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Addressing the challenges of establishing quality wastewater or non-sewered sanitation-based surveillance, including laboratory and epidemiological considerations, in Malawi

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ABSTRACT

Learning from clinical laboratories, wastewater or environmental (including non-sewered sanitation) environmental microbiology laboratories can be established in resource-limited settings that focus on pathogen detection and pandemic prevention. Transparent discussions on the laboratory challenges and adaptations required for this can help meet the future requirements of health research and surveillance. This report aims to describe the challenges encountered when setting up a wastewater or environmental laboratory for multipathogen surveillance in Malawi, a resource-limited setting, as well as the lessons learnt. We identified nine unifying themes: what to monitor, human resource capacity, indicators of data quality, equipment availability, supply of consumable goods, ongoing operation and maintenance of the laboratory, application of localised guidelines for laboratory operations, lack of real-time clinical correlation for calibration and localised ethical considerations. Over our 6-month timeline, only *Salmonella* typhi, *Vibrio cholerae* and severe acute respiratory syndrome coronavirus 2 analyses were set-up. However, we were unable to set-up measles and tuberculosis analyses owing largely to supply delays. By establishing this system at a public higher education academic laboratory in Malawi, we have ensured that ongoing capacity building and piloting of public health work is conducted in the country, rather than relying on non-governmental organisations or reference laboratory support beyond national borders. This work is not intended to replace clinical testing but rather demonstrates the potential for adapting higher education academic laboratory infrastructure to add wastewater or environmental (including non-sewered sanitation) samples, where appropriate, as additive epidemiological data for better pandemic preparedness.

INTRODUCTION

More than 4500 sites globally have analysed samples for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in

SUMMARY BOX

- ⇒ Wastewater or environmental (including non-sewered sanitation)-based epidemiology offers the potential for better pandemic preparedness.
- ⇒ The existing literature has identified key factors and practical solutions for the set-up and operation of clinical laboratories in resource-limited settings but not for wastewater or environmental public health laboratories.
- ⇒ Lessons learnt from ongoing experiences are often not considered widely.
- ⇒ We demonstrated that it is possible to set-up a wastewater or environmental public health laboratory over a 6-month period by adapting an existing public higher education academic laboratory.
- ⇒ There is an ongoing role for public higher education researchers and the government to build sustainable wastewater or non-sewered sanitation surveillance programmes to ensure timely and quality epidemiological data in low-resource settings.

wastewater since the start of the coronavirus disease 2019 (COVID-19) pandemic,¹ thereby providing data that complement clinical data relevant to the pandemic. Laboratory standards for SARS-CoV-2 wastewater surveillance have largely focused on sewer systems with supporting PCR-based analyses.² Additional surveillance efforts are increasingly moving beyond SARS-CoV-2 to other public health diseases of concern, such as influenza and polio, which can also be detected in wastewater.^{3 4} Long-term and ongoing WHO environmental surveillance efforts for poliovirus were established before the recent pandemic⁵; in this respect, detection is not necessarily a target for expansion, but rather provides a model for replication. The existing

literature has identified key factors and practical solutions for setting up and operating clinical laboratories in resource-limited settings,^{6,7} and other studies on higher education have focused on strengthening the sanitation and hygiene research workforce in such settings.⁸ Wastewater surveillance systems may include numerous households in an area by sampling a sewer connection, but surveillance may also include non-sewered sanitation sampling through individual or community-based household pit latrines or septic tanks or environmental samples where human waste is also known to occur.^{9,10} In resource-limited settings, there is a need for strategies and systems ensuring the robust monitoring of sanitation systems, including the role of laboratories. Wastewater or environmental (including non-sewered sanitation) based surveillance, where appropriate, based laboratory and epidemiological considerations in resource-limited settings can focus on pathogen detection and pandemic prevention; however, transparent discussions on the challenges and adaptations needed in operationalisation can help meet the needs of future health research surveillance.

We adapted an existing public higher education academic laboratory for wastewater or environmental public health analyses focused on pathogen detection in Malawi. Laboratory construction, infrastructure improvements and retrofitting were avoided. Pathogens were agreed on during consultation with various national environmental health sector stakeholders, including the Ministry of Health and the United Nations Children's Fund. Pilot samples and analyses were introduced weekly in 2022 for culture-based methods used to monitor *Vibrio cholerae* and *Salmonella typhi*, followed by confirmation analyses of isolates using PCR-based methods and SARS-CoV-2 was also analysed using PCR. We were unable to perform measles or tuberculosis analyses. The three sampling locations, all of which were close to the laboratory, included: a large government healthcare facility, a small private healthcare facility and the higher education campus where the laboratory was located. This report aims to describe the challenges encountered over a 6-month period of setting up a wastewater or environmental public health laboratory for multipathogen surveillance in a resource-limited setting. Furthermore, this article contributes to the ongoing discussion on the African perspective for future public health wastewater surveillance^{10,11} and how to ensure more timely and higher quality epidemiological data.

WHAT TO MONITOR

Our initial monitoring priorities were a combination of diseases with known faecal shedding, diseases endemic to our particular region mostly affecting the health of women and children under the age of 5, pathogens with an existing local clinical testing capacity, the availability of validated protocols and targets for environmental sample processing and diseases prioritised in consultation with

key stakeholders in the environmental health sector. The available infrastructure and existing laboratory expertise at Malawi University of Science and Technology were also reviewed to consider feasibility; existing laboratory biosafety systems were crucial, which included biosafety level 2 (BSL-2) facilities. This is relevant to pathogens endemic in our particular region and may differ between culturing and direct extraction; tuberculosis requires biosafety level 3 (BSL-3) for culturing; however, with direct extraction then BSL-2 laboratory operations become suitable. In addition to BSL-3, some pathogens (eg, SARS-CoV-2) under sanitation system surveillance are listed as BSL-2+ and not solely BSL-2.¹² Accordingly, we wanted to monitor measles, *S. typhi*, SARS-CoV-2, tuberculosis and *V. cholerae*.

WORKFORCE

The number of staff with wastewater or non-sewered sanitation laboratory analysis experience in Malawi was limited¹³; therefore, some degree of training was required for the personnel involved in the project. Our project-training model focused on direct hands-on mentoring. The laboratory was supervised by an academic staff member (holding a PhD in Molecular Biology), and analyses were conducted by a laboratory technician (holding a Diploma in laboratory technology) and three interns who had obtained in the preceding 2 years Bachelor of Science degrees in either medical microbiology or biotechnology. Laboratory occupational health and safety procedures for staff were in place prior to the pilot study and were based on a precedent of the existing clinical testing capacity, such as the handwashing sinks, emergency eye wash stations and good laboratory waste management systems. We adopted readily available SARS-CoV-2 clinical personal protective equipment protocols for all pathogens studied. The staff understood the inherent risk of working with wastewater samples compared with clinical samples, and their comfort levels and questions were considered. While Parsons *et al.*⁶ recommended technicians performing clinical tuberculosis analyses in resource-limited settings receive 1 week of training, our experience was that wastewater analysis required 1 month of training. We also found it helpful to have standard operating procedures in plain language, accompanied by photographs. As motivation, staff were provided with supplemental pay from the project budget, as the research was beyond their regular academic duties. This work also enhanced professional development opportunities for the team through participation in the writing of peer-reviewed manuscripts. Long-term workforce training and occupational safety goals should be prioritised in this rapidly evolving field as more pathogens and analysis methods are added.

INDICATORS FOR MONITORING QUALITY

Quality data was of importance to the project. We collected wastewater or environmental samples using the

grab sample method once a week for 6 weeks as a proof of concept. Shipping a positive control to Malawi was unrealistic; therefore, we collected samples from sites likely to test positive for the pathogens being monitored, such as the tuberculosis and COVID-19 hospital wards. We used locally available *S. typhi* isolates as a standard for comparison with the results obtained from positive cultures. Furthermore, to check for contamination of the culture media, which was prepared at least once every 2 weeks, 5% of media was incubated at 37°C for 18 hours. Since the laboratory windows were not sealed, contamination of the samples was avoided as thoughtfully as possible by keeping the work surfaces clean with regular glove changes. Glove changes were not made for each sample but were made at least hourly and in any event of suspected or confirmed sample spillage. Culture samples were processed in a biosafety cabinet. Our initial culture method results were reported in terms of presence or absence, which may be of sufficient quality as early warning indicators during non-outbreak periods to reflect background prevalence of the disease in the population and that the pathogen in the environmental matrix are really what drive the determination of the appropriateness of reporting.

During the pilot study, only wastewater samples were processed in the laboratory space to provide an additional step to ensure minimal contamination of clinical samples in the same general area. While adaptation of clinical laboratories for one-off projects is a stopgap at best, it is not in accordance with best practices, as it may carry the risk of cross-contamination and threaten existing clinical workflows, among other concerns, while still offering an opportunity to build the environmental laboratory capacity. In our case, a microbiological clinical laboratory that handles most target pathogens was considered.

EQUIPMENT

Our work focused on adapting the clinical testing capacity and available equipment in the academic laboratory to build wastewater or non-sewered sanitation surveillance system testing capacity. A centrifuge and an orbital shaker were key equipment needed but they were not available for local purchase. An existing centrifuge broke early in the study, and the only alternative equipment was a centrifuge from another research institution in the same region. However, this would require considerable travel time, and because the samples could not be transported at lower temperatures as this would denature the milk (the key flocculation ingredient used in the laboratory protocol for SARS-CoV-2), thus affecting data quality. Therefore, a manual (hand-crank) centrifuge was purchased locally. We further adapted an existing vortex machine as a shaker.

A PCR machine was available, along with a small centrifuge, pipettes, pipette tips and PCR kits; however, primers and probes for all the pathogens were not. For the culture

and isolation of *S. typhi* and *V. cholera*, most equipment and consumables were readily available in the laboratory based on the academic clinical testing capacity. These included an incubator, autoclave, heat block, water bath, glassware (conical flasks, measuring cylinder and test tubes), scale, automatic pipette and tips, Petri dishes, Pasteur pipettes, loops and spreaders. A generator needed to maintain the BSL-2 during frequent power outages was already available in the laboratory prior to this pilot study, as were computers and a pH adjustment kit. Equipment was sterilised using either ultraviolet exposure for the smaller units or a 70% ethanol spray for the larger units. Unfortunately, while reported to be cost-effective, easy to use or compact, equipment alternatives including the laboratory-on-a-chip platform¹⁴ and the suitcase laboratory¹⁵ for low-resource settings are not currently commercially available in Malawi and would, therefore, need to be imported. As collaborations increase and the team gains vital experience, there will be a need to ensure the availability of dedicated equipment and space for sample processing to minimise cases of cross-contamination and to prioritise multipathogen capable equipment.

SUPPLY OF CONSUMABLES

Coordination of the ordering and delivery of consumables was a major bottleneck. Generally, the consumables for the culture-based methods were obtainable from well-established local suppliers. The team worked with five local suppliers for planned PCR-based methods, with the exception of SARS-CoV-2. Despite some being well established, they provided no response to our team after several order requests. Therefore, to save time, we initially focused on two pathogens (*S. typhi* and *V. cholerae*) using culture-based methods and SARS-CoV-2 using PCR while consumable supply alternatives were sought. For the available stocks, there was one instance where oxidase discs were in stock, but they had expired and self-reacted because of prolonged and poor local storage conditions. Therefore, we had to return the consumable item immediately after it was located.

On project commencement and with prior approval, reagents for SARS-CoV-2 were made partially available as stock donated to the academic laboratory by the Government of Malawi, whereas other reagents had to be sourced from other laboratories and later replaced. The PCR-based supplies for measles, tuberculosis, *S. typhi* and *V. cholerae* that had to be imported took 2 weeks to 2 months to respond to requests for quotes, and some never responded. Extensive staff member time was lost during this circular process. We were not able to bundle or combine purchases in bulk to obtain loyalty discounts from manufacturers to help minimise the costs and staff time required; instead, we had to purchase what we could find among the limited supply. The external supply of reagents and consumables was further affected by a nationwide foreign exchange shortage (US\$ or South

African rand). Ultimately, the remaining primers, probes and reagents had to be sourced off-label from outside the country because in-brand manufacturers would not commit to supplying for our project. This poses a quality risk in terms of the risk of counterfeit supplies. Ultimately, a final hurdle was the primers and probes were shipped, lyophilised from South Africa, and kept in a temperature-controlled room (with air conditioning) at the local Blantyre airport for 38 days, awaiting customs clearance.

As a result of consumable supply issues, after collection, the samples were processed for RNA and DNA extraction and kept at -80°C and -20°C , respectively, while awaiting the arrival of primers and probes. Ultimately, over our 6-month timeline, only *S. typhi*, SARS-CoV-2 and *V. cholerae* analyses were performed, and we were unable to perform measles and tuberculosis analyses because of consumable supply delays.

OPERATION AND MAINTENANCE OF LABORATORY

Funding, power supply and security are key to long-term laboratory operations in resource-limited settings. Between June and November 2022, the laboratory was set-up with financial support from external partners. In a low-resource setting, internal research funding at academic institutions is less likely to be available. Malawi experiences frequent power blackouts that can disrupt laboratory operations and consequently increase sample processing and data reporting turnaround times. For example, PCR requires a continuous power supply for an uninterrupted run once the samples are loaded. To avoid interruptions, the PCR had an existing dedicated battery-based backup system to complement a university-wide diesel generator to which most laboratory equipment, including the BSL-2, was connected. To ensure security, the laboratory in this pilot had controlled (keyed) access only accessible to the project staff.

GUIDELINES FOR LABORATORY OPERATIONS IN MALAWI

Currently, Malawi does not have an all-encompassing policy for laboratories, and proper approvals are difficult to obtain or do not exist. Although the National Medical Laboratory Policy¹⁶ provides general guidance for medical laboratories in regional and district health facilities, it has not yet been applied in academic laboratories. Currently, the Malawi Government's National Commission for Science and Technology recommends that higher education institutions have their laboratories licensed and accredited by international accreditation bodies. However, if a laboratory offers public services, the Medical Council of Malawi must certify it. The laboratory in our study was approved as BSL-2 by the Public Health Institute of Malawi during the COVID-19 pandemic, when it was approved as a testing centre. Finally, the Malawi Bureau of Standards requires instruments and equipment to be calibrated; however, the guidelines for higher education institutional research for pandemic preparedness are undefined. National guidance and standards

for environmental microbiology laboratories, versus the existing landscape of policy which covers the majority clinical/medical laboratories earlier discussed, would be warranted as another priority gap to be addressed.

CLINICAL AND WASTEWATER OR NON-SEWERED SANITATION DATA CORRELATION

Perhaps the most serious limitation in performing wastewater or non-sewered sanitation-based epidemiology in a resource-limited setting is the lack of reference disease burden data. Even though the government has implemented a Health Information Management System, contradictions were still observed in the clinical data availability for some pathogens, with some officials indicating data availability, while others indicated no clinical data availability. Ultimately, our researchers in some cases were required to obtain records using paper-based case files. For COVID-19 and *V. cholerae*, due to the current outbreak conditions, daily clinical case data were widely reported at a national level to health stakeholder groups daily via WhatsApp messages. This contrasts with *S. typhi* data, which were reported monthly, making our weekly wastewater samples difficult to correlate. This highlights the need to define how a wastewater or non-sewered sanitation surveillance system will complement the clinical surveillance of an outbreak-prone pathogen where regular clinical surveillance may be episodic at best. This disconnection means we retrospectively calibrated only once, at the end of the project. That clinical surveillance and reporting are not necessarily adequate—another challenge in resource-limited settings—hinders wastewater or non-sewered sanitation surveillance system laboratories in public health research. Other factors also make this type of data difficult to correlate; for example, sewer-sheds may not be compatible with how clinical cases are classified or the demographics of a population sampled seeking care at a healthcare facility far from their home.

ETHICAL CONSIDERATIONS

This pilot study received ethical clearance from Malawi University of Science and Technology Research Ethics Committee and the University of Louisville, and we would advocate for this practice to obtain research ethical clearance. This step took less than a month.

At the government hospital, despite providing an academic research ethics committee certificate, it was not clear who was responsible for approval and communication among departments was poor. Project briefings were conducted multiple times. As well, the private hospital initially suspected that our pilot study was part of the inspection and quality control processes of the Ministry of Health, and as such, permission was delayed. Site approval ultimately took approximately 2 months.

In addition, other ethical considerations included the sustainability of this work, access to and use of data for both the short-term and long-term, the public opinion of the sampled populations and that health services in

Box 1

Key messages for wastewater or non-sewered sanitation surveillance system laboratory set-up in resource-limited settings

- ⇒ Focus: Prioritise pathogens endemic to the particular region and targets that are accessible based on the available infrastructure, existing laboratory expertise and validated methods.
- ⇒ Human resources: Start with a workforce trained in medical microbiology and provide hands-on training; the laboratory must have standard operating procedures.
- ⇒ Site coverage: Initial sample sites should be locations known to test most likely positive, with several weeks of analysis.
- ⇒ Method equipment and supplies: Adapt existing equipment and, where needed, order consumable supplies as far in advance as possible; have alternative analysis methods selected.
- ⇒ Finance: Have external funding for proof-of-concept research; long-term laboratory operation and maintenance should include wider infrastructure needs such as a power backup system for key equipment.
- ⇒ Regulation: Guidelines on wastewater or non-sewered sanitation laboratory surveillance system operation may be challenging to obtain, or do not exist.
- ⇒ Data use: Complementary role of wastewater data with regard to clinical data should be defined before the system is set-up to clarify the goals and the complementarity of the wastewater data to clinical data.
- ⇒ Ethics: Ethical considerations include both academic research approval and approval from sample collection sites.

Malawi prioritise women and children under 5 years old such as those visiting childhood vaccination clinics or accessing family planning services; thus, our pilot samples at healthcare facilities may under-represent men and other age groups in the community.

CONCLUSIONS

Setting up and operating a quality wastewater-based or non-sewered sanitation-based epidemiology laboratory in a resource-limited setting is possible. Lessons learnt from ongoing experiences are not often widely considered; thus, our article attempts to fill this gap (box 1). By establishing this system at a public higher education academic laboratory in Malawi, we have ensured that ongoing capacity building and piloting of public health work is conducted in the country, rather than relying on non-governmental organisations or reference laboratory support beyond national borders. There is an ongoing role for academic researchers¹⁷ and the government in building sustainable wastewater surveillance programmes in resource-limited settings. Although this study focused on laboratory set-up, there is a need for further thought leadership on sample design unique within resource-limited settings, such as using torpedo-style passive sampling devices¹⁸ and the highly necessary integration of combined wastewater or non-sewered sanitation surveillance system laboratory and epidemiology approaches to guide public health intervention. Future studies should prioritise careful and systematic collection

of laboratory operations data that can systematically highlight confounders in resource-limited settings; for example, some PCR supplies are more available than others, and we constantly learn new things as more pathogens and analysis methods are added. Long-term work could require fluid analytical methods due to changing equipment and supply availability, which may need to be factored into data interpretation. Malawi and other resource-limited settings will continue clinical pathogen testing. This work is not intended to replace clinical testing but rather demonstrates the potential for adapting existing higher education laboratory infrastructure to add wastewater or environmental (including non-sewered sanitation) samples, where appropriate, as additive epidemiological data for better pandemic preparedness.

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Contributors Conceptualisation: RHH, TS, PC. Methodology: PC, RHH. Formal analysis: PC, RN, RHH. Writing—original draft preparation: RHH. Writing—review and editing: RHH, RN, TS, PC. Supervision: RHH. Project administration: RHH. All the authors have read and agreed to the published version of the manuscript.

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