Household water storage compromises drinking water safety in a "safely managed" system

Yarrow Linden[1], Erin Kowalsky[1], Kevin Zhu[1], Christine Fagnant-Sperati[2], Sandra McGunegill[3], Arnaldo Cumbana[4], Magalhães Mangamela[5], Rassul Nalá [6], Matthew C. Freeman[3], Karen Levy[2], Joe Brown[1] and The PAASIM Study Authorship Consortium

1. Department of Environmental Sciences and Engineering, University of North Carolina-Chapel Hill, Chapel Hill, North Carolina, United States
2. Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, United States
3. Gangarosa Department of Environmental Health, Emory University, Atlanta, Georgia, United States
4. Laboratório de Higiene de Águas e Alimentos, Beira, Mozambique
5. Autoridade Reguladora de Água e Saneamento (AURA, IP), Beira, Mozambique
6. Instituto Nacional de Saúde, Ministério de Saúde, Maputo, Mozambique

Corresponding Author: J. Brown

Email: joebrown@unc.edu

Abstract

While nearly three-quarters of the globe use safely managed drinking water services, water quality can deteriorate between the point of service provision and point of consumption due to intermittent supply and the need to store water at the household level. To test if water storage contributes to waterborne pathogen hazards, we estimated prevalence and concentration of enteric pathogens in piped and stored drinking water samples in Beira, Mozambique, using large-volume sampling methods. We assessed water sample concentrates for microbial contamination from bacterial, protozoan, viral, helminthic, antimicrobial resistance (AMR), and microbial source tracking targets with RT-qPCR using a custom TaqMan Array Card. We found that enteric pathogens, AMR targets, and human mtDNA were more prevalent in stored drinking water compared to household tap water. We also detected enteric pathogens—including *Cryptosporidium, Giardia,* pathogenic *Escherichia coli*, and rotavirus— directly from 13% (11/87) of piped sources connected to a well-managed, but intermittent, water supply system, albeit less frequently than in stored water. These findings suggest that without continuity in service delivery combined with effective filtration treatment in piped supplies and safe storage microbial contamination of drinking water at the point of use may continue to occur.

1. Introduction

An estimated 2.2-4.4 billion people lack access to safely managed drinking water (i.e., a drinking water source that is available on premises when needed and free from chemical and biological hazards), and instead rely on sources that are susceptible to contamination with fecal pathogens (1,2). Unsafe drinking water is responsible for an estimated 35% of diarrhea-related deaths in low-and-middle income countries (LMICs) (3). While approximately 73% of the global population used safely managed drinking water services in 2022, this is likely an overestimation as monitoring systems for chemical and microbial contaminants are often inadequate (1,2,4,5). Furthermore, even populations with access to “safely managed” piped water sources are susceptible to waterborne contaminant exposures, especially at the point of use. Water from improved sources has been shown to contain lower levels of fecal contamination compared with water from unimproved sources, but there is evidence of microbial contamination even in improved drinking water samples (6,7). Improved sources consistently fail to meet normative water safety guidelines in large-scale surveys (8). Therefore, it is important to understand specific microbial risks attributable to drinking water exposures in these types of settings by characterizing pathogen specific hazards in these conditions to inform science-backed solutions.

Poor water quality in piped water supplies may result from inadequate or inconsistent treatment, degradation of water quality in distribution systems, and intrusion during low-pressure events or intermittent service (9,10). Intermittent water supplies (IWSs) are common in many LMICs that have piped water systems, with an estimated 925 million people relying on IWSs for water delivery (11). These IWS systems become susceptible

to intrusion from non-treated water during periods of pressure-loss and create an environment suitable for microbial regrowth (12).

Importantly, intermittent service creates a need for household storage of water, so that water is available for use when needed. Multiple studies have shown that stored water contains significantly higher levels of *Escherichia coli* fecal indicator bacteria (FIB) compared to samples taken directly from a tap receiving improved water, resulting in impaired water quality at the point of consumption (8,13,14).

While household water storage is the norm in many LMIC communities, most studies of water storage effects on water quality in LMICs have only evaluated FIB (8,13,14). Understanding which pathogens play a role in household water contamination can inform control strategies, as not all are preventable with the same means. Additionally, much of what we know on pathogens in drinking water supplies are from studies based in high-income countries and may not be globally representative (15–18). FIB serve as a proxy for recent fecal contamination but may not represent the full set of microbial contamination in a water sample, as different organisms have different survival rates in the environment. Detection of a more comprehensive set of specific enteric pathogens, rather than reliance on indicator organisms, can be helpful for estimating more realistic health hazards. In addition, microbial source tracking (MST) can help identify the origin of enteric pathogens and inform their control (19).

Some important enteric pathogens can be resistant to antibiotics. Antibiotic resistance (AMR) can be naturally found in the environment but is more prevalent in areas with anthropogenic pollution, particularly from wastewater contamination (20,21). AMR in drinking water supplies can impact public health by direct exposure to AMR bacteria or

via horizontal gene transfer, potentially leading to risk of treatment failure when using antibiotics to treat infections. Presence of antibiotic resistance genes (ARGs) in drinking water supplies can indicate contamination from wastewater or agriculture and may suggest that current water treatment methods are insufficient. Co-occurrence of ARGs and pathogens in drinking water may confer resistance in pathogens due to horizontal gene transfer (22).

Seasonality can also influence pathogen hazards in the environment. In many settings, there are higher levels of fecal contamination present in drinking water supplies during the rainy season (23).

In this study, we evaluated the prevalence and concentration of enteric pathogens, ARGs, and MST targets in piped and stored drinking water sources used by households served by a safely managed drinking water supply. Our aim was to understand specific microbial hazards associated with water storage and seasonality. To achieve this aim, we compared contamination between stored and tap drinking water from the same households and neighborhoods, and between the rainy and dry seasons, hypothesizing that water storage and wet season conditions contribute to increased waterborne pathogen hazards in this setting.

2. Materials and Methods

2.1 Study Context

This study was nested within the *Pesquisa Sobre o Acesso à Água e a Saúde Infantil em Moçambique* (PAASIM; Research on Access to Water and Children's Health in Mozambique) trial, a prospective matched controlled birth cohort assessing if and how

improvements in water supply in low-income urban areas of Beira, Mozambique impact health outcomes in children (24). Enrolled households were visited every three months from the end of pregnancy until the child was 12 months old. At each visit, enumerators conducted a household survey, took anthropometric measurements, and collected child stool, drinking water samples, and dried capillary blood spots (6, 9, and 12 month visits only). For this sub-study, we collected additional large volume water samples from households and community water points and assayed them via culture and molecular methods to detect specific enteric pathogens and other targets of interest.

Beira is the fourth largest municipality in Mozambique, with a population of over half a million people and much of the population resides in informal settlements (25). It is classified as a tropical savanna climate with distinct rainy and dry seasons. Figure 1 shows locations of water sampling for this sub-study. Beira receives water from the Pungwe River, around 80 km outside the city, where water is transferred via pipeline to a longitudinal canal for sedimentation pre-treatment before entering the water treatment plant (26). Treated water is transferred to the distribution center via pipeline. As of April 2024, municipal water was supplied to households approximately 14 hours per day on average (27). It should be noted that the water regulatory agency does not have the goal of supplying water 24 hours a day 7 days a week due to water losses.

Figure 1. Study area, showing community water sample collection points. All samples were collected in Beira, Mozambique, within the PAASIM study neighborhoods.

2.2 Sample Selection and Sampling Methods

To evaluate associations between water storage and detection of waterborne pathogen hazards we collected both stored (point-of-use) and tap water (point-of-collection)

drinking water samples. Household point-of-use samples were identified based on stored water that the mother said she would give to her child to drink. In addition to the household samples, we collected water from public community taps as a proxy for contaminants in the household water supply as it is from the same distribution system. Details of number of samples collected from each sample type (household stored water, household water sampled from a tap, and piped water collected from public community taps) are presented in Table 1.

Table 1. Description of drinking water samples collected, method for processing, total volume sampled, number of samples in each season, and data generated from each sample type

Sample Type	**Method**	**Volume sampled**	**Dry season n**	**Rainy season n**	**Total n**	**Data generated**
Household Stored	Membrane Filtration	0.5-2.5 L	51	24	75	Culturable *E. coli* detection & quantification from Most Probable Number (MPN) Enteric pathogen, antimicrobial resistance, and microbial source tracking gene target detection & quantification from RT-qPCR
Household Tap	Membrane Filtration	0.5-2.5 L	26	12	38	
Community Tap	Dead-End-Ultrafiltration	15-55 L	24	25	49	

To understand the year-round waterborne hazards and assess differences between the seasons, we collected samples at different points in the year (28–30), first during the dry months (May-June 2022) and then during the rainy months (January-February 2023) to

account for seasonal variations in water quality. To dichotomize rainy verse dry seasons, the PAASIM study used a data driven approach incorporating daily rainfall, temperature, and specific and relative humidity using a principal component analysis (PCA) and k-means clustering to group months as rainy or dry (31). This approach resulted in the rainy season during our study timeframe defined as October through April, while the dry season ran from May through September, aligning with usual rainfall and temperature trends.

During the sampling effort, we selected a subset of 75 PAASIM households (of 548 total households included in the study) at random in equal proportions between intervention and comparison neighborhoods, based on which houses were being visited at the time of sample collection for this sub study. These household sampling visits took place at any of the 3-, 6-, 9-, or 12-month visits. Different households were visited in the wet and dry season. For sampling of community taps, we sampled all taps under surveillance for PAASIM longitudinal monitoring. This included 25 community taps within the study neighborhoods, selected from a total of 43 that were functioning at the beginning of the study using probability proportional to size sampling based on household density (24). For our large volume dead-end-ultrafiltration (DEUF) sampling, we visited these 25 community taps twice, once during the rainy season and again during the dry season. During the dry season, one of the 25 taps had no water available so we were not able to collect a sample.

2.3 Membrane Filtration

We used membrane filtration to concentrate household drinking water samples (tap and stored). In this method water is passed through a membrane filter paper by vacuum

pump and organisms in the sample are trapped on the membrane for downstream analysis. At each household, a stored and/or tap water sample was collected by the project enumerators during scheduled household visits. Due to limited household water supplies, we did not take more than 2.5 L of stored or tap water from any home. Household samples were collected using a large Whirl-Pak bag (Nasco, Pleasant Prairie, Wisconsin) containing sodium thiosulphate for dechlorination, and transported on ice to the Beira Water Laboratory to be processed by membrane filtration on a 47mm mixed cellulose ester HA filter with a pore size of 0.45μm (MilliporeSigma, Burlington, Massachusetts) using an electric vacuum pump. Filters were stored in individual cryovials containing 1 mL of Zymo DNA/RNA Shield (Zymo, Irvine, California). Cryovials were stored in the freezer at -20° C until transfer to UNC-Chapel Hill, where they were transported and stored at -80° C until nucleic acid extraction and molecular analyses were performed.

2.4 Culture-Based Methods

We used IDEXX Colilert-18 Quanti-Tray 2000 (IDEXX, Westbrook, Maine) to detect and quantify *E. coli* in all three drinking water types. We followed the EPA Standard Method 9223 B Colilert-18® by combining 100 mL of undiluted sample with Colilert-18 reagent and incubating at 37° C for 18-22 hours. Following incubation, we read the results under a UV chamber (IDEXX, Westbrook, Maine). IDEXX methods were performed by local research staff at the Beira Water laboratory within 6 hours of collection, and stored on ice or in the refrigerator prior to analysis.

We measured culturable *E. coli* in addition to direct pathogens as a proxy indicator for viability, since molecular methods measure nucleic acids from both viable and non-viable organisms. Enteric pathogens, especially unenveloped viruses and protozoan cysts, are more persistent and resistant to a wide range of inactivation processes compared with vegetative *E. coli,* making it a potentially conservative proxy for enteric pathogen viability overall (32–34).

2.5 Dead-End Ultrafiltration (DEUF)

To sample the public community taps, we used DEUF, a technique for sampling and concentrating large volumes of water, where water is passed through a hemodialyzer by peristaltic pump and organisms in the sample are trapped in the ultrafilter and retrieved when the filter is backflushed (15,35). These larger volume (15-55 L) samples increase the probability of pathogen detection, which can be scarcer in piped supplies than in stored water (9). Briefly, we drew water from the community tap into a sterilized 5-L bucket and pumped the sample through a Rexeed 25S dialysis filter (Asahi Kasei Medical Co., Ltd, Tokyo, Japan) using a Geotech GeoPump peristaltic pump (Geotech Environmental Equipment, Inc., Denver, Colorado) and fitted tubing sterilized in a bleach bath and rinsed with sodium thiosulphate between uses, while monitoring the filtered volume with a water totalizing meter (Clark Solutions, Hudson, Massachusetts). Following collection, we collected 500 mL of purified outflow into a sterilized bottle containing 5g sodium thiosulphate to pump through the filter and dechlorinate any remaining chlorine from the supply. Used filters were capped and transported on ice. Immediately upon arrival to the Beira Water laboratory, we backflushed the dead-end-ultrafilters with a solution containing 0.5% Tween 80, 0.01% sodium polyphosphate,

0.001% antifoam Y-30, to a volume of approximately 500 mL. Backflush was frozen at -20° C until transport to the Polana Caniço Health Research and Training Center (CISPOC) laboratories in Maputo on cold chain, where we further concentrated the backflush using polyethylene glycol (PEG) precipitation. Briefly, the backflush solution was adjusted to 0.9 M NaCl, 12% PEG 8000, and 1% bovine serum albumin, refrigerated overnight, and then centrifuged at 10,000xg and 4° C for 30 minutes. The pellet was resuspended in 1-4 mL of phosphate buffered saline (PBS). All samples were shipped on dry ice at -80° C to our laboratory at UNC-Chapel Hill for nucleic acid extraction and molecular analyses.

2.6 Molecular Analysis

We extracted nucleic acids from 200 µL of resuspended pellet (DEUF samples) or eluted filter (membrane filtration samples) using the automated Qiagen QIAamp 96 Virus QIAcube HT Kit on the QIAcube HT (Qiagen, Hilden, Germany). We included a validated pre-treatment step using the Qiagen TissueLyser involving beat beating in Powerbead Pro tubes for 5 minutes on each side at 25 Hz. As a positive extraction control, we used 10 µL of Inforce3, which contains both a DNA virus (BHV) and an RNA virus (BRSV), spiked into each sample prior to the pre-treatment step. Extractions were completed in batches, and each batch included a negative extraction control of molecular grade water. We stored extracts at -80° C until further analysis.

We analyzed all samples using real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) via a TaqMan Array Card (TAC) with 48 gene targets related to waterborne exposures. We tested for 31 pathogen targets, including 11 bacterial (16

genes), 6 viral (7 genes), 4 protozoan, and 4 soil-transmitted helminths, and 8 AMR markers (11 genes), 1 human MST marker, and 5 control targets (Table S1). TACs were prepared using AgPath One-Step RT-PCR mastermix (ThermoFisher Scientific, Waltham, Massachusetts) with a hepatitis G process control and run on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR System (ThermoFisher Scientific, Waltham, Massachusetts). All PCR experiments were performed in accordance with MIQE guidelines (36) (Table S2). Array cards were analyzed in QuantStudio™ Real-Time PCR Software (ThermoFisher Scientific, Waltham, Massachusetts) using standard curves to estimate gene copies of each target (Table S3).

To estimate gene copies of each target in our water samples, we used Cq values in combination with standard curves to produce gene copies per PCR reaction and accounted for sampling concentration steps and dilutions during processing to estimate gene copies per 100 mL of drinking water. We determined the lower limit of detection (LOD) based on a set of repeated measurements around the estimated limit and used a probit analysis to estimate the level of concentration corresponding to a 95% probability of detection (Table S3). Samples that were below the LOD were deemed non-detects. Non-detect concentrations were set to 50% of the LOD value for their specific assay.

2.7 Data Analysis

We compared prevalence and concentration of pathogens and other targets in drinking water between stored versus tap samples and rainy versus dry seasons. To determine differences in prevalence, we calculated unadjusted and adjusted prevalence ratios using Poisson regression with robust standard errors. Models were adjusted for intervention status and rainy season, determined *a priori*. With our concentration data,

we calculated mean differences using robust linear regression, with non-detects imputed to 50% of the LOD (37). To assess seasonal differences in prevalence, we used Poisson regression with robust standard errors. We used Microsoft Excel 365 for data management, conducted all analyses in STATA version 18.5, and created figures using R version 4.4.2. Full details on molecular assays are provided in Table S2 (36).

2.8 Ethics Approval

This study protocol, informed consent forms, and data collection tools was approved by the Mozambique National Bio-Ethics Committee for Health (IRB00002657) and Emory University's Institutional Review Board (IRB00098584). Credential letters and permissions were obtained from Beira municipality and municipal district administrations from study neighborhoods for study conduct in those areas. Study participants were given written informed consent forms at their homes during enrollment visits, which were signed or marked with a thumbprint by the primary caregiver or a parent or guardian over 18 years old. Mothers were recruited and enrolled in the study in their third trimester of pregnancy between February 19th 2021 and October 9th 2022.

3. Results

We collected a total of 162 water samples, including household stored (n=75), household tap (n=38) and community tap (n=49) sample types. Results from the TAC analysis for individual targets (i.e. not aggregated) are shown in the supplemental information (Table S4).

3.1 Cultured *E. coli*

Household stored water had the highest concentrations of culturable *E. coli* (mean = 83 CFU/100 mL, sd=321), followed by household piped water (mean = 64 CFU/100 mL, sd=393), and community tap water (mean = 1.6 CFU/100 mL, sd=10) (Table 2).

Table 2. Seasonal prevalence and mean concentration of culturable E. coli (most probable number (MPN) of E. coli per 100 mL of drinking water) from IDEXX analysis

		Household Stored (n=75)		**Household Tap (n=38)**		**Community Tap (n=49)**	
Organism	**Season**	% Detects (n)	Mean (sd) MPN/100 mL water	% Detects (n)	Mean (sd) MPN/100 mL water	% Detects (n)	Mean (sd) MPN/100 mL water
Culturable *E. coli*	Both seasons	44% (33/75)	83 (321)	18% (7/38)	64 (393)	22% (11/49)	1.6 (10)
	Rainy season	54% (13/24)	52 (212)	17% (2/12)	202 (699)	36% (9/25)	2.9 (14)
	Dry season	39% (20/51)	98 (361)	19% (5/26)	0.23 (0.52)	8% (2/24)	0.13 (0.45)

3.2 Molecular Targets

3.2.1 Community Tap Water

Of the 49 community taps sampled, we detected an enteric pathogen in seven (14%) samples. We detected *Cryptosporidium* spp. in water from three (6%) community taps, rotavirus in three (6%), and *E. coli* (EAEC aatA) in one (2%) sample.

In one community tap, we detected five different genes (CTX M1, CTX M2, CTX M8, SHV, TEM) associated with extended-spectrum beta-lactamases (ESBL) that may

confer resistance to beta-lactams. We found int1, a mobile genetic element, in 19 (39%) of community taps (38,39). We detected human mtDNA, a marker of human fecal contamination (40), in samples from four (8%) community taps.

3.2.2 Household Tap Water

Of the 38 households sampled, we detected *Cryptosporidium* spp. in three (8%) household taps, and *Giardia* spp. in one (3%).

In taps from four households, we detected one or more ESBL genes (CTX M8, CTX M9, SHV, TEM). We detected the intl1 gene in 11 (29%) household tap water samples, and human mtDNA in five (13%).

3.2.3 Household Stored Water

Stored drinking water had the greatest prevalence and diversity of enteric pathogens detected. Of the 75 households tested, we detected at least one enteric pathogen in 20 (27%) stored water samples. Enteroaggregative *E. coli* (EAEC aaiC or EAEC aatA) was detected in four (5%) samples, enteropathogenic *E. coli* (EPEC eae and EPEC bfpA) in one (1%), enterotoxigenic *E. coli* (ETEC LT or ETEC STp) in two (3%), and diffusely adherent *E. coli* (DEAC afaB) in two (3%) samples. Non-toxigenic *Vibrio* spp. was found in six (8%) samples. We detected *Cryptosporidium* spp. in 10 (13%) stored water samples, and *Giardia* spp. in four (5%). No viruses or helminths were detected in any stored water sample.

We detected an AMR ESBL gene in 54 (72%) of stored household water samples, including CTX M1, CTX M2, CTX M8, CTX M9, SHV, and TEM. In one stored household water sample we detected mcr-1, a gene associated with colistin resistance

(41). We found intl1 in 62 (83%) household stored water samples, and human mtDNA in 49 (65%) samples.

3.2.4 Culturable *E. coli* co-detection

Of the 162 samples of all types that were evaluated, we detected at least one pathogen in 29 samples, 62% (18) of which also contained culturable levels of *E. coli*. Co-detection of *E. coli* by culture in the same sample as a pathogen target indicates potential for viability and therefore exposure risk to people consuming this water. Of the 16 samples where *Cryptosporidium* spp. was detected, 8 (50%) also tested positive for culturable *E. coli*. Similarly, culturable *E. coli* was detected alongside *Giardia* in 20% (1/5), *Vibrio* in 67% (4/6), EAEC (aaiC) in 50% (2/4), EAEC (aatA) in 50% (2/4), ETEC (LT) in 50% (1/2), and DAEC in 50% (1/2).

3.3 Detection and quantification of enteric pathogens and other targets across sample types and seasons

Detection and quantification of enteric pathogens and other targets from the molecular analysis are shown in Table 3.

Table 3. Seasonal prevalence and mean concentration of molecular targets in gene copies (gc) of target per 100 mL of different sample types of drinking water from TaqMan Array Card RT-qPCR analysis. Values used as input into regression analyses.

		Household Stored (n=75)		**Household Tap (n=38)**		**Community Tap (n=49)**	
Organism	**Season**	% Detects (n)	Mean (sd)	% Detects (n)	Mean (sd)	% Detects (n)	Mean (sd)

			gc/100 mL water		gc/100 mL water		gc/100 mL water
Any pathogen (n=31)	Both seasons	2% (34/2,325)	$9.0x10^2$ ($2.4x10^4$)	0.3% (4/1,178)	$1.9x10^1$ ($3.1x10^2$)	0.5% (7/1,519)	$1.7x10^1$ ($3.4x10^2$)
	Rainy season	2% (12/744)	$3.4x10^2$ ($7.1x10^3$)	0.3% (1/372)	$1.1x10^1$ ($1.9x10^2$)	0.5% (4/775)	$7.7x10^0$ ($1.2x10^2$)
	Dry season	1% (22/1,581)	$1.2x10^3$ ($2.9x10^4$)	0.4% (3/806)	$2.3x10^1$ ($3.6x10^2$)	0.4% (3/744)	$2.7x10^1$ ($4.7x10^2$)
Any protozoa (n=4)	Both seasons	5% (14/300)	$3.8x10^3$ ($6.2x10^4$)	3% (4/152)	$1.4x10^2$ ($8.6x10^2$)	0.8% (3/196)	$2.1x10^1$ ($2.4x10^2$)
	Rainy season	4% (4/96)	$8.2x10^1$ ($4.1x10^2$)	2% (1/48)	$7.8x10^1$ ($5.3x10^2$)	3% (3/100)	$4.0x10^1$ ($3.3x10^2$)
	Dry season	5% (10/204)	$5.6x10^3$ ($7.5x10^4$)	3% (3/104)	$1.7x10^2$ ($9.8x10^2$)	0% (0/96)	$1.6x10^0$ ($0.89x10^0$)
Any AMR (n=11)	Both seasons	22% (184/825)	$5.6x10^5$ ($9.2x10^6$)	4% (18/418)	$3.0x10^2$ ($1.9x10^3$)	5% (27/539)	$2.3x10^3$ ($4.6x10^4$)
	Rainy season	25% (66/264)	$1.6x10^6$ ($1.6x10^7$)	5% (7/132)	$5.0x10^2$ ($2.6x10^3$)	6% (16/275)	$4.4x10^3$ ($6.5x10^4$)
	Dry season	21% (118/561)	$6.8x10^4$ ($5.6x10^5$)	4% (11/286)	$2.0x10^2$ ($1.4x10^3$)	4% (11/264)	$7.5x10^1$ ($5.9x10^2$)
Human fecal contamination (mtDNA) (n=1)	Both seasons	65% (49/75)	$1.6x10^4$ ($2.8x10^4$)	13% (5/38)	$8.5x10^2$ ($2.5x10^3$)	8% (4/49)	$2.7x10^2$ ($1.3x10^3$)
	Rainy season	71% (17/24)	$1.6x10^4$ ($2.7x10^4$)	17% (2/12)	$1.0x10^3$ ($2.8x10^3$)	12% (3/25)	$4.0x10^2$ ($1.7x10^3$)
	Dry season	63% (32/51)	$1.5x10^4$ ($2.9x10^4$)	12% (3/26)	$7.7x10^2$ ($2.5x10^3$)	4% (1/24)	$1.5x10^2$ ($7.0x10^2$)

AMR=Antimicrobial resistance

Stored household water was associated with increased prevalence of pathogen targets (PR=4.3, aPR=4.2, p<0.05), AMR genes (PR=5.2, aPR=5.2, p<0.05), and human fecal contamination as measured by mtDNA (PR=5.0, aPR=4.9, p>0.05) compared to household tap water. There were no statistically significant differences in prevalence of protozoa only between water types. Bacteria and viruses were not analyzed between water types as they were not detected frequently between the water types. Culturable *E. coli* was also more prevalent in stored water compared to household tap water. There were no differences in prevalence between household tap water and community tap water for any indicator (Table 4, Figure 2).

Table 4. Prevalence ratios, adjusted for season and intervention status. Household tap water as reference. Bolded p-values indicate statistical significance at alpha 0.05.

	Water Type	**PR (95% CI)**	**p-value**	**aPR (95% CI)**	**Adjusted p-value**
Any pathogen present	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	1.4 (0.40-4.6)	0.625	1.4 (0.42-4.8)	0.574
	Household Stored	**4.3 (1.5-12)**	**0.006**	**4.2 (1.5-12)**	**0.006**
Any protozoa present	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	0.58 (0.13-2.6)	0.474	0.58 (0.14-2.4)	0.456
	Household Stored	1.8 (0.59-5.3)	0.305	1.7 (0.58-5.2)	0.316
Any AMR genes present	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	1.0 (0.57-1.9)	0.913	1.0 (0.55-1.8)	0.991
	Household Stored	**5.2 (3.2-8.3)**	**<0.001**	**5.2 (3.2-8.3)**	**<0.001**
Human fecal contamination	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	0.62 (0.18-2.2)	0.454	0.60 (0.17-2.1)	0.421
	Household Stored	**5.0 (2.2-11)**	**<0.001**	**4.9 (2.2-11)**	**<0.001**

(mtDNA MST marker) present					
Culturable *E. coli* present	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	1.2 (0.52-2.9)	0.649	1.1 (0.48-2.5)	0.816
	Household Stored	**2.4 (1.2-4.9)**	**0.018**	**2.4 (1.2-4.9)**	**0.018**

AMR=Antimicrobial resistance, MST=microbial source tracking

Figure 2. Prevalence ratios of water quality indicators in unadjusted and adjusted regression models with household tap water as reference

Household stored water was marginally associated with greater concentrations of enteric pathogens in the unadjusted (p=0.08) and adjusted (p=0.08) models, and AMR genes in the unadjusted (p=0.08) and adjusted (p=0.09) models, compared to household tap water. Household stored water had significantly greater concentrations of human fecal contamination (p<0.05) compared to household tap water (Table 5, Figure 3).

Table 5. Mean difference in concentration between water types, adjusted for rainy season and intervention status. Household tap water as reference.

	Water Type	**Mean Difference (95% CI)**	**p-value**	**Adjusted Mean Difference (95% CI)**	**p-value**
Concentration of any pathogen	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	-2.4 (-27-22)	0.847	188 (-87-463)	0.181
	Household Stored	885 (-110-1880)	0.081	841 (-99-1780)	0.079
Concentration of protozoa	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	-119 (-260-22)	0.099	874 (-1110-2858)	0.387

	Household Stored	3675 (-3305-10656)	0.302	3483 (-3115-10081)	0.300
Concentration of AMR genes	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	1964 (-1950-5878)	0.325	-167141 (-415673-81392)	0.187
	Household Stored	555068 (-70975-1181113)	0.082	566836 (-814550-1215126)	0.087
Concentration of human mtDNA	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	-571 (-1462-320)	0.208	383 (-1968-2733)	0.748
	Household Stored	**14672 (8194-21149)**	**<0.001**	**14276 (8052-20500)**	**<0.001**
Concentration of culturable *E. coli*	Household Tap	Ref	Ref	Ref	Ref
	Community Tap	-62 (-188-63)	0.327	-72 (-223-78)	0.344
	Household Stored	19 (-126-164)	0.793	21 (-122-165)	0.771

AMR=Antimicrobial resistance

Figure 3. Forest plot of mean difference of water quality indicators with household tap water as reference, adjusted for rainy season and intervention status. Log transformed.

There was no difference in prevalence of all pathogens, protozoa only, AMR targets, or human fecal contamination in any drinking water type between the dry and rainy seasons (Table 6). In community tap water, culturable *E. coli* was more prevalent during the rainy season compared to the dry season (PR=4.3, p<0.05).

Table 6. Differences in prevalence between seasons, dry season as reference

	Water Type	**PR (95% CI)**	**p-value**
Any pathogen present	Household Tap	0.72 (0.075-6.9)	0.778

	Community Tap	1.3 (0.29-5.7)	0.746
	Household Stored	1.2 (0.58-2.3)	0.679
Any protozoa present	Household Tap	0.72 (0.077-6.8)	0.776
	Community Tap	NA	NA
	Household Stored	0.85 (0.27-2.6)	0.779
Any AMR present	Household Tap	1.4 (0.55-4.5)	0.497
	Community Tap	1.1 (0.52-2.5)	0.753
	Household Stored	1.2 (0.91-1.5)	0.199
Human fecal contamination present	Household Tap	1.4 (0.27-7.7)	0.667
	Community Tap	2.9 (0.31-26)	0.349
	Household Stored	1.1 (0.81-1.6)	0.478
Culturable *E. coli* present	Household Tap	0.87 (0.19-3.9)	0.853
	Community Tap	**4.3 (1.0-18)**	**0.047**
	Household Stored	1.4 (0.83-2.3)	0.211

AMR=Antimicrobial resistance

4. Discussion

Using large-volume sampling, we detected pathogens in both stored and tap drinking water supplies in informal settlements in Beira, Mozambique. We most consistently found *Cryptosporidium* but also detected other pathogens. More than half of the drinking water samples were accompanied by co-detection of *E. coli*, indicating potential for viability and therefore exposure risk to people consuming this water. We found that enteric pathogens, AMR targets, human mtDNA, and culturable *E. coli* were all more prevalent in stored water compared to household tap water. We did not find any differences between pathogen presence or concentration in drinking water between the rainy and dry seasons.

370 Beira has an improved water system, as defined by the Joint Monitoring Programme
371 (JMP). However, it can be characterized as an intermittent water supply as it does not
372 deliver water for 24 hours a day, seven days a week. PAASIM study staff surveyed
373 each enrolled household on water intermittency and found that those with a household
374 water connection had access to their piped water an average of 6.3 days a week
375 (sd=1.5) and 11.4 hours per day (sd=6.0)(42). Water intermittency can impact water
376 quality even in a highly chlorinated system. A study in India comparing intermittent
377 supply to continuous supply found that water served by an intermittent supply was more
378 likely to be positive for culturable *E. coli* and waterborne pathogen gene targets
379 including ETEC, *Shigella*/EIEC, norovirus GI and GII, adenovirus, *Cryptosporidium*, and
380 *Giardia* (9). Furthermore, 99% of households enrolled in PAASIM reporting storing
381 drinking water (43). Intermittency not only impacts the water quality coming out of the
382 taps but also necessitates water storage, which is associated with decreased water
383 quality.

384 Water supply characteristics in Beira could influence the pathogen concentrations we
385 detected in the drinking water. The piped water in this study comes from a centralized
386 water distribution system that was undergoing improvements to certain areas during the
387 sampling period. Water samples were collected from neighborhoods that had already
388 been connected to the upgraded distribution system and those that were still on the
389 older system. Illegal tapping of these networks is not uncommon, allowing for infiltration
390 during periods of heavy rain leading to flooding (44).

391

We detected *Cryptosporidium* in community and household taps, possibly as a result of inadequate filtration processes at the water treatment facility or infiltration from illegal connections, as a consequence of intermittent water supply. PAASIM study staff monitored free and total chlorine at the community taps on a longitudinal basis and found consistent levels of residual free chlorine to protect against bacterial growth (0.2-2.0 mg/L) in 80% of samples over a one-year period, which was associated with lower *E. coli* contamination (45). However, *Cryptosporidium* is highly resistant to chlorine and requires filtration or additional disinfection techniques (e.g. UV light or ozonation) for efficient removal or inactivation. *Cryptosporidium* oocysts are also highly infectious, with a low dose required for infection (46). Detection of *Cryptosporidium* in a water supply has historically been affiliated with city-wide cryptosporidiosis outbreaks (47,48). *Cryptosporidium* oocysts can cause severe gastrointestinal illness with diarrhea lasting 10-14 days (49). In immunocompromised individuals, illness can become chronic or even fatal (50). Infection in children under 5 can have long-term health impacts and has been associated with a decrease in height-for-age, weight-for-age, and weight-for-height Z scores (51).

Exposure to enteric pathogens in drinking water can result in gastrointestinal illnesses, but direct transmission pathways are difficult to assess. Concordance between enteric pathogen findings in drinking water and those detected in stool from exposed individuals could potentially demonstrate the relevance of the waterborne exposure pathway. The PAASIM trial assayed for the same pathogens in stool that we tested for in their drinking water, from children aged 3-12 months enrolled in the study and found that 15% of stool samples (91/600) were positive for *Cryptosporidium*, reflecting a similarly high

prevalence to our findings in stored drinking water (13%,10/75 were positive)(43). *Cryptosporidium* prevalence in our study population is higher compared with other studies measuring *Cryptosporidium* in child stool in similar settings (52–55). Detecting *Cryptosporidium* in both drinking water and stool samples from children exposed to the drinking water is a notable finding in Beira, suggesting the need for further understanding its local pathways of transmission and to identify intervention strategies to prevent infections.

Besides *Cryptosporidium*, other frequently detected pathogens in child stool samples were DAEC (73%), EAEC (44%), atypical EPEC (36%), *C. jejuni/coli* (26%), typical EPEC (20%), *Giardia* spp. (20%), EIEC/*Shigella* (16%), norovirus (11%), ETEC (10%), sapovirus (10%), rotavirus (7%), *C. difficile* (7%), and astrovirus (5%) (43). In our water samples, we detected some but not all of these, consistent with the hypothesis that waterborne exposures may present infection risk in this setting, but no direct test of this was possible.

Contrary to what we expected, we did not find seasonal differences between pathogen presence or concentration in drinking water. However, this may have been affected by the particular conditions of the rainy and dry season in the year that we carried out sampling. We defined rainy and dry seasons based on historical averages, but The months we sampled for the dry season had more precipitation (7 days, 50-85 mm per month) than historical averages (2-3 days, 22-25 mm per month), while our rainy season months (14 days, 150-221 mm per month) were more aligned with historical averages (12-14 days, 187-244 mm per month) (56).

437 We detected genes associated with AMR and human fecal contamination in samples
438 across all three water types. We frequently detected ESBL genes (CTX-M, SHV, TEM),
439 which encode beta-lactamases that confer resistance to third-generation cephalosporin
440 antibiotics (57). Finding these genes in drinking water can elevate risks of human
441 exposure to bacteria and could contribute to antibiotic treatment failures, especially in
442 immunocompromised individuals (58). One stored household water sample contained
443 mcr-1, which confers resistance to colistin, another last-resort antibiotic used to treat
444 multi-drug resistant infections (41). Colistin resistance is capable of horizontal gene
445 spreading, making it particularly concerning to find in an environmental sample (41). We
446 frequently detected intI1, the class 1 integron gene, in all three water types. This
447 integron is a marker of AMR, as it encodes for mobile genetic elements associated with
448 the transfer of AMR genes between bacteria (38). The presence of these AMR genes in
449 drinking water in Beira suggests contamination from wastewater or agriculture runoff, or
450 possible reservoirs in biofilms within the drinking water distribution system (59,60). The
451 combined presence of human mtDNA markers suggests human fecal contamination.
452 Conventional disinfection methods like chlorine may not fully inactivate AMR genes, and
453 advanced methods like UV or advanced oxidation could be more effective (61).

454 Many LMICs have intermittent water supply due to a range of factors including water
455 scarcity, limited energy supply, water governance, population growth, operation and
456 management, institutional, and socio-economic variables (62). Classifying safely
457 managed supplies requires risk characterization, which can be costly for municipalities
458 in LMICs. Following the JMP drinking water services ladder, and without continued
459 water quality testing and risk characterization, might overestimate the population that

truly receives safe drinking water. Our findings highlight the need to focus on continuity of service – including maintaining positive pressure 24 hours per day, every day - in water supply systems to meet Sustainable Development Goal 6, because intermittent water supplies cannot reliably be “free from contamination” (63) . If continuous water supply is not achievable, household water practices like safe storage and handling become necessary to protect consumers.

4.1 Limitations

Measuring pathogens in environmental matrices can be difficult, as they occur in lower concentrations than in clinical samples, requiring concentration and sensitive methods for detection. For our methods, we calculated a high lower limit of detection (LOD) for our molecular targets, meaning that we may have missed detecting pathogens that were at lower concentrations. However, our findings represent a conservative estimate of the true water quality of our samples. When using molecular detection methods, we are only able to determine the amount of genetic material present in the sample, which does not always correlate to pathogen viability. Viability could be estimated indirectly based on culturable *E. coli* proportions relative to genetic material concentrations. However, there are limitations of using *E. coli* as a reliable indicator of fecal contamination. In tropical or sub-tropical environments, as Beira can be characterized, environmental factors including temperature, pH, humidity, and salinity can all affect the persistence of *E. coli* in the environment, and some species may have the potential to naturalize in tropical waters (64). Additionally, bacteriological indicators such as *E. coli* may be less suitable indicators for viruses or protozoa (65).

We detected *Cryptosporidium* in all three water types, but it should be noted that the molecular assay used was a generalized assay encompassing multiple strains of the organism. There are at least 44 species of *Cryptosporidium* with over 120 genotypes, and 19 of these species and 4 genotypes have been found in humans (66). Around 95% of human cases are from *C. parvum* (zoonotic) and *C. hominis* (anthroponotic), but other zoonotic species have also resulted in cryptosporidiosis cases (66,67). The *Cryptosporidium* spp. assay that we used came from Liu *et al* 2016 and targets the 18s rRNA gene (68).

Members of the *Vibrio genus* were detected in stored water samples, and the assay, targeting the *hylA* gene, was originally published as *Vibrio cholerae* (68). Upon further investigation using digital PCR, we found that none of the *Vibrio* contained the ctxA toxin gene and have reclassified this assay as *Vibrio* spp. in our results because these are probably detections of non-choleragenic *Vibrio* species.

We detected rotavirus in 3 community taps, as the only viral detections across our sample set. Rotavirus is highly diverse, containing 10 species (A-J), with group A as the most common species to infect humans (69). Our rotavirus assay targeted the NSP3 gene, a protein that is not specific to human strains (68). Since 2015, live-attenuated rotavirus vaccines have been routinely administered at ages 2 and 3 months in Mozambique, with high compliance rates (73% in 2021) (70). There is evidence of viral shedding post vaccination (71). Apart from 3 samples positive for rotavirus in community tap samples, we did not detect viral targets in water; enteric virus transmission may be dominated by person-to-person exposures (54,72–74).

5. Conclusions

Our findings show that microbial contamination of drinking water with enteric pathogens and AMR targets can occur even in a well-managed piped water supply system. The need for household water storage increases microbial hazards for users at the point of consumption. Without maintaining continuous water pressure, systems cannot reliably provide safe water: contamination opportunities within the system and subsequent risks from water storage are well known. Eliminating intermittent water supply is an important goal that can improve water safety. Continuity of service should be incorporated as a criterion for “safely managed” water supplies in the context of SDG6. Furthermore, our finding of *Cryptosporidium* in the water supply, in combination with infection in children, shows a possible exposure route that should be addressed with proper filtration removal at the water treatment facility or through household water filtration combined with safe storage.

6. Acknowledgements

This work was supported by National Institute of Allergy and Infectious Diseases (NIAID) Grant #R01AI130163, National Institute of Environmental Health Sciences (NIEHS) Grant #5T32ES12870 and a contract from Autoridade Reguladora de Água, Instituto Público (AURA, IP), Mozambique. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. We would like to acknowledge the PAASIM data collection field staff, as well as those in the PAASIM study authorship consortium who are listed below, for their contributions to this research.

Joshua Garn[1], Konstantinos Konstantinidis[2], Tom Clasen[3], Nicolette Zhou[4], Molly Miller-Petrie[4], Sydney Hubbard[3], Courtney Victor[3], Rebecca Kann[4], Lilly O'Brien[2]

1. Department of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Nevada, Reno, Nevada, United States of America
2. School of Civil & Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia, USA
3. Gangarosa Department of Environmental Health, Emory University, Atlanta, Georgia, United States
4. Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, United States

7. References

1. UNICEF, WHO. Progress on household drinking water, sanitation and hygiene 2000–2022: special focus on gender. New York; 2023.

2. Greenwood EE, Lauber T, van den Hoogen J, Donmez A, Bain RES, Johnston R, et al. Mapping safe drinking water use in low- and middle-income countries. Science. 2024 Aug 15;385(6710):784–90.

3. Wolf J, Johnston RB, Ambelu A, Arnold BF, Bain R, Brauer M, et al. Burden of disease attributable to unsafe drinking water, sanitation, and hygiene in domestic settings: a global analysis for selected adverse health outcomes. The Lancet. 2023 Jun;401(10393):2060–71.

4. Peletz R, Kisiangani J, Bonham M, Ronoh P, Delaire C, Kumpel E, et al. Why do water quality monitoring programs succeed or fail? A qualitative comparative analysis of regulated testing systems in sub-Saharan Africa. Int J Hyg Environ Health. 2018 Jul;221(6):907–20.

5. Shaheed A, Orgill J, Montgomery MA, Jeuland MA, Brown J. Why “improved” water sources are not always safe. Bull World Health Organ. 2014 Apr 1;92(4):283–9.

6. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal Contamination of Drinking-Water in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. PLOS Med. 2014 May 6;11(5):e1001644.

7. Shaheed A, Orgill J, Ratana C, Montgomery MA, Jeuland MA, Brown J. Water quality risks of “improved” water sources: evidence from Cambodia. Trop Med Int Health TM IH. 2014 Feb;19(2):186–94.

8. Bain R, Johnston R, Khan S, Hancioglu A, Slaymaker T. Monitoring Drinking Water Quality in Nationally Representative Household Surveys in Low- and Middle-Income Countries: Cross-Sectional Analysis of 27 Multiple Indicator Cluster Surveys 2014–2020. Environ Health Perspect. 2021 Sep;129(9):097010.

9. Bivins A, Lowry S, Wankhede S, Hajare R, Murphy HM, Borchardt M, et al. Microbial water quality improvement associated with transitioning from intermittent to continuous water supply in Nagpur, India. Water Res. 2021 Aug;201:117301.

10. Besner MC, Prévost M, Regli S. Assessing the public health risk of microbial intrusion events in distribution systems: Conceptual model, available data, and challenges. Water Res. 2011 Jan 1;45(3):961–79.

11. Bivins AW, Sumner T, Kumpel E, Howard G, Cumming O, Ross I, et al. Estimating Infection Risks and the Global Burden of Diarrheal Disease Attributable to Intermittent Water Supply Using QMRA. Environ Sci Technol. 2017 Jul 5;51(13):7542–51.

574 12. Kumpel E, Nelson KL. Intermittent Water Supply: Prevalence, Practice, and
575 Microbial Water Quality. Environ Sci Technol. 2016 Jan 19;50(2):542–53.

576 13. Wright J, Gundry S, Conroy R. Household drinking water in developing countries:
577 a systematic review of microbiological contamination between source and point-of-
578 use. Trop Med Int Health. 2004;9(1):106–17.

579 14. Clasen TF, Bastable A. Faecal contamination of drinking water during collection
580 and household storage: the need to extend protection to the point of use. J Water
581 Health. 2003 Sep 1;1(3):109–15.

582 15. Smith CM, Hill VR. Dead-End Hollow-Fiber Ultrafiltration for Recovery of Diverse
583 Microbes from Water. Appl Environ Microbiol. 2009 Aug;75(16):5284–9.

584 16. Lu J, Struewing I, Vereen E, Kirby AE, Levy K, Moe C, et al. Molecular Detection
585 of Legionella spp. and their associations with Mycobacterium spp., Pseudomonas
586 aeruginosa and amoeba hosts in a drinking water distribution system. J Appl
587 Microbiol. 2016 Feb 1;120(2):509–21.

588 17. Polaczyk AL, Narayanan J, Cromeans TL, Hahn D, Roberts JM, Amburgey JE, et
589 al. Ultrafiltration-based techniques for rapid and simultaneous concentration of
590 multiple microbe classes from 100-L tap water samples. J Microbiol Methods. 2008
591 May 1;73(2):92–9.

592 18. Liu P, Hill VR, Hahn D, Johnson TB, Pan Y, Jothikumar N, et al. Hollow-fiber
593 ultrafiltration for simultaneous recovery of viruses, bacteria and parasites from
594 reclaimed water. J Microbiol Methods. 2012 Jan 1;88(1):155–61.

595 19. Holcomb D. Human fecal contamination of water, soil, and surfaces in
596 households sharing poor-quality sanitation facilities in Maputo, Mozambique | Elsevier
597 Enhanced Reader. Int J Hyg Environ Health [Internet]. 2020 May [cited 2022 Oct
598 30];226. Available from:
599 https://reader.elsevier.com/reader/sd/pii/S1438463919309873?token=3ACB5840AD2
600 0C59BE4320D448F68E4A554360F4C84A014FA9EFFEC865057CC20E2B67639D6
601 50916408F3643A461DAF7E&originRegion=us-east-
602 1&originCreation=20221030131824

603 20. Rothrock Jr. MJ, Keen PL, Cook KL, Durso LM, Franklin AM, Dungan RS. How
604 Should We Be Determining Background and Baseline Antibiotic Resistance Levels in
605 Agroecosystem Research? J Environ Qual. 2016 Mar;45(2):420–31.

606 21. Berkner S, Konradi S, Schönfeld J. Antibiotic resistance and the environment—
607 there and back again. EMBO Rep. 2014 Jul;15(7):740–4.

608 22. Li S, Ondon BS, Ho SH, Zhou Q, Li F. Drinking water sources as hotspots of
609 antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs):
610 Occurrence, spread, and mitigation strategies. J Water Process Eng. 2023 Jul
611 1;53:103907.

612 23. Kostyla C, Bain R, Cronk R, Bartram J. Seasonal variation of fecal contamination
613 in drinking water sources in developing countries: A systematic review. Sci Total
614 Environ. 2015 May 1;514:333–43.

615 24. Levy K, Garn JV, Cumbe ZA, Muneme B, Fagnant-Sperati CS, Hubbard S, et al.
616 Study design and rationale for the PAASIM project: a matched cohort study on urban
617 water supply improvements and infant enteric pathogen infection, gut microbiome
618 development and health in Mozambique. BMJ Open. 2023 Mar;13(3):e067341.

619 25. World Bank, Africa Urban & Water Unit. Municipal ICT Capacity and its Impact on
620 the Climate-Change Affected Urban Poor: The Case of Mozambique. Washington,
621 DC; 2012 p. 65.

622 26. The World Bank. Water Services and Institutional Support Project II. 2016.

623 27. The World Bank. Water Services & Institutional Support II. 2024 Jun.

624 28. Kumpel E, Nelson KL. Comparing microbial water quality in an intermittent and
625 continuous piped water supply. Water Res. 2013 Sep;47(14):5176–88.

626 29. Eisenhauer IF, Hoover CM, Remais JV, Monaghan A, Celada M, Carlton EJ.
627 Estimating the Risk of Domestic Water Source Contamination Following Precipitation
628 Events. Am Soc Trop Med Hyg. 2016 Jun 1;94(6):1403–6.

629 30. Phan NK, Sherchan SP. Microbiological Assessment of Tap Water Following the
630 2016 Louisiana Flooding. Int J Environ Res Public Health. 2020 Feb 17;17(4):1273.

631 31. Kann RS, Garn J, Fagnant-Sperati CS, Snyder JS, Kowalsky E, Brown J, et al.
632 Variability of enteric pathogen infections by season and meteorological conditions in
633 a low-income, urban setting in Mozambique [Internet]. medRxiv; 2025 [cited 2026 Jan
634 26]. p. 2025.10.02.25337035. Available from:
635 https://www.medrxiv.org/content/10.1101/2025.10.02.25337035v1

636 32. Murphy H. Global Water Pathogen Project. Michigan State University, UNESCO;
637 2017 [cited 2025 Feb 26]. Persistence of Pathogens in Sewage and Other Water
638 Types. Available from: https://www.waterpathogens.org/book/persistence-in-sewage

639 33. Aw. Global Water Pathogen Project. Michigan State University, UNESCO; 2016
640 [cited 2025 Feb 26]. Environmental Aspects and Features of Critical Pathogen
641 Groups. Available from: https://www.waterpathogens.org/book/environmental-
642 aspects-and-features-of-critical-pathogen-groups

643 34. Korich DG, Mead JR, Madore MS, Sinclair NA, Sterling CR. Effects of ozone,
644 chlorine dioxide, chlorine, and monochloramine on Cryptosporidium parvum oocyst
645 viability. Appl Environ Microbiol. 1990 May;56(5):1423–8.

646 35. Mull B, Hill VR. Recovery of diverse microbes in high turbidity surface water
647 samples using dead-end ultrafiltration. J Microbiol Methods. 2012 Dec;91(3):429–33.

36. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin Chem. 2009 Apr 1;55(4):611–22.

37. McCall MN, McMurray HR, Land H, Almudevar A. On non-detects in qPCR data. Bioinformatics. 2014 Aug 15;30(16):2310–6.

38. Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, et al. Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob. 2015 Oct 20;14:45.

39. Ali N, Ali I, Din AU, Akhtar K, He B, Wen R. Integrons in the Age of Antibiotic Resistance: Evolution, Mechanisms, and Environmental Implications: A Review. Microorganisms. 2024 Dec;12(12):2579.

40. Zhu K, Suttner B, Pickering A, Konstantinidis KT, Brown J. A novel droplet digital PCR human mtDNA assay for fecal source tracking. Water Res. 2020 Sep 15;183:116085.

41. Ling Z, Yin W, Shen Z, Wang Y, Shen J, Walsh TR. Epidemiology of mobile colistin resistance genes mcr-1 to mcr-9. J Antimicrob Chemother. 2020 Nov 1;75(11):3087–95.

42. Victor C, Ocasio DV, Cumbe ZA, Garn JV, Hubbard S, Mangamela M, et al. Spatial heterogeneity of neighborhood-level water and sanitation access in informal urban settlements: A cross-sectional case study in Beira, Mozambique. PLOS Water. 2022 Jun 9;1(6):e0000022.

43. Freeman M, Victor C, Garn J, Kann R, Fagnant-Sperati C, Kowalsky E, et al. The Impact of Urban Water Supply Improvements on Infant Enteric Pathogen Infection, Diarrhea, and Growth: Results from the PAASIM Matched Cohort Study [Internet]. Research Square; 2025 [cited 2025 Sep 10]. Available from: https://www.researchsquare.com/article/rs-6697339/v1

44. African Development Bank. Project Performance Evaluation Reports for Mozambique: Maputo Water Supply Rehabilitation Project - Zambia:Central Province Water Supply and Sanitation Project - Approach Paper. 2011 Oct;

45. Kann R, Hubbard S, Snyder J, McGunegill S, Muneme B, Manuel JL, et al. Impact of weather and season on stored water contamination and infant diarrhea in climate-vulnerable, urban Mozambique. 2025 Nov 18 [cited 2026 Jan 26]; Available from: https://eartharxiv.org/repository/view/10831/

46. Messner MJ, Berger P. Cryptosporidium Infection Risk: Results of New Dose-Response Modeling. Risk Anal. 2016;36(10):1969–82.

47. Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, et al. A Massive Outbreak in Milwaukee of Cryptosporidium Infection Transmitted through the Public Water Supply. N Engl J Med. 1994 Jul 21;331(3):161–7.

685 48. Mason BW, Chalmers RM, Carnicer-Pont D, Casemore DP. A Cryptosporidium
686 hominis outbreak in North-West Wales associated with low oocyst counts in treated
687 drinking water. J Water Health. 2010 Jun 1;8(2):299–310.

688 49. Chalmers RM, Davies AP. Minireview: Clinical cryptosporidiosis. Exp Parasitol.
689 2010 Jan 1;124(1):138–46.

690 50. Yang X, Guo Y, Xiao L, Feng Y. Molecular Epidemiology of Human
691 Cryptosporidiosis in Low- and Middle-Income Countries. Clin Microbiol Rev. 2021
692 Feb 24;34(2):10.1128/cmr.00087-19.

693 51. Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, et al. Morbidity,
694 mortality, and long-term consequences associated with diarrhoea from
695 Cryptosporidium infection in children younger than 5 years: a meta-analyses study.
696 Lancet Glob Health. 2018 Jul 1;6(7):e758–68.

697 52. Korpe PS, Valencia C, Haque R, Mahfuz M, McGrath M, Houpt E, et al.
698 Epidemiology and Risk Factors for Cryptosporidiosis in Children From 8 Low-income
699 Sites: Results From the MAL-ED Study. Clin Infect Dis Off Publ Infect Dis Soc Am.
700 2018 Dec 1;67(11):1660–9.

701 53. Delahoy MJ, Omore R, Ayers TL, Schilling KA, Blackstock AJ, Ochieng JB, et al.
702 Clinical, environmental, and behavioral characteristics associated with
703 Cryptosporidium infection among children with moderate-to-severe diarrhea in rural
704 western Kenya, 2008–2012: The Global Enteric Multicenter Study (GEMS). PLoS
705 Negl Trop Dis. 2018 Jul 12;12(7):e0006640.

706 54. Knee J, Sumner T, Adriano Z, Anderson C, Bush F, Capone D, et al. Effects of
707 an urban sanitation intervention on childhood enteric infection and diarrhea in
708 Maputo, Mozambique: A controlled before-and-after trial. Lewnard J, Franco E,
709 Lewnard J, Platts Mills J, editors. eLife. 2021 Apr 9;10:e62278.

710 55. Kowalsky E, Monteiro V, Holcomb D, Mataveia E, Chiluvane M, Cumbane V, et
711 al. Effects of urban sanitation upgrades on enteric pathogen ... | VeriXiv [Internet].
712 2025 [cited 2025 Jun 12]. Available from: https://verixiv.org/articles/2-103/v1?src=rss

713 56. Beira Climate, Weather By Month, Average Temperature (Mozambique) -
714 Weather Spark [Internet]. 2025 [cited 2025 Mar 4]. Available from:
715 https://weatherspark.com/y/98048/Average-Weather-in-Beira-Mozambique-Year-
716 Round

717 57. Bush K, Jacoby GA. Updated functional classification of beta-lactamases.
718 Antimicrob Agents Chemother. 2010 Mar;54(3):969–76.

719 58. Beshiru A, Isokpehi NA, Igbinosa IH, Akinnibosun O, Ogofure AG, Igbinosa EO.
720 Extended-spectrum beta-lactamase (ESBL)- and non-ESBL producing Escherichia
721 coli surveillance in surface water sources in Edo State, Nigeria: a public health
722 concern. Sci Rep. 2024 Sep 17;14(1):21658.

59. Nolan TM, Reynolds LJ, Sala-Comorera L, Martin NA, Stephens JH, O'Hare GMP, et al. Land use as a critical determinant of faecal and antimicrobial resistance gene pollution in riverine systems. Sci Total Environ. 2023 May 1;871:162052.

60. Gholipour S, Shamsizadeh Z, Gwenzi W, Nikaeen M. The bacterial biofilm resistome in drinking water distribution systems: A systematic review. Chemosphere. 2023 Jul 1;329:138642.

61. Kalli M, Noutsopoulos C, Mamais D. The Fate and Occurrence of Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes during Advanced Wastewater Treatment and Disinfection: A Review. Water. 2023 Jan;15(11):2084.

62. Machimana LI, Gumbo AD, Moyo H, Mugari E. The Impact of Load-Shedding on Scheduled Water Delivery Services for Mohlaba-Cross Village, Greater Tzaneen, South Africa. Water. 2024 Jan;16(14):2033.

63. SDGs [Internet]. [cited 2025 Jul 2]. Available from: https://data.unhabitat.org/pages/sdgs

64. Rochelle-Newall E, Nguyen TMH, Le TPQ, Sengtaheuanghoung O, Ribolzi O. A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. Front Microbiol [Internet]. 2015 Apr 17 [cited 2025 Feb 24];6. Available from: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2015.00308/full

65. Motlagh AM, Yang Z. Detection and occurrence of indicator organisms and pathogens. Water Environ Res. 2019 Oct;91(10):1402–8.

66. Ryan U, Zahedi A, Feng Y, Xiao L. An Update on Zoonotic Cryptosporidium Species and Genotypes in Humans. Animals. 2021 Nov;11(11):3307.

67. Wang D, Jiang P, Yang X, Zhang J, Chen T, Hu M, et al. Novel strategy to quantify the viability of oocysts of *Cryptosporidium parvum* and *C. hominis*, a risk factor of the waterborne protozoan pathogens of public health concern. Water Res. 2024 Jul 1;258:121788.

68. Liu J, Gratz J, Amour C, Nshama R, Walongo T, Maro A, et al. Optimization of Quantitative PCR Methods for Enteropathogen Detection. PLOS ONE. 2016 Jun 23;11(6):e0158199.

69. Crawford SE, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, et al. Rotavirus infection. Nat Rev Dis Primer. 2017 Nov 9;3(1):1–16.

70. Manjate F, Quintó L, Chirinda P, Acácio S, Garrine M, Vubil D, et al. Impact of rotavirus vaccination on diarrheal hospitalizations in children younger than 5 years of age in a rural southern Mozambique. Vaccine. 2022 Oct 19;40(44):6422–30.

759 71. Hsieh YC, Wu FT, Hsiung CA, Wu HS, Chang KY, Huang YC. Comparison of
760 virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine.
761 Vaccine. 2014 Feb 26;32(10):1199–204.

762 72. Holcomb DA, Monteiro V, Capone D, António V, Chiluvane M, Cumbane V, et al.
763 Long-term impacts of an urban sanitation intervention on enteric pathogens in
764 children in Maputo city, Mozambique: study protocol for a cross- sectional follow-up
765 to the Maputo Sanitation (MapSan) trial 5 years postintervention. Open Access. 2025;

766 73. Sinharoy SS, Pittluck R, Clasen T. Review of drivers and barriers of water and
767 sanitation policies for urban informal settlements in low-income and middle-income
768 countries. Util Policy. 2019 Oct 1;60:100957.

769 74. Vickerstaff V, Ambler G, Omar RZ. A comparison of methods for analysing
770 multiple outcome measures in randomised controlled trials using a simulation study.
771 Biom J. 2021;63(3):599–615.

772

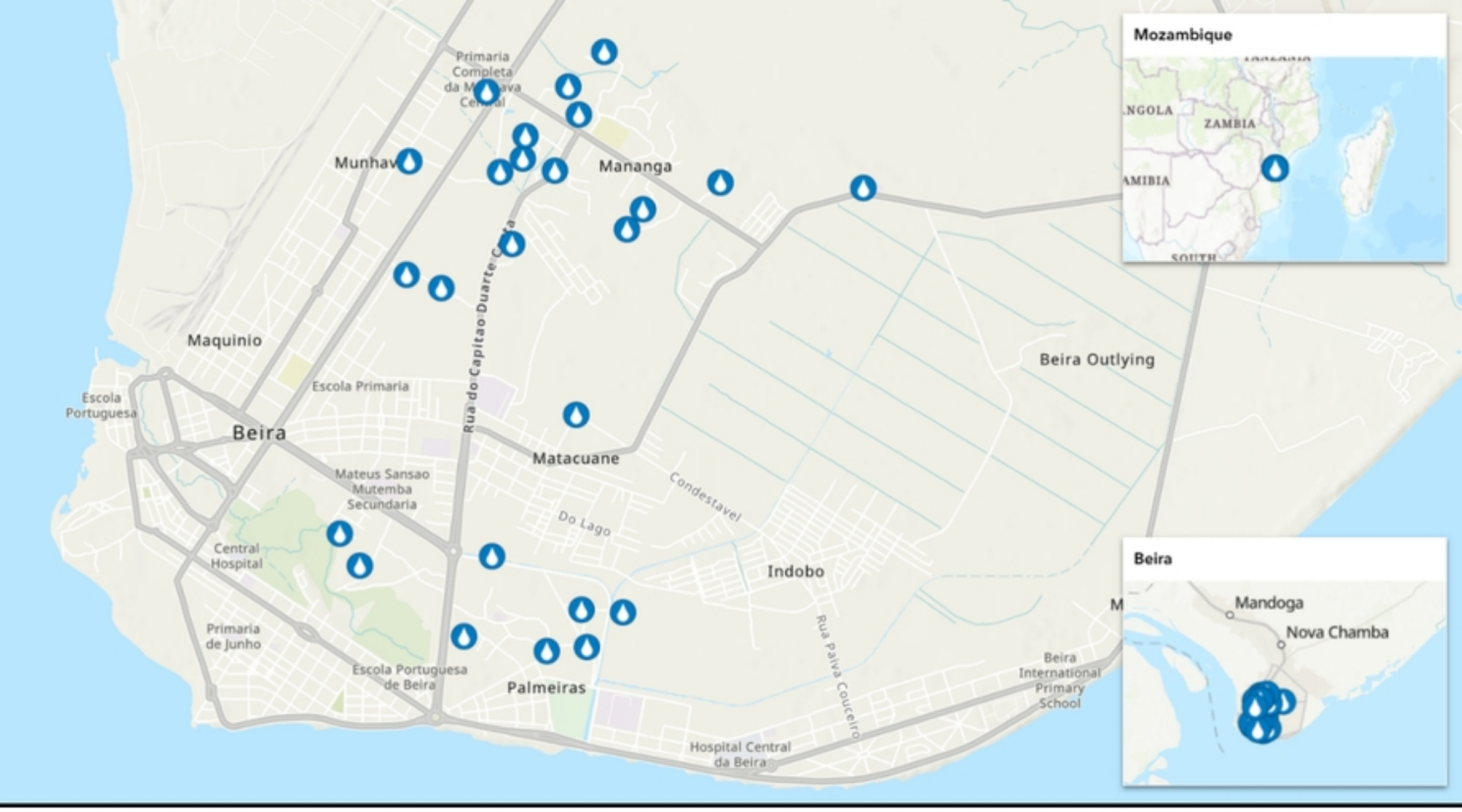
Mozambique
NGOLA
ZAMBIA
AMBIA
SOUTH
Beira
Mandoga
Nova Chamba
Munhava
Mananga
Primaria Completa da Munhava Central
Rua do Capitao Duarte Costa
Maquinio
Escola Portuguesa
Escola Primaria
Beira
Matacuane
Beira Outlying
Mateus Sansao Mutemba Secundaria
Condestavel
Do Lago
Central Hospital
Indobo
Primaria de Junho
Escola Portuguesa de Beira
Palmeiras
Rua Paiva Couceiro
Beira International Primary School
Hospital Central da Beira

Figure 1

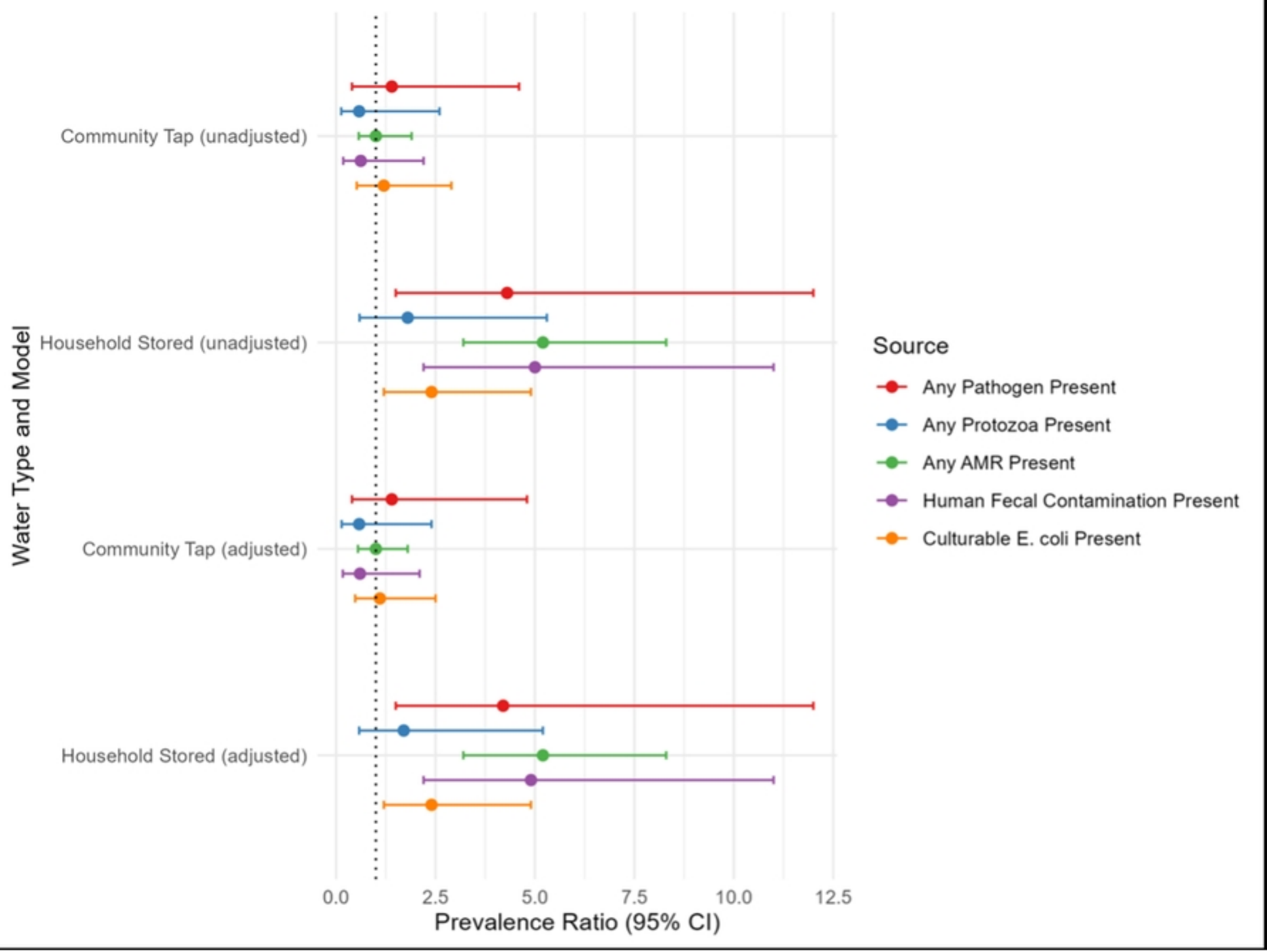
Water Type and Model
Community Tap (unadjusted)
Household Stored (unadjusted)
Community Tap (adjusted)
Household Stored (adjusted)
0.0
2.5
5.0
7.5
10.0
12.5
Prevalence Ratio (95% CI)
Source
Any Pathogen Present
Any Protozoa Present
Any AMR Present
Human Fecal Contamination Present
Culturable E. coli Present

Figure 2

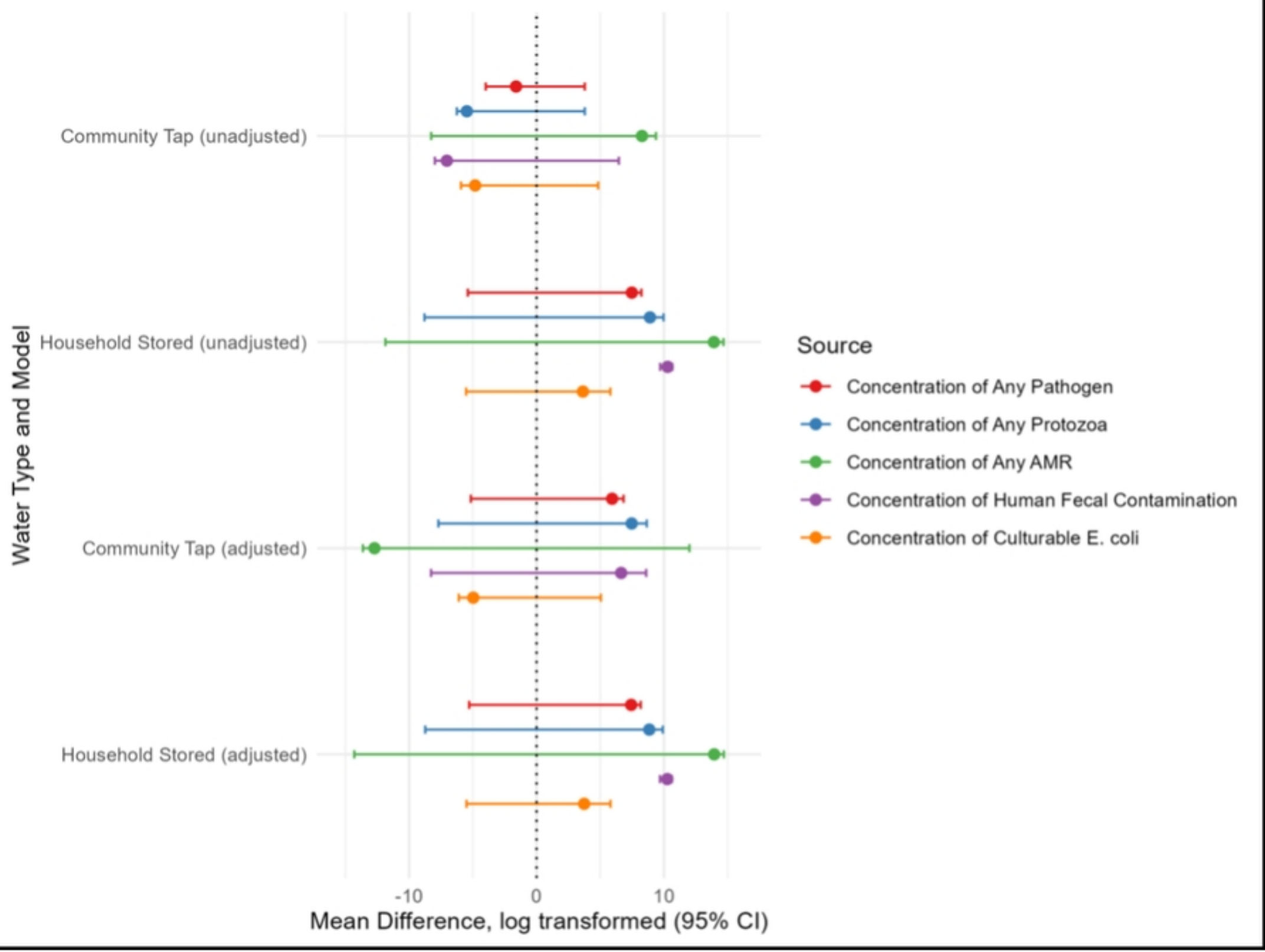
Water Type and Model
Community Tap (unadjusted)
Household Stored (unadjusted)
Community Tap (adjusted)
Household Stored (adjusted)
-10
0
10
Mean Difference, log transformed (95% CI)
Source
Concentration of Any Pathogen
Concentration of Any Protozoa
Concentration of Any AMR
Concentration of Human Fecal Contamination
Concentration of Culturable E. coli

Figure 3