Quality of Piped and Stored Water in Households with Children Under Five Years of Age Enrolled in the Mali Site of the Global Enteric Multi-Center Study (GEMS)

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Abstract. Water, sanitation, and hygiene information was collected during a matched case-control study of moderate and severe diarrhea (MSD) among 4,096 children < 5 years of age in Bamako, Mali. Primary use of piped water (conditional odds ratio [cOR] = 0.45; 0.34–0.62), continuous water access (cOR = 0.30; 0.20–0.43), fetching water daily (cOR = 0.77; 0.63–0.96), and breastfeeding (cOR = 0.65; 0.49–0.88) significantly reduced the likelihood of MSD. Fetching water in > 30 minutes (cOR = 2.56; 1.55–4.23) was associated with MSD. Piped tap water and courier-delivered water contained high (> 2 mg/L) concentrations of free residual chlorine and no detectable *Escherichia coli*. However, many households stored water overnight, resulting in inadequate free residual chlorine (< 0.2 mg/L) for preventing microbial contamination. Coliforms and *E. coli* were detected in 48% and 8% of stored household water samples, respectively. Although most of Bamako's population enjoys access to an improved water source, water quality is often compromised during household storage.

INTRODUCTION

An estimated 1.7 billion episodes of diarrheal illness occur annually in children < 5 years of age in developing countries and account for ~15% of deaths in this age group.[1] Limited access to improved water and sanitation and poor hygiene behaviors facilitate the transmission of enteric pathogens and contribute to the high burden of diarrheal disease morbidity and mortality. In sub-Saharan Africa, 83% of urban and 49% of rural populations have access to improved water sources,[2] whereas 43% of urban and 23% of rural populations have access to improved sanitation facilities. Improvements in water, sanitation, and hygiene (WASH) would decrease transmission of enteropathogens and reduce the pediatric diarrheal disease burden.[3,4]

Provision of potable water and point-of-use treatment and safe storage of drinking water are cost-effective.[5–7] Although household-based methods are cheaper, centralized facilities that deliver piped treated water are more efficient for urban areas and offer larger overall health benefits at the population level.[5–7] Nonetheless, in poor countries logistical hurdles and economic shortfalls often compromise maintenance and operation of centralized water treatment, delivery systems, and water quality monitoring. Contaminated source catchments, inadequate treatment methods and system management, intermittent supply or insufficient pressure in the water distribution system, and groundwater infiltration through leaking pipes or cross-connections all contribute to water supply system failures.[8,9] Failed municipal piped water systems delivering contaminated water have led to outbreaks and been correlated with greater diarrheal disease endemicity.[10–18] However, few studies have systematically measured and reported health impacts attributable to use of centralized water and sanitation services in developing countries.

Mali has one of the world's highest under-five child mortality rates and a high incidence of diarrheal disease.[19] The capital, Bamako, with 1.8 million inhabitants, is one of the fastest growing cities in Africa.[20,21] Although a substantial proportion of Bamako's population has access to improved source water from a centralized piped water system, population growth and in-migration from rural areas threaten to outpace the capacity of the municipal water supply network.[22] We used data collected during the Global Enteric Multicenter Study (GEMS) to examine associations between drinking water and moderate and severe diarrhea (MSD) in children < 5 years of age living in the Bamako, Mali site. The GEMS is a matched case-control study of the etiology, burden, and risk factors of moderate-to-severe diarrhea in children < 5 years of age carried out in three sites in South Asia and four sites in sub-Saharan Africa, including Bamako.[23,24] At all seven GEMS sites, WASH data were collected by questionnaires at enrollment from the caretakers of case children presenting at health facilities and at home for matched control children. Observations of WASH conditions were recorded by trained health workers at a single household visit ~60 days after enrollment.

Based upon the observed associations, we designed a nested water quality risk assessment study, carried out in two Bamako quartiers, to investigate two hypotheses: 1) that piped water from "improved" sources in Bamako contains lower levels of fecal contamination than water that is bought from couriers ("unimproved"); and, 2) that household stored water collected by caretakers who fetched and stored water overnight would contain significantly more fecal bacteria than source water.

MATERIALS AND METHODS

Subjects. Subjects (2,033 case and 2,063 control children) were enrolled between December 3, 2007 and December 2, 2010 into the GEMS Mali case-control study.[23] Each GEMS site is linked to a defined population under a demographic surveillance system (DSS) that captures census data 2–3 times per year. The GEMS Mali DSS included the total population

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in Banconi and Djikoroni Para quartiers (210,425 people in a 2007 baseline census). Cases were children < 5 years of age from the DSS seeking care for MSD at one of the seven sentinel health centers serving the DSS. Control children without diarrhea were randomly selected from the DSS population within 14 days of presentation of the case and were matched by age, gender, and quartier. The MSD was defined by adapting the World Health Organization (WHO) definition of diarrhea (passing ≥ 3 loose stools within 24 hours) to include clinical signs of moderate-to-severe dehydration (sunken eyes, loss of skin turgor, or administration of INTRAVENOUS fluids) or dysentery or diarrheal illness leading to a clinical decision to admit the child to a health center or hospital.[23] Stool specimens were collected from all children at enrollment and examined for many etiologic agents.[25]

The WASH data were collected at enrollment from the caretakers of case children presenting at health facilities and at home for matched control children, using a standardized questionnaire. Approximately 60 (range, 50–90) days after enrollment, a trained field worker visited each case and control household to collect follow-up health information and record WASH observations. All households that had completed the 60-day follow-up visits before March 12, 2010, and where the caretakers had identified either a private (water piped into the home or yard) or a public water tap as their primary water source, were eligible for participation in the nested water quality study; this comprised 1,279 case and 1,295 eligible control households. Twenty-two case and 26 control GEMS households were randomly selected from a list of eligible case and control households to be visited between March and April of 2010 for the sub-study of water storage practices. Bought water was collected directly from randomly selected couriers within the residential area, rather than from caretakers who use bought water because we could not identify and collect samples from the specific source (courier) of household water stored and sampled during the visit. Neighborhoods have coverage from many different couriers who are extremely mobile. Thus, caretakers tend to use whichever courier is closest.

Water sample collection and chlorine testing. Water quality of primary drinking water sources was evaluated by sampling the primary source of piped drinking water for the 48 (22 case, 26 control) selected GEMS caretakers, and water supplied by 15 randomly selected professional water couriers servicing the GEMS neighborhoods (7 from Banconi and 8 from Djicoroni Para), who were in the process of transporting water within a residential area.

During household visits, caretakers were asked to guide the field team to their primary drinking water source where a 300 mL sample was collected. "Bought" water was collected by asking couriers to provide a 300 mL sample of water from a storage container on their cart. To determine the role of courier water storage practices on water quality between procurement at the source and delivery to the household, couriers were also asked to identify the source that they used for procuring water for sample collection and their water collection and storage practices were recorded. A 300 mL sample was collected for courier sources. To determine the role of household water storage practices on household drinking water quality after collection at the source, caretakers were asked to provide a 300 mL sample of stored drinking water. Field workers recorded the type of container from which the

stored water sample was provided (either a wide [≥ 6 cm]- or narrow [< 6 cm]-mouthed container), whether the container was covered, and whether water was removed by dipping (with cup or ladle) or by pouring. The caretaker was asked the length of time that had passed because their stored water had been collected, and whether it was given to the GEMS child. No incentive was provided.

Free residual chlorine (FRC) concentrations were measured for all water samples at the time of collection using the Extech CL200 portable meter (Waltham, MA). All 300 mL water samples were collected into sterile autoclaved containers. Samples were kept in coolers on ice packs and transported to the laboratory for microbiological analysis within a maximum of 4 hours of collection.

Microbiological analysis of coliforms. Assays conformed to the U.S. Environmental Protection Agency (USEPA) Approved Method 1604: *Total Coliforms and* Escherichia coli *in Water by Membrane Filtration Using a Simultaneous Detection Technique* (*MI Medium*).[26] Upon delivery to the laboratory, 50 mg/L of sodium thiosulfate (Sigma, St. Louis, MO) was immediately added to neutralize residual chlorine and the samples were microbiologically processed. Duplicate serial dilutions of each sample were vacuum-filtered through a 0.22 μm mixed cellulose esters filter (Millipore Corporation, Billerica, MA) using an autoclaved vacuum filter apparatus. Filters were incubated overnight on BBL MI coliform indicator medium (Becton Dickerson and Company, Atlantic City, NJ) at 37°C. After 18 hours, plates were inspected for *Escherichia coli* and total coliform units (TCU), per manufacturer's recommendations. If no TCU or *E. coli* were identified, the water sample was considered negative for coliform contamination. If the concentration of bacteria exceeded 250 colony forming unit (cfu)/100 mL in the highest dilution, it was recorded as the maximum recordable number of 25,000 cfu/100 mL.

Data analysis. Data were analyzed using SAS Version 9.3 (SAS Institute, Inc., Cary, NC). Descriptive statistics of study subjects or variables were reported as proportions, means, and ranges. Means were compared using a two-sample t-test. Categorical variables were compared using χ^2 or Fisher's exact test. Reported P values are two-tailed; $P \leq 0.05$ was considered significant. Conditional logistic regression was conducted using case status as the dependent variable to determine the unadjusted matched odds ratios (cOR) for independent variables.[27] Backward, forward, and stepwise selection was used to screen all variables with $P < 0.10$, including "primary use of a piped water source," "the primary source is always available," "the caretaker fetches water daily," "the caretaker requires > 30 minutes to fetch water," "the child is breastfed," "water is poured from a container," and "the container aperture > 6 cm," and variables for confounding (wealth quintile index, both parents living in the home, and caretaker's education). Variables and terms for interaction between variables were retained in the final multivariate model if product terms met criteria for significance ($P \leq 0.05$). The final model included primary use of a piped water source, the primary source is always available, the caretaker fetches water daily, the caretaker requires > 30 minutes to fetch water, the child is breastfed, the variables for confounding described previously, and a term for interaction between both parents being in home and use of piped water. The COLLIN and variance inflation factor (VIF) option was used in the model statement to test for collinearity. Significant

collinearity was not detected for model variables (greatest condition index = 7.79 for both parents in the home and an interaction term between both parents in the home and use of piped water.

Ethical considerations. Written informed consent was obtained from adult caretakers of all children enrolled in GEMS. The Ethics Committee of the Faculté de Medécine, Pharmacie et Odonto-Stomatologie, University of Bamako, and Institutional Review Board of the University of Maryland, Baltimore approved the protocol and consent forms.

RESULTS

Epidemiologic findings from the case-control study. *Characteristics of GEMS households in Bamako.* Households of GEMS case and control children were similar with respect to gender, age, number of overall individuals and children < 5 years of age in the household, and the level of caretaker's education (Table 1). Although *P* values indicated significant differences in mean age in weeks among case and control children in the youngest two age groups (0–11 months and 12–23 months), the sample size is large, and the proportions are similar representing a difference of only 6 to 9 days, which is not considered clinically relevant. Breastfeeding was more common among the control children than case children, particularly in the 12–23 month age group (cOR = 0.57; 95% confidence interval [CI] 0.43–0.75) (Table 1). Both parents living in the home was also protective against MSD (80.8% cases, 87.2% controls, cOR = 0.60, CI 0.51–0.72) (Table 1). Surprisingly, a higher proportion of case children lived in the wealthiest quintile (22.0% cases versus 18.0% controls, cOR = 1.31, CI 1.08–1.59) (Table 1).

Drinking water sources used by GEMS caretakers and odds of MSD. Overall, the municipal piped system was the primary drinking water source for > 99% of GEMS Bamako households, collected either directly (household or public taps) or indirectly (bought from couriers). Caretakers reported that public taps (76.2% of cases versus 79.0% of controls) or private taps in the house or yard (10.6% cases versus 9.8% controls) were the primary sources of drinking water for enrolled children (Table 2). One in 10 caretakers reported use of water bought from a courier (12.0% cases versus 10.7% controls). Other sources were uncommon as primary sources (< 1%). However, use of secondary water sources was reported by 21.8% of case and 22.4% of control caretakers, which included unimproved private wells (48.6% case, 55.9% control), public taps (25.9% case, 21.6% control), courier water (14.2% case, 14.7% control), and unimproved public wells (9.2% case, 8.6% control) (Table 2). Univariate analysis suggested that the odds of MSD were slightly lower for children in households who relied primarily on a private or public tap (86.8% cases versus 88.8% controls; cOR = 0.83, CI 0.68–1.00), compared with those who primarily used other sources like bought water (12.0% case versus 10.7% control) or other less common sources (Table 2).

Water collection and water storage practices among GEMS caretakers and odds of MSD. Compared with caretakers of controls, case caretakers were less likely to report that water was available from their primary water source "all the time" (91.2% versus 97.3%, cOR 0.28, CI 0.20–0.38), and to fetch water every day (76.1% versus 80.4%, cOR 0.76, C.I. 0.63–0.91, (Table 3). Case caretakers were more likely to report the round trip to their primary water source took > 30 minutes (4.3% versus 1.5%; cOR 2.96, CI 1.84–4.75) (Table 3). Lacking access to a primary source "all the time" did not influence use of a secondary source (22.2% versus 18.9%, *P* = 0.45). Fetching drinking water daily was more frequent among case (84.8%) and control (86.3%) caretakers who relied on private or public taps than among case (20.5%) and control (33.7%) caretakers

TABLE 1
Socio-demographic characteristics and univariate associations with moderate and severe diarrhea (MSD) in case and control children matched by age, gender, and quartier and enrolled between 2008 and 2010 in the GEMS in Bamako, Mali*

	Case, N = 2,033	Control, N = 2,064	cOR (95% CI)†	P
Household socio-demographic characteristics				
Male child‡	910 (44.8%)	923 (44.7%)	–	0.98
Age category, mean number months‡				
0 to 11 months, N = 1,453	7.3 (2.7 SD)	7.0 (2.5 SD)		0.02
12 to 23 months, N = 1,378	16.8 (3.5 SD)	16.3 (3.2 SD)		0.02
24 to 59 months, N = 1,266	35.5 (9.7 SD)	35.1 (9.5 SD)	–	0.84
Mean number of people in household	15.0 (9.7 SD)	15.0 (9.7 SD)	–	0.47
More than 1 child < 5 years of age in household	1,618 (79.6%)	1,660 (80.5%)	0.94 (0.81–1.10)	0.47
Both parents live in home§	1,986 (80.8%)	1,779 (87.2%)	0.60 (0.51–0.72)	0.0001
Caretaker's education§				
None or some primary	1,726 (84.9%)	1,756 (85.1%)	Ref.	Ref.
Completed primary	209 (10.3%)	184 (8.9%)	1.15 (0.93–1.41)	0.21
Primary or greater	97 (4.8%)	124 (6.0%)	0.80 (0.61–1.05)	0.11
Breastfed				
0 to 11 months, N = 1,453	716 (98.6%)	727 (100.0%)	–	–
12 to 23 months, N = 1,378	495 (72.5%)	565 (81.3%)	0.57 (0.43–0.75)	0.0001
24 to 59 months, N = 1,266	15 (2.4%)	22 (3.4%)	0.68 (0.35–1.31)	0.25
Wealth index quintile§				
1	394 (19.4%)	426 (20.6%)	Ref.	Ref.
2	382 (18.8%)	436 (21.1%)	0.95 (0.78–1.15)	0.59
3	399 (19.6%)	421 (20.4%)	1.04 (0.85–1.26)	0.74
4	411 (20.2%)	409 (19.8%)	1.08 (0.89–1.31)	0.44
5	447 (22.0%)	372 (18.0%)	1.31 (1.08–1.59)	0.006

*Values are shown as numbers (percent), or means (SD).
†Conditional logistic regression (cOR) of variables collected from matched case-control pairs, 95% confidence interval (CI).
‡Cases and controls matched. Ref. refers to the reference group for logistic regression.
§Variables included as potential confounders in multivariate model.

TABLE 2

Distribution and odds of MSD for primary source and any source of drinking water used by Bamako caretakers (based on information collected at child's enrollment in GEMS)*

PRIMARY drinking sources	Cases, N = 2,033 n (%)	Controls, N = 2,064 n (%)	cOR (95% CI)*	P
Municipal piped water tap	1,765 (86.8%)	1,833 (88.8%)	0.83 (0.68–1.00)	0.05
Private tap	215 (10.6%)	203 (9.8%)	1.10 (0.89–1.35)	0.40
Public tap	1,550 (76.2%)	1,630 (79.0%)	0.85 (0.73–0.99)	0.03
Improved well	16 (0.8%)	6 (0.3%)	2.33 (0.90–6.07)	0.08
Unimproved private well	1 (0.1%)	2 (0.1%)	0.5 (0.05–5.51)	0.57
Unimproved public well	0	0	–	–
Protected spring	7 (0.3%)	2 (0.1%)	3.5 (0.73–16.85)	0.12
Bought from courier (municipal supply)	244 (12.0%)	220 (10.7%)	1.15 (0.94–1.40)	0.17
SECONDARY drinking water sources	Cases, N = 444	Controls, N = 463	cOR (95% CI)*	P
Municipal piped water tap	115 (25.9%)	100 (21.6%)	0.67 (0.11–3.99)	0.66
Private tap	2 (0.4%)	2 (0.4%)	0.50 (0.05–5.51)	0.57
Public tap	113 (25.5%)	98 (21.2%)	1.69 (0.85–3.36)	0.13
Improved well	2 (0.4%)	2 (0.4%)	0.94 (0.21–2.73)	0.82
Unimproved private well	216 (48.6%)	259 (55.9%)	0.76 (0.60–0.97)	0.03
Unimproved public well	41 (9.2%)	40 (8.6%)	1.05 (0.66–1.66)	0.85
Protected spring	2 (0.4%)	3 (0.5%)	0.67 (0.11–3.99)	0.66
Bought from courier (municipal supply)	63 (14.2%)	68 (14.7%)	1.14 (0.76–1.70)	0.54

*Conditional logistic regression (cOR) of variables collected from matched case-control pairs, 95% confidence interval (CI).
MSD = moderate and severe diarrhea.

who primarily bought water from couriers (Table 3). Caretakers who required > 30 minutes to fetch water were less likely to have constant access to a water source (2.3% versus 12.8%, P < 0.0001) and to fetch daily from their primary source (62.3% versus 78.8%, P < 0.0001) than caretakers who spent < 30 minutes retrieving water.

Drinking water was stored by nearly all GEMS caretakers (Table 3). Field workers observed traditional wide-mouthed earthen water storage containers with lids in 99.9% of case and 99.7% of control households (Table 3). Household stored water was reportedly not treated by additional filtration, chlorination, flocculation, or other methods in over 99.8% of case and control households (Table 3). The vast majority of caretakers used a short-handled cup for scooping water from the household storage container for drinking. A higher propor-

tion of caretakers in control households than in case households reported removing water from storage containers by pouring (cOR = 0.61, CI 0.41–0.90) (Table 3).

Predictors for MSD in a multivariate model. Modeling procedures (forward, backward, and stepwise) were compared. Forward and stepwise models both suggested that always having access to water, fetching water, requiring > 30 minutes to fetch water, and breastfeeding were all independently associated with MSD. Backward selection identified the same variables, plus primary use of piped water, interaction between piped water and fetching water daily, and interaction between piped water and both parents living in the home. Model fit was conducted by using the forward/stepwise model and adding piped water alone, and with its interaction terms to check for significant contribution to the model. The use of

TABLE 3

Household water access and water handling practices among GEMS caretakers*

	Case	Control	cOR (95% CI)*	P
Reported access and use of primary drinking water source				
	N = 2,033	N = 2,064		
Water always available from primary source	1,855 (91.2%)	2,009 (97.3%)	0.28 (0.20–0.38)	0.0001
	N = 1817	N = 1847		
Fetch water daily†	1,383 (76.1%)	1,485 (80.4%)	0.76 (0.63–0.91)	0.003
Public tap, N = 3180	1,314 (84.8%)	1,406 (86.3%)	0.84 (0.66–1.08)	0.17
Bought, N = 452	50 (20.5%)	70 (33.7%)	0.75 (0.17–3.35)	0.71
Time to fetch > 30 minutes†	78 (4.3%)	28 (1.5%)	2.96 (1.84–4.75)	0.0001
Observations of storage conditions in household‡				
	N = 1,786	N = 1,891		
Observed container for storing drinking water	1,786 (100%)	1,891 (100%)	–	–
Aperture of storage container > 6 cm	1,773 (99.3%)	1,886 (99.7%)	0.39 (0.14–1.08)	0.07
Containers are covered	1,785 (99.9%)	1,885 (99.7%)	–	–
Water obtained by§:				
Scooping with cup	1,783 (99.8%)	1,890 (100%)	0.33 (0.04–3.21)	0.34
Pour	52 (2.9%)	84 (4.4%)	0.61 (0.41–0.90)	0.01
Stored water was not treated	1,784 (99.9%)	1,887 (99.8%)	0.67 (0.11–3.99)	0.66

*Conditional logistic regression (cOR) of variables collected from matched case-control pairs, 95% confidence interval (CI).
†Data was not collected for caretakers with water piped into the house or yard based upon the assumption that they had daily access to water (N = 1817 for cases and N = 1847 for controls).
‡Data was collected during follow-up visits in N = 1786 case households and N = 1891 control households.
§Indicates where caretakers could select more than one answer.
GEMS = Global Enteric Multicenter Study.

Factors independently associated with increased or decreased risk of moderate and severe diarrhea (MSD) in a multivariable logistic regression analysis*

Predictive factor	Adjusted cOR (95% CI)
Primarily uses piped water source	0.45 (0.34–0.62)
Water source is always available	0.30 (0.20–0.43)
Caretaker fetches water daily	0.77 (0.63–0.96)
Requires > 30 minutes to fetch drinking water from primary source	2.56 (1.55–4.23)
Breastfed	0.65 (0.49–0.88)

*Variables included in the final multivariate model were primary use of piped water, always have access to primary water source, fetch water daily, fetching water requires > 30 minutes, breastfeeding, and an interaction term for use of piped water and both parents being in the home. Confounding variables included both parents being in the home, wealth index quintile, and a categorical ordinal variable for caretaker's education. Adjusted conditional logistic regression (cOR) of variables collected from matched case-control pairs, whereby all odds ratios control for other factors in the model; 95% confidence interval (CI).

piped water and interaction between piped water and both parents living in the home improved model fit (P = 0.02) and were retained. Model fit was not improved by removing any variables. Variables that remained predictors of decreased likelihood of MSD in the final multivariate regression model after adjustment for confounding by wealth quintile index, both parents living in the home, and caretaker's education included use of private or public tap water (cOR = 0.45, CI 0.34–0.62), always having access to the primary source (cOR = 0.30, CI 0.20–0.43), fetching water daily (cOR = 0.77, CI 0.63–0.96), and breastfeeding (cOR = 0.65, CI 0.49–0.88) (Table 4). Spending > 30 minutes per trip to fetch water was associated with increased risk of MSD (cOR = 2.55, CI 1.55–4.23), Table 4.

Nested study of water quality. *Chlorine concentrations in water from Bamako piped water sources, couriers, and household storage containers.* To test the hypothesis that piped water quality is superior to bought water quality, we first tested chlorine concentrations in water from the two primary observed sources of water used by GEMS case and control caretakers: municipal piped water and couriers that sell water from mobile carts on the street. A survey of professional couriers (N = 15) found that all couriers collected water from public taps into 20-L plastic narrow-mouthed containers, which were then transported to households on hand-pushed or donkey-pulled carts. To determine whether courier water management practices contributed to degradation of water quality, we also identified and tested the sources where couriers collected water. These water sources were identified as being the same municipally supplied public taps used by caretakers. The FRC concentrations in water collected from 63 piped water outlets varied significantly day to day, but all, with one exception, consistently exceeded WHO recommended minimum FRC concentrations for piped, treated water (0.5 mg/L chlorine).[28] Median FRC concentrations from 48 household sources (2.45 mg/L, range 0.39–5.88 mg/L) and 15 courier sources (2.6 mg/L, range 1.54–5.39 mg/L) were similar (Figure 1, 1st and 3rd boxes). The median FRC in courier-delivered bought water was 2.32 mg/L (range 1.24–4.93 mg/L) (Figure 1, 4th box). The observed free residual chlorine concentration in these sources suggested that piped drinking water would not harbor bacterial contamination.

We also tested chlorine concentrations of water stored in households for drinking to determine whether water quality significantly degraded after collection. In comparison to the median FRC of 2.45 mg/L at the tap, the median FRC in household stored water samples was 0.74 mg/L (range 0.02–
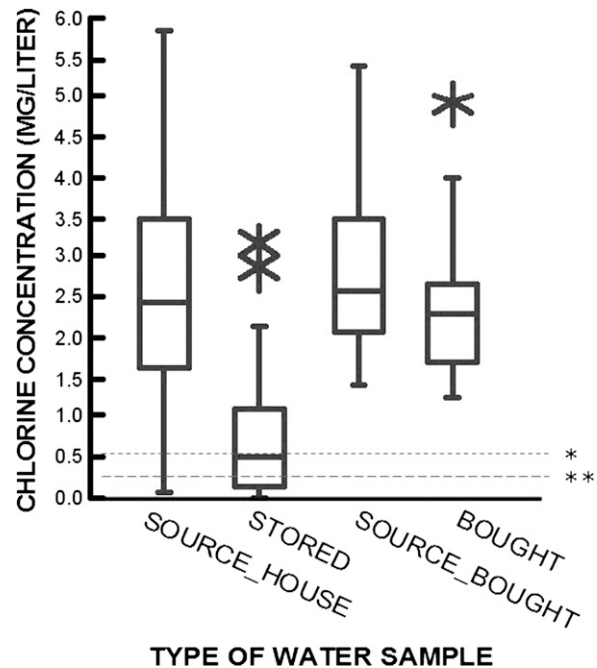

FIGURE 1. Box plot displaying median, 1st and 3rd quantiles, minimum and maximum data points, and outliers for the residual chlorine concentrations found in primary water sources (1st box) and stored water containers (2nd box) for 48 households, and water sources (3rd box) and storage containers (bought, 4th box) for 15 couriers. The box displays the range of data points lying within the first (lower line) to third (upper line) quartile, with the median in between. The distribution of free residual chlorine (FRC) concentrations detected were 0.39 mg/L to 5.88 mg/L in public taps used by caretakers, 0.02 mg/L to 3.17 mg/L in household stored water, 1.54 mg/L to 5.39 mg/L in public tap water used by couriers, and 1.24 mg/L to 4.93 mg/L in water bought from couriers. * WHO standard for FRC concentrations (0.5 mg/L) in treated piped source water. ** WHO recommended FRC concentration (0.2 mg/L) for controlling contamination in stored drinking water.

2.12 mg/L) (Figure 1, 2nd box, Table 5). A total of 35% of households did not meet the WHO recommended minimum FRC concentration of 0.2 mg/L for preventing microbial contamination in stored drinking water (Table 5).[28]

Microbial quality of drinking water from piped water sources and couriers. Most samples of water from piped sources and couriers lacked microbial contamination. One public tap sample out of the 63 tested contained > 2 × 10^4 TCU/100 mL

Average concentration of total coliform units (TCU), *Escherichia coli*, and free residual chlorine (FRC) in household drinking water stored by caretakers either on the day of testing or overnight (or longer) in households in Bamako, Mali

Day	Collected and stored on day of testing, N = 14	Collected and stored on previous day(s), N = 34	Average
Percent of households with > 0.2 mg/L FRC	0%	50%	35%
FRC (mg/L) [range]	1.27 mg/L [0.22–3.17]	0.53 mg/L [0.02–2.88]	0.74 mg/L [0.02–3.17]
Percent of households with TCU, N = 23	14%	62%	48%
TCU/100 mL	2.98×10^1	4.54×10^3	3.22×10^3
Percent of households with E. coli	0%	12%	8.3%
E. coli/100 mL	0	1.61×10^1	1.1×10^1

of water, despite the presence of 2.33 mg/L of residual chlorine. The blank conducted for the sample did not reveal any background contamination from the filter, glassware, or wash solution, suggesting that chlorine-resistant bacteria may have been introduced through a fault in a nearby underground pipe. One of the 15 samples from water couriers also contained 10^2 TCU/100 mL volume, despite a 2.14 mg/L chlorine concentration. The TCU were not identified from the paired water source, so the chlorine-resistant TCU could have been introduced post-procurement by the courier's hands or from the transport container. No tested water sources contained fecal *E. coli*.

The role of water collection and household storage practices on household drinking water quality. Water storage practices among caretakers enrolled in the nested study were identical to those observed at the follow-up visits in the overall study population. All 48 caretakers used a public or private piped water tap, stored drinking water in a traditional wide-mouthed earthen container, and used a short-handled cup to scoop water from the container. All caretakers reported giving stored water to their children for drinking. Thirty-three caretakers (69%) provided their children with drinking water that had been stored overnight and one (2%) indicated that the water had been stored for 2 days. The remaining 14 caretakers (29%) reported gathering and storing fresh water during the morning of the visit.

Chlorine concentrations in water that was stored overnight were significantly lower than for freshly collected water. Water from all households that had collected water on the day of our visit (N = 14) exceeded the WHO minimum recommended concentration of 0.2 mg/L FRC for preventing microbial growth in stored water (Table 5, FRC concentration range of 0.22–3.17 mg/L). In comparison, only 17 (50%) of 34 samples from households who had stored drinking water overnight contained greater than 0.2 mg/L chlorine residual (Table 5, FRC concentration range of 0.02–2.88 mg/L). Increased storage time and declining chlorine concentrations were associated with increased microbial contamination in stored water. The TCU contamination was identified in 23 (48%) of 48 household stored water samples that contained an average FRC of 0.74 mg/L (range 0.02–3.17 mg/L) FRC (Figure 2). *Escherichia coli* were found in 8% of stored water samples, all of which

contained > 10^4 TCU/100 mL and < 0.2 mg/L FRC. Among 14 households where fresh water had been collected that day, 1 (7%) had TCU contamination ranging between 11 and 100 cfu/100 mL, and 1 (7%) had between 101 and 1,000 cfu/100 mL; neither sample contained *E. coli* (Figure 2). Among the 34 households that had collected and stored water for a day or longer, TCU were detected in 21 (62%) stored water samples various concentrations and 4 (12%) contained *E. coli* (Figure 2). Concentrations of *E. coli* in water from these households varied between 1 and 540 per 100 mL (Figure 2). Three (3) households met criteria for low risk of fecal contamination (< 10 cfu/100 mL), whereas only 1 household was at high risk (101–1,000 cfu/mL) (Figure 2).[29] Water stored overnight or longer had a ~186-fold increase in TCU concentration (P = 0.004) compared with water that was stored < 24 hours (Table 5).

DISCUSSION

Almost nine-tenths of the caretakers in the Bamako, Mali site of the GEMS case-control study, used a municipal piped, treated water supply for their primary drinking water source. By WHO and UNICEF standards, this is an improved water source.[2] Our study also found it was a safe source, with samples from all 63 randomly selected taps meeting the minimum recommended guidelines for free residual chlorine in piped, treated water (0.5 mg/L), and, with one exception, free from microbiologic contamination.[28] Chlorine-resistant TCU were detected in water from one public tap, suggesting that biofilm contamination may be present in pipes supplying that tap. Biofilms are notorious in the water supply industry for growing on pipe surfaces and contributing to the survival and persistence of microbial contamination.[30–32]

Over a tenth of caretakers bought water from couriers who delivered water from this same municipal source. Because of the potential for introduction of contamination during collection and transport, water from couriers is considered by the WHO to be an unimproved water source. Water collected from couriers contained similar FRC levels as piped source water and 93% of samples were free from microbial contamination, suggesting that high FRC concentrations can help offset contamination risks posed by water vendors. The fact
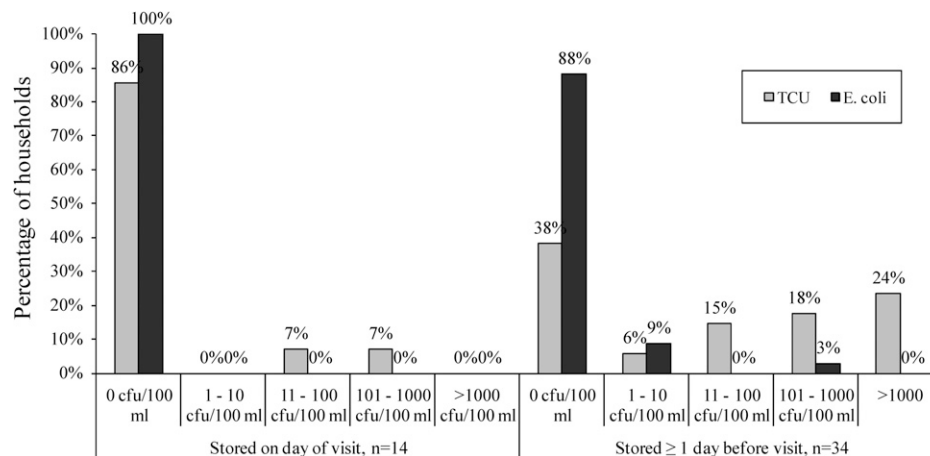

FIGURE 2. Percent of households with none (0 colony forming unit [cfu]/100 mL), low (1–10 cfu/100 mL), moderate (11–100 cfu/100 mL), high (101–1,000 cfu/100 mL), or very high risk (> 1,000 cfu/100 mL) of drinking household drinking water contaminated with total coliform bacteria (TCU) or *Escherichia coli* after storage for < 1 day (N = 14) vs. ≥ 1day (N = 34).

that one courier did introduce chlorine-resistant TCU contamination into the vended water supply is a reminder though that couriers could also introduce and transmit other chlorine-resistant fecal organisms such as *Cryptosporidium*. Overall, GEMS data suggest that piped water delivered directly from a tap or by courier to ~99% of the GEMS study population in two quartiers of urban Bamako meets criteria for safety and is an unlikely source of introduction of chlorine-sensitive diarrheal pathogens into households.

Although high quality piped water sources were generally pervasive throughout this community, a small subset of households did lack consistent access to their primary water source. Case-households had significantly less dependable access to their primary water source and had longer travel times to their water source than control-households. Longer travel times to a water source have been previously linked with increased diarrhea rates in children, and WHO and UNICEF define access to an improved water source as round-trip travel time of no more than 30 minutes from the household.[2,33–35] Dependability and ease of access to a water source can impact water collection and storage practices. Dependable access to a nearby water source increases the volume of water available for household needs, and the frequency with which water is likely to be collected.[34,36] Caretakers who had inconsistent access or had to walk > 30 minutes to fetch water were less likely to fetch water daily. Infrequent collection of water means that water must be stored in the home for longer periods of time, which in turn leads to lower FRC levels and increased risks of in-home contamination. Additionally, caretakers may be more likely to supplement water needs from poorer quality sources. Caretakers who use a piped source, who have daily access, or who require < 30 minutes to fetch water may also use a greater volume of water daily for washing and cleaning, which can also help reduce the risk of diarrheal diseases.[37]

Water collection and storage behaviors can also be influenced by motivations and perceptions caused by difficulties in accessing a safe water source.[38] Caretakers with regular access to a nearby water source may still elect to spend less time, energy, or money going to the source to fetch and store water, resulting in less water used, longer water storage times, and possibly increased use of poorer quality secondary sources. All 48 caretakers in the nested sub-study reported having dependable access to their piped source, yet one-third still provided their child with drinking water that had been stored overnight or longer. In our multivariate model piped, treated water, dependable access to a water source within 30 minutes of the home, and fetching water daily, contributed independently to reduced risk of MSD in children. The nested study confirmed that fetching water less than once per day contributed to increased risk of fecal contamination in stored drinking water. Thus, access to an improved and safe water source alone, especially one that is not continuously available is insufficient to minimize diarrheal disease risk if safe behaviors for water collection, transport and storage are not practiced.

Data from the nested microbiologic sub-study shows how chemically and microbiologically improved source water can quickly deteriorate between point-of-collection and point-of-use, and how high FRC concentrations in piped treated water can partially mitigate that effect.[39] Water storage in wide-mouthed containers that allow introduction of hands and objects, such as those used in virtually all GEMS Mali house-

holds, has been incriminated as a risk for microbial contamination of drinking water and for diarrheal disease.[11,39–45,46,47] In our study, piped water that was stored in these containers showed a decrease in WHO recommended FRC concentrations and an 186-fold increase in microbial contamination in household water stored overnight. Despite this, water stored overnight in many households in unsafe storage containers still had > 0.2 mg/L of FRC (50%) and no detectable contamination (38%). *Escherichia coli* was detected in stored water from only 4 (8%) of 48 households in the nested water quality study, all of which had stored water at least overnight and with FRC concentrations ≤ 0.2 mg/L. The low prevalence of fecal bacteria contamination is most likely caused by the lasting residual effects of originally high FRC concentrations in piped source water.[28,29] The isolation of fecal *E. coli* from stored water samples containing < 0.2 mg/L FRC reinforces current WHO standards for safe levels of disinfectant in stored drinking water.[28]

Improving access and availability of improved water sources, promoting more frequent water collection and the use of safe water storage containers by caretakers, and educating them about safe water handling could further reduce contamination of water in the home and the concomitant risk of waterborne disease transmission by pathogens, including those that are chlorine-resistant, within households.[48–50] Modifying locally produced containers to include a smaller aperture and a spigot prevents the introduction of hands and objects into stored water, and may reduce the chlorine decay rate, thereby protecting the water from contamination during storage.[51]

Urban poor tend to pay more for water, in part because of a lack of access to private taps and subsequent dependency upon water vendors.[52,53] The GEMS caretakers who bought water from vendors were more likely to belong to the poorest socioeconomic strata, even though water bought from a courier in Bamako costs twice as much as public tap water. These caretakers were particularly likely to fetch water less than once a day, suggesting that their water may be stored for even longer time periods than in households that collect water from a tap. Caretakers who pay more for water may be unable to afford to purchase water as often, leading to longer water storage times in the household and an increased risk of MSD among their children. For logistical reasons, we did not visit households using vended water during this sub-study. However, this population seems to be at increased risk for MSD and further studies should investigate the impact of economic and behavioral factors on water collection and storage practices in these households.[52,53]

Our findings have several limitations. First, water sampling was conducted during the dry season in Bamako. Piped distribution systems can experience a greater burden on chlorine demand during periods of high precipitation and ground saturation, so piped water quality in Bamako may worsen during the rainy season.[9] Second, the nested environmental microbiology sub-study was conducted in GEMS households in two quartiers of Bamako after the child's enrollment in the case-control study, and therefore constitutes cross-sectional data in a portion of the city that cannot be causally linked to the original recorded health outcomes or generalized to the entire population of Bamako. Third, TCU and *E. coli* indictor assays are useful for evaluating treatment efficacy for piped water and for tracking the microbiological deterioration of treated

water in the home after procurement. However, the absence of coliforms is not a reliable indicator for the absence of contamination by chlorine-resistant microorganisms, such as *Cryptosporidium*.[28] Fourth, the use of secondary sources for drinking water may be an important cause of exposure to waterborne pathogens. Although > 96% of caretakers reported having daily access to their primary water source, wells or other sources might be used if the primary source was non-functional, or if the caretaker lacked either the financial resources to pay for water or the time to fetch it. Caretakers may elect to save money and time by using secondary water sources for drinking, cooking, bathing, or hand washing. Data were not collected on the frequency with which water from secondary water sources was used for drinking. Nevertheless, this is an important issue that could influence the success of behavioral interventions. In addition, we did not collect data on the quantity of water collected or used per person in study households. Where water is scarce, hand washing and hygiene suffer and the risk of diarrheal disease transmission may increase.

Finally, even with broad access to treated source water, pediatric diarrheal morbidity and mortality in Bamako remain high. In addition to contamination of water during household storage, other routes of transmission of enteric pathogens (e.g., by contaminated food vehicles, fomites, flies, and direct fecal oral contact) are common in poor urban communities in the developing world, and are likely important contributors to the high rates of pediatric disease that persist in Bamako.[54] Conversely, behaviors like breastfeeding can help protect infants and toddlers from disease through passive protection from antibodies and other factors in the mother's milk,[55] and/or by reducing the likelihood that an infant will consume contaminated food or water. Our results concur with many other studies that children who are breastfed are protected against diarrhea, including when unsafe water practices are used. Environmental and behavioral interventions to diminish the pediatric diarrheal disease burden must be directed toward all of these modes of transmission.

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