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Antibiotic-Resistant *Escherichia coli* in Drinking Water Samples from Rural Andean Households in Cajamarca, Peru

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Abstract. Antibiotic resistance in pathogenic bacteria is a serious public health issue. The growing threat is a cause for concern and action to prevent the emergence of new resistant strains and the spread of existing ones to humans via the environment. This study aimed at identifying fecal pathogens in drinking water obtained from rural Andean households from Cajamarca, Peru, and measuring the antibiotic resistance profile of *Escherichia coli*. The study was embedded within a community-randomized controlled trial among 102 communities in the northern highlands of the Cajamarca region, Peru. Of 314 samples, 55.4% (95% CI [49.7, 61.0], n = 174) were identified as thermotolerant coliforms. Among the samples positive for thermotolerant coliform, *E. coli* was isolated in 37.3% (n = 117), *Klebsiella* spp. in 8.0% (n = 25), *Enterobacter* spp. in 5.1% (n = 16), and *Citrobacter* spp. in 2.5% (n = 8). Of the 117 *E. coli* samples, 48.7% (95% CI [39.4, 58.1], n = 57) showed resistance to any antibiotic. The *E. coli* antibiotic resistance profile showed highest resistance against tetracycline (37.6%), ampicillin (34.2%), sulfamethoxazole–trimethoprim (21.4%), and nalidixic acid (13%). Some 19.7% (95% CI [12.9, 28.0], n = 23) of the *E. coli* isolates displayed multidrug resistance, defined as resistance to at least three classes of antibiotics. The CTX-M-3 gene, which encodes extended-spectrum resistance to beta-lactamase antibiotics, was found in one isolate. The high prevalence of fecal contamination in drinking water highlights the importance of household water treatment methods. Likewise, the high levels of antibiotic resistance found indicate a need for further research to identify the origins of potential environmental contamination, misuse, or inadequate disposal of antibiotics.

INTRODUCTION

Antibiotic resistance in pathogenic bacteria is a serious public health issue.[1] The growing threat is a cause for concern and action to prevent the emergence of new resistant strains and the spread of existing ones from humans and animals in the environment.[2] Although the use of antibiotics for treatment represents one of the most significant therapeutic advances in history, the appearance of antibiotic-resistant bacteria (ARB) now threatens our ability to manage common conditions, resulting in public health implications of longer illness durations, disability, and death.[3] Global rates of antibiotic use in humans were estimated to increase by more than 30% in the first decade of the 21st century,[4] with corresponding increases in worldwide antibiotic use in agriculture, the food industry, and aquaculture,[5] facilitating the spread of antibiotic resistance genes by the release and accumulation of antibiotics in the entire human–animal–environment sphere.

Antibiotic resistance in wastewater, surface water, and drinking water is well documented.[6,7] Animal and human fecal flora and the environment, including water sources, serve as natural habitats and reservoirs of antibiotic-resistant bacteria and resistance genes. A study investigating human fecal samples, household environmental samples including samples from household animals and household water samples, and wastewater treatment samples from a peri-urban area in Peru found links between the resistomes of bacteria of human, animal, and environmental origins.[7] Within a community, resistant bacteria circulate among humans directly as well as between humans, animals, and the environment. The epidemiology of antibiotic-resistant microorganisms at the human–animal–environmental interface involves complex and largely unpredictable systems that include transmission routes of resistant bacteria, as well as resistance genes, and the impact of antibiotic-selective pressures in various reservoirs: animals, humans, and the environment.

Previous research in remote communities in Peru has illuminated the gaps in our understanding of environmental exposures to antibiotic resistance. The unexpected finding of carriage of antibiotic-resistant *Escherichia coli* among animals and humans in an isolated Amazonian community in Peru in the absence of high levels of antibiotic use represents a quintessential example of the complexity of antibiotic resistance transmission pathways.[8,9] Research has found that remote, high-altitude Andean regions of South America harbor antibiotic-resistant bacteria in water bodies in the natural environment,[10] but there are very little data addressing antibiotic-resistant bacteria in the immediate or household environment for remote, high-altitude Peruvian communities. Several studies have taken place in Peru regarding antibiotic resistance carriage and profile, but only one (Kalter et al.) included remote, high-altitude regions.[6,7,11–13] Kalter et al. investigated risk factors for antibiotic resistance among children in multiple regions of Peru and concluded that environmental contamination was as important as prior antibiotic use in increasing children's carriage of antibiotic-resistant *E. coli*.[12] As a result, there is a need for more targeted studies that assess household environmental exposure to ARB and risk factors that promote or favor ARB in rural and high-altitude areas of Peru.

In 2015, the WHO developed a *Global Action Plan for Antimicrobial Resistance* with the overarching goal of ensuring global access to safe and effective treatment for infectious diseases through strategies including research, surveillance, and infection reduction.[14] A workshop on the WHO Action Plan held at the 2015 International Water Association Health-Related Water Microbiology Symposium was convened to

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focus on the issue of antimicrobial resistance in water, wastewater, and feces, addressing the role of water, sanitation, and hygiene. The primary conclusions of this workshop stipulated the need to further examine water and the environment as human exposure pathways for AMR, determine the health impact of AMR in water and compare it with other exposure routes, generate guidelines for intervention methods to reduce the spread of antimicrobial resistance (AMR) to humans via the environment, and finally, create a global surveillance system strategy that can be used in low-income countries to monitor the degree and the dissemination of AMR.[3]

In an effort to contribute to understanding of transmission pathways in the rural environment, our study aimed to identify the fecal pathogens in drinking water obtained from rural Andean households from Cajamarca, Peru, and to measure the antibiotic resistance profile and extended-spectrum beta-lactamase (ESBL) activity and genetic determinants in *E. coli* isolates in drinking water.

METHODOLOGY

Study setting. The study was located in the provinces of San Marcos and Cajabamba, located in the northern Andean region of Peru (Department of Cajamarca), with altitudes between 2,200 and 3,900 m above sea level. Most of the population are small-scale farmers, living in houses with earthen floors, adobe walls, and clay tile roofs, and use unventilated traditional stoves or open fires for cooking.[15] Water supply for homes in San Marcos and Cajabamba comes from a piped gravity system that transports untreated water captured from central community reservoirs or springs through individual or small-scale collective plastic piping to a tap in the courtyard.[15]

Study design. The present study is embedded within an integrated community-randomized controlled trial (health and development effectiveness of an integrated home-based intervention package (IHIP-2) in rural Andean communities: a randomized trial (IHIP-2), registered at www.isrctn.com under ISRCTN-26548981), among 102 communities in the northern highlands of the Cajamarca Region, Peru. In brief, the IHIP-2 trial assessed the effectiveness of an integrated home-environmental intervention package comprising the installation of a kitchen sink with running water, ventilated improved cookstove and general kitchen, hand and food hygiene education, and early child development.[16] For the parent study, participant families were eligible if they met the following criteria: 1) have at least one child aged < 1.5 months living at home, 2) use solid fuels as main energy source for cooking/heating, 3) have access to non-treated piped water in the yard or the community (15 m maximum), 4) do not plan to move within the next 24 months, and 5) are not participating in another program or intervention. All data presented here are from the baseline phase of the IHIP-2 trial.

Identification of fecal pathogens in drinking water. Water sampling took place between October 2015 and January 2016. Field-workers visited each household and collected approximately 125 mL of water using sterile bottles (Labsystems S.A.C., Peru). Samples were obtained from the main source the child commonly used for drinking. If the researcher did not observe the drinking process, mothers were asked, "if your child was thirsty right now, what water would you give him to drink?" and a sample was collected from the indicated source. We performed socioeconomic surveys and collected water samples from a total of 314 households. Household surveys to collect socioeconomic data, which included water source and treatment, were collected between 0 and 190 days before the water sampling was performed, with a median difference of 65 days. The samples were transported from the field to the research station laboratory located in the city of San Marcos, using thermal bags with ice packs to conserve the samples. Training in the correct handling of the sample, transportation, and quantitative data collection was provided to the field-workers. The field supervisor reviewed all completed questionnaires on the day of collection.

Sample analysis. We tested all samples within 8 hours after their collection. The water samples were analyzed for thermotolerant (fecal) coliforms using the membrane-filtration method of the Oxfam DelAgua Water Testing Kit (DelAgua, England), product code 14867. We incubated the samples at 44 ± 0.5°C, from 14 to 16 hours in lauryl sulfate broth. We read the samples according to the instructions of the kit, counting the yellow colony-forming units in the first 15 minutes, as indicative of thermotolerant bacterial growth. For further pathogen identification, five colonies per sample were saved in peptone media vials and were transported to the Enteric Diseases and Nutrition Laboratory at the Tropical Medicine Institute, Cayetano Heredia University, Lima, for analysis. Samples in which the coliform level was assigned too numerous to count were represented as 500 colonies for statistics and data analysis.[17] Enterobacteriaceae isolates were identified using conventional media with standard methods.[18]

Antibiotic susceptibility testing. The antibiotic resistance pattern was determined against 12 commonly used antibiotics using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines[19,20]: nalidixic acid (30-μg disk), chloramphenicol (30-μg disk), nitrofurantoin (300-μg disk), ciprofloxacin (5-μg disk), gentamicin (10-μg disk), tetracycline (30-μg disk), trimethoprim–sulfamethoxazole (25-μg disk), amoxicillin–clavulanic acid (30-μg disk), ampicillin (10-μg disk), cefotaxime (30-μg disk), azithromycin (15-μg disk), and cefoxitin (30-μg disk). Antibiotic susceptibility testing was performed for all isolated bacteria but is presented in aggregate only for *E. coli* because of low sample sizes in other bacteria.

Extended-spectrum beta-lactamases detection and confirmation. *Phenotypic detection of ESBL bacteria.* Antibiotic susceptibilities for all isolated strains of bacteria were tested using the Jarlier method[21] for the following antibiotics: aztreonam (5-μg disk), ceftazidime (30-μg disk), cefotaxime (30-μg disk), ceftriaxone (30-μg disk), amoxicillin–clavulanic acid (30-μg disk), and cefepime (30-μg disk).

Molecular confirmation of ESBL genes. Escherichia coli displaying phenotypic ESBL activity were tested for the presence of SHV, TEM, OXA-1-like, CTX-M-2, CTX-M-3, CTX-M-8, CTX-M-9, and CTX-M-10 genes using conventional polymerase chain reaction.[22] Identified ESBL genes were not sequenced for allelic variants.

Data analysis. Data were analyzed using STATA 14.0 (StataCorp., College Station, TX) and R version 3.5.1 (R Foundation for Statistical Computing, Austria).[23] We present descriptive statistics and prevalence of coliform contamination and specific bacterial types. Using the binom.test() command in R, 95% CIs for prevalence of specific bacterial types and antibiotic resistance among *E. coli* isolates were calculated.

Ethics. The project was registered on the ISRCTN registry (ISRCTN26548981). Community leaders and local authorities

from the study signed a collaborative agreement with the Universidad Peruana Cayetano Heredia before study implementation. The parent participating in the study signed a written informed consent form.

RESULTS

Characteristics of study sample. Almost all households providing water samples in this study have access to tap water piped into the home or building (99.4%). The gravity-based piped water supply system provides water to each household. However, this water is untreated and chlorination is uncommon. Drinking water at home is typically either consumed directly without treatment (40.8%) or boiled (57.6%) among households providing water samples this study, with a small minority (1.6%) treating water with chlorine or bleach.

Bacterial contamination of water sources. Of 314 samples collected, 55.4% (n = 174) were positive for thermotolerant coliforms (Table 2); 35.0% (n = 110) of samples had more than 20 colonies. We classified the type of bacteria (*E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*, or a nonfermenter) in 144 of these samples. We isolated multiple distinct species of thermotolerant bacteria in 7.3% of the 314 samples. Samples were provided from different water sources or containers depending on the household. *Escherichia coli* was isolated in 37.3% (n = 117) of all households, *Klebsiella* spp. in 8.0% (n = 25), *Enterobacter* spp. in 5.1% (n = 16), and *Citrobacter* spp. in 2.5% (n = 8) (Table 2). Of the 314 households tested for fecal coliforms, 280 (89.2%) reported using the same container in which the sample was collected to give water to children in the household, including 156 of the households in which thermotolerant fecal coliforms were isolated.

Antibiotic resistance of bacterial isolates. Of 117 *E. coli* samples (one per household), 48.7% displayed resistance to at least one antibiotic (Table 3). The *E. coli* antibiotic resistance profile showed highest resistance against tetracycline (32.5%), followed by ampicillin (28.2%), trimethoprim–sulfamethoxazole (17.9%), and nalidixic acid (9.4%) (Table 3). Multidrug resistance was displayed in 19.7% (23) of the *E. coli* isolates from this study (Table 3). Multidrug resistance was defined as resistance to three or more of the following classes of antibiotics: penicillins, quinolones, nitrofurans, aminoglycosides, tetracyclines, folate inhibitors, cephalosporins, macrolides, and phenicols.

Detection of ESBL resistance genes. Eight strains of bacteria, including six *E. coli* and two *Klebsiella* isolates, displayed phenotypic ESBL activity. One isolate was found to carry the CTX-M-3 gene. All of the other strains displaying phenotypic ESBL activity were found to be negative for carriage of all tested ESBL genes.

TABLE 1
Descriptive statistics of household water supply and treatment

Household water sources	Households, % (*N*)
Public system/piped water—inside the home or building	99.3% (304)
Other/surface water	0.65% (2)
Water treatment	
Boiling	57.2% (175)
Chlorine or bleach	1.63% (5)
None*	41.1% (126)

*One hundred twenty-seven households reported no water treatment, but one subsequently reported use of boiling.

TABLE 2
Descriptive statistics of bacterial contamination by colony-forming units (CFU/mL), frequency, and type of thermotolerant coliform identified

Coliform levels	
Thermotolerant coliform count (CFU/mL)—median (IQR 1st–3rd Quantile)	2 (0–85)
Thermotolerant coliform count (CFU/mL)—mean (SD)	100.0 (177.9)
Bacterial types	**Households, % (95% CI) *N***
Thermotolerant coliform (any level)	55.4% (49.7,61.0) 174
Escherichia coli	37.3% (31.9, 42.9) 117
Klebsiella	8.0% (5.2, 11.5) 25
Enterobacter	5.1% (95% CI: 2.9, 8.1) 16
Citrobacter	2.5% (95% CI: 1.1, 5.0) 8

DISCUSSION

This study was among the first to examine environmental contamination with antibiotic-resistant bacteria in remote Andean regions of Peru. The findings of thermotolerant coliform in the drinking water given to children in a majority of households and widespread antibiotic resistance among water contamination suggest that drinking water represents a potential transmission route for carriage and infection with ARB.

The high prevalence of fecal contamination indicates that lack of access to safe drinking water is an area of concern for the households in the region under study. A majority of the households in this study (55.4%) contained thermotolerant coliforms, indicating fecal contamination.[24] Both Peruvian national standards for drinking water and the WHO guidelines indicate that no fecal or thermotolerant fecal coliforms should be detectable in a treated drinking water sample (the WHO specifies a 100-mL sample).[24,25] Many households in this study (35.0%) were also found to have thermotolerant coliform levels in drinking water samples in excess of Peruvian legal standards for water that could be made potable for disinfection.[26] These results indicate that research and interventions to address water contamination in this setting cannot rely solely on household and community disinfection measures, and should also identify and target sources of water contamination.

The finding of resistance to at least one antibiotic in more than half (51%) of *E. coli* isolates from drinking water indicates a potentially important role for drinking water in contributing to the carriage of antibiotic-resistant *E. coli* among rural communities in Peru. Antibiotic resistance in *E. coli* was most commonly identified among older generations of antibiotics, foremost in tetracycline (35.0%), ampicillin (30.5%), trimethoprim–sulfamethoxazole (20.7%), and chloramphenicol (11.9%). These results are consistent with studies of *E. coli* in fecal samples of children[6,9,12,27] and adults[9] in other areas of Peru, where resistance to tetracycline, ampicillin, trimethoprim–sulfamethoxazole, and chloramphenicol are consistently among the most commonly identified resistance phenotypes. Previous studies in Peru have explored the relationship between fecal carriage of antibiotic-resistant bacteria in humans and environmental and animal sources of bacteria in Peru,[7,9,12] and suggested that the source and storage of drinking water may play an important role in antibiotic-resistant infections.[13] The present study provides direct evidence that antibiotic-resistant bacteria are present in

TABLE 3

Escherichia coli antibiotic resistance profile to a panel of antibiotics, proportion of isolates resistant to any antibiotic, and proportion of multidrug-resistant isolates, where multidrug resistance was defined as resistance to three or more classes of antibiotics

Antibiotic	Susceptible, % (*N*)	Intermediate, % (*N*)	Resistant, % (*N*)	% Resistant (95% CI)
Amoxicillin–clavulanic acid	90.6% (106)	6.0% (7)	3.4% (4)	(0.94, 8.5)
Ampicillin	48.7% (57)	23.1% (27)	28.2% (33)	(20.3, 37.3)
Azithromycin	93.2% (109)	–	6.8% (8)	(3.0, 13.0)
Cefotaxime	94.9% (111)	1.7% (2)	3.4% (4)	(0.94, 8.5)
Cefoxitin	96.8% (30)	–	3.2% (1)	(0.08, 16.7)
Chloramphenicol	88.9% (104)	0.0% (0)	11.1% (13)	(6.1, 18.3)
Ciprofloxacin	90.6% (106)	5.1% (6)	4.3% (5)	(1.4, 9.7)
Gentamicin	97.4% (114)	–	2.6% (3)	(0.53, 7.3)
Nalidixic acid	85.5% (100)	5.1% (6)	9.4% (11)	(4.8, 16.2)
Nitrofurantoin	94.9% (111)	3.4% (4)	1.7% (2)	(0.21, 6.0)
Trimethoprim–sulfamethoxazole	81.2% (95)	0.9% (1)	17.9% (21)	(11.5, 26.1)
Tetracycline	67.5% (79)	–	32.5% (38)	(24.1, 41.8)
Any antibiotic	–	–	48.7% (57)	(39.4, 58.1)
Multidrug resistance	–	–	19.7% (23)	(12.9, 28.0)

drinking water and will enable the evaluation of household risk factors, including animals and water storage, in future analyses.

The finding of moderate resistance to the quinolone antibiotics nalidixic acid (12.9%) and ciprofloxacin (5.9%), in particular, among drinking water bacteria, may contribute to explaining the phenomenon of unexpectedly high quinolone resistance in populations with limited exposure to this class of antibiotics. In previous studies by Bartoloni and others,[9] a rapid increase in quinolone resistance was observed in a remote community in the Peruvian Amazon following a rise in quinolone use in other areas of Peru despite the absence of antibiotic selection pressure within that community. In addition, previous studies have noted a high prevalence of resistance to nalidixic acid among *E. coli* isolates in young children in Peru (28% to diarrheagenic *E. coli* and 32% to commensal strains), despite quinolone antibiotics not being historically recommended for pediatric use.[28] Drinking water may represent one mechanism of transmission of quinolone resistance carriage to children and populations that are not expected to be exposed to high levels of quinolone use.

Eight isolates of bacteria showed phenotypic indications of ESBL resistance and one *E. coli* isolate was found to carry the CTX-M-3 gene, a relatively rare variant of the CTX-M type of beta-lactamase genes.[29] Beta-lactam antibiotics are a class of antibiotics that are valuable and widely used in human and veterinary medicine alike. Bacteria carrying CTX-M genes for ESBL production are known agents of nosocomial and community infections and have been identified across human, animal, and environmental sources.[30] They pose a particular threat to children, given that antibiotic treatment options for infections in children are limited,[31] and it is critically important to preserve the efficacy of those that exist, such as cephalosporins. *Escherichia coli* with resistance to extended-spectrum cephalosporins were recently shown to be rapidly increasing in fecal carriage by Peruvian children, and this trend is highly related to the spread of CTX-M–type ESBL genes.[30] Extended-spectrum beta-lactamase–producing Enterobacteriaceae, including CTX-M producers among others, have been identified as frequent agents of bacteremia among patients in Peruvian hospitals,[32–34] and the presence of ESBL production is associated with higher mortality in cases of bacteremia.[32] Previous studies have demonstrated that ESBL-producing bacteria can be found worldwide in environmental water reservoirs, such as in hospital and municipal sewage and wastewater, including posttreatment waters.[35,36] There is evidence of the presence of ESBL-producing bacteria in drinking water in both low- and high-income countries worldwide.[37–39] The presence of a strain of ESBL-producing bacteria in the present study suggests that drinking water may serve as a route of transmission and potential source of community-acquired infections with ESBL-producing Enterobacteriaceae.

Drinking water contamination presents a risk for enteric disease, and the prevalence of thermotolerant bacteria identified in the present study indicates the need for research into waterborne illness and interventions to improve water quality among the study community. Interventions to improve water quality are associated with reductions in diarrheal disease.[40,41] Previous research in rural Peru found that both using an improved water source and water storage in a container with a properly fitted lid were protective factors against shigellosis infection in children.[13] However, it is important to note that the bacterial contamination identified in the present study is not necessary directly associated with illness. Although some previous studies found an association between bacterial contamination of drinking water and diarrheal disease incidence,[42] studies using coliform levels or *E. coli* to assess water quality show considerable heterogeneity.[40] The relationship between thermotolerant coliforms or even specific indicator bacteria and illness is complex, where illness outcomes of interest may be caused by multiple pathogens from various exposure routes.[40]

The prevalence of bacterial contamination and antibiotic resistance described here among household water sources given to children aged 5 years and younger emphasizes the need for comprehensive research into causes and prevention strategies for contamination of drinking water. In particular, it will be important to explore the transmission pathway for bacteria, especially antibiotic-resistant bacteria, from the source to the point of exposure and evaluate the extent to which drinking water directly contributes to carriage and infection with antibiotic-resistant bacteria. Further analyses should directly investigate sources and risk factors for drinking water contamination with ARB and quantify the relationship between drinking water contamination and illness in remote, high-altitude settings.

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