Semi-quantitative estimation of Escherichia coli levels in public drinking water sources in northern Haiti

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ABSTRACT

Prior research in Milot, Haiti, documented that public water sources are commonly fecally contaminated, as indicated by the presence of Escherichia coli. However, the degree of contamination was not assessed. In this study, the degree of fecal contamination in public drinking water sources was determined. Further, the usefulness of sanitary inspection surveys to predict fecal contamination was evaluated. A convenience sample of public water sources was tested using a semi-quantitative assay, which estimates the most probable number (MPN) of E. coli/100 mL of water. Each source was evaluated using the World Health Organization sanitary inspection score and classified as improved or unimproved. Sixty-three water sources were tested, of which 27 (43%) had <1 MPN/100 mL, 19 (30%) were contaminated from 1 to 100 MPN/100 mL, and 17 (27%) were contaminated with >100 MPN/100 mL. Some improved water sources were contaminated with >100 MPN/100 mL. The sanitary inspection score did not distinguish between sources that were and were not contaminated with E. coli. In Milot, Haiti, public water sources can be highly contaminated with E. coli. Since neither the categorization of a water source as improved or unimproved nor the sanitary inspection score can predict contamination, routine microbiological testing is justified.

Key words | drinking water, Escherichia coli, Haiti

INTRODUCTION

With the 2010 emergence of cholera in Haiti, water safety became a pressing issue that drew both local and international attention (Periago et al. 2012). As of December 2014, there have been 720,524 cases of cholera, including 8,774 deaths (Ministere de la Sante Publique 2014). Cholera is transmitted via a fecal–oral route, through the consumption of food and water that has been contaminated with feces from an infected individual. Fecally contaminated drinking water is commonly found in areas of Haiti that have a heavy burden of cholera and other gastrointestinal diseases.

The best approach to eliminating this epidemic and at the same time reducing the frequency of other water-borne diseases is through improving access to safe water and sanitation (Waldman et al. 2013). Chlorination, which is a common method for water decontamination, is found in <50% of piped public water systems throughout Haiti, according to studies done by Direction Nationale de l’Eau-potable et de l’Assainissement (Blanc 2013). Current efforts to reduce the burden of cholera in Haiti have focused on improving access to safe water through the construction of new water sources and providing point of use water purification methods, such as household water filtration and chlorination (United Nations Children’s Fund 2011).

In a setting with such high frequency of contaminated water and such a low frequency of use of chlorination, it is critically important to identify which water sources are safe and which are unsafe. This knowledge would allow...
for the prioritization of remediation efforts and determines the areas in most need of point of use water purification.

There are three commonly used ways of determining the relative safety of a water source: microbiological testing, sanitary inspection of the source, and categorization of the source as improved or unimproved. Microbiological testing is the gold standard for determining the contamination of water sources and does so by identifying the presence of microbes associated with fecal contamination such as *Escherichia coli* and thermotolerant coliforms (World Health Organization 2011). This testing can either be quantitative, where the number of microbes in a given volume is determined; semi-quantitative, where the number of microbes in a given volume is estimated statistically; or qualitative, where only the presence or absence of microbial contamination is determined (Bain et al. 2012a).

Water source safety can also be characterized by visual inspection. The World Health Organization (WHO) has promoted the use of a sanitary inspection score, which is derived from a questionnaire that assesses possible routes of contamination of the water source (WHO 1997). This score, however, has not been validated.

Finally, sources can be categorized as improved or unimproved, which is a proxy for safety that is based on the type of water source and the degree to which it is protected from routes of contamination. Improved water sources include the following: piped water on premises, public taps or standpipes, boreholes or tubewells, protected dug wells, protected springs, and rainwater collection. Unimproved water sources include the following: unprotected dug wells, unprotected springs, cart with small tank/drum, tanker truck, bottled water, and surface water (WHO/UNICEF 2011). This method of classification is currently being used by the WHO for the assessment of Target 7.C of the Millennium Development Goals (United Nations 2014). However, this proxy has been shown to overestimate the safety of sources (Godfrey et al. 2011; Bain et al. 2012b; Clasen 2012; Onda et al. 2012; WHO/UNICEF 2012; Patrick et al. 2013).

Our prior research, which took place in the Milot Valley, located in the northern region of Haiti, had determined that many public water sources were contaminated with *E. coli* (Hwang et al. 2012). This testing for *E. coli* was conducted using a qualitative (presence/absence) test that detected a minimum of 1 *E. coli* per 100 mL of water.

Limitations of this qualitative testing were that it required extensive training, an incubator and a UV light source. Further, it did not distinguish between the water sources that have low levels of *E. coli* (1–10 colony forming units (cfu) per 100 mL) and the sources that have very high levels of *E. coli* (>100 cfu/100 mL). In the United States the current standard for safe drinking water is <1 cfu of *E. coli*/100 mL (USEPA 2014). The WHO has proposed a recommended drinking water quality standard of <10 cfu of *E. coli*/100 mL (WHO 2013).

The current study took place in the Milot Valley of Haiti, in partnership with Hôpital Sacré Coeur. We used a semi-quantitative test to determine the extent to which the water sources in the region were contaminated with *E. coli*. Semi-quantitative testing can be used to provide an estimate of the number of *E. coli* present in a water source, and therefore may be helpful to community health workers in identifying the highest risk sources in order to prioritize those that need remediation and identify areas that should be targeted for point of use water purification. Due to the costliness of this microbiological testing, however, we also assessed the ability of the WHO sanitary inspection score to predict *E. coli* contamination of improved and unimproved water sources.

**METHODS**

**Site selection**

Public water sources were tested in seven communities in the Milot Valley of the Northern Department of Haiti. The communities were selected for their size, distance from Milot, and accessibility. Convenience (i.e., nonrandom) sampling was used to select the public water sources for testing. Community members helped the authors to locate the water sources that served multiple families. Water sources were not tested if they served only individual families. Using this method, we were able to identify and sample 63 water sources.

**Sample and data collection**

The water samples were collected in the wet season between June 1 and July 20, 2013. At each public water source,
altitude, ambient temperature, and a general description of the water source were recorded, and the location was recorded with GPS coordinates. A single 100 mL sample was collected once from each public water source. Samples were collected from piped sources and wells with hand pumps by letting the water run for several seconds to clear standing water, then catching a mid-stream sample with a sterile collection bottle. For open dug wells, water was collected using the supplied collection bucket when available, or our own clean collection bucket when a local method was unavailable.

Microbiological testing

Samples from the sources were each tested once using the semi-quantitative compartmentalized bag test (CBT) (Aquagenx, LLC, Chapel Hill, NC) to determine the most probable number (MPN) of \textit{E. coli} present. The MPN is a numeric result of a dilution method for estimating, without a direct count, the density of organisms in a liquid (McCray 1955; Cochran 1950). Each kit consisted of a sterile 100 mL collection bottle, a growth medium pellet, and a compartmentalized bag sufficient to test a single water sample.

Samples were processed within 6 hours of collection and incubated in the compartmentalized bag at ambient temperature following the manufacturer's instructions. The bags were observed for an additional 24 hours following the incubation period; no changes in the estimated MPN were observed. The limits of detection of the CBT are <1 MPN/100 mL and >100 MPN/100 mL. All tests were from a single manufacturer's lot, and prior to using the kits for data collection, the quality of the lot was tested with chlorinated water (negative control) and samples from the hospital drainage system (positive control).

Sanitary inspection score

Each water source was categorized using the WHO’s 2nd edition \textit{Guidelines For Drinking-water Quality} (WHO 1997). Sources identified in the field included open dug wells (unimproved), wells with hand pumps and piped distribution sources (both considered improved) (WHO/UNICEF 2011). Where an individual piped distribution system had multiple water sources (for example, multiple tap stands), we tested all of the accessible water sources from that system and reported the results under the category of piped distribution sources. Two of the authors (WL, DG) scored each water source using the provided instructions for the source type-specific WHO sanitary inspection tool (WHO 1997), which is a questionnaire composed of a series of 10–12 ‘yes’ or ‘no’ questions (see Supplemental data, which is available in the online version of this paper). When discrepancies in scoring occurred, agreement was reached through discussion. The questions in the inspection tool pertain to the structural integrity and the presence of possible routes of contamination. The number of ‘yes’ answers, indicating a possible route of contamination, is scored as a fraction of the total number of questions. A value of zero is the best possible score and a value of one is the worst. We compared the average sanitary inspection score in sources with and without \textit{E. coli} contamination.

Fieldwork

Fieldwork was conducted by two of the authors (WL, DG), who have training in laboratory measures and the sanitary handling of specimens. Sanitary inspection scores were generated by consensus based on the instructions provided in the WHO 2nd edition \textit{Drinking-water Quality Guidelines}. \textit{E. coli} testing results were reported back to the community through community health workers employed by Hôpital Sacré Coeur.

Statistical analyses

Analyses to evaluate the ability of the sanitary inspection score to predict \textit{E. coli} contamination were conducted using logistic regression using STATA 12 (StataCorp LP, College Station, TX) in which the dependent variable was the presence or absence of \textit{E. coli} and the independent variable was the sanitary inspection score expressed as a continuous variable. The questions that comprise the sanitary inspection score are specific to the source type and thus, scores across source type are not completely comparable. For this reason, the analyses were stratified by source type.

In a separate analysis, we assessed the ability of source type to predict contamination with \textit{E. coli}. In this analysis, the comparison was limited to wells with hand pumps and
piped distribution sources, as all open dug wells were contaminated.

RESULTS

It was found that the water sources in the Milot Valley generally fall into three categories: open dug wells, wells with hand pumps, and piped distribution systems. We observed that open dug wells predominated in more rural areas and tended to be located in the yards of homes. Wells with hand pumps were often located along main roads and at crossroads. Piped distribution sources tended to be present in more densely populated areas. In addition, piped distribution sources had significant variability in the hours in which water was available. Although samples were collected during the rainy seasons, no samples were collected within 24 hours of a rainstorm.

We tested 63 water sources: 28 wells with hand pumps, 17 outlets of piped distribution sources, and 18 open dug wells. Of these 63 water sources, 27 (43%) tested negative for E. coli (<1 MPN/100 mL). The remaining 36 (57%) tested positive for at least 1 MPN/100 mL. Of the 36 E. coli-positive water sources, 17 (47%) were contaminated at >100 MPN/100 mL and 19 (53%) were between 1 and 100 MPN/100 mL (Table 1).

All of the open dug wells were contaminated with ≥1 MPN/100 mL, and the majority (11/18, 61%) were contaminated to a level >100 MPN/100 mL (Table 1). In contrast, 2 of the 28 (7%) wells with hand pumps were contaminated to a level >100 MPN/100 mL, and the majority (21/28, 75%) had E. coli at <1 MPN/100 mL. The contamination levels in the piped distribution sources were highly variable. Of the 17 piped distribution sources, 4 (24%) were contaminated to a level >100 MPN/100 mL, while 6 (35%) had <1 MPN/100 mL. Piped distribution sources were six times as likely to be contaminated with E. coli compared to wells with hand pumps (OR = 6.0, 95% confidence interval = 1.6–22.0, p = 0.007). Of the 45 improved water sources, 18 (40%) were contaminated, while all of the unimproved water sources were contaminated. Furthermore, 6 (13%) of the improved water sources were contaminated to >100 MPN/100 mL, which is similar to the results of a recent systematic review of data from five countries that reported 10% of improved water sources were contaminated to >100 MPN/100 mL (Bain et al. 2014).

For all source types combined, the WHO sanitary inspection score ranged from 0.25 to 0.92 (median = 0.55). The wells with hand pumps had sanitary inspection scores that ranged from 0.25 to 0.92 (median = 0.50). The open dug wells had sanitary inspection scores that ranged from 0.36 to 0.82 (median = 0.68). The piped distribution systems had sanitary inspection scores that ranged from 0.36 to 0.82 (median = 0.61).

When all source types were combined in a single analysis, an increase in sanitary inspection score by 0.1 increased the likelihood of a water source being contaminated (defined as >1 MPN/100 mL) by 50% (OR = 1.50, 95% confidence interval 1.05–3.18, p = 0.024). However, as we were concerned that this association of water contamination to sanitary inspection score was unduly influenced by the uniform contamination in the open dug wells, we repeated the analysis within each source type. For the analyses within source type, the sanitary inspection score was not predictive of contamination for either wells with hand pumps (OR = 1.05, p = 0.87) or piped distribution systems (OR = 1.06, p = 0.90). The analysis could not be conducted for open dug wells as all sources were contaminated.

The median sanitary inspection score in relation to water source type and the presence or absence of E. coli is shown in Table 2. Within water source type, the median

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**Table 1** Number of water sources at each level of MPN of E. coli per 100 mL

<table>
<thead>
<tr>
<th>Source type</th>
<th>MPN of E. coli per 100 mL</th>
<th>Total number of sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well with hand pump</td>
<td>&lt;1  1  2  5  14  48  &gt;100</td>
<td>28</td>
</tr>
<tr>
<td>Piped distribution sources</td>
<td>6  2  3  0  1  1  4</td>
<td>17</td>
</tr>
<tr>
<td>Open dug well</td>
<td>0  0  0  1  2  4  11</td>
<td>18</td>
</tr>
<tr>
<td>Total number of sources</td>
<td>27  4  4  2  4  5  17</td>
<td>63</td>
</tr>
</tbody>
</table>

*The MPN was determined using the Aquagenx Compartmentalized Bag Test (Aquagenx, LLC, Chapel Hill, NC). The categories of MPN of E. coli/100 mL are derived from a chart of estimated MPN values provided in the manufacturer’s instructions.*
sanitary inspection score was similar in those sources that were contaminated with E. coli and those that were not.

## DISCUSSION

The majority of public water sources tested were contaminated with E. coli, and at least one of each source type was contaminated to a level of >100 MPN/100 mL, including some that met the WHO criteria for improved water sources. Results of our microbiological testing in the Milot Valley are similar to those recently reported from the Artibonite department of Haiti, where 51% of improved water sources were contaminated with E. coli. (Patrick et al. 2015)

It is of concern that the piped distribution sources were so frequently contaminated, as they often supply a higher density population, and therefore put a large number of people at risk for diarrheal disease. Of all of the departments in Haiti, the Northern Department, where the Milot Valley is located, has the lowest rates of centralized chlorination of water systems (Blanc 2013). Priority should be placed on centralized chlorination of piped water systems in the Northern Department.

As open dug wells (considered unimproved) were uniformly contaminated, the areas where they are prevalent should be targeted for the promotion of point of use water purification—such as chlorination or filtration—until safe drinking water sources are constructed. Household water chlorination interventions have been tried in other parts of Haiti and have been shown to improve the microbiological quality of stored household water and reduce the burden of diarrheal disease (Harshfield et al. 2012).

In our study, the WHO sanitary inspection score did not distinguish between sources of the same type that were and those that were not contaminated. It should be noted that when all sources were combined in a single analysis, the sanitary inspection score was predictive of the presence of contamination in a source. However, it proved on further analyses that this association was driven by the high prevalence of contamination of the open dug wells. This suggests that if the source type, and therefore the categorization of a source as improved versus unimproved, is known, the sanitary inspection score does not provide additional information about the probability of E. coli contamination.

Our results are similar to those from a study conducted in Nicaragua, which also did not find an association between microbiological contamination and the sanitary inspection score (Aldana 2010). It is not clear why the sanitary inspection score was not predictive of E. coli contamination in these studies. It is unlikely that this is due to a lack of statistical power, as a useful tool would have been able to distinguish between contaminated and uncontaminated sources with a sample size such as the one in this study. A tool requiring a larger sample size would be of little use to communities.

The inability of the sanitary inspection score to predict E. coli contamination may be related to the poor applicability of individual items on the questionnaire to our environment or weaknesses in the design of the items and their weighting. As an example, water source contamination by distant surface water would not be identified by any of the items on the current questionnaire. Overall, our data imply that the items in the questionnaire do not capture the route of contamination of these water sources. Since there would be value in a validated sanitary inspection checklist as an inexpensive surveillance tool for community healthcare workers, and therefore a realistic way to identify mechanisms to remediate contaminated water sources, future research should be done to develop and validate improved items.

### Table 2

<table>
<thead>
<tr>
<th>Water source type</th>
<th>Median WHO sanitary inspection score (min-max)*b</th>
<th>Water sources with MPN</th>
<th>Water sources with MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well with hand pump (n = 28)</td>
<td>0.50 (0.33–0.75)</td>
<td>0.50 (0.25–0.92)</td>
<td></td>
</tr>
<tr>
<td>Piped distribution sources (n = 17)</td>
<td>0.55 (0.36–0.82)</td>
<td>0.64 (0.36–0.82)</td>
<td></td>
</tr>
<tr>
<td>Open dug well (n = 18)</td>
<td>N/A</td>
<td>0.68 (0.36–0.82)</td>
<td></td>
</tr>
</tbody>
</table>


*Possible scores range from 0 to 1 with 0 as the best possible score and 1 as the worst. N/A: No open dug wells in the category of E. coli absent.

Within water source type, WHO score was not predictive of the presence/absence of E. coli.
Given the high prevalence of contamination of improved water sources and the inability of the sanitary inspection score to differentiate between sources of the same type that were and those that were not contaminated, our data support the previous assertion that routine microbiological testing of water sources in Haiti is justified.

There are several advantages to quantifying the level of contamination with *E. coli* in public water sources. It would identify the water sources that are most contaminated, permitting the prioritization of the water sources that require immediate remediation. The identification of the most contaminated water sources would also permit the prioritization of households using those sources for point of use water purification interventions.

With training, community healthcare workers could estimate the level of contamination with *E. coli* in their local water sources using a kit like the CBT utilized in this study. This kit was easy to use and does not require extensive training in laboratory measures or sophisticated equipment such as an incubator, since incubation is done at ambient temperature. Having the community test its water sources might be less costly than having the testing done by outside organizations. Further, involving the community in monitoring the quality of its public water sources may motivate households to employ point of use water purification methods if community members observe that their water sources are contaminated.

There were limitations to this study. The public water sources were a convenience sample identified by the community. We did not use government supplied information or GIS data to identify all water sources within a community, but rather used community knowledge to identify which water sources were used by multiple families. Water sources were only tested once, which did not allow us to assess the variability of *E. coli* contamination over time. In addition, we did not measure water sources within 24 hours of a rainfall, a time frame over which they would be most susceptible to contamination. As a result, we may have underestimated the number of water sources that were contaminated. It would be useful in future studies to estimate the number of people using each water source in order to prioritize the water sources that require remediation based both on population density and the extent of contamination. Also, some metrics of water sample quality, such as pH and the presence of other contaminants (e.g., arsenic), were not recorded. Future studies should include these important parameters of water quality. Finally, we did not assess the local understanding of the safety of water systems, such as beliefs about purity of various water sources and collection methods, which would be important data to gather in order to shape future interventions.

**CONCLUSIONS**

There is a high prevalence of contamination of public water sources in Haiti, including those that meet the WHO criteria for improved water sources. In this environment, the WHO sanitary inspection score does not predict the safety of a water source. Since neither the categorization of a water source as improved or unimproved nor the sanitary inspection score can reliably predict contamination, routine microbiological testing is justified. Semi-quantitative microbiological testing to identify the most contaminated sources may permit the prioritization of water source remediation and the targeting of household water purification interventions.

**ACKNOWLEDGEMENTS**

Funding for this project was provided by the Tisch College of Citizenship & Public Service at Tufts University and Tufts University School of Medicine. We thank the support staff, translators, and community health workers of Hôpital Sacré Cœur for helping us to execute our research. In particular we thank Lumarc Pierre, the volunteer coordinator, for arranging in-country travel and translators and for serving as a cultural liaison. We also thank Mme Fils-Aime Nirva, the Director of Community Health, for helping to distribute the results of our research to the community. We would like to thank Daniele Lantagne and Andrew Karp for their extensive comments on the manuscript and guidance in framing our research. Finally, we thank Andrew Hwang and Rose Yu, students from Tufts University School of Medicine, for providing a groundwork onto which we built our research. Daniel I. Gerges and William G. LaPlant are co-first authors of this paper.
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First received 7 January 2015; accepted in revised form 8 February 2016. Available online 17 February 2016.