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Article

Strengthening Pathogen and Antimicrobial Resistance Surveillance by Environmental Monitoring in Sub-Saharan Africa: A Stakeholder Survey

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Highlights

- Explored infectious disease surveillance systems in Tanzania, Burkina Faso, and the Democratic Republic of the Congo.
- Prioritized waterborne and respiratory pathogens and antimicrobial-resistant targets for wastewater and environmental surveillance (WES).
- The explored countries utilize centralized electronic systems for clinical data, supplemented by Excel and paper-based registries.
- WES could serve as a valuable tool to prevent and detect outbreaks and for data-driven epidemic response.
- WES is capable of identifying circulating pathogens, underscoring the importance of effective issue sanitation for human, animal, and environmental health.

Abstract: Background: Waterborne diseases pose a significant global public health threat, compelling enhanced comprehensive surveillance. This study investigates the current infectious disease and antimicrobial resistance (AMR) surveillance systems, including wastewater and environmental surveillance (WES), in three sub-Saharan African countries: Tanzania, Burkina Faso, and the Democratic Republic of the Congo (DRC). The three countries have their specificities regarding wastewater networks and logistic systems. So, emphasizing the need to strengthen existing disease surveillance, the paper advocates for incorporating WES systems specifically designed for countries' context to monitor waterborne and re-emerging pathogens, as well as AMR. **Methods:** National workshops were conducted to assess the current clinical and environmental surveillance systems and identify priority pathogens for new environmental monitoring. Data were collected through surveys from experts in academia, research, policy, and healthcare. **Results:** Prioritized pathogens for WES include waterborne (poliovirus, *Salmonella Typhi*, *Vibrio cholerae*), respiratory (influenza A&B, SARS-CoV-2), other (Measles and Rubella, *Mycobacterium tuberculosis*). Recommended AMR pathogens include drug-resistant *Mycobacterium tuberculosis*, *Salmonella* spp., methicillin-resistant *Staphylococcus aureus*, and extended-spectrum beta-lactamase and carbapenemase-producing *E. coli*. Tanzania, Burkina Faso, and the DRC employ DHSI2-based

centralized electronic systems for clinical data collection, complemented by Excel and paper-based registries. The WES approach is commonly employed for monitoring poliovirus and rarely for AMR and other pathogens. **Discussion and conclusions:** WES is a valuable tool for early detection of locally circulating human-derived pathogens, aiding in outbreak detection, data-driven epidemic response, and prevention. The availability of WES results underscores the importance of effective sanitation for safeguarding human, animal, and environmental health. WES is a pivotal tool for integrated risk management, preventing waterborne outbreaks, protecting drinking water sources, and ultimately gaining various UN Sustainable Development Goals. The study highlights the importance of customized WES systems in line with each country's context, emphasizing localized approaches for effective monitoring of waterborne pathogens and AMR.

Keywords: wastewater; waterborne pathogen; sub-saharan african countries; environmental surveillance; prioritization

1. Introduction

Despite progress in water access, 27% of the global population, particularly in low and middle-income countries (LMIC), lacks access to clean drinking water [1]. Diarrheal disease is a major contributor, causing over one million deaths and 55 million disability-adjusted life years [2]. Microbiologically contaminated drinking water can transmit diseases like diarrhea, cholera, dysentery, typhoid, and polio [1]. Antimicrobial drugs are effective for managing and preventing many of these diseases [3]. However, their use may accelerate antimicrobial resistance (AMR). Misuse and overuse of antimicrobials in humans, and animals are primary causes of AMR development [3,4]. Inadequate water, sanitation, and hygiene (WASH) can contribute to AMR spread in communities [5]. AMR is a significant global threat to clinical treatment in human and animal health and overall human development. In 2019, AMR caused 1.27 million direct deaths and contributed to an additional 4.95 million deaths globally [6]. This surpasses the annual death toll from COVID-19, which stands at about seven million deaths between 2019 and 2023 [7].

In order to tackle the spread of infectious disease and AMR, robust epidemiological surveillance is crucial for timely detection and monitoring, and thereafter guide resource allocation [8,9]. Surveillance methods can vary based on the pathogen, surveillance objective, AMR targets, and available resources [10]. Surveillance evaluates pathogen diversity, variants, and outbreak impact to inform mitigation strategies [11]. Traditional disease surveillance relies on clinical testing and reporting [12–14], involving the collection and analysis of individual test reports over time to confirm and assess spatial and temporal outbreak patterns [13,15]. However, clinical testing often focus on symptomatic cases in severe clinical stages, potentially underestimating morbidity from asymptomatic or self-limiting infections, and under-reporting cases in areas with limited healthcare access [14,16–19].

Wastewater and Environmental Surveillance (WES) is an emerging approach for monitoring multiple pathogens and AMR targets, complementing clinical surveillance [15,20–22]. WES offers nearly real-time evidence, often providing early warning signals for disease outbreaks at a population level [21–24]. There is a growing focus on enhancing disease surveillance by integrating WES into existing clinical surveillance systems [14,20,22,25]. WES can detect pathogens and AMR targets in early infections, as soon as an infected individual releases biomarkers (from its bodily fluids, feces, urine, sneezing), into the sewage system. This allows for timely interventions, and actions to mitigate potential outbreaks [10,23,26,27]. It yields results with minimal personal intervention, addressing ethical challenges and independent of healthcare facility availability or individuals' willingness to undergo testing [28,29]. The approach informs evidence-based public health responses, with a specific focus on high-risk areas, optimizing resource allocation to enhance the effectiveness of disease control measures [15,20–22].

In LMIC, many infected individuals may not undergo clinical testing due to reasons like limited healthcare access and diagnostic tool constraints [16]. In these settings, WES can be an effective alternative to accurately estimate disease outbreak burdens at the community level [30–35]. When properly implemented, WES provides additional evidence of potential infection outbreaks, enabling timely and appropriate responses to emerging public health issues [36–38]. However, its adoption still lags, particularly in LMICs [33–35,39].

The present study, part of ODIN consortium project, aims to improve WES of waterborne pathogens and AMR in sub-Saharan Africa [40]. The project also strives to strengthen genomics and bioinformatics capacities in sub-Saharan countries, foster international multidisciplinary research to reduce disease cases from contaminated drinking water in the region. Within the project scope, this paper compiles data on potential pathogens for WES and the existing surveillance network. The study explores clinical and environmental surveillance for communicable disease epidemiology in three Sub-Saharan countries (Tanzania, Burkina Faso, and the Democratic Republic of the Congo - DRC). It evaluates the current state of clinical and environmental surveillance systems and assesses the potential for establishing a new WES system in the region. Additionally, to identify priority pathogens for WES, this study examines recent infectious disease outbreaks in sub-Saharan Africa, with a specific focus on waterborne pathogen transmission and AMR in the environment.

2. Methodology

We arranged stakeholder workshops (November-December 2023), in each country (Tanzania, Burkina Faso, and the DRC), covering four key themes: (a) current clinical surveillance, (b) existing environmental surveillance, (c) challenges in current clinical surveillance, and (d) potential of wastewater and environmental surveillance. The participants in the workshops comprised distinguished experts in clinical and environmental fields, including academicians, researchers, policymakers, healthcare authorities, and representatives from national and international, governmental, and non-governmental organizations (NGOs), such as the West African Health Organization, WHO country office, National Office for Water and Sanitation, Water Analysis Laboratories, and experts affiliated with WHO Polio Eradication Program (**Table 1**). Before the main surveys, the questionnaire was piloted to ensure comprehensiveness in terminologies across participating countries.

Among the participants, we conducted two surveys. In both surveys, responses were collected in English in Tanzania and French in Burkina Faso and the DRC. The Mentimeter.com platform was used for mapping priority pathogens and AMR targets for creating the new WES system in each country. In the Mentimeter survey, priority pathogens were grouped into three categories: (a) waterborne pathogens requiring enhanced public health surveillance, (b) other than waterborne pathogens suitable for monitoring through WES, and (c) antimicrobial resistance (AMR) targets requiring increased surveillance and response for public health significance. A predetermined list of pathogens and AMR targets, based on local epidemiological evidence and prior knowledge of regional outbreaks, was provided to unify the options. Also, a free-text field was included in the survey after each category, allowing respondents to prioritize other pathogens not covered by the survey organizers.

The second survey was conducted covering four key themes, as same as the workshop themes: (a) current clinical surveillance, (b) existing WES, (c) challenges in current clinical surveillance, and (d) potential of WES. The second survey, utilizing the Webropol platform, featured both open-ended and multiple-choice questions in the questionnaire.

To the survey responses from the three separate country-specific workshops, Mentimeter.com data were transferred to Microsoft Excel (MS Excel) and visualized using OriginPro software. Quantitative analyses of Webropol responses were also conducted in MS Excel. To provide a comprehensive sub-Saharan overview, responses from all three countries (Tanzania, Burkina Faso, and the DRC) were aggregated. Textual questionnaire responses were thematically organized and marked with expertise fields using MS Word. We employed thematic analysis for qualitative data analysis.

Table 1. National-level workshop participants.

Tanzania	Burkina Faso	DRC
Academicians and researchers	Academicians, Researchers	Academicians and researchers
Policymakers and representatives from ministry of Health, and National Public Health Laboratory	Policymakers and representatives from five-ministries of the One health program: - Health and public hygiene - Ministry of the Environment, Energy, Water and Sanitation - Livestock - Agriculture - High education, research and innovation	Policymakers and representatives from ministries of Health and Environment
Healthcare providers and authorities (local)	Head of the Nanoro District Head of the CMA Saint Camille de Nanoro	-
Representatives from national and international non-governmental organizations and community organizations such as healthcare providers and regional water and sewerage authorities (local)	WaterAid, Directorate-General for Water Resources, the National Office for Water and Sanitation (ONEA), Director General of Wastewater and Excreta Disposal, and Water and environment quality analysis laboratory	Water Analysis Infrastructure (REGIDESO), and the National Institute of Biomedical Research

3. Results

3.1. Priority Pathogens

The prioritization of the pathogens based on the responses from the workshop participants are shown in Figures 1-3. Among waterborne pathogens, poliovirus, *Salmonella Typhi*, and *Vibrio cholerae* (toxigenic O1/O139) were rated as the top three priority pathogens in all three countries (Figure 1). There was no such consensus when other than waterborne pathogens were considered. Of other than waterborne pathogens, influenza A&B, and SARS-CoV-2 were among the top priorities in Tanzania and the DRC, and dengue virus, *Mycobacterium tuberculosis*, and respiratory syncytial virus (RSV) were considered as the priority pathogens in Burkina Faso (Figure 2). Among AMR targets, multidrug-resistant *Mycobacterium tuberculosis*, multidrug-resistant *Salmonella* spp., and extended-spectrum beta-lactam (ESBL) pathogens including carbapenem-resistant Enterobacteriaceae (*E. coli*, and *Klebsiella pneumoniae*) had the highest priority by respondents in all three countries (Figure 3). Based on local outbreak evidence, Burkina Faso survey used additionally multidrug-resistant *Streptococcus pneumoniae* as a predetermined option, where it was prioritized as high as the 3rd priority.

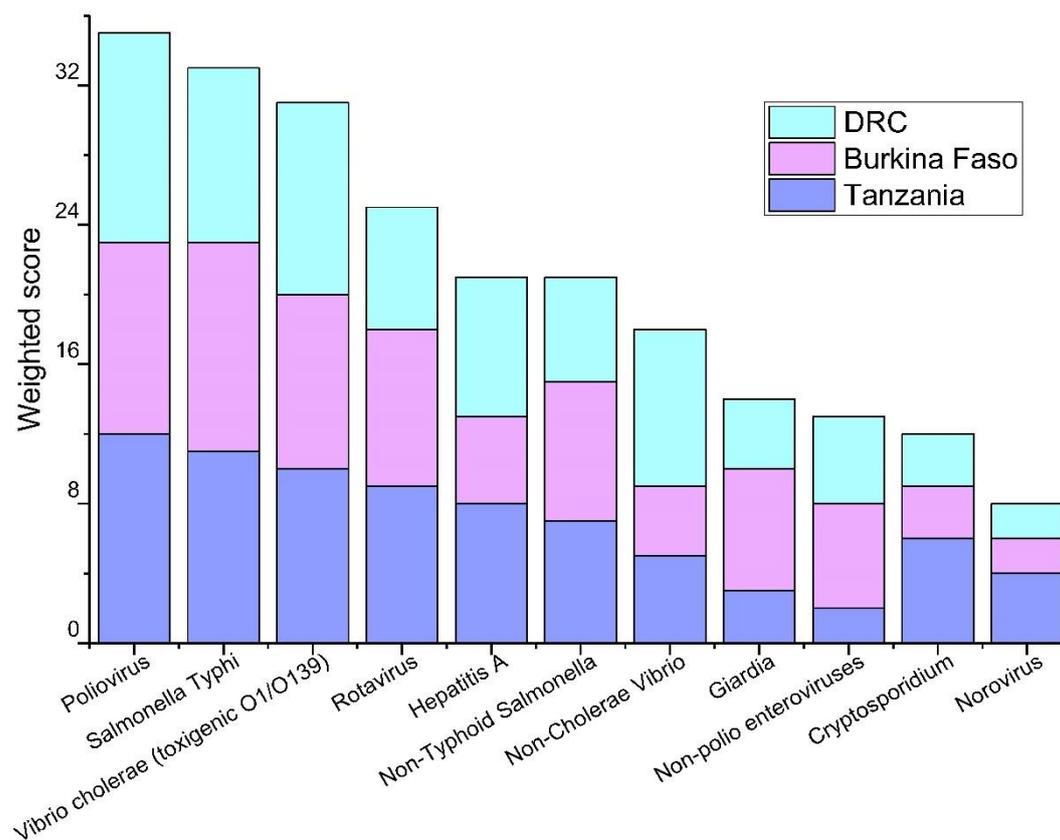


Figure 1. Priority order of waterborne pathogens from the Mentimeter.com survey among workshop participants (Tanzania N=22, Burkina Faso N=40, Democratic Republic of the Congo N=20). Following other waterborne pathogens were specified by the respondents as open end-question: (a) Tanzania (pathogenic *E. coli*, *Campylobacter* spp., *Shigella* spp., *Schistosoma*, harmful algal blooms, helminths); (b) Burkina Faso (pathogenic *E. coli*, *Campylobacter* spp., *Klebsiella pneumoniae*, *Shigella dysenteriae*, Amoebas, *Schistosomiasis*, helminths, Streptococci, *Entamoeba coli*); (c) the DRC (pathogenic *E. coli*, *Campylobacter* spp., *Shigella* spp., hepatitis A, Amoebas).

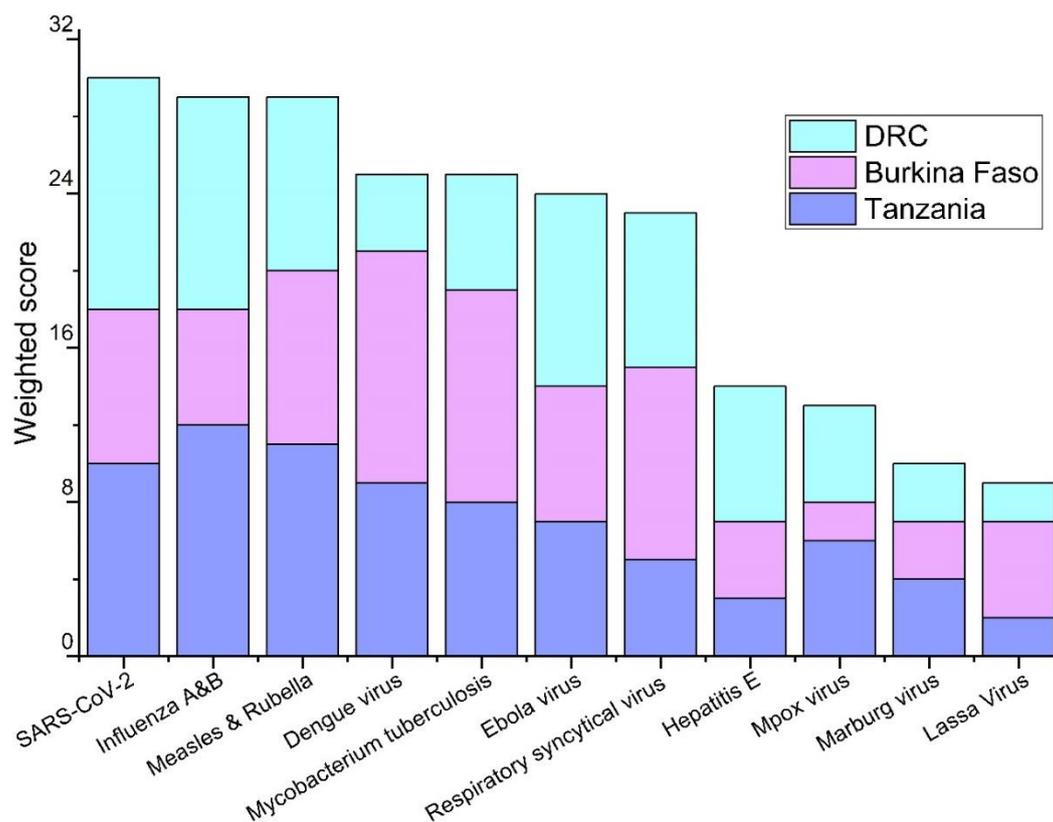


Figure 2. Priority order for other than waterborne pathogens in the Mentimeter.com survey among workshop participants (Tanzania N=19, Burkina Faso N=28, Democratic Republic of the Congo N=20). Respondents specified the following non-waterborne pathogens: (a) Tanzania: smallpox, tinea, plasmodium (malaria), aflatoxins, and rabies. (b) Burkina Faso: *Helicobacter pylori*, *Mycobacterium ulcerans*, *Bacillus anthracis*. (c) the DRC: Chikungunya virus, Arbovirus group.

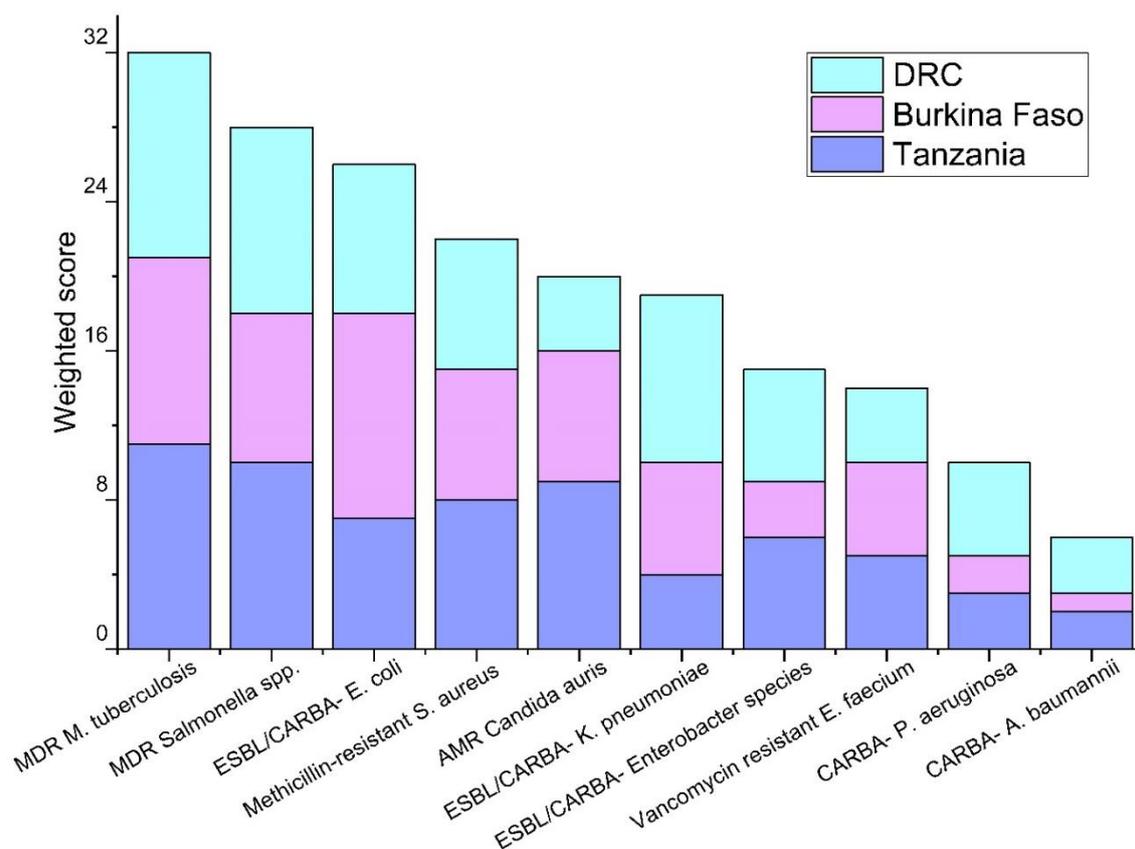


Figure 3. Prioritization of antimicrobial-resistant pathogens in the Mentimeter.com survey among workshop participants (Tanzania N=17, Burkina Faso N=27, Democratic Republic of the Congo N=20). Respondents specified the following AMR pathogens: (a) Tanzania: Drug-resistant viruses and fungi, *Neisseria gonorrhoeae*, MDR genes. (b) Burkina Faso: Drug-resistant HIV. (c) the DRC: Drug-resistant Malaria, clarithromycin-resistant *H. pylori*, fluoroquinolone-resistant *Campylobacter*, cephalosporin, and fluoroquinolone-resistant *N. gonorrhoea*. ESBL = extended-spectrum beta-lactamases. # Multidrug-resistant *Streptococcus pneumoniae* was included to the predetermined list of options in the survey in Burkina Faso and prioritized there as the third significant pathogen for WES.

3.2. Existing Clinical and Epidemiological Surveillance

The number of participants responding to the Webropol questionnaire in Tanzania's workshop was 20, 24 in Burkina Faso, and 16 in the DRC (Table 2). Notably, as most of the questions were optional, not all respondents answered every question. The response rates varied, and the percentage shows the lowest response rate with 90% (18/20) in Tanzania, 88% (21/24) in Burkina Faso, and 88% (14/16) in the DRC.

In all three countries, the respondents indicated that the primary approach for gathering clinical information involves various electronic systems like Integrated Disease Surveillance and Response (IDSR), Health Management Information System (HMIS), and Public Health Emergency Operations Centre (PHEOC) in Tanzania, DHSI2, Health data warehouse (EnDoS), Telegram weekly official letter (TLOH), and System for Tracking Epidemiological Data and Laboratory Specimens (STELaB) in Burkina Faso and demographic and health survey (DHSID), and national health information system (*Système National d'Information Sanitaire*, SNIS) in the DRC as the official methods for clinical data collection. DHSI2 is one of the largest health information management systems in the world, commonly used in LMICs (<https://dhis2.org/>). In the DRC, the reports are entered into the SNIS, the DRC's national DHIS2 platform for health information management, in the health zones (<https://dhis2.org/drc-data-use/>). Additionally, the option to gather information through Excel and paper-based registries was reported to be available in all these countries for cases where electronic systems are not feasible.

Considering the clinical diagnostic methods used within the clinical surveillance, all three countries acknowledged capacity for conventional PCR and quantitative PCR, virus culture, serology, microscopy, rapid diagnostic tests, bacterial culture, and Sanger and Next Generation Sequencing (NGS).

Table 2. Fields of expertise among the respondents of Webropol questionnaire for clinical and environmental surveillance themes.

Characteristic	Tanzania	Burkina Faso	DRC
	Number (%) Total Number of Respondents, N=20	Number (%) Total Number of Respondents, N=24	Number (%) Total Number of Respondents, N=16*
Clinical surveillance/epidemiologist	6 (30 %)	4 (17 %)	1 (6 %)
Environmental surveillance/water supply	6 (30 %)	3 (13 %)	3 (19 %)
Healthcare professional	2 (10 %)	9 (38 %)	3 (19 %)
International organization	1 (5 %)	2 (8 %)	-
Non-governmental organization (NGO)/civil society organization (CSO)	1 (5 %)	1 (4 %)	-
Researcher	3 (15 %)	4 (17 %)	5 (31 %)
Other	1 (5 %)	1 (4 %)	3 (19 %)

*For the DRC, one respondent did not define the field of expertise.

3.3. Existing Environmental Surveillance

All three countries operate an existing wastewater-based poliovirus surveillance system, managed by the Ministry of Health. Moreover, all three countries conduct limited AMR monitoring for academic research. In Tanzania, there is limited SARS-CoV-2 WES initiated in Dar es Salaam under the National Public Health Laboratory and in Burkina Faso respondents mentioned WES for other pathogens like enterovirus. Respondents from the DRC mentioned WES for SARS-CoV-2 and cholera. There was uncertainty among respondents about key WES practical aspects, such as sampling locations, frequency, analyzing laboratories, and sharing analyzed results. In Tanzania, the Ministry of Health manages an infectious disease database, which includes WES of poliovirus. In Burkina Faso, respondents did not specify entities for WES operations. The Institute of National Research Biomedical (INRB) is handling wastewater database in the DRC. For collecting supplementary environmental information, such as climatic data, the Tanzania Meteorological Authority (TMA) database and The Météo database maintained by the National Geographic Institute for the DRC were mentioned by multiple respondents. One respondent from Burkina Faso suggested Station Synoptique for such purposes in Burkina Faso.

3.4. Challenges and Limitations in the Current Surveillance Systems

The workshop participants were asked to prioritize the challenges and limitations within the current clinical surveillance systems. The limited laboratory infrastructure was ranked as the biggest challenge, followed by inadequate access to diagnostic tools (Figure 4). Open-ended responses from all three countries emphasized resource constraints, including a shortage of trained healthcare professionals, inadequate training, and insufficient financial resources. Communication issues between stakeholders and surveillance systems were mentioned, including concerns about the poor linkage between laboratory and surveillance data, and a lack of data sharing or linking among existing systems. In the DRC, ensuring the cold chain for laboratory samples was noted as a challenge. The identified major challenges in all three countries for detecting infectious disease outbreaks and

AMR spread were infrastructure deficiencies (e.g., laboratory resources), absence of electronic databases, and insufficient human resources. Open-ended responses for Tanzania and Burkina Faso underscored the importance of written regulations and policies during outbreaks.

There is limited existing WES system working in these countries, except wastewater-based poliovirus surveillance, so the respondents were asked to prioritize the major infrastructural weaknesses in their countries' current water systems to have information for creating the new WES. Limited sanitation and limited access to clean water supply were chosen as the top weaknesses. In the open-ended responses, respondents from all three countries emphasized the issue of limited coverage in the sewerage system, both in rural and urban settings, along with constrained laboratory capacity for analyzing environmental samples.

Considering the actual implementation of the new WES of infectious diseases and AMR, challenges for limited knowledge of opportunities in WES or on the environmental transmission of pathogens, as well as inadequate monitoring practices of water sources and environmental reservoirs, such as data or sample collection from the area, stood out among other options (Figure 5). The open-ended responses highlighted the same concern about limited awareness of WES, which can affect funding opportunities and resource allocation. Considering the limited laboratory capacity, concerns about the quality and management of the samples and data were mentioned. Also, as WES involves multiple stakeholders, including public health and environmental experts, concerns about the effective communication between the different entities were presented. Respondents from the DRC raised a concern about the lack of legal regulation in this new environmental surveillance field.

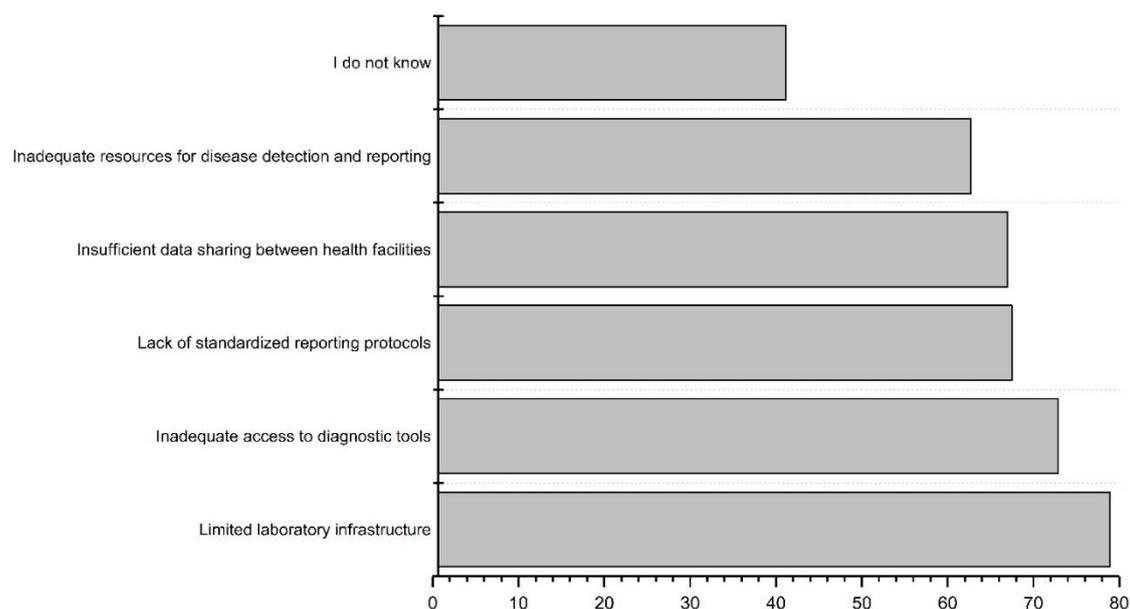


Figure 4. Perceptions of workshop participants on the identified gaps or challenges in the current clinical surveillance system in Tanzania, Burkina Faso and the DRC. The respondents selected five options in a prioritized order (1st, 2nd, 3rd, 4th and 5th). The responses were ranked with the scores: first priority received 0.9, second priority received 0.8, third priority received 0.7, fourth priority received 0.6, and fifth priority received 0.5. The total score for each option was then calculated by adding the individual scores.

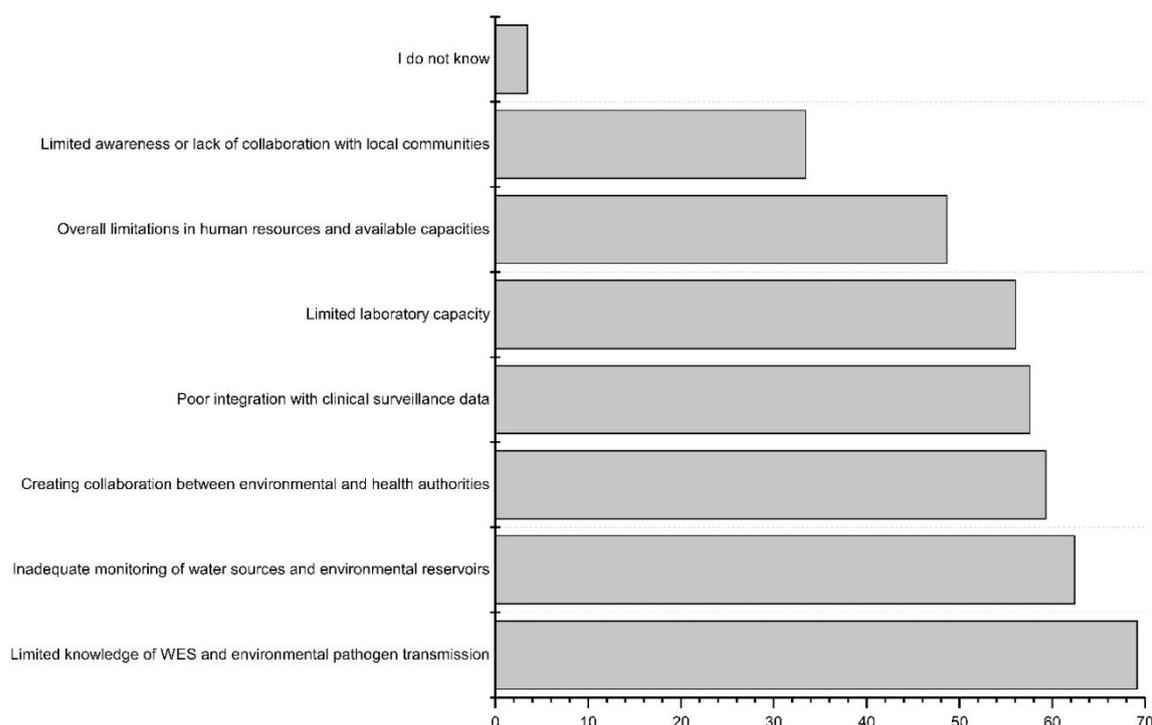


Figure 5. Perception of workshop participants on the major challenges in implementing WES of infectious diseases and AMR in Tanzania, Burkina Faso and the DRC. The respondents selected five options in a prioritized order (1st, 2nd, 3rd, 4th and 5th).

3.5. Potential of Wastewater and Environmental Surveillance Systems

The workshop participants were asked to prioritize the potential benefits of the new WES system (Figure 6). The foremost benefit, considered by respondents, was the early detection of outbreaks and data-driven epidemic response, with the potential for outbreak prevention through early warning. The second highest considered potential benefit was describing disease distribution, spread, trends, modality, and risk factors, followed by estimating community disease burden and providing early signals for focusing sentinel surveillance, clinical testing, or vaccination.

In the open-ended responses from Tanzania, it was emphasized that the increased awareness brought about by the planning of the new WES could lead to the growth of funding and resources, thus expanding the surveillance system network in sub-Saharan region. Additionally, the respondent from Tanzania underscored that environmental surveillance could provide more accurate data on AMR distribution within sub-Saharan Africa countries. In the responses from Burkina Faso, the knowledge of circulating environmental pathogens was seen as a potential benefit, as well as the research stimulated by the environmental surveillance topic.

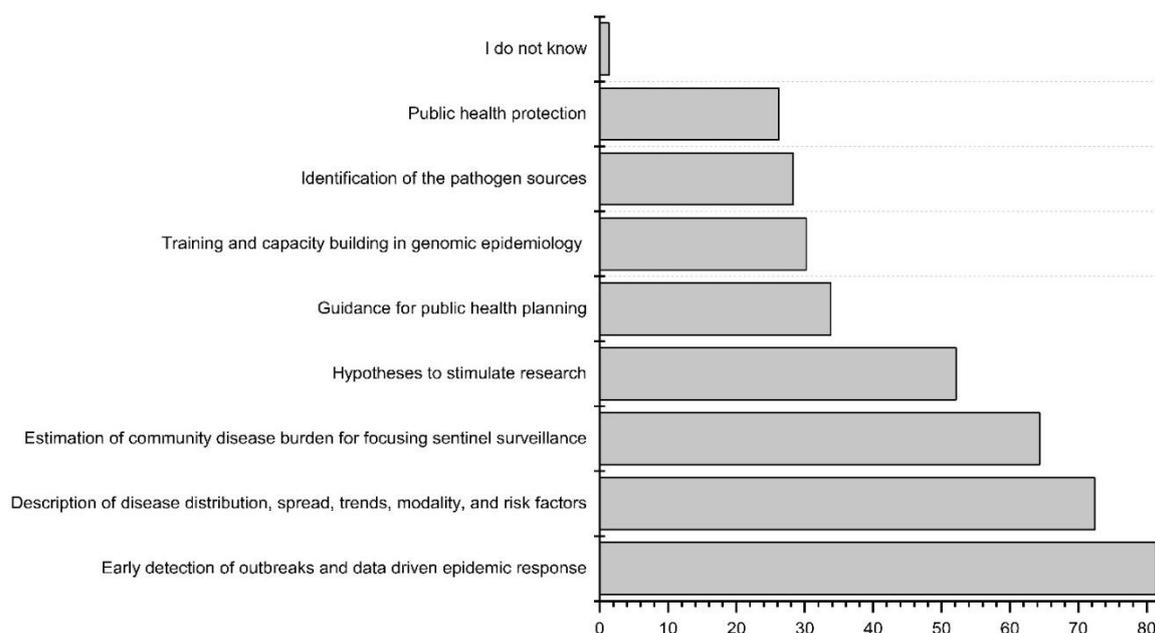


Figure 6. Perceptions of workshop participants on the potential benefits of WES in Tanzania, Burkina Faso and the DRC. The respondents were asked to select five options in a prioritized order (1st, 2nd, 3rd, 4th and 5th)". The responses were ranked with the following scores: first priority received 0.9, second priority received 0.8, third priority received 0.7, fourth priority received 0.6, and fifth priority received 0.5. The total score for each option was then calculated by adding the individual scores.

4. Discussion

This study evaluates for the first-time priority pathogens for environmental surveillance and examines existing epidemiological surveillance systems in three sub-Saharan countries: Tanzania, Burkina Faso and the DRC. Among waterborne pathogens, respondents from the three countries selected poliovirus, *Salmonella Typhi*, and *Vibrio cholerae* (toxigenic O1 and O139) as priorities for WES. As the survey included only a limited number of pre-chosen pathogens, also the open-ended responses were evaluated. In these responses, participants from all three countries noted the importance of pathogenic *E. coli* and *Campylobacter* spp. as waterborne pathogens to be monitored.

WES is an important component of the global poliovirus eradication program, and it has been extensively used [15,41–44]. WES of poliovirus evaluates the effectiveness of mass vaccination campaigns, and the detection of poliovirus in wastewater samples may trigger immediate mass vaccination programs in the community [41,44,45]. While the wastewater monitoring for the poliovirus eradication program has been widely implemented with significant progress in Africa, most countries have not yet tapped on the potential to establish a coordinated system for monitoring and surveillance of other priority pathogens to improve regional preparedness and response to disease outbreaks [44].

Salmonella Typhi [46–48], and toxigenic *Vibrio cholerae* (O1 and O139) [49–53], are major public health burdens in many LMIC including sub-Saharan African regions, and thus potential priority pathogens for WES. Due to the typhoid fever, an illness caused by *Salmonella Typhi*, as of 2019, WHO estimated that globally about nine-millions people are ill and about 110 000 people die annually [54]. Sub-Saharan Africa has one of the highest burdens of typhoid fever in the world with about 762 deaths per 100 000 population each year [55]. In Tanzania, annually about 79 thousand cases and about 1700 deaths due to typhoid fever were estimated [56]. Antimicrobial resistance on commonly

used antibiotics (amoxicillin, chloramphenicol, and trimethoprim-sulfamethoxazole), is a major concern connected with typhoid cases in recent years, up to 89% of *Salmonella* spp. isolates collected from typhoid patients were recorded resistant to antibiotics [56].

The actual global burden of toxigenic *Vibrio cholerae*, serotypes O1 and O139 known as causative agents for cholera, is often underestimated and underreported due to limited epidemiological surveillance and laboratory capacity, along with social, political, and economic disincentives for reporting [57,58]. As prioritized in the present study, WES of *V. cholerae* O1/O139 can be an effective way for estimating the actual burden at the population level [59–61]. From January 1, 2023, to December 18, 2023, there has been reported a total of 879,177 cholera cases, resulting in 5,045 deaths worldwide [50]. Cholera is still a serious public health concern in sub-Saharan regions, both Tanzania, Burkina Faso, and the DRC have reported cholera outbreaks in 2022 [53,62,63]. In 2023, a neighboring country Malawi, declared cholera outbreaks exacerbated by natural disasters (cyclones and floods), resulting in over 59,075 cases and 1,769 deaths as of October 2023 [64]. In October 2023, Zambia reported a new cholera outbreak that spread in many provinces, putting millions of people at risk [58,65]. Also a next East African country, Zimbabwe reported a cholera outbreak in February 2023, with a total number of 6,686 suspected and 1,127 confirmed cases confirmed by November 2023 [58]. The use of WES for monitoring of toxigenic *V. cholerae* is in the early stage of development [59–61], and is closely connected with ensuring the safe clean water supply, where together with monitoring of drinking water quality, also Water Safety Planning (WSP) could provide awareness and management tools for outbreak prevention and mitigation of the on-going outbreaks [66,67].

Diarrheal diseases pose a significant public health challenge in LMIC, notably contributing to child mortality (around 9% globally) [68]. The prevalence of pathogens in wastewater can be influenced by ongoing outbreaks. Simultaneously, multiple waterborne outbreaks may be occurring concurrently. In a recent study by Hugbo et al. (2023), fecal samples from 146 children (below age 5) with diarrhea were investigated in Moshi, Kilimanjaro region, Tanzania, for finding the causative pathogens [69]. Predominant clinical symptoms were diarrhea (100%), including vomiting (88.36%) and fever (60.27%). At least one diarrhea-associated pathogen was detected in 80.14% (n = 117) of the children. Major diarrhea-causing pathogens were rotavirus 38.36% (n = 56), adenovirus 40/41 19.86% (n = 29), *Shigella*/EIEC 12.33% (n = 18), norovirus GII 11.44% (n = 17), and *Cryptosporidium* 9.59% (n = 14) [69]. In the same way as by studying a huge number of fecal samples from individuals, the use of WES can provide already from a couple of samples representative information of pathogens circulating in the communities. However, the WES samples do not provide information to be used for patient care, as the WES sampling sites represent the population level with a certain coverage depending on the sampling site characteristics.

For pathogens other than the waterborne transmission route, respondents in this study prioritized SARS-CoV-2, influenza A&B, measles and rubella, and *Mycobacterium tuberculosis*. Of these, the existing use cases of WES have already been reported for SARS-CoV-2 [70–74], influenza A&B [75–78], and dengue virus [33,79,80]. Measles and rubella, viral diseases, transmitted through respiratory and airborne droplets, are relatively less often monitored in wastewater [15,80]. Many infection cases of both measles and rubella may go asymptomatic, and these are detected in urine, so these have high potentiality to be monitored through WES. Rubella is often mild; up to 50% of cases exhibit symptoms [81]. Measles and rubella viruses have not achieved a 100% vaccination rate in developing countries, which holds significant practical implications for WES. Further, TB is the ninth leading cause of death globally and over 25% of TB deaths occur in the African Region [82]. The emergence of multidrug-resistant TB (MDR-TB) poses a significant health security threat and jeopardizes progress made in the fight against TB. TB in wastewater is monitored earlier [83].

Among the group of antimicrobial-resistant pathogens, respondents prioritized multidrug-resistant *Mycobacterium tuberculosis* and *Salmonella* spp., and extended spectrum beta lactamase (ESBL) and carbapenemase-producing *E. coli* as being potentially important to be monitored through WES. Multidrug-resistant *S. pneumoniae* was exclusively listed in Burkina Faso's workshop and was ranked there among the top three prioritized AMR pathogens for WES. Antimicrobial resistance poses a serious challenge in LMIC, the situation is exacerbated by poor sanitation [3,84]. Poor

sanitation practices contribute to the proliferation of pathogens, that need huge amounts of antibiotics for treatment, and ultimately create selective pressure for the development of AMR pathogens [3]. The synergy between AMR and poor sanitation is particularly concerning, as developing countries often face limited resources, healthcare infrastructure, and education on proper use of antibiotics [85–87]. This combination increases the risk of infectious diseases, hampers effective treatment, and compromises public health outcomes. WES can be an effective way for monitoring the situation of AMR in regional and local levels [27,85–87].

4.1. Current Clinical Surveillance

Disease surveillance involves four essential steps: specimen collection, analysis, result dissemination to stakeholders, and taking appropriate public health actions [15]. Each of the three countries has a three-level of hospital system: (a) primary level, consisting of dispensary health centers and district hospitals, (b) secondary level, comprising regional referral hospitals, and (c) tertiary level, encompassing zone hospitals and national hospitals. Reference laboratories collect and confirm specimens for major pathogens like yellow fever, tuberculosis, antimicrobial resistance, and typhoid fever. Reference laboratories play a pivotal role in collecting and providing data for reporting, requiring active involvement from clinicians and epidemiologists.

Presently, electronic laboratory information-sharing systems are partially employed in urban hospitals with adequate facilities. In all three countries, the respondents revealed that the primary approach for gathering clinical information involves electronic systems based on DHIS2 (<https://dhis2.org/>). DHIS2 has been used globally at least in 80 countries, impacting 3.2 billion individuals (40% of the world's population), including many African countries. However, the prevalence of these modern electronic and automated data collection systems has not yet been fully adopted. Android phone-based apps, viper and WhatsApp could have been widely used for communicating health-related data across different units in these countries.

Health information systems in many developing countries face challenges, whether due to insufficient infrastructure, trained manpower, or lack of hundred percent electrification [88]. Consequently, information may not always be reported uniformly across all health facilities, with comprehension gaps evident at the community and health center levels [88]. The workshop participants of this study reported the continued use of paper-based registries and Excel sheets, along with telephone reporting, indicating that these traditional methods persist.

Clinical surveillance is a continuous process for systematically gathering, analyzing, interpreting, and providing feedback on outcome-specific data. In all these countries (Tanzania, Burkina Faso and the DRC), a central body such as the Ministry of Health or the Prime Minister's office oversees the administration of the surveillance system. This includes revising priority pathogens, formulating policies, and collecting data. The African CDC, WHO, and West African Health Organization (WAHO) play a crucial role in providing guidelines to ensure harmonization of surveillance systems at an international level.

In Tanzania, policy guidelines for clinical reporting are governed by the Public Health Act 2009 [89], and Tanzania Personal Data Protection Law 2022 [90]. Key stakeholders in clinical surveillance systems include the Ministry of Health, the Prime Minister's Office- Disasters Management Department (DMD), and local district and regional health management teams and hospitals. In Burkina Faso, the Central National Laboratory, overseen by the Ministry of Health, supervises clinical laboratories and conducts external quality assessments for selected pathogen surveillance. In the DRC, key stakeholders in pathogen surveillance are the Ministry of Health (MoH), local private sector, head physicians in health districts, and professionals at various levels, including the providential chef and Head of Direction Générale de Lutte contre la Maladie. All national hospitals and reference laboratories are interconnected through the Integrated Disease Surveillance and Response (IDSR) framework, guided by the National Plan de Développement Sanitaire as the governing legislation for pathogen surveillance and waterborne outbreak monitoring. While both public and private hospitals in districts are mandated to utilize DHIS2 for patient data management, there exist

significant gaps in the system's implementation. These challenges impede seamless integration and hinder the optimal utilization of DHIS2 across healthcare facilities.

Despite having infrastructure and disease surveillance systems, highlighted in the responses to the survey conducted during this study, challenges persist due to resource constraints, incomplete electrification, frequent power cuts, and, in some cases, a shortage of skilled human resources. Also, the workshop results highlighted limited resources, including both laboratory infrastructure and human resources, as the main challenges. This emphasizes the necessity for a thorough examination of these aspects in the development of new surveillance systems. This could be facilitated by engaging experts and infrastructures provided by The Africa Center for Disease Control and Prevention (African CDC) that is a regional organization in Africa contributing to the surveillance of pathogens and antimicrobial resistance and responding to outbreaks (<https://africacdc.org/>). The primary mission of the African CDC is to ensure public health across the African continent by collaborating with member states to address health challenges and emergencies. Harmonizing disease control and prevention policies and surveillance systems among member states is considered as a key aspect for implementing successful public health actions.

4.2. Wastewater and Environmental Surveillance

The workshop outcomes reveal that Tanzania, Burkina Faso, and the DRC have each implemented WES to some extent, with a specific focus on poliovirus surveillance. Respondents showed limited awareness of alternative wastewater surveillance initiatives in their countries, reflecting a general lack of knowledge about environmental surveillance opportunities. This underscores the necessity for increased awareness on this subject. Despite this, the recognition of environmental surveillance's potential, especially as an early warning system for outbreak detection and in estimating community disease burden through continuous surveillance, indicates a promising basis for initiating a new environmental surveillance system.

WES have been used for monitoring waterborne, respiratory, multidrug-resistant, and many other pathogens [10,15,19,86,91–94]. However, the choice of pathogens for WES is influenced by their epidemiological relevance, microbiological evidence, and practical feasibility. The surveillance outcomes should be able to trigger an available public health action that could be implemented to mitigate the adverse health effects. Similarly, a prerequisite condition for an effective WES of a pathogen is that the pathogen needs to have a consistent shedding rate to the sewage system from infected individuals. In many nations, WES of health-related pathogens and AMR primarily emphasizes water quality and public health risks assessment. For example, the revised Urban Wastewater Treatment Directive (UWWTD) of the European Union, in Article 17, includes a provision for urban wastewater surveillance [25]. Member States are mandated to establish a national system facilitating collaboration between health authorities and wastewater treatment authorities. This system aims to identify critical public health parameters, such as SARS-CoV-2 and its variants, poliovirus, influenza virus, emerging pathogens, and any other relevant factors, to be monitored at wastewater treatment plants, primarily in wastewater inlets [25].

Various human enteric pathogens such as adenovirus, enterovirus, norovirus, and rotavirus, contribute to diarrheal diseases, often undiagnosed based solely on symptoms [68,95]. As many infections are asymptomatic or mild, clinical testing is typically sought only in severe conditions. Similarly, diseases like ebola virus, malaria, cholera, typhoid fever, meningitis, and viral hemorrhagic fevers share clinical symptoms like fever, sore throat, muscle pain, headaches, vomiting, and diarrhea [96]. Also, three common arboviruses; dengue, zika virus, and chikungunya share similar primary clinical symptoms such as fever, vomiting, severe headache, and muscle and joint pains [79,97]. Theoretically, a single wastewater test can confirm the actual agent causing the outbreak and can assist in identifying circulating pathogens in the community. Integrating genomics approaches to WES further allows even more specific implementation of effective monitoring and surveillance strategies and identification of multiple disease pathogens and its evolution, variants of concern, AMR and other emerging infectious threats.

However, establishing WES schemes in developing countries remains challenging also due to lack of centralized sewage systems. Besides wastewater, monitoring hospital wastewater and urban rivers can also provide important sampling materials for environmental surveillance [33,98–100]. Because of the gaps and challenges in implementing effective clinical surveillance systems and effective diagnostic approaches, many disease outbreaks can be highly underreported, based on current surveillance approaches [47,48]. Clinical surveillance may not account for cases if individuals do not seek testing or lack access, more likely in many low and middle-income countries, where universal health insurance is not practiced, and testing facilities are scanty. Thus, the combination of environmental surveillance with the current clinical surveillance system may comprehensively account for the spread of pathogens at a community level.

A major implication of current study is its direct benefit for gaining the Sustainable Development Goals (SDGs) [101]. A previous study indicated that WES for pathogens contributes to more than half of the 17 UN Sustainable Development Goals (SDGs) in the 2030 agenda [39]. It plays a vital role in disease prevention, water quality assessment, and sanitation improvement—integral elements of sustainable development, addressing social, economic, and environmental challenges. There is growing evidence suggesting a decrease in pathogens incidence, coinciding with the profound impacts on poverty alleviation, economic development of a nation, and overall social and infrastructure development. Waterborne diseases pose a significant global health threat, exerting a substantial toll on morbidity, mortality, and economic well-being.

4.3. Challenges and Future Directions in WES

Wastewater surveillance, though promising, faces significant developmental challenges, requiring additional empirical evidence to enhance its practicality. Respondents primarily considered deficiencies in infrastructure, coordination gap between WES authorities and epidemiologists, and a lack of human resources as major challenges for WES. In broader context, currently, standard monitoring procedures for numerous pathogens, including wastewater sample collection, population sampling coverage of sample sites, methods for target concentration, nucleic acid extraction, and data interpretation, are lacking [14,77,102,103]. However, certain pathogens, such as SARS-CoV-2 and poliovirus, are more frequently studied in WES, accumulating practical experiences and evidence compared to other pathogens [15]. Still, each pathogen's distinct characteristics, such as distribution in wastewater, epidemiology, shedding rate in clinical individuals, and fate and decay rate in the ambient environment, demand specific contexts for sample collection, analysis, and result interpretation [14,77,102,103]. Respondents primarily considered deficiencies in infrastructure, coordination gap between WES authorities and epidemiologists, and a lack of human resources as major challenges for WES.

One interpretation challenge might arise from the use of live-attenuated vaccines, as observed especially with poliovirus and rotavirus vaccines, which have been linked to enhanced virus shedding in wastewater [104]. Further, data on pathogen loads in symptomatic and asymptomatic carriers of many pathogens by various individuals, pathogen persistence, fate, and decay kinetics in wastewater and in the environment remain mostly unknown. Understanding that such gaps in data occur is crucial for interpreting WES results. Various factors, such as infection rate, detection limits of the laboratory methods, representativeness and population coverage of the selected WES sampling locations, water flow characteristics, wastewater matrix complexity, and viral shedding, influence the detection of microbial targets in wastewater and from other environmental samples. The persistence of many pathogens in the ambient environment needs more information. A low signal may be attributed to a limited number of cases, underscoring the importance of estimating the minimum number of infected individuals required to detect viral markers in sewage systems. Lastly, ethical considerations, including cultural and community values, are essential before disseminating results to avoid the potential stigmatization of sampled communities.

4.4. One Health Perspective for Waterborne Pathogens

Many of the pathogens included in the current survey for example, bacterial pathogens (e.g. *Salmonella* spp., pathogenic *E. coli*, *Campylobacter* spp.), protozoan parasites (e.g. *Cryptosporidium* spp., *Giardia* spp.) and other pathogens shedding antimicrobial resistance are zoonotic, i.e. infecting both humans and other animal species [105–109]. WES for zoonotic pathogens may require a comprehensive One Health approach in designing the surveillance scheme, interpreting results, and planning management actions [38,86,110]. Some zoonotic pathogens identified through WES could be originated from animal sources. If these pathogens escape into the environment from the wastewater environment, subsequently infecting animal hosts, there is a potential for them to circulate back into human hosts, perpetuating the chain. The monitoring of pathogens in wastewater and environmental waters has potential to reveal deficiencies in the sanitation systems and raise awareness of fecal pathogen distribution and thus could lead to significant public health interventions.

The risks and impact of pathogens depend on factors like persistence, prevalence, dose, invasiveness, virulence, and an individual's immune status, leading to acute and chronic health consequences. Infections can be asymptomatic, with the proportion of carriers varying based on pathogen and population characteristics. Even asymptomatic individuals can contribute to pathogen transmission. Integrated risk management, involving safeguarding drinking water sources, optimizing drinking treatment processes, and effectively managing drinking water distribution systems, is crucial to prevent waterborne outbreaks [111]. Environmental surveillance of pathogens is vital for protecting drinking water sources and managing outbreaks.

4.5. Limitation of Current Study

The list of pathogens designed for multiple-choice questionnaires for the workshops relied on microbiological evidence and the epidemiological severity of various pathogens. However, biases in the results gained may arise due to the predetermined lists of pathogens and other answering options provided. Respondent biases could also be present; for example, perceptions of a pathogen may be influenced by its seasonality. Visibility in the media can also shape perceptions of the stakeholders; for example, SARS-CoV-2 received extensive attention during the COVID-19 pandemic, potentially affecting the prioritization. Existing surveillance programs and personal experience may also influence responses, e.g., a poliovirus expert might more likely nominate this pathogen as a priority without further thought. Personal stigma may cause hesitation; for instance, Mpox has been widely communicated as a gay-related disease, and may have caused hesitation in mentioning it [14,28]. Pathogens vary in microbiology, incubation period, outbreak progression, infectivity, and fatality rates. Consequently, interpreting pathogen monitoring in wastewater requires careful consideration.

5. Conclusions

This study highlights the importance of customized WES systems in three sub-Saharan countries (Tanzania, Burkina Faso, and the DRC), emphasizing localized approaches for effective monitoring of waterborne pathogens and AMR. Based on various surveys and existing knowledge on pathogens, this study evaluates priority pathogens for WES including, poliovirus, *Salmonella Typhi*, *Vibrio cholerae*, influenza A&B, measles and rubella, SARS-CoV-2, and *Mycobacterium tuberculosis*. Recommended AMR pathogens include drug-resistant *Mycobacterium tuberculosis*, *Salmonella* spp., and extended spectrum beta lactamase (ESBL) and carbapenemase-producing *E. coli*. However, many factors could affect practical use of these pathogens for WES, majorly microbiological evidence and practical feasibility.

Currently these countries employ centralized electronic systems for clinical data collection, complemented by Excel and paper-based registries. However, WES can be a valuable tool for early detection of locally circulating human-derived pathogens, aiding in outbreak detection, data-driven epidemic response, and timely intervention. Integrating WES with the current disease surveillance system can be crucial for monitoring waterborne pathogens, respiratory pathogens, and pathogens with AMR. The WES results can be also important for measuring effectiveness of existing sanitation practices, and safeguarding human, animal, and environmental health. Thus, WES can be an

important tool for risk management, reducing waterborne outbreaks, and promptly achieving UN Sustainable Development Goals.

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