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Feasibility of the Hydrogen Sulfide Test for the Assessment of Drinking Water Quality in Post-Earthquake Haiti

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Abstract

In 2010 a magnitude 7.0 earthquake struck Haiti, severely damaging the drinking and waste water infrastructure and leaving millions homeless. Compounding this problem, the introduction of *Vibrio cholera* resulted in a massive cholera outbreak that infected over 700,000 people and threatened the safety of Haiti's drinking water. To mitigate this public health crisis, non-government organizations installed thousands of wells to provide communities with safe drinking

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Competing Interests

The authors of this study declare that they have no competing interests.

Authors' contribution

TAW and MTA carried out the microbiological assays including the membrane filtration and hydrogen sulfide tests. TAW carried out the statistical analyses, generated the map and drafted the manuscript. JW and DM were responsible for the coordination and implementation of the sampling with the community health workers and generating a geographic information system (GIS) database of the water sources. MHR and VMB coordinated the study at the field location and were involved in critical revision of the manuscript. JAJ and AA helped draft the manuscript, provided technical support with the microbiological assays, and performed critical revisions of the manuscript. JGM and JW conceived of the study, participated in its design and coordination, and helped to draft the manuscript.

water. However, despite increased access, Haiti currently lacks the monitoring capacity to assure the microbial safety of any of its water resources. For these reasons, this study was designed to assess the feasibility of using a simple, low cost method to detect indicators of fecal contamination of drinking water that could be implemented at the community level. Water samples from 358 sources of drinking water in the Léogâne flood basin were screened with a commercially available hydrogen sulfide test and a standard membrane method for the enumeration of thermotolerant coliforms. When compared with the gold standard method, the hydrogen sulfide test had a sensitivity of 65% and a specificity of 93%. While the sensitivity of the assay increased at higher fecal coliform concentrations, it never exceeded 88%, even with fecal coliform concentrations greater than 100 colony forming units per 100 milliliters. While its simplicity makes the hydrogen sulfide test attractive for assessing water quality in low resource settings, the low sensitivity raises concerns about its use as the sole indicator of the presence or absence of fecal coliforms in individual or community water sources.

Keywords

hydrogen sulfide test; water quality assessment; fecal coliforms; Haiti

1. Introduction

Every year in developing nations around the World, over 1.7 billion cases of diarrheal illness occur, leading to 760,000 deaths in children less than five years of age (WHO 2012). In countries where safe drinking water, hygiene, and sanitation are suboptimal, diarrheal diseases acquired from drinking water contaminated with human fecal material remain one of the leading causes of morbidity and mortality (Leclerc et al. 2002; WHO 2011). In 2010, a magnitude 7.0 earthquake in Haiti caused over 200,000 deaths, billions of dollars of damage to infrastructure, and led to the introduction of *Vibrio cholerae* (Cavallo et al. 2010; Frerichs et al. 2012). Following this tragedy, hurricane Tomas provided record amounts of rainfall that facilitated the spread of the cholera outbreak to the entire country, claiming thousands of lives and threatening the safety of the Haiti's drinking water resources (Tappero and Tauxe 2011).

To provide water to the millions of Haitians displaced by the earthquake and mitigate the deadly effects of cholera, thousands of wells were installed by non-government organizations. However, despite ongoing cholera transmission, a national water quality monitoring program to ensure adequate drinking water quality has not been established (MSPP 2012). Routine analysis of drinking water sources for the absence of bacteria indicating fecal contamination is a critical component of any water monitoring program and one of the most basic public health necessities (WHO 2011). Most standard methods require lab equipment, trained technicians, and can be expensive, which precludes their use during emergencies or in low resource settings such as developing nations; both of which apply to post-earthquake Haiti (APHA 1992; WHO 2011). For these reasons, there is a great need for the development of low cost, technically simple water quality tests for indicators of fecal contamination that can be implemented at the community level (Pillai et al. 1999; Sobsey and Pfaender 2002; Pathak and Gopal 2005; Gupta et al. 2008).

One low-cost method for the detection of fecal contamination is the hydrogen sulfide (H_2S) test. This test was originally developed after the observation that “fecal” or thermotolerant coliforms (TTC), a group of bacteria indicating fecal contamination, are often accompanied by sulfate-reducing bacteria in water contaminated with feces (Manja et al. 1982). Using a H_2S test, enrichment media containing a sulfate source and ferric ammonium citrate as an indicator is added directly to the water sample and incubated. In the presence of sulfate-reducing bacteria, the hydrogen sulfide produced reacts with ferric ammonium citrate to form a black precipitate (Widdel 1988). Previous evaluations of the H_2S test indicated variability in performance between studies and recommended that the H_2S test be benchmarked against a gold standard before deployment in a new country. Furthermore, the performance of the test at different concentrations of fecal coliforms and comparisons between different types of water sources needed further characterization (Wright et al. 2012).

As part of a larger study examining the presence of water-borne pathogens and thermotolerant coliforms in the Ouest Department of Haiti, a subset of water samples were used to compare the performance of the hydrogen sulfide screening test to a standard membrane filtration methodology (Widmer et al. 2014). The objective of the study was to evaluate the feasibility of using H_2S tests to detect the presence or absence of fecal coliforms in community drinking water resources. If performance were to be adequate, this low cost method could empower communities to determine the safety of their water resources, in order to prevent diarrheal diseases caused by exposure to water contaminated with pathogens of fecal origin and to aid in the prevention of the ongoing cholera epidemic.

2. Methods

The study area (Figure 1) lies in the region between Léogâne and Gressier, approximately 16 miles due West of the capital, Port-au-Prince. Inhabited by approximately 250,000 people, the study area is traversed by three rivers that lead to seasonal flooding, and was ground zero for the 2010 earthquake. Samples were collected from water sources that met the Joint Monitoring Programme’s definition of both improved and unimproved water sources (WHO 2013). The majority of improved sources ($n=296$) were comprised by India Mark II tube wells with hand pumps ($n=186$) and deep borehole systems with individual and communal standpipes ($n=85$). Unimproved water sources ($n=82$) were comprised of unprotected spring water ($n=43$) and wells dug by hand ($n=39$). Water samples ($n=358$) were collected in sterilized Nalgene® containers (500 ml) by a trained team of local community health workers and brought to the field laboratory at ambient temperature within 3 hours. A commercial H_2S test containing Pathoscreen™ media (Hach Company, Loveland, CO) was used to screen each water sample. Following manufacturer specifications, the contents of one media “pillow” was added to 100 ml of the water sample inside a sterile 100 ml clear polyethylene bag (Whirl-pack®, Nasco, Fort Atkinson, WI) containing 10 mg of active sodium thiosulfate (HACH 2012). The bags were incubated at ambient temperature between 25–29°C and the color (black or gold) was observed after 24 and 48 hours. H_2S test results were compared with results obtained by using a standard method of membrane filtration (50 ml water sample) followed by overnight growth at 44.5°C on mFC agar (Difco™) and direct

enumeration of blue colonies (APHA 1992). Using a sample volume of 50 ml of water gave a limit of detection of TTC at 2 colony forming units (CFU) per 100 ml.

3. Results and Discussion

Test results are shown in Table 1 below. Using the membrane filtration method as the “gold standard,” the H₂S assay had a sensitivity of 64.9% and a specificity of 93.3% to detect the presence or absence of fecal coliforms. The H₂S test gave an estimated prevalence of 28.5%, compared to the 37.4% samples that actually contained detectable TTC based on membrane filtration. At this prevalence, the positive predictive value of the H₂S assay was 85.3%, with a negative predictive value of 81.6%.

While positive results with the H₂S test were obtained from some samples with fecal coliform counts at the limit of detection, 40% of the false negatives had fecal coliforms/TTC at a concentration of less than 10 CFU per 100 ml. The proportion of tests that were positive at fecal coliform concentrations from the limit of detection to greater than or equal to 300 CFU per 100 ml and the effect of incubation time are presented in Figure 2 below. As the TTC concentration approached 50 CFU per 100 ml the sensitivity increased, however did not exceed 88%, even with concentrations greater than or equal to 300 CFU per 100 ml. In the H₂S assay, samples with TTC concentrations greater than 10 CFU per 100 ml also had 25 times the likelihood of turning black within the first 24 hours of incubation compared to samples with lower concentrations.

Much like the concentration and incubation time, the water source type also affected the performance of the H₂S test (Figure 3). As the proportion of samples with detectable fecal coliforms and the concentrations present in those water sources increased, the sensitivity increased. Improved water sources including India Mark II and gravity fed communal standpipes, as well as unimproved spring water had fewer sources with detectable fecal coliforms and on average lower concentrations compared to all unimproved water sources or wells dug by hand (Table 2). This translated into lower sensitivity and higher specificity, whereas the opposite effect was observed with all unimproved water sources and wells dug by hand, which had the lowest specificity and the highest sensitivity. The increased sensitivity could simply be due to the higher prevalence and concentrations of fecal coliforms as demonstrated in Figure 2; however the decrease in specificity for superficial, unprotected sources could be caused by the presence of sulfate-reducing bacteria commonly found in tropical soil and surface waters that are not associated with fecal coliforms (Gupta et al. 2008). This explanation would support the observation that water from high output springs or deeper groundwater sources like the India Mark II wells gave fewer false positive results.

Compared to any other study using H₂S tests with similar prevalence of fecal contamination, this study achieved the highest reported positive predictive value (85.3%) and is the first field trial of a hydrogen sulfide test to detect the presence or absence of fecal coliforms from drinking water sources in post-earthquake Haiti. Results indicated a screening test that is more specific than it is sensitive which performs better at fecal coliform concentrations much higher than those recommended for drinking water. As suggested previously, the false

negatives (47/358) could reflect the lack of adequate sensitivity at concentrations of fecal coliforms less than 10 CFU per 100 ml, or could be due to the presence of TTC that are not associated with sulfate-reducing bacteria (Tewari et al. 2003; Vasudevan and Tandon 2008; Wright et al. 2012). Likewise, false positive results (15/358) could have been due to environmental hydrogen sulfide producing bacteria that are not associated with TTC, which can be also present in tropical groundwater (Kaspar et al. 1992; Pant et al. 2002; Janda and Abbott 2006; Wright et al. 2012). None of the false positive samples turned black within the first hour of incubation, which could have indicated the presence of naturally occurring hydrogen sulfide in the water samples.

Recently, more advanced molecular methods such as Terminal Restriction Fragment Length Polymorphism (TRFLP) have been utilized along with culture-based techniques to evaluate the performance of the H₂S test and indicated that it was able to correctly identify fecal indicator bacteria and enteric pathogens in water contaminated with sewage (McMahan et al. 2011). Furthermore, a subsequent study conducted to determine if positive H₂S test results corresponded with bacterial pathogens of fecal origin revealed that a “false positive” result obtained using the H₂S test that lacked *Escherichia coli* was actually contaminated with organisms that were a concern to human health (McMahan et al. 2012). Since the objective of this study was to compare the performance of the H₂S test to a standard membrane filtration method for the enumeration of fecal coliforms, we were unable to determine if the false positive results actually contained pathogenic organisms. However, due to the potential for human exposure to pathogens of fecal origin, the larger proportion of false negative results observed in this study where the H₂S test failed to detect indicators of fecal contamination represent a greater public health concern if the H₂S test were to be used for community drinking water quality assessments in Haiti. An interesting observation made during this study was that despite the inadequate performance for individual water sources, the H₂S test exactly estimated the prevalence of water sources containing greater than or equal to 10 CFU per 100 ml. This indicates the potential of this test to be a low cost method to determine regional prevalence of fecal contamination, which could be used to prioritize efforts to improve water and sanitation infrastructure as Haiti recovers from the earthquake.

4. Conclusions

In the absence of any national water quality monitoring program, it has been proposed by both NGOs and academics that the H₂S test could be a feasible method of monitoring community water sources for microbial safety in Haiti. Indeed, the H₂S test was easy to transport, store, administer, and replicate by the community health workers at a relatively low cost per test (2.50 USD). However the results from this study suggest that the predictive values of the test to determine the presence or absence of fecal indicator organisms in individual water samples were too low to guarantee a reasonable level of public safety. In a country that is looking for solutions to the monitoring of its drinking water, H₂S tests could be used to assess water quality on a regional level, however are not recommended as the sole detection method to ensure the absence of fecal contamination from community potable water sources.

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Figure 1.

“Map of study area in the Léogâne flood basin and location of water sources tested.”

The study was located in a flood basin between the cities of Léogâne and Gressier, in the Ouest Department of Haiti, approximately 16 miles due West of the capital Port-au-Prince (map inset). The locations of the major cities (black dots), water sources sampled for fecal coliforms (blue dots), and the University of Florida field laboratory (red dot) appear in the enlarged map with respect to the national highway system (red and green lines).

Figure 2.

“Proportion of positive hydrogen sulfide test results obtained after 24 and 48 hour incubation periods.”

The proportion of positive test results obtained after either 24 or 48 hours of incubation (triangles and square markers, respectively) are shown stratified by the concentration of fecal coliforms (CFU per 100 ml) present in the water samples.

Figure 3.

“Hydrogen sulfide test performance, average concentration of fecal coliform bacteria, and prevalence of fecal coliform bacteria isolated from different water sources.”

The relationship between the test performance (sensitivity and specificity) and both the prevalence (%) and average concentration of fecal coliforms (CFU per 100 ml) isolated from the water samples is presented with respect to the different classifications of drinking water sources defined using guidelines from the WHO Joint Monitoring Programme.

“Performance of the hydrogen sulfide test compared to a standard membrane filtration method for the enumeration of fecal coliform bacteria.”

Table 1

	Membrane Filtration		Performance Measures	
	Positive	Negative	Total	(%)
Sulfide Test	Positive	87	15	102
	Negative	47	209	256
	Total	134	224	358
				64.9
				93.3
				82.7
				37.4

^aSensitivity = True Positive results/(True Positive results + False Negative results)

^bSpecificity = True Negative results/(True Negative results + False Positive results)

^cAccuracy = (True Positive results + True Negative results)/Grand Total

^dThe prevalence was determined by the number of water samples containing fecal coliforms at the limit of detection (2 CFU per 100 ml) divided by the total number of samples.

“Hydrogen sulfide test performance and average concentration of fecal coliforms from different water sources.”

Table 2

Water Source Classification ^a	Improved	Mark II	Gravity	Unimproved	Spring	Hand Dug
Sample Size (n)	296	186	85	81	43	39
Sensitivity (%)	79.2	37.1	50.0	84.7	71.4	96.8
Specificity (%)	83.1	95.4	92.7	82.6	93.3	62.5
Accuracy (%)	82.4	84.4	77.6	84.1	79.1	89.7
Pos. Pred. Value ^b (%)	50.6	65.0	78.9	92.6	95.2	90.9
Neg. Pred. Value ^c (%)	94.8	86.7	77.3	67.9	63.6	83.3
Prevalence (%)	17.9	18.8	35.3	72.0	65.1	79.5
Avg. Concentration ^d	21.6	15.1	16.6	137.3	73.1	208.0

^aWater sources were classified using World Health Organization (WHO) Joint Monitoring Programme guidelines.

^bPositive Predictive Value = True Positive results/(True Positive results + False Positive results)

^cNegative Predictive Value = True Negative results/(True Negative results + False Negative results)

^dThe average concentration of fecal coliforms (CFU per 100 ml) was determined using a standard membrane filtration technique.