quarry Road West informal settlement	Surface	-29.804122, 30.966965	bi-weekly	20 weeks
17		eThekwini	eThekwini	Umhlangane River upstream Johanna Road informal settlement	River	-29.796499, 30.993136	bi-weekly	20 weeks
18		eThekwini	eThekwini	Umhlangane River Downstream Johanna Road informal settlement	River	-29.799888, 30.991315	bi-weekly	20 weeks
19		eThekwini	eThekwini	Johanna Road informal settlement	Surface	-29.797288, 30.994522	bi-weekly	20 weeks
20		eThekwini	eThekwini	Urine Diversion Toilet composite samples in peri-urban eThekwini (TBC)	Unsewered on site sanitation	Various	5 samples	May to August
21	Mpumalanga	Kanyamazane	Mbombela	Crocodile River at Kanyamazane Upstream	River	-25.483761, 31.147015	bi-weekly	20 weeks
22		Kanyamazane	Mbombela	Crocodile River at Kanyamazane Downstream	River	-25.488409, 31.171980	bi-weekly	20 weeks

2.2 Sample Processing and Analysis

Sample processing and analysis for SARS-CoV-2 were established as part of the Proof-of-Concept Study (Pocock, Coetzee, Mans, Taylor, & Genthe, 2020). In summary, the recovery method for the surface water samples is the skimmed milk method as it is fast, less expensive than other methods and requires less expensive equipment (Pocock, Coetzee, Mans, Taylor, & Genthe, 2020). Further details of the nucleic acid extraction and viral amplification can be found in the Proof-of-Concept study (Pocock, Coetzee, Mans, Taylor, & Genthe, 2020).

2.3 Passive Sampling Overcomes Sampling Challenges

Through project, passive sampling devices were adapted from Australia (Schang, et al., 2021) and applied to non-sewered settlements. Instead of dangling device into sewers as was done in Australia, the passive sampling devices were placed in run-off, streams and rivers nearby the settlements. The passive sampling devices solved many issues related to sampling in these areas: low yield during high dilution periods, easier and cheaper transport of samples, and quicker sample processing. This method of sampling became standardised practice as the programme progressed. The optimal time for deployment was found to be 24 hours. Longer times can lead to fouling of device and gauze.

Figure 2. Passive sampling devices adapted for sampling non-sewered settlements

CASE LOADS VS SAMPLES FROM NON-SEWERED SETTELEMENTS

We have been able to detect SARS-CoV-2 in non-sewered community run-off, surface water and in the rivers which lie downstream of these communities. The data also showed that the incidence of COVID-19 in these communities is reflected in the Ct values obtained in the rivers and surface run-off samples. In Gauteng province where the informal settlements are dense and the rivers are highly polluted by faecal matter from these communities, the trends were even more evident (Figure 3).

Cts <29 (grey shaded area) are strong positive reactions indicative of abundant target nucleic acid in the sample
Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid
Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination.
Red dashed line is the trend
Shaded blue area give 95% confidence of the trend line
Circle point is the number of cases (x100)

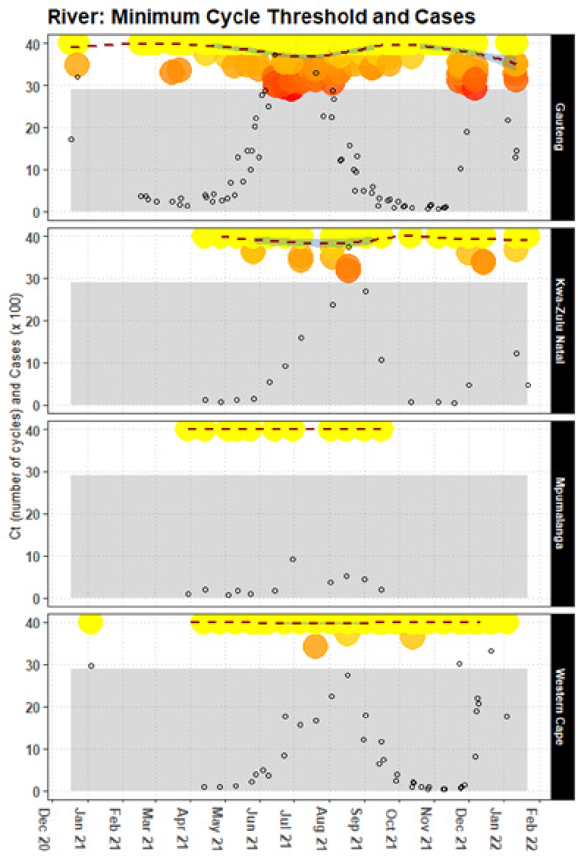


Figure 3. Daily cases and Ct values in rivers downstream of unsewered settlements across four provinces in South Africa (Pocock, Coetzee, Mans, & Genthe, Development of a Framework for Water Quality-Based COVID-19 Epidemiology Surveillance for Non-Sewered Communities, (in prep))

While the non-sewered surveillance programme focused on SARS-CoV-2, we have also been able to demonstrate that the same sample collection, recovery and extraction techniques can be successfully applied to collect contextual and public health information on other pathogens and indicators. Norovirus is an enteric virus that is very commonly present in South Africa's population and shed via stool, often used as reference viruses for sewage surveillance. Norovirus was found to be almost ubiquitous across sites sampled, with almost all samples assayed testing positive for norovirus. Similarly, samples positive for Hepatitis E virus were found in all four provinces tested; Gauteng, Mpumalanga, KwaZulu-Natal KZN and the Western Cape.

ESTABLISHING A CITY-WIDE ENVIRONMENTAL SURVEILLANCE PROGRAMME

To establish City-Wide Environmental Surveillance, the following will be required:

- A sewer-based WBE programme. The methods and tools are fairly well understood, and many countries have adopted the approach to pandemic response. Lessons from other countries can be used in a developing an approach (Tlhagale, et al., 2022).
- A non-sewered surveillance programme. The tools developed through the WRC can be adapted to local context. Local partnerships and collaborations were seen as key to the success of the programme enabling sampling capacity and co-ordination in non-sewered settlements across the country. More cost-effective methods for sampling and viral recovery were also developed and the techniques were shown to equally applicable for other pathogens of concern. The frequency of sampling and analysis needs to be determined by stakeholders. For example, routine monthly samples can be taken and sampling efforts sampled up during the onset of a disease.

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