Research Paper

Bacterial contamination of reusable bottled drinking water in Ecuador

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ABSTRACT

In northern coastal Ecuador, water is routinely sold in 20 L reusable bottles for household consumption. These bottles are filled at central treatment facilities and distributed by private water companies. Similar bottled water markets are found in countries around the world. Commercially available bottled water offers an alternative source of drinking water in locations where piped infrastructure may be unsafe or non-existent. In this study we found that 73% ($n = 94/128$) of water sold in reusable containers in the Esmeraldas province of Ecuador was contaminated with coliform bacteria. In comparison, 25% ($n = 9/36$) of non-reusable bottles and 9% ($n = 2/22$) of water samples taken directly from the water treatment system contained coliform, suggesting that most observed bacterial contamination occurred due to inadequate cleaning of reusable bottles between use. The coliform contamination may pose a health risk to the Esmeraldas population. The present study may be indicative of similar situations in low- and middle-income countries around the world, given the widespread use of reusable bottles for water.

Key words | bottled water, developing countries, drinking water quality, *Escherichia coli*, public health, total coliform

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INTRODUCTION

Poor water, sanitation and hygiene (WASH) practices account for at least 9.1% of the total global disease burden (Prüss-Üstün *et al.* 2008), and 1.8 billion people worldwide use a drinking water source that is fecally contaminated (Prüss-Ustün *et al.* 2016). In low- and middle-income countries (LMICs), inadequate drinking water is responsible for 34% of all diarrheal illness (Prüss-Ustün *et al.* 2016). Diarrheal illness accounts for 842,000 deaths annually and is the main contributor to global child mortality (Prüss-Ustün *et al.* 2014, 2016).

While piped, clean water is usually readily available for urban dwellers in wealthy countries, these systems often do not reach lower-income or rural areas in LMICs (WHO/ UNICEF 2012). Large-scale water treatment centres with piped infrastructure are frequently deemed too costly for small rural populations (Hutton & Haller 2004). In areas with limited water infrastructure, people may turn to bottled water. The number of users of bottled drinking water globally rose from 37 million in 1990 to 228 million in 2010 (WHO/UNICEF 2012).

The global market for bottled water is substantial. In 2013, 227.4 billion L of bottled water were consumed worldwide. This number has been growing at approximately 5% annually, and is expected to reach 310.2 billion L by 2018 (MarketLine 2015). Following the global trend, bottled water has grown in popularity in Ecuador (Kjellén & McGranahan 2006). While available data are limited, existing reports suggest that people in rural parts of Ecuador are increasingly dependent on private water purification companies for drinking water (d'Ozouville 2008; Liu & d'Ozouville 2013).

Reusable 20 L bottles of water are ubiquitous and can be perceived to be safe by local consumers. However, an informal report from the Galápagos region of Ecuador found variable levels of contamination in drinking water from these reusable 20 L bottles (Liu & d'Ozouville 2013), and a study in South Africa found two out of ten bottled water samples contained bacterial counts above local guidelines (Ehlers *et al.* 2004).

The majority of private water companies in northern coastal Ecuador sell drinking water in 20 L reusable bottles, which resemble the bottles used for water coolers in the USA and other wealthy countries, and are routinely used for household consumption. The 20 L plastic bottles are typically cylindrical and have a narrow mouth. A first-time consumer purchases a 20 L plastic bottle filled with purified water and exchanges this for another full bottle when it is empty. The empty bottle is cleaned and filled with purified water at the treatment plant, and then redistributed to other consumers. However, the cleaning process is unregulated and water companies have no control over how the bottles are used and stored between refills.

Bottles may become contaminated due to inadequate treatment of source water, inadequate cleaning and sealing of reusable bottles (Liu & d'Ozouville 2013) or improper storage techniques (Wright *et al.* 2004; Trevett *et al.* 2005; Günther & Schipper 2012). Large bottled water companies in the United States are regulated by the US Food and Drug Administration (FDA 2016), and large companies, such as Nestlé's Poland Spring, have a thorough process for inspecting and disinfecting bottles prior to reuse (Nestlé Waters North America Inc. 2010). However, despite nationwide drinking water guidelines (INEN 2011), no available evidence suggests that there is active regulation of the distributors in Ecuador.

Given the growing reliance on bottled water networks and early reports of bottle contamination, we designed a pilot study to evaluate bottle purity focusing on a province in coastal Ecuador. The aim of this study was to measure levels of *Escherichia coli* (*E. coli*) and total coliform in commercially available drinking water sold in reusable 20 L bottles to determine overall frequency of contamination and potential sources of contamination. The study focused on treatment plants that distribute drinking water in the Esmeraldas province of Ecuador.

METHODS

Sample collection and survey procedure

Six bottled water companies in Esmeraldas Province were identified through an informal survey of end users in Muisne. All were visited at least once, and four were visited twice with at least a week between visits. At each visit, 20 L reusable water bottles ($n = 10$–12) and 4 L non-reusable (single-use) water bottles ($n = 4$) were purchased on the same day they were bottled and transported via truck to the testing site. In addition, two sterile 125 mL plastic bottles were rinsed and filled with the distributor's purified water prior to bottling. Upon arrival at each testing site, each 20 L bottle was labelled with a number from 1 to 12, and details of date, time of purchase, time of arrival, colour, cap type, and seal type were recorded along with comments on any leaks or bottle damage. To simulate typical storage conditions, bottles were stored on the tiled floor inside an open-air building, where the temperature varied from 23.5 to 27 °C.

A brief survey was also conducted with each distributor to determine how many bottles were sold, whether sales
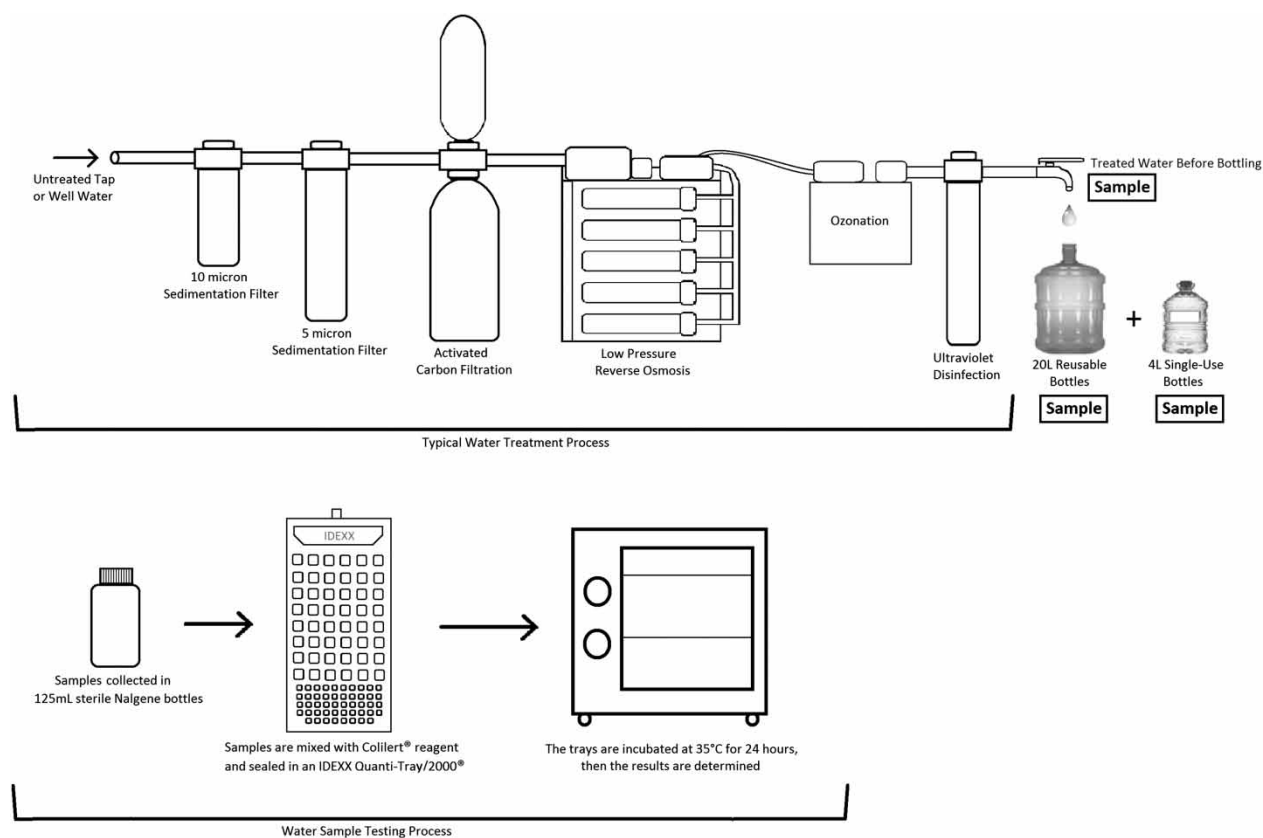
varied throughout the year, and where/to whom the bottles were sold. A tour of the facilities was also requested to view the treatment and cleaning procedures, and overall cleanliness of the facility. Participation in the survey and tour was completely voluntary and all distributors were made aware that identifiable information would be kept confidential. The study was approved by the Yale University Institutional Review Board (IRB) as well as the Esmeraldas Ministry of Public Health. Contact details were left with the distributors should they have any questions or be interested in receiving their results.

Testing procedures

In accordance with the World Health Organization Guidelines for Drinking-water Quality, as well as the US Environmental Protection Agency's standards, *E. coli* and total coliform were used as indicators of bacterial fecal contamination (EPA 2015; WHO 2016). The two samples from the purification system were tested immediately on day 0, while two 20 L reusable bottles from each collection round were tested 0, 3, 7, 14, 21, and 28 days from the initial collection date, and one 4 L non-reusable bottle was tested 0, 7, 21, and 28 days from the initial collection date. Samples were examined over the course of 28 days because most water bottles encountered included a label that directs the consumer to drink its contents within one month of bottling.

Bottles were opened and inspected inside for damage and the presence of mould, insects, or other visible particles or contamination. Then, 100 mL of water from each bottle was prepared and incubated per manufacturer's instructions for coliform contamination (IDEXX Laboratories, Inc. 2013). All bottles were tested within 30 minutes of opening. Total coliform and *E. coli* were determined using the corresponding IDEXX laboratories procedures and Most Probable Number (MPN) tables (IDEXX Laboratories, Inc. 2013) (Figure 1).

Figure 1 | Typical treatment and testing schematic. Typically, distributors used a combination of reverse osmosis, ozonation, and ultraviolet processes, but not all three.

Two negative controls were undertaken: one with a 500 mL Dasani-brand bottled water and one with pharmacy-purchased distilled water. The positive control was a mixture of sewer and borehole water.

Data analysis

Data were analyzed using R 3.2.2 (R Core Team, Vienna, Austria). We used z-tests of proportions and analysis of variance (ANOVA) to measure differences in contamination, including differences in contamination levels by company and the difference in proportion of samples contaminated in 20 L vs. 4 L jugs vs. source water samples.

We also used logistic regression to examine the relationship between jug type and contamination. Our outcome variable was coliform and/or *E. coli* presence. The main effect of interest was whether the sample had been taken from a 20 L jug. Covariates included storage time, and whether a sample was leaking. We ran both an unadjusted regression and a regression adjusted for covariates. Both regressions included company fixed effects and clustered standard errors at the company level.

RESULTS

Company characteristics

All six distributors were located in northern Ecuador and distributed water primarily in Esmeraldas province. Distributors had operated for a mean of 9 years (range: 3–18 years). There was substantial variation between the estimated number of bottles sold per day, with a range of 150–1,100 (mean 670). Companies reported selling water primarily for resale to small businesses, at a price of $0.75–1.00 per bottle for 20 L reusable bottles and $0.63–1.00 per bottle for 4 L non-reusable bottles.

The water purification processes were similar across all companies. All distributors reported using a combination of activated carbon and/or sand filters, ozone purification and ultraviolet decontamination. Companies A, D and E also use reverse osmosis. Bottle decontamination processes were also similar – all distributors reported washing bottles with water plus a detergent or chlorine disinfectant, and half used a high-pressure hose.

20 L bottle contamination

Visual inspection

One hundred and twenty-eight 20 L bottles were analyzed. Seventy of these bottles (55%) leaked due to an inadequate seal. Six (5%) had noticeably weak plastic, and one (1%) was visibly contaminated with insect larvae. We also analyzed thirty-six 4 L bottles, of which one had noticeable defects.

Total coliform

Overall, 73% ($n = 94$) of the 20 L reusable water bottles were contaminated with coliform. Fifty-four of these bottles (57%) had 1–100 coliform/100 mL, while 24 (26%) had 100–500 coliform/100 mL, and 16 (17%) had more than 500 coliform/100 mL. Of the 16 samples that contained >500 coliform/100 mL, 11 (69%) of them contained >1,000 coliform/100 mL, including one bottle which contained >2,400 coliform/100 mL (Table 1). Drinking water

Table 1 | Level of coliform and *E. coli* contamination in 20 L bottles by company

Level of contamination/100 mL (%)	Company A $n = 24$	Company B $n = 12$	Company C $n = 24$	Company D $n = 24$	Company E $n = 22$	Company F $n = 22$	Total $n = 128$
<1 coliform	13/24 (54%)	6/12 (50%)	6/24 (25%)	5/24 (21%)	3/22 (14%)	1/22 (5%)	34/128 (26%)
1–99 coliform	10/24 (42%)	3/12 (25%)	10/24 (42%)	9/24 (38%)	9/22 (41%)	13/22 (59%)	54/128 (42%)
100–500 coliform	1/24 (4%)	2/12 (17%)	4/24 (17%)	6/24 (25%)	4/22 (18%)	7/22 (32%)	7/128 (19%)
>500 coliform	0/24 (0%)	1/12 (8%)	4/24 (17%)	4/24 (17%)	6/22 (27%)	1/22 (5%)	16/128 (13%)
E. coli present	0/24 (0%)	1/12 (8%)	0/24 (0%)	1/24 (4%)	1/22 (5%)	0/22 (0%)	3/128 (2%)

n = number of bottles tested.

guidelines state that total coliform should be zero and presence indicates contamination (EPA 2015; WHO 2016).

There was a significant difference in the percentage of contaminated bottles by company, from 46 to 95% ($p = 0.006$).

In logistic regression analysis, with standard errors clustered at the company level, we found no relationship between presence of contamination and storage time ($p = 0.23$), sample collection round ($p = 0.24$), or leakage ($p = 0.27$). This indicates that while contamination levels varied among distributors, they did not significantly change when a repeat visit was made to the same distributor, and that storage time or leakage did not affect contamination.

E. coli

Three 20 L bottle samples (2%) from three separate companies contained *E. coli*. *E. coli* contamination levels ranged from 12 to 25 *E. coli*/100 mL.
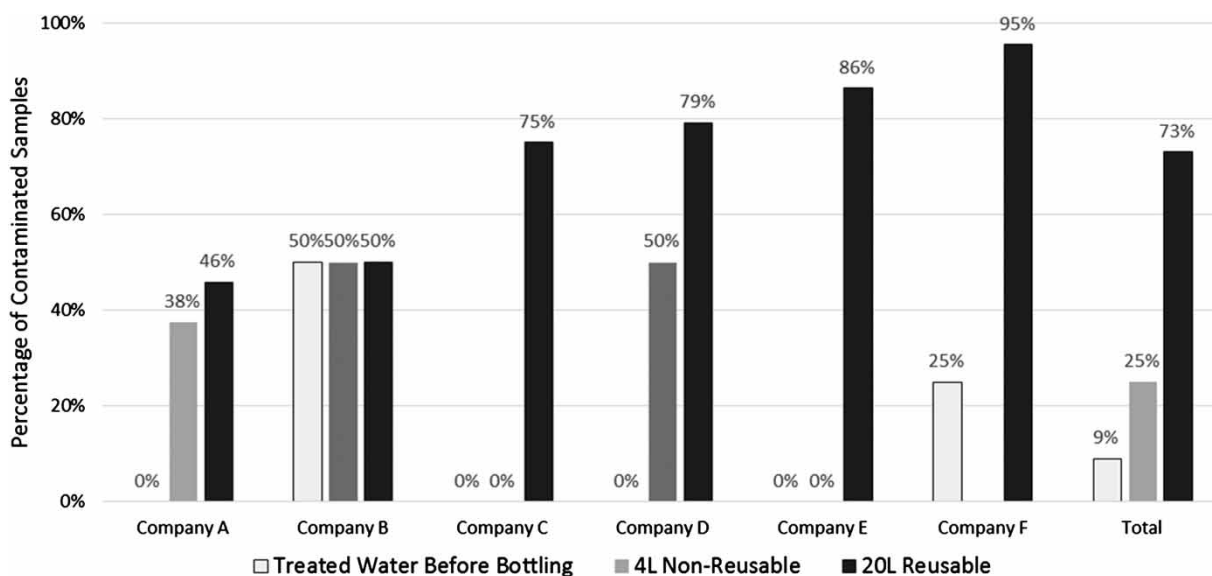
Comparison of source types

We found no association between level of coliform contamination and storage time for all bottles ($p = 0.70$). Therefore, we used aggregate data for comparisons.

We compared the level of contamination observed in the 20 L bottles to that observed in the 4 L bottles and source water samples taken before bottling (Figure 2). Compared to the 73% ($n = 94/128$) of 20 L bottles that tested positive for coliform, 25% ($n = 9/36$) of 4 L non-reusable bottles tested positive for coliform, and 9% ($n = 2/22$) of the treated, pre-bottled water samples tested positive for coliform. Coliform contamination was found in the treated, pre-bottled water for both companies B and F. These companies also use carbon activated filters, UV and ozone treatment, however companies B, F and C are the only facilities that do not use reverse osmosis.

The percent coliform contamination by company and source type is given in Table 2. Distribution of coliform counts were found to be right-skewed; the 25th percentiles were 0 coliform/100 mL for the 20 L bottles, 4 L bottles, and the pre-bottled samples. The median levels of total coliform contamination were 23 coliform/100 mL for the 20 L bottles, and 0 coliform/100 mL for both the 4 L bottles and the pre-bottled samples. The 75th percentiles were 149 coliform/100 mL for the 20 L bottles, and 0 coliform/100 mL for both the 4 L bottles and the pre-bottled samples (Table 3).

Additionally, there was no statistically significant difference between the coliform concentration of the water before

Figure 2 | Percentage of coliform bacteria contamination among different water samples by company. Note: Company F did not sell 4 L non-reusable bottles.

Table 2 │ Percent coliform contamination by company and source type (company F did not sell 4 L non-reusable bottles)

	20 L reusable bottles (%)	4 L non-reusable bottles (%)	Treated water before bottling (%)
Company A	46	38	0
Company B	50	50	50
Company C	75	0	0
Company D	79	50	0
Company E	86	0	0
Company F	95	NA	0

bottling and the contamination levels of the 4 L non-reusable bottles ($p = 0.27$).

DISCUSSION

Contamination in the treatment and distribution process

This study is, to our knowledge, the first to systematically measure levels of *E. coli* and total coliform contamination in commercially available drinking water sold in Esmeraldas province of Ecuador. We found contamination throughout the entire treatment and bottling process. Levels of coliform in 20 L reusable water bottles were significantly higher than either 4 L non-reusable bottles or water samples taken prior to bottling. Additionally, this study found no significant association between the level or presence of coliform contamination and duration of bottle storage.

Based on these observations, it seems likely that the primary cause of contamination is inadequate cleaning of reusable water bottles between uses. In comparison to the 20 L reusable bottles, the treated water and 4 L non-reusable bottles had very low levels of contamination. Another likely cause is contamination during the sealing process, as contamination in 4 L non-reusable bottles was still an order of magnitude higher than in the treated water. Given that the treatment and bottling process is the same for both 4 and 20 L bottles, we suggest that the majority of bacteria introduced into 20 L reusable bottles occurs while they are in the possession of the consumer. While both bottle types risk acquiring bacteria during the bottling process, since the 4 L bottles are only used once, there are fewer sources of potential contamination. There are several other differences between the two bottle types tested (e.g. volume, shape, type of plastic and seal, and ability to be reused) that may also be related to different contamination levels.

The sealing process is slightly different for each bottle type: reusable water bottles use pull-tab caps and shrink wrap seals, whereas non-reusable bottles use a twist-off seal. While one may be more effective than the other (reusable bottle caps often leaked), it appears unlikely that this variation would account for such a large contamination difference, particularly in samples tested only a few hours after bottling. The bottling and storage processes were the same for reusable and non-reusable bottles in this study; therefore, these factors do not explain the observed difference in contamination levels between bottle types. Ultimately, all parts of the water treatment and distribution process should be scrutinized, however, instigation of proper cleaning and sealing techniques are likely simple fixes that would dramatically reduce contamination levels.

Table 3 │ Coliform counts (No. coliform/100 mL) as median, 25th and 75th percentiles by company and source type (company F did not sell 4 L non-reusable bottles)

No. coliform/100 mL	20 L reusable bottles			4 L non-reusable bottles			Treated water before bottling		
	25th Percentile	Median	75th Percentile	25th Percentile	Median	75th Percentile	25th Percentile	Median	75th Percentile
Company A	0	0	10	0	0	4	0	0	0
Company B	0	1	60	0	2	18	0	1	1
Company C	1	13	160	0	0	0	0	0	0
Company D	9	58	414	0	25	177	0	0	0
Company E	22	75	753	0	0	0	0	0	0
Company F	15	49	135	NA	NA	NA	0	0	2

Limitations

This study has several limitations. It is a sample from a single region of Ecuador during the start of the rainy season and from a fraction of the total private bottled water companies. Water quality may exhibit seasonal variation and likely also varies from one region to another. The study also does not account for different types of storage and transportation conditions between bottling and consumption. Although we did attempt to reproduce realistic transportation and storage conditions (transported by truck and stored indoors at ambient temperature), we were unable to account for all differences in conditions that exist between the numerous locations where water is sold in Esmeraldas. Additionally, many of the water bottles are not labelled with the bottling date and may be stored in local shops for several months or longer.

Health risk and global standards

Although most coliform are not considered direct human pathogens, total coliform are used as a global standard to determine whether a water sample has been exposed to a source of contamination such that there could be pathogenic organisms in the water (EPA 2013). Total coliform represent one of the most ubiquitous bacterial genus, so while it is possible for coliform to be present in a sample without the presence of pathogenic organisms, it is highly unlikely that a sample would contain pathogens yet test negative for coliform (EPA 2013; Gruber *et al.* 2014).

E. coli is another pathogen indicator. We found a lower percentage of contamination with *E. coli* than with total coliform, with only 2.3% of samples testing positive for *E. coli*. *E. coli* concentrations also occurred at lower levels than those for total coliform. However, at the moderate concentrations observed in this study, *E. coli*-contaminated water samples do pose a 'moderate' health risk (Bain *et al.* 2014).

Current Ecuadorean regulations state that there must be zero total coliform for any given 100 mL sample of water from a treatment centre, and if a sample tests positive for total coliform, a bottler must pass several subsequent tests in order for the facility to continue operating. All companies tested in this investigation would have failed to meet US EPA regulations, which are considered to be a gold standard. These prescribe a Maximum Contaminant Goal Level (MCGL) of 0 total coliforms, and for water systems that collect fewer than 40 routine samples per month, no more than one sample can be coliform-positive per month (INEN 2011; EPA 2014).

National and global implications

The results of this study may have implications for national policy targeting drinking water quality in Ecuador and countries with similar water and treatment conditions. Despite marketing appealing to a desire for good health and much higher prices than alternative water sources, the bottled water tested in this study demonstrated coliform contamination that may represent a substantial health risk to the population of Esmeraldas. The present study may reflect similar situations in LMICs and rural communities that use bottled water around the world. Further research should be undertaken to determine the percentages and concentrations of reusable bottled water contamination and usage globally, as well as the impact this may have on local health such as diarrhea levels.

Recommendations

To combat contamination in bottled water, improved bottle cleaning methods are needed, alongside better regulation of the industry as a whole and penalties for non-compliance with approved methods. Each private water company from which we collected samples reported using a cleaning method for their reusable water bottles prior to refilling them. However, it is unclear how closely companies adhere to these procedures. The variability in contamination levels suggests that there may be an opportunity to identify companies that have better cleaning methods and disseminate these practices across the industry. However, given that even the company with the lowest percentage of contamination still had coliform in 46% of sampled bottles, there may be no local company to serve as a model of best practices.

One approach is to look to bottled water companies operating in high-income countries, which use advanced cleaning techniques for their large reusable water bottles.

These companies often employ a pre-screening process where bottles that test positive for any kind of contamination or deformity are destroyed (Nestlé Waters North America Inc. 2010). However, this process is not financially feasible for smaller companies operating in LMICs such as Ecuador, where the bottles themselves represent a significant cost to both consumers and water producers.

Perhaps a more pragmatic strategy would involve devising novel cleaning methods designed for lower resource settings through original research. This research could be targeted toward identifying lower cost and small-scale cleaning solutions that comply with country-specific regulations. It could also focus on the addition of residual disinfectants and would work to combat contamination that escapes the cleaning process and/or is introduced through sealing and handling.

CONCLUSIONS

Contamination was present throughout the entire treatment and bottling process, indicating that many people are purchasing water that is contaminated with *E. coli* and total coliform. Seventy-three percent of 20 L reusable water bottles tested were contaminated with coliform bacteria and 2% were contaminated with *E. coli*. Contamination levels were significantly higher in reusable bottles than either non-reusable bottles or water samples taken prior to bottling. Based on the findings of this study, it seems likely that the primary cause of contamination is inadequate cleaning of reusable bottles between uses. A randomized, controlled trial would further elucidate this finding. The results of this study may have implications for national policy aimed at improving quality control for bottled water in Ecuador and countries with similar water conditions and there is scope for inexpensive, small-scale technologies that could reduce contamination in bottles.

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