Original Paper

Establishing Antimicrobial Resistance Surveillance in the Water and Environment Sector in a Resource-Limited Setting: Methodical Qualitative and Quantitative Description of Uganda's Experience From 2021 to 2023

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Abstract

Background: Antimicrobial irrational use and poor disposal in the human and animal sectors promote antimicrobial resistance (AMR) in the environment as these antimicrobials and their active ingredients, coupled with resistant microbes, are released into the environment. While AMR containment programs in the human and animal sectors are well established in Uganda, those in the water and environment sector still need to be established and strengthened. Therefore, the Ministry of Water and Environment set out to establish an AMR surveillance program to bolster the One Health efforts for the containment of AMR under the National Action Plan 2018-2023.

Objective: This study aims to describe Uganda's experience in establishing AMR surveillance in the water and environment sector.

Methods: A methodical qualitative and quantitative description of the steps undertaken between August 2021 and March 2023 to establish an AMR surveillance system in the water and environment sector is provided. The Uganda Ministry of Water and Environment used a stepwise approach. Governance structures were streamlined, and sector-specific AMR surveillance guiding documents were developed, pretested, and rolled out. The National Water Quality Reference Laboratory infrastructure and microbiology capacity were enhanced to aid AMR detection and surveillance using conventional culture-based methods. A passive and targeted active surveillance hybrid was used to generate AMR data. Passive surveillance used remnants of water samples

collected routinely for water quality monitoring while targeted active surveys were done at selected sites around the Kampala and Wakiso districts. Excel and Stata 15 statistical software were used for data analysis.

Results: A sector-specific technical working group of 10 members and focal persons is in place, providing strategic direction and linkage to the national AMR surveillance program. The National Water Quality Reference Laboratory is now at biosafety level 2 and conducting microbiology testing using conventional culture-based techniques. Up to 460 water samples were processed and 602 bacterial isolates were recovered, of which 399 (66.3%) and 203 (33.7%) were priority pathogens and nonpriority pathogens, respectively. Of the 399 priority pathogens, 156 (39.1%), 140 (35.1%), 96 (24.1%), and 7 (1.8%) were *Escherichia coli*, *Klebsiella* species, *Enterococcus* species, and *Salmonella* species, respectively. *E coli* showed resistance to ampicillin (79%), ciprofloxacin (29%), and ceftriaxone (29%). Similarly, *Klebsiella* species showed resistance to ampicillin (100%), ciprofloxacin (17%), and ceftriaxone (18%). *Enterococcus* species showed resistance to ciprofloxacin (52%), vancomycin (45%), and erythromycin (56%). Up to 254 (63.7%) of the priority pathogens recovered exhibited multiple and extensive resistance to the different antibiotics set.

Conclusions: Initial efforts to establish and implement AMR surveillance in the water and environment sector have succeeded in streamlining governance and laboratory systems to generate AMR data using conventional culture-based methods.

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KEYWORDS

antimicrobial resistance; surveillance system; water and environment sector

Introduction

Antimicrobial resistance (AMR) occurs when microorganisms survive after exposure to antimicrobials that would normally kill them, inhibit their metabolism, or stop their growth [1]. This also includes antibiotic resistance in bacteria [2]. As a result, antibiotics become ineffective against disease-causing bacteria, leading to AMR evolving into a silent pandemic [2,3], which is projected to account for over 10 million human deaths by 2050 [3]. This imparts a substantial financial burden on the health care system [4]. AMR is a "One Health" issue, highlighting the complex interconnectedness between the health and well-being of animals, people, plants, and their shared environment [5]. This results from the transmission of resistant microorganisms, their genes, or mobile genetic elements between these compartments [6]. Thus, making AMR one of the top threats to global health, with increasing trends in resistant infections in humans and animals, tending toward a postantibiotic era [7].

Globally, AMR in the environment has been a neglected issue whose public health importance, burden, and implication are yet to be explored comprehensively [8], especially in resource-limited settings [9]. Over the past decades, global health research has increasingly shown that the environment plays a key role in the proliferation and exacerbation of AMR and its effects [9-12]. Most antimicrobials used in humans and animals are excreted or indiscriminately disposed of into the environment in their raw or active forms [13]. Sublethal levels of antimicrobials, contaminants, and resistant bacteria in effluents from pharmaceutical industries, households, agricultural runoffs, and health care settings are released into the environment [3,14]. This creates an unnatural selective pressure in the environment [3,15] that, coupled with direct contact between natural bacterial communities and the discharged resistant bacteria, drives the evolution, selection, and emergence of resistant strains within the environment [3,13,16].

In Uganda, AMR surveillance and containment efforts have mainly focused on the human and animal sectors [17], partly due to the lack of a structured surveillance program with consensus on standard methodologies and targets in the water and environment sector on both the national and global scale [7]. Currently, there is no benchmarking or threshold data in the sector to inform epidemiological, evolutionary, and risk modeling efforts [7,12]. The Uganda Ministry of Water and Environment (MWE) and its partners, therefore, undertook efforts to establish an AMR surveillance program in the water and environment sector to bolster a One Health approach in curbing AMR, guided by the AMR National Action Plan 2018-2023. Instituting AMR surveillance in the sector was done in cognizance of the country's environmental concerns, local contexts, and a structured framework [13,18-20]. We, therefore, present Uganda's experience in establishing an AMR surveillance program and the emerging data on the status of AMR in the environment sector between August 2021 and March 2023.

Methods

Overview

A methodical qualitative and quantitative description of the steps undertaken between August 2021 and March 2023 to establish an AMR surveillance system in the water and environment sector is provided. Several surveillance documents, guidelines, and reports were reviewed to collect data appropriately aligned to AMR surveillance in a low-resource setting [21]. The description below provides the steps (processes and methods) undertaken to set up the program.

AMR Governance Establishment and Enhancement

The Government of Uganda, through the MWE, with support from the Infectious Diseases Institute at Makerere University through the Fleming Fund Country Grant 2 project, instituted a stepwise approach with incremental targets and sequential phases from August 2021. This involved establishing a

foundation, consolidating and refining gains, scaling up, and further expanding the surveillance system.

A 10-member sector-specific AMR technical working group (TWG) with focal persons was established to coordinate the AMR containment efforts, including surveillance activities in the sector. The TWG developed AMR surveillance documents (plan, protocol, and standard operating procedures) focused on the monitoring of priority environmental bacteria (*Escherichiacoli*, *Klebsiella* species, *Enterococcus* species, and *Salmonella* species) in water samples. These documents were aligned to the different international and national guidelines [22-25]. The National Water Quality Reference Laboratory (NWQRL) was identified as the sentinel site for AMR surveillance in the sector using conventional culture-based techniques. One sample type (water samples) was also designated for the initial AMR surveillance efforts. The TWG also reviewed the generated AMR data to inform AMR programming and policy formulation in the sector.

Enhancement of the Microbiology Capacity of the NWQRL

The NWQRL microbiology section was equipped and its infrastructure was enhanced. The human resource capacity was enhanced through in-service microbiology, biosecurity and biosafety, and laboratory quality management system trainings and mentorships. This capacity enhancement was done through a One Health approach, leveraging the established AMR surveillance capacity in the human and animal sectors. The NWQRL was also enrolled in the national laboratory external quality assessment and proficiency testing scheme of the Uganda National Health Laboratory and Diagnostic Services to enhance the microbiology testing quality.

Pretest and Rollout of the AMR Surveillance Documents

The developed sector-specific AMR surveillance documents were pretested through an active survey. This involved the collection of samples from the Kampala-Wakiso region and analyzing them at the NWQRL. A total of 9 strategic surface water (nonpoint sources) and wastewater (point sources) sampling sites were identified in Kampala and Wakiso, and 15 grab samples were collected using the standard procedures as stipulated in the different surveillance documents. The samples were transported to the NWQRL under appropriate conditions and analyzed using standard, conventional, culture-based procedures. The lessons learned during the pretest were used to refine the surveillance documents.

The documents were then rolled out to generate AMR surveillance data through a hybrid of passive and active surveillance. The initial efforts focused on nonpoint sources or surface water sources (rivers, streams, and other open channels), drinking water (national water grid and other potable water), and point sources or wastewater (sewer, wastewater treatment plants and ponds, and septic tanks) samples. Passive surveillance used remnants of samples routinely referred from across the country to the NWQRL for water quality monitoring. Active surveillance used samples collected through quarterly targeted surveys from strategic sites (shown in the map provided in Multimedia Appendix 1) in the Kampala-Wakiso region as stipulated in the surveillance protocol. This region was chosen because it has vast human economic activities that involve the intense use of antibiotics and thus provided an ideal setting for integrated AMR surveillance in the sector.

Conventional culture-based bacteriology techniques were used for the enumeration, isolation, and identification of priority bacteria. Water sample enrichment was done by inoculating 2-5 ml of the sample into 10 ml of brain heart infusion and incubating for 16-18 hours at 37 °C. Following the enrichment, culture media and biochemical tests were used to isolate and identify the bacteria.

For *E coli* and *Klebsiella* species, MacConkey agar with crystal violet was used for the primary culture and purity plating. The distinct colonies (*E coli*: pink/red lactose-fermenting colonies; *Klebsiella* species: pink-yellow mucoid lactose-fermenting colonies) that grew after 18-24 hours of incubation at 37 °C were subjected to Gram stain (microscopy) and biochemical tests including oxidase, urea, citrate, triple sugar iron, and sulfide indole motility tests using standard procedures.

For *Salmonella* species, MacConkey agar with crystal violet was used for the primary culture. The distinct colonies (colorless colonies) that grew after 18-24 hours of incubation at 37 °C were purity plated on xylose lysine deoxycholate media. The distinct colonies (red colonies with black centers) that grew after 18-24 hours of incubation at 37 °C were subjected to Gram stain and biochemical tests including oxidase, urea, citrate, triple sugar iron, and sulfide indole motility tests using standard procedures. For *Enterococcus* species, Slanetz and Bartley agar was used for the primary culture. The distinct colonies (red or purple colonies) that grew after 18-24 hours of incubation at 37 °C were purity plated on 5% sheep blood agar media. The distinct colonies (white or gray colonies) that grew after incubation were subjected to Gram stain (microscopy) and biochemical tests including catalase and bile esculin tests.

Other than the priority bacterial isolates, we also recovered other bacteria species including *Citrobacter freundii*, *Enterobacter*, *Pseudomonas*, *Proteus*, *Providencia*, and *Raoultella* species.

AMR Data Generation

Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method with the appropriate isolate-antibiotic combinations and techniques as stipulated in the 31st edition of the Clinical and Laboratory Standards Institute guidelines [26]. Penicillins, fluoroquinolones, cephalosporins, aminoglycosides, carbapenems, glycopeptides, macrolides, oxazolidinones, folates (sulphonamide-trimethoprim), tetracycline, and phenicols were the antibiotic classes considered. Isolates were cryopreserved in a 20% glycerol and brain heart infusion and archived at the Uganda National Biorepository at the Central Public Health Laboratories and National Animal Diseases Diagnostics and Epidemiology Center laboratory at –80 °C.

Data Analysis

Excel 2016 (Microsoft Corporation) and Stata 16 (StataCorp) were used for data entry, cleaning, and analysis. The percentage

resistance of the isolates to each antibiotic was generated and visuals (charts and graphs) were developed. The chi-square test and binary logistic regression were used to test whether the resistance of the priority pathogens (*Ecoli*, *Klebsiella*, and *Enterococcus* species) to the different antibiotics was significantly different across the point and nonpoint sources. A *P* value <.05 indicated a significant statistical difference.

Ethical Statement

The work reported here is part of the Uganda National AMR surveillance program that was approved by the National AMR Sub-Committee of the National One Health Platform.

Results

Streamlining of AMR Governance in the Water and Environment Sector

The sector-specific TWG was constituted and comprised of 10 members from the three directorates of the MWE, including the directorates of water resources management, water development, and environmental affairs. Focal persons for AMR surveillance in the sector were identified. The TWG finalized the sector surveillance documents that stipulate the target priority pathogens and sample type, designated the NWQRL as the sentinel site, designed the routine and targeted surveys, and reviewed and reported the AMR data generated to the MWE. The TWG also published a report on the AMR burden in the sector in the annual Natural Resources, Environment, Climate Change, Land and Water Management Program Performance Report 2022 [27]. Further, the TWG functioned as a linkage for the sector surveillance program to the national AMR surveillance program under the National AMR Sub-Committee of the National One Health Platform, the human and animal sector-specific AMR surveillance programs, the academia, and implementing partners.

Microbiology Laboratory Capacity Enhancement in the Sector

Following the infrastructural and equipment enhancement, the NWQRL now houses a fully-fledged biosafety level 2 microbiology laboratory, the minimum level required for AMR surveillance. The laboratory has staff trained in microbiology, including antimicrobial susceptibility testing, biosafety biosecurity, AMR data management, and laboratory quality management systems. These staff have supported the laboratory analysis of environmental samples to generate AMR data.

Bacterial Isolates Recovered

Up to 460 samples were collected and processed at the NWQRL between August 2021 and March 2023, of which 363 (78.9%) were from passive surveillance while 97 (21.1%) were from active surveillance. Of the 460 samples, 158 (34.3%) were from point sources (wastewater samples), while 284 (61.7%) were from nonpoint sources: 108 (23.5%) ground, 95 (20.7%) surface, and 81 (17.6%) drinking water samples. Up to 328 (71.3%) samples had significant growth and yielded 602 bacterial isolates, of which, 399 (66.3%) and 203 (33.7%) were priority and nonpriority pathogens, respectively. Of the 602 isolates, 223 (37%), 166 (27.6%), 132 (21.9%), and 67 (11.1%) were from waste, surface, ground, and drinking water sources, respectively. Of the 602 isolates, 399 (66.3%) were priority pathogens: 156 (39.1%) *E coli*, 140 (35.1%) *Klebsiella* species, 96 (24.1%) *Enterococcus* species, and 7 (1.8%) *Salmonella* species. The 203 nonpriority isolates were *Citrobacter* species (n=57, 28.1%), *Enterobacter* species (n=52, 25.6%), *Proteus* species (n=23, 11.3%), *Pseudomonas* species (n=19, 9.4%), *Acinetobacter* species (n=8, 3.9%), and other bacterial species (n=44, 21.7%).

The Burden of AMR in the Water and Environment Sector

E coli (n=156) had a resistance of 79% to ampicillin, 55% to trimethoprim/sulfamethoxazole, 29% to ceftriaxone and ciprofloxacin, 18% to cefepime and chloramphenicol, 11% to imipenem, and 0% to amikacin and meropenem. Other antibiotic resistance is shown in Table 1.

Klebsiella species isolates (n=140) had a resistance of 100% to ampicillin, 33% to trimethoprim/sulfamethoxazole, 28% to amoxicillin-clavulanate, 27% to cefuroxime, 17% to ciprofloxacin, 2% to imipenem, and 0% to amikacin. *Salmonella* species isolates (n=7) had a resistance of over 50% to ampicillin, ciprofloxacin, ceftriaxone, trimethoprim/sulfamethoxazole, and tetracycline. *Enterococcus* species (n=96) had a resistance of 45% to vancomycin, 56% to erythromycin, 54% to tetracycline, and 52% to ciprofloxacin (Table 1).

Overall, there was no significant difference between the resistance observed in *E coli* and *Klebsiella* species isolates recovered from point and nonpoint sources. Among the *Enterococcus* species isolates, a significant difference (odds ratio 5.318182, 95% CI 1.793498-15.76977; *P*=.003) was observed in the resistance to chloramphenicol between the isolates recovered from point and nonpoint sources. The *Enterococcus* isolates recovered from point sources were 5 times more likely to be resistant to chloramphenicol than those recovered from nonpoint sources.

Several isolates recovered exhibited nonsusceptibility to more than one antibiotic and to two or more antibiotic classes, a phenomenon referred to as multidrug resistance (MDR) and extensive drug resistance, respectively. Up to 254 (63.7%) of the 399 priority pathogens recovered exhibited MDR or extensive drug resistance, of which 99 (39%), 85 (33.5%), 65 (25.6%), and 5 (2%) were *E coli*, *Enterococcus* species, *Klebsiella* species, and *Salmonella* species, respectively. Of the MDR *E coli* isolates (n=99), 40 (40%), 25 (25%), 18 (18%), 12 (12%), 1 (1%), and 3 (3%) showed resistance to 2, 3, 4, 5, 6, and 7 antibiotics from different classes, respectively. Of the MDR *Enterococcus* species isolates (n=85), 27 (32%), 34 (40%), 16 (19%), and 8 (9%) showed resistance to 2, 3, 4, and 5 antibiotics from different classes, respectively. Of the MDR *Klebsiella* species isolates (n=65), 36 (55%), 20 (32%), 2 (3%), 6 (9%), and 1 (2%) showed resistance to 2, 3, 4, 5, and 7 antibiotics from different classes, respectively (Table 2).

Table 1. Antimicrobial resistance profiles for the different isolates recovered between August 2021 and March 2023

Isolate name, antibiotic class, and antibiotic name	Isolates tested for AST[a], n	Resistance (%)
Escherichia coli **(n=156)**		
Penicillins		
Ampicillin	151	79
Fluoroquinolones		
Ciprofloxacin	141	29
Levofloxacin	38	21
Cephalosporins		
Cefuroxime	38	39
Ceftriaxone	115	29
Ceftazidime	38	26
Cefepime	66	18
Aminoglycosides		
Gentamicin	150	9
Amikacin	38	0
Carbapenems		
Meropenem	38	0
Imipenem	148	11
Beta-lactamase		
Piperacillin-tazobactam	53	4
Amoxicillin-clavulanate	38	13
Sulfonamide-trimethoprim		
Trimethoprim/sulfamethoxazole	146	55
Tetracyclines		
Tetracycline	50	30
Amphenicols		
Chloramphenicol	94	18
Glycyclines		
Tigecycline	50	30
Klebsiella **species (n=140)**		
Penicillins		
Ampicillin	137	100
Fluoroquinolones		
Ciprofloxacin	89	17
Levofloxacin	71	7
Cephalosporins		
Ceftriaxone	92	18
Ceftazidime	71	15
Cefepime	80	11
Aminoglycosides		
Gentamicin	134	4
Amikacin	71	0

Isolate name, antibiotic class, and antibiotic name	Isolates tested for AST[a], n	Resistance (%)
Carbapenems		
Meropenem	71	1
Imipenem	134	2
Beta-lactamase		
Piperacillin-tazobactam	75	3
Amoxicillin-clavulanate	71	28
Sulfonamide-trimethoprim		
Trimethoprim/sulfamethoxazole	135	33
Tetracyclines		
Tetracycline	10	30
Amphenicols		
Chloramphenicol	56	4
Glycyclines		
Tigecycline	71	4
***Salmonella* species (n=7)**		
Penicillins		
Ampicillin	7	86
Fluoroquinolones		
Ciprofloxacin	7	71
Cephalosporins		
Ceftriaxone	5	60
Carbapenems		
Imipenem	7	29
Sulfonamide-trimethoprim		
Trimethoprim/sulfamethoxazole	7	57
Tetracyclines		
Tetracycline	4	75
Amphenicols		
Chloramphenicol	7	43
***Enterococcus* species (n=96)**		
Penicillins		
Ampicillin	74	30
Fluoroquinolones		
Ciprofloxacin	60	52
Gentamicin-Syn	51	0
Glycopeptides		
Vancomycin	88	45
Macrolides		
Erythromycin	88	56
Oxazolidinones		
Linezolid	51	2
Sulfonamide-trimethoprim		

Isolate name, antibiotic class, and antibiotic name	Isolates tested for AST[a], n	Resistance (%)
Trimethoprim/sulfamethoxazole	66	89
Tetracyclines		
Tetracyclines	80	54
Amphenicols		
Chloramphenicol	40	50
Glycyclines		
Tigecycline	71	0

[a]AST: antimicrobial susceptibility testing.

Table 2. Isolates that exhibited multidrug resistance and extensive drug resistance tendencies among the priority isolates recovered.

Number of antibiotics for which resistance is shown	Frequency of resistant isolates
Two antibiotics	
Enterococcus species	27
Klebsiella species	36
Escherichia coli	40
Three antibiotics	
Enterococcus species	34
Klebsiella species	20
Escherichia coli	25
Four antibiotics	
Enterococcus species	16
Klebsiella species	02
Escherichia coli	18
Five antibiotics	
Enterococcus species	8
Klebsiella species	6
Escherichia coli	12
Six antibiotics	
Enterococcus species	0
Klebsiella species	0
Escherichia coli	1
Seven antibiotics	
Enterococcus species	0
Klebsiella species	1
Escherichia coli	3

Discussion

AMR surveillance in the Uganda water and environment sector is taking shape, including the streamlining of the sector AMR governance structures. This has expanded the One Health approach to AMR surveillance in the country. A TWG, a national AMR reference laboratory, and an AMR focal person were constituted and provided with terms of reference. This is in alignment with the road map for the participation of low- and middle-income countries in the Global Antimicrobial Surveillance System [21]. This program has been established using a phased/stepwise approach where one sentinel site, the NWQRL, was identified and its capacity enhanced and is now used for AMR surveillance. This is a prerequisite for establishing targeted and well-monitored public health surveillance of AMR in low-income settings [21,28]. The alignment of the efforts in the sector with national and international guidelines allows national and international

comparisons of established surveillance systems to identify areas of improvement [29].

The program has succeeded in profiling the resistance patterns of the bacterial pathogens recovered from different water types from both point and nonpoint sources of AMR determinants in the environment as categorized by Khurana and Sinha [30]. Wastewater samples represent the point sources as the resistant microorganisms in these samples either emerge directly or after antimicrobials reach and contaminate the different environment compartments. The ground, drinking, and surface water samples constitute the nonpoint sources of resistant organisms since these indicate the interface and spillage of resistant microorganisms from the environment to the human and animal populations [30].

The program used culture-based methods as these were better aligned with the current laboratory infrastructure in the Uganda water and environment sector and the country at large. Liguori et al [7] have described the methods as fairly standardized and an avenue for further analysis of the recovered isolates including sensitivity testing, sequence-based typing, and whole genome sequencing, which aid in detecting and identifying antibiotic-resistant genes and genetic elements. However, these culture-based methods recover fewer microorganisms as the majority of the environmental microbes are not readily cultured, yet they may pose public health challenges [31]. Thus, the AMR surveillance systems in the sector require appropriate expansion to include whole genome sequencing and environmental wastewater sequencing.

E coli, *Klebsiella* species, *Enterococcus* species, and *Salmonella* species used for surveillance in the Ugandan sector are globally recommended for the environment sector [32,33]. An expert survey conducted among 105 experts from different fields and parts of the world found that Enterobacterales (mainly *E coli* and *Salmonella* species) and *Enterococcus* species were the most selected culture target microorganisms for AMR surveillance in the environment [7]. These microorganisms are similar to those recommended for AMR surveillance in the human [34,35] and animal health [36] sectors. This makes the comparison, quantification, and evaluation of the occurrence of these organisms across the different sectors in low-resource settings feasible [3,37]. They are classified as the major clinically relevant multidrug-resistant pathogens prevalent in the different environmental compartments [3,32,33,38] that can cause life-threatening illnesses in humans and animals [24].

The high *E coli* resistance to ampicillin and trimethoprim/sulfamethoxazole observed in our evaluation is similar to that observed in South Africa, where *E coli* had high resistance to sulfamethoxazole (100%) and ampicillin (90%) [39]. Moges et al [40] in Ethiopia observed that the recovered *E coli* had 100% resistance to ampicillin and 38% resistance to trimethoprim/sulfamethoxazole. This ampicillin resistance is similar to that found by Nabadda et al [41] among recovered *E coli* isolates from human samples in Uganda, which had more than 90% resistance to ampicillin. Amaya et al [42] also found that some *E coli* recovered from groundwater from wells in León, Nicaragua showed very high resistance of 100% to ampicillin, ciprofloxacin, and trimethoprim/sulfamethoxazole. This indicates a similarity in the resistance of *E coli* recovered from humans and the environment, which requires further assessment using whole genome sequencing techniques to elucidate the interrelatedness.

The high resistance of *Klebsiella* species against ampicillin observed in the Uganda program correlates to the high ampicillin intrinsic resistance (100%) observed in the *Klebsiella* species isolates from environmental samples in a study in Pakistan from 2017 to 2019 [43]. Holt et al [44] similarly found 100% resistance to ampicillin among the different *Klebsiella* species recovered [45]. This phenomenon of *Klebsiella* species' intrinsic resistance to ampicillin is demonstrated in the Clinical and Laboratory Standards Institute guidelines [26] and the European Committee on Antimicrobial Susceptibility Testing guidelines [46,47]. The moderate *Klebsiella* species resistance (below 50%) to gentamicin, imipenem, and trimethoprim/sulfamethoxazole observed in the program is consistent with that observed in an Iraqi study on the resistance patterns of *Klebsiella* species isolates from clinical and environmental samples [48].

Resistant *Salmonella* species isolated from some of the samples indicate contamination of these sources by human and animal waste [49,50]. This points to a potential risk for an outbreak of salmonellosis within the animal or human populations residing in the surroundings of the sample source [51,52]. Such an observation can be used to predict the health profile of the human and animal populations in a given catchment area using environmental samples [20].

Enterococcus species resistance to tetracycline (54%), vancomycin (45%), erythromycin (56%), and ciprofloxacin (52%) in this program were all higher (in some instances double) than the resistance of the *Enterococcus* species from environmental samples in an area of intensive poultry production in Canada [53]. Another study in France also showed high resistance of *Enterococcus* species isolates to erythromycin (100%), vancomycin (85.7%), and tetracycline (57.1%) [54].

The MDR exhibited by the isolates recovered in the program is consistent with the findings of studies conducted in several parts of the world. For example, a 96.4% MDR occurrence was observed in the bacterial strains recovered from samples collected from different aquatic environments in a study in France [54]. Another study in Ghana by Odonkor and Addo [55] found that 63% of the *E coli* strains recovered were resistant to at least 3 antibiotics from different classes. Further, a study conducted in the United States also found that 65% of the isolates recovered from combined sewage overflows (wastewater) were resistant to 6 or more antibiotics from different classes [56]. Human and animal exposure to and infection with such highly resistant bacteria in the environment through complex interactions impacts the economy of countries as such infections are harder to treat. This increases medical costs, hospital stays, the risk of infection spread, severity, and mortality rates [57].

Our evaluation had one major limitation. The representativeness of the AMR data generated is still limited as the active surveys are conducted in only the Kampala-Wakiso region. Therefore, the data may not be sufficient to generalize the prevalence of

AMR in Uganda's water and environment sector. However, the data marks the first efforts to generate AMR data in the sector, but more efforts are required to increase the quantity of the sector AMR data.

Efforts to implement AMR surveillance in the water and the environment sector succeeded in streamlining AMR surveillance governance and isolating resistant pathogens from different water types (waste, drinking, surface, and groundwater). The program needs to be consolidated and expanded to include more sentinel sites, sample types, advanced AMR surveillance methodologies and techniques, and the surveillance of antimicrobial residues. Sustained surveillance in the sector and interlinkages with the human and animal sectors' surveillance systems are also required to inform concerted strategies to control AMR in the country.

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Data Availability

The data generated from the water and environment sector is a preserve of the Uganda MWE. It can be accessed by placing a request and concept on use to the Commissioner Water Quality Management Department in the Directorate of Water Resources Management, MWE.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Map showing the environment sector antimicrobial resistance surveillance sampling points in Kampala and Wakiso.
[PNG File , 656 KB-Multimedia Appendix 1]

References

1. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-318. [doi: 10.1179/2047773215Y.0000000030] [Medline: 26343252]
2. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10(4):369-378. [doi: 10.1016/j.jiph.2016.08.007] [Medline: 27616769]
3. Samreen, Ahmad I, Malak HA, Abulreesh HH. Environmental antimicrobial resistance and its drivers: a potential threat to public health. J Glob Antimicrob Resist. Dec 2021;27:101-111. [doi: 10.1016/j.jgar.2021.08.001] [Medline: 34454098]
4. Tillotson GS, Zinner SH. Burden of antimicrobial resistance in an era of decreasing susceptibility. Expert Rev Anti Infect Ther. Jul 2017;15(7):663-676. [doi: 10.1080/14787210.2017.1337508] [Medline: 28580804]
5. Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, et al. Antibiotic resistance is the quintessential One Health issue. Trans R Soc Trop Med Hyg. Jul 2016;110(7):377-380. [doi: 10.1093/trstmh/trw048] [Medline: 27475987]
6. Larsson DJ, Andremont A, Bengtsson-Palme J, Brandt KK, de Roda Husman AM, Fagerstedt P, et al. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environ Int. Aug 2018;117:132-138. [doi: 10.1016/j.envint.2018.04.041] [Medline: 29747082]
7. Liguori K, Keenum I, Davis BC, Calarco J, Milligan E, Harwood VJ, et al. Antimicrobial resistance monitoring of water environments: a framework for standardized methods and quality control. Environ Sci Technol. Jul 05, 2022;56(13):9149-9160. [doi: 10.1021/acs.est.1c08918] [Medline: 35732277]
8. Taneja N, Sharma M. Antimicrobial resistance in the environment: the Indian scenario. Indian J Med Res. Feb 2019;149(2):119-128. [doi: 10.4103/ijmr.IJMR_331_18] [Medline: 31219076]
9. Fletcher S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. Environ Health Prev Med. Jul 2015;20(4):243-252. [doi: 10.1007/s12199-015-0468-0] [Medline: 25921603]
10. Musoke D, Namata C, Lubega GB, Niyongabo F, Gonza J, Chidziwisano K, et al. The role of Environmental Health in preventing antimicrobial resistance in low- and middle-income countries. Environ Health Prev Med. Oct 05, 2021;26(1):100. [doi: 10.1186/s12199-021-01023-2] [Medline: 34610785]
11. Vassallo A, Kett S, Purchase D, Marvasi M. Antibiotic-resistant genes and bacteria as evolving contaminants of emerging concerns (e-CEC): is it time to include evolution in risk assessment? Antibiotics (Basel). Sep 03, 2021;10(9):1066. [doi: 10.3390/antibiotics10091066] [Medline: 34572648]

12. Larsson DGJ, Flach C. Antibiotic resistance in the environment. Nat Rev Microbiol. May 2022;20(5):257-269. [doi: 10.1038/s41579-021-00649-x] [Medline: 34737424]
13. Frontiers 2017: emerging issues of environmental concern. United Nations Environment Programme. 2017. URL: https://www.unep.org/resources/frontiers-2017-emerging-issues-environmental-concern [accessed 2023-02-01]
14. Puvača N, Vapa Tankosić J, Ignjatijević S, Carić M, Prodanović R. Antimicrobial resistance in the environment: review of the selected resistance drivers and public health concerns. J Agron Technol Eng Manag. Sep 30, 2022;5(5):793-802. [doi: 10.55817/CSCQ3326]
15. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. Jan 09, 2016;387(10014):176-187. [doi: 10.1016/S0140-6736(15)00473-0] [Medline: 26603922]
16. Irfan M, Almotiri A, AlZeyadi ZA. Antimicrobial resistance and its drivers-a review. Antibiotics (Basel). Oct 05, 2022;11(10):1362. [doi: 10.3390/antibiotics11101362] [Medline: 36290020]
17. Kivumbi M, Standley C. Efforts to identify and combat antimicrobial resistance in Uganda: a systematic review. Trop Med Infect Dis. May 24, 2021;6(2):86. [doi: 10.3390/tropicalmed6020086] [Medline: 34074031]
18. Food and Agriculture Organization of the United Nations, World Organisation for Animal Health, World Health Organization, United Nations Environment Programme. Strategic Framework for Collaboration on Antimicrobial Resistance: Together for One Health. Rome, Italy. Food & Agriculture Organization; Apr 6, 2022.
19. McEwen S, Collignon PJ. Antimicrobial resistance: a One Health perspective. Microbiol Spectr. Mar 2018;6(2):A. [doi: 10.1128/microbiolspec.ARBA-0009-2017] [Medline: 29600770]
20. Huijbers PM, Flach C, Larsson DJ. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ Int. Sep 2019;130:104880. [doi: 10.1016/j.envint.2019.05.074] [Medline: 31220750]
21. Seale AC, Gordon NC, Islam J, Peacock SJ, Scott JAG. AMR surveillance in low and middle-income settings - a roadmap for participation in the Global Antimicrobial Surveillance System (GLASS). Wellcome Open Res. 2017;2:92. [doi: 10.12688/wellcomeopenres.12527.1] [Medline: 29062918]
22. Global action plan on antimicrobial resistance. World Health Organization. 2015. URL: https://apps.who.int/iris/rest/bitstreams/864486/retrieve [accessed 2020-12-12]
23. Strategic and operational guidance for national action plan on antimicrobial resistance for developing countries focusing on animal and environment aspects. Centre for Science and Environment. 2016. URL: https://cdn.cseindia.org/userfiles/strategic-and-operational-guidance-NAP-on-AMR-for-Developing-Countries.pdf [accessed 2023-02-01]
24. National action plan for antimicrobial resistance. Central Public Health Laboratories of the Republic of Uganda Ministry of Health. 2018. URL: https://www.cphl.go.ug/sites/default/files/2020-02/Uganda%20National%20Action%20Plan%20for%20Antimicrobial%20Resistance%202018-%202023-compressed_0.pdf [accessed 2023-02-01]
25. Uganda One Health strategic plan 2018-2022. Ministry of Health Republic of Uganda. 2018. URL: https://health.go.ug/sites/default/files/Uganda%20OHSP%20Final%20Launched%2015-02-2018%20%281%29.pdf [accessed 2023-02-01]
26. M100 performance standards for antimicrobial susceptibility testing. Clinical & Laboratory Standards Institute. Mar 2021. URL: https://clsi.org/media/z2uhcbmv/m100ed31_sample.pdf [accessed 2024-04-23]
27. NRECCLWM programme management report 2022. Ministry of Water and Environment Republic of Uganda. 2022. URL: https://www.mwe.go.ug/library/nrecclwm-programme-management-report-2022 [accessed 2023-02-01]
28. Nsubuga P, White ME, Thacker SB, Anderson MA, Blount SB, Broome CV, et al. Public health surveillance: a tool for targeting and monitoring interventions. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al, editors. Disease Control Priorities in Developing Countries. 2nd edition. Washington, DC. The International Bank for Reconstruction and Development; 2006.
29. Queenan K, Häsler B, Rushton J. A One Health approach to antimicrobial resistance surveillance: is there a business case for it? Int J Antimicrob Agents. Oct 2016;48(4):422-427. [doi: 10.1016/j.ijantimicag.2016.06.014] [Medline: 27496533]
30. Khurana A, Sinha R. Tackling AMR starts with the environment. Sanitation Environment. 2019.:78-80.
31. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ. Mar 01, 2013;447:345-360. [doi: 10.1016/j.scitotenv.2013.01.032] [Medline: 23396083]
32. Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ Pollut. Nov 2009;157(11):2893-2902. [doi: 10.1016/j.envpol.2009.05.051] [Medline: 19560847]
33. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, et al. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol. May 2015;13(5):310-317. [doi: 10.1038/nrmicro3439] [Medline: 25817583]
34. Iskandar K, Molinier L, Hallit S, Sartelli M, Hardcastle TC, Haque M, et al. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. Antimicrob Resist Infect Control. Mar 31, 2021;10(1):63. [doi: 10.1186/s13756-021-00931-w] [Medline: 33789754]
35. Pandey R, Mukherjee R, Chang CM. Antimicrobial resistance surveillance system mapping in different countries. Drug Target Insights. 2022;16:36-48. [doi: 10.33393/dti.2022.2482] [Medline: 36479338]

36. Amin MA, Pasha MH, Hoque MN, Siddiki AZ, Saha S, Kamal MM. Methodology for laboratory-based antimicrobial resistance surveillance in animals. Vet World. Apr 2022;15(4):1066-1079. [doi: 10.14202/vetworld.2022.1066-1079] [Medline: 35698528]
37. Jorge M, Awa AK. THE ESBL tricycle amr surveillance project: a simple, ONE HEALTH approach to global surveillance. AMR Control. 2017. URL: http://resistancecontrol.info/2017/the-esbl-tricycle-amr-surveillance-project-a-simple-one-health-approach-to-global-surveillance/ [accessed 2022-02-01]
38. Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. Science. Jul 18, 2008;321(5887):365-367. [doi: 10.1126/science.1159483] [Medline: 18635792]
39. Genthe B, Ndlela L, Madlala T. Antimicrobial resistance screening and profiles: a glimpse from the South African perspective. J Water Health. Dec 2020;18(6):925-936. [doi: 10.2166/wh.2020.034] [Medline: 33328364]
40. Moges F, Endris M, Belyhun Y, Worku W. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. BMC Res Notes. Apr 05, 2014;7:215. [doi: 10.1186/1756-0500-7-215] [Medline: 24708553]
41. Nabadda S, Kakooza F, Kiggundu R, Walwema R, Bazira J, Mayito J, et al. Implementation of the World Health Organization Global Antimicrobial Resistance Surveillance System in Uganda, 2015-2020: mixed-methods study using national surveillance data. JMIR Public Health Surveill. Oct 21, 2021;7(10):e29954. [doi: 10.2196/29954] [Medline: 34673531]
42. Amaya E, Reyes D, Paniagua M, Calderón S, Rashid M, Colque P, et al. Antibiotic resistance patterns of Escherichia coli isolates from different aquatic environmental sources in León, Nicaragua. Clin Microbiol Infect. Sep 2012;18(9):E347-E354. [doi: 10.1111/j.1469-0691.2012.03930.x] [Medline: 22738232]
43. Aslam B, Chaudhry TH, Arshad MI, Muzammil S, Siddique AB, Yasmeen N, et al. Distribution and genetic diversity of multi-drug-resistant at the human-animal-environment interface in Pakistan. Front Microbiol. 2022;13:898248. [doi: 10.3389/fmicb.2022.898248] [Medline: 36147844]
44. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A. Jul 07, 2015;112(27):E3574-E3581. [doi: 10.1073/pnas.1501049112] [Medline: 26100894]
45. Bouza E, Cercenado E. Klebsiella and enterobacter: antibiotic resistance and treatment implications. Semin Respir Infect. Sep 2002;17(3):215-230. [doi: 10.1053/srin.2002.34693] [Medline: 12226801]
46. Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, et al. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. Feb 2013;19(2):141-160. [doi: 10.1111/j.1469-0691.2011.03703.x] [Medline: 22117544]
47. Sánchez-Bautista A, Coy J, García-Shimizu P, Rodríguez JC. From CLSI to EUCAST guidelines in the interpretation of antimicrobial susceptibility: What is the effect in our setting? Enferm Infecc Microbiol Clin (Engl Ed). Apr 2018;36(4):229-232. [doi: 10.1016/j.eimc.2017.03.003] [Medline: 28479139]
48. Jabbar Ibrahim IA, Kareem Hameed TA. Isolation, characterization and antimicrobial resistance patterns of lactose-fermenter Enterobacteriaceae isolates from clinical and environmental samples. Open J Med Microbiology. 2015;05(04):169-176. [doi: 10.4236/ojmm.2015.54021]
49. Abakpa G, Umoh V, Ameh J, Yakubu S, Kwaga J, Kamaruzaman S. Diversity and antimicrobial resistance of Salmonella enterica isolated from fresh produce and environmental samples. Environ Nanotechnology Monitoring Manage. May 2015;3:38-46. [doi: 10.1016/j.enmm.2014.11.004]
50. Singh S, Yadav AS, Singh SM, Bharti P. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res Int. Oct 2010;43(8):2027-2030. [doi: 10.1016/j.foodres.2010.06.001]
51. Bell RL, Kase JA, Harrison LM, Balan KV, Babu U, Chen Y, et al. The persistence of bacterial pathogens in surface water and its impact on global food safety. Pathogens. Oct 27, 2021;10(11):1391. [doi: 10.3390/pathogens10111391] [Medline: 34832547]
52. Dewey-Mattia D, Manikonda K, Hall AJ, Wise ME, Crowe SJ. Surveillance for foodborne disease outbreaks - United States, 2009-2015. MMWR Surveill Summ. Jul 27, 2018;67(10):1-11. [doi: 10.15585/mmwr.ss6710a1] [Medline: 30048426]
53. Furtula V, Jackson C, Farrell E, Barrett J, Hiott L, Chambers P. Antimicrobial resistance in Enterococcus spp. isolated from environmental samples in an area of intensive poultry production. Int J Environ Res Public Health. Mar 12, 2013;10(3):1020-1036. [doi: 10.3390/ijerph10031020] [Medline: 23481592]
54. Pérez-Etayo L, González D, Leiva J, Vitas AI. Multidrug-resistant bacteria isolated from different aquatic environments in the North of Spain and South of France. Microorganisms. Sep 16, 2020;8(9):1425. [doi: 10.3390/microorganisms8091425] [Medline: 32947947]
55. Odonkor ST, Addo KK. Prevalence of multidrug-resistant isolated from drinking water sources. Int J Microbiol. 2018;2018:7204013. [doi: 10.1155/2018/7204013] [Medline: 30210545]
56. Balasa G, Levengood E, Battistelli J, Franklin R. Diversity of multidrug-resistant bacteria in an urbanized river: a case study of the potential risks from combined sewage overflows. Water. Aug 01, 2021;13(15):2122. [doi: 10.3390/w13152122]
57. Pezzani MD, Tornimbene B, Pessoa-Silva C, de Kraker M, Rizzardo S, Salerno ND, et al. Methodological quality of studies evaluating the burden of drug-resistant infections in humans due to the WHO Global Antimicrobial Resistance Surveillance

System target bacteria. Clin Microbiol Infect. Jan 13, 2021;27(5):687-696. [doi: 10.1016/j.cmi.2021.01.004] [Medline: 33450389]

Abbreviations

AMR: antimicrobial resistance
MDR: multidrug resistance
MWE: Ministry of Water and Environment
NWQRL: National Water Quality Reference Laboratory
TWG: technical working group

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