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Abstract

Angela M. Hardin

The Test for H_2S Production: Analysis of Correlation to Fecal Indicators and Risk of Diarrheal Disease in Bonao, Dominican Republic.

(Under the direction of Christine Stauber, Faculty Member)

Background: Access to improved water and sanitation are key measures of the World Health Organizations. However, while a community can be classified as having access to improved water and sanitation, the possibility of microbiological contaminations exists. Globally, there is a need to assess the quality of drinking water to better classify levels of microbiological quality in attempts to reduce diarrheal disease burden. Utilizing the test for hydrogen sulfide (H_2S) producing bacteria test is a cost effective and easy to use method that may be comparable to the traditional yet more costly method (IDEXX Colilert Quantitray). Due to a paucity of data on the test for H2S producing bacteria, this study was performed to examine how well the test for hydrogen sulfide (H_2S) producing bacteria compared to traditional measure of fecal indicator bacteria total coliforms and *E. coli* in drinking water. Furthermore, an analysis of the ability of the test for H2S producing bacteria to predict diarrheal disease was also examined.

Methods: The following conditions for the H2S were examined in the study: 2 volumes (10mL or 90mL), 2 incubation times (24 and 48 hours) and the use of a semi-quantitative scoring system that measured the intensity of the black precipitate formed (H_2S). To examine how well these conditions compared to E. coli and total coliform results, the following analyses were performed: 1) analysis of sensitivity and specificity to examine presence/absence of bacteria in both samples, 2) linear regression to examine how well a semi-quantitative H2S scoring system predicted bacterial concentrations and 3) logistic regression to examine how well the H_2S test predicted risk of diarrheal disease.

Results: Within the dataset, there were 816 observations among the 7 communities involved in the study. The H_2S test condition that had the highest sensitivity and specificity (94.23% and 36.07% respectively) for total coliforms was 90mL volume at 48 hours. This test condition also produced the highest sensitivity and specificity for E. coli (97.82% and 78.67%, respectively). An analysis using linear regression demonstrated that a semi-quantitative H_2S scoring system was able to predict both total coliform and *E. coli* concentrations in the same samples. In a logistic regression analysis of diarrheal disease, the test of H_2S producing bacteria suggested an increase in diarrheal disease risk for higher levels of H_2S (OR of 1.18 (p=0.03; 1.02 – 1.35)).

Discussion: The initial results here suggest that the use of the test for H_2S producing bacteria has potential with high sensitivity (>90%) for E. coli and total coliforms. The application of the semi-quantitative scoring system may also have applications in predicting concentration of E. coli and total coliforms and well as possibly predicting diarrheal disease. However, more work needs to be completed to standardize the semi-quantitative approach to reduce subjectivity of scoring as well as examine the role of the test in additional epidemiologic studies.

INDEX WORDS: waterborne disease, *E. coli*, Dominican Republic, microbial testing

THE TEST FOR H_2S PRODUCTION: ANALYSIS OF CORRELATION TO FECAL INDICATORS AND RISK OF DIARRHEAL DISEASE IN BONAO, DOMINICAN REPUBLIC

BY

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B.S. SPELMAN COLLEGE

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THE TEST FOR H_2S PRODUCTION: ANALYSIS OF CORRELATION TO FECAL INDICATORS AND RISK OF DIARRHEAL DISEASE IN BONAO, DOMINICAN REPUBLIC

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Chapter I. Introduction

The environment impacts the health of people in many ways through exposures to various physical, chemical and biological risk factors. Environmental exposures enumerated account for nearly 10% of deaths and disease burden globally and around one quarter of morbidity and mortality burden in children under 5 years of age (WHO, 2004).

Diarrheal disease is the second most widespread cause of death among children under the age of 5(need citation). Being one of the most preventable and treatable illnesses, it kills approximately 1.5 million children every year as compared to HIV/AIDS, tuberculosis, and malaria (Global Health Council, 2000-2011, WHO, 2009). While high rates of morbidity and mortality associated with diarrheal disease in developing nations are acknowledged, they are still not well documented, nor measured correctly (Black, 1984).

Almost one tenth of the global disease burden could be prevented by improving water supply, sanitation, hygiene and management of water resources (Fewtrell et al., 2007). According to WHO (World Health Organization), improved sanitation (e.g. pit latrines, septic tanks, and composting toilets) can decrease diarrheal morbidity by 32 percent; improved water supply can reduce diarrheal disease as much as 25 percent (e.g. protected dug wells, public taps, and tube wells) (UNICEF/WHO, Meeting the MDG Drinking Water and Sanitation Target).

The United Nations Statistics Division reported that 95% of the total population in the Dominican Republic is using improved drinking-water sources and 79% have access to improved 0water sanitation (WHO, 2006). While possessing the ability to access clean water and sanitation, there can still be a possibility of water contamination through storage. It is imperative

that people have the ability to test their water and ensure safe consumption. The rationale behind this study is to determine the efficacy of using hydrogen sulfide (H_2S) producing bacteria as an indicator of fecal contamination compared to usual means of detecting total coliforms and *E. coli* in drinking water. Also, it important to gather information determining whether there is a relationship between the presence of hydrogen sulfide producing bacteria and household self-reporting diarrheal disease in children under 5. With the limited researching surrounding this association, this study will provide more insight into the following questions: 1) How well does the test for hydrogen sulfide (H_2S) producing bacteria compared to traditional measure of fecal indicator bacteria, total coliforms, and E. coli in drinking water? 2) Will the utilization of the H_2S test help predict diarrheal disease?

Chapter II: Review of the Literature

2.1 Overall global burden of disease from lack of access to water, sanitation and hygiene

Global Lack of Access

According to the UNICEF and the WHO, 780 million people lack access to improved water supplies and 2.4 billion people lack adequate sanitation facilities (2012). Inadequate water, sanitation, and hygiene plague the world accounting for 5.7% of the total disease burden (Prüss-Üstün and Corvalán 2007). This lack of access has been at the crux of many deaths annually, most significantly in children under 5. Waterborne diseases, most specifically diarrhea, plague developed and underdeveloped nations alike.

Infectious agents tend to account for most of the mortality and morbidity associated with waterborne diseases in developing countries. The four main routes by which water-related infections are transmitted are water-borne route, water-washed route, water based route and insect vector route (Ako, Nkeng, & Takem, 2009). Waterborne infectious diseases are caused by consumption of contaminated water containing a pathogen, or causative organism. These diseases are initiated by drinking water tainted by human or animal feces which have pathogenic microorganisms. A number of pathogens implicated in this are from human feces, and contracted by ingesting fecally contaminated water (fecal-oral diseases). These fecal-oral diseases can also be spread through media other than water, such as contaminated food, fingers or utensils (UNEP/WHO, 1997). The prime fecal-oral diseases include cholera, typhoid, shigellosis, amoebic dysentery, hepatitis A and various types of diarrhea. Approximately, 1.8 million people die every year from diarrheal diseases (including cholera); 90% are children

under 5, mostly in developing countries. Eighty-eight percent of diarrheal disease is attributed to unsafe water supply, inadequate sanitation and hygiene (WHO, 2004).

Combating microbial waterborne disease is a daily burden for the developing world. Water treatment plants, their facilities, and effective delivery networks are vital in providing safe drinking water along with wastewater collection and treatment. In many developing nations' cities where infrastructure exists, watershed protection is not effective, water treatment is generally insufficient and water supply is irregular through failing delivery networks. With such an inefficient system, it is hard for protective measure to implemented, in turn increasing the probability for the burden of diarrheal disease (Choffnes & Mack, 2009).

Types of Waterborne Exposures

Water-related diseases are caused by pathogenic microorganisms and weigh heavily on impoverished nations – placing a major burden on developing countries and their healthcare systems (WHO, 1999). Diarrheal diseases associated with waterborne exposure consist of Giardiasis (Protozoan), Cryptosporidiosis (Bacteria), Campylobacteriosis (Bacteria), and Shigellosis (Bacteria). Water washed exposure is an infection that occurs when an individual has poor hygiene and inadequate access to clean water; often for domestic use (bathing, washing clothes, etc.). Diseases such as trachoma and scabies are associated with this transmission route. Water contact and vector-borne diseases require interaction with insects and parasites that are found in or reside near contaminated water. Water contact can be linked to diseases such as Schistosomiasis and Dracunculiasis (guinea worm disease). Health complications such as malaria and dengue are usually correlated with vector-borne exposure (WHO, 2012). While this type of exposure is considered a water-related exposure, the dynamic of association is different. Vector-borne diseases are not as dependent on access to clean and sanitation, but are more

dependent on vector control. With proper access to clean water and improved sanitation techniques, there is an increased possibility of reducing the burden of these water-related diseases and exposures (WHO, 2012).

Burden of Diarrheal Disease

There is a wide range of viral, bacterial, and parasitic agents that are most commonly associated with diarrheal disease. The usual cause of death is dehydration. Most cases of diarrheal illness and death occur in developing countries because of unsafe water, poor sanitation, and insufficient hygiene. Other waterborne diseases do not cause diarrhea; instead these diseases can cause malnutrition, skin infections, and organ damage (Prüss, Kay, Fewtrell, & Bartram, 2002).

Diarrhea is an infection which varies in duration and is classified as acute or chronic. Deciphering whether the episode is considered acute or chronic is determined by the type of stool produced, as well as the length of time associated with the occurrence. Acute diarrhea is stereotyped as loose stools passed at least three times a day. Chronic diarrhea has a longer duration of approximately four weeks or longer and can be associated with a chronic disease. More severe cases of diarrhea may be life threatening due to fluid loss, particularly those who are immune-compromised, malnourished, or infants and adolescents (Lima *et al*., 2000).

The ability to access clean water, efficient sanitation, and proper hygiene is essential to everyday wellbeing. While most developed regions have this under control, developing countries struggle to tackle this feat. The Environmental Institute approximated that the proportion of the global population residing in areas with great water distress will increase from roughly 34% in 1994 to 63% in 2025, including large areas of Africa, Asia, and Latin America (Mara, 2003).

These increases will have a significant impact on the lives and livelihood of many.

As a consequence of inadequate water and sanitation, 780 million people lacked access to improved water sources representing 8% of the global population (JMP, 2012). Without the growth of populations receiving clean water, evidence confirms that water, sanitation and hygiene-related diseases account for 2,213,000 deaths annually and an annual loss of 82,196,000 disability adjusted life years (DALYs) (WHO, 2000). Approximately 88% of people in developing countries are estimated to have access to a water supply, greater in urban than rural areas (JMP, 2012). However, a significantly less amount of individuals have access to improved sanitation facilities in urban and rural areas, 79% and 46%, respectively (JMP, 2012). Improved drinking-water sources are classified to best piped water to the house or yard, public taps or standpipes, boreholes, protected dug wells, protected springs and rainwater collection (WHO/UNICEF, 2009).

DRINKING-WATER SOURCES

IMPROVED

Piped water into dwelling, plot or yard
Public tap/standpipe
Tube well/borehole
Protected dug well
Protected spring
Rainwater collection

UNIMPROVED

Unprotected dug well
Unprotected spring
Cart with small tank/drum
Tanker truck
Surface water (river, dam, lake, pond, stream, canal, irrigation channel)
Bottled water[a]

SANITATION FACILITIES

IMPROVED[b]

Flush/pour flush to:
- piped sewer system
- septic tank
- pit latrine

Ventilation improved (VIP) latrine
Pit latrine with slab
Composting toilet

UNIMPROVED

Flush/pour flush to elsewhere[c]
Pit latrine without slab/open pit
Bucket
Hanging toilet/hanging latrine
No facilities or bush/field

[a] Bottled water is considered to be improved only when the household uses water from an improved source for cooking and personal hygiene.
[b] Only private facilities are considered to be improved.
[c] Excreta are flushed to the street, yard or plot, open sewer, ditch, drainage way, channel river or stream.

Figure 1. Classifications on drinking water and sanitation facilities (UNICEF, 2006).

Representing 63% of the population, 2.6 billion people lack access to improved sanitation (UNCIEF, 2006). Improved sanitation facilities include flush or pour-flush toilets connected to a piped sewer system, septic tanks or pit latrines, and composting toilets. Of these facilities, they are considered improved if there are private and not communal (UNICEF, 2006). Global sanitation coverage increased from 49% in 1990 to 59% in 2004, equating to approximately 1.2 billion people gaining access to improved sanitation facilities (Mara, 2003). But despite the many advances the world is making in achieving the goals set forth by the UN, it is not sufficient enough progress to the meet the target. Meeting the target would require the improvement rate to increase and double its current progress until 2015 (UNICEF, 2006). With population treads growing, it can be estimated that 2.4 billion people, will be without basic sanitation by 2015 (UNICEF, 2006).

2.2 Focus on Latin America and Caribbean

Coverage of improved water supply sources is approximately 90% or more in Latin America and the Caribbean (JMP, 2012). In developing regions, improved drinking water coverage has increased by 16% since 1990. More specifically, Latin American and Caribbean regions have increased by 9% with improved water and sanitation (JMP, 2012). In spite of the water crisis globally and difficulty meeting MDG aims, some places are on target. Regions such as Latin America and the Caribbean (LAC) are making advances in improving their access to water and sanitation facilities. Mortality rates for children under 5 in these countries dropped 43% from 1990 to 2004 (Mitra & Rodriguez-Fernandez, 2010).

As with any territory, the delivery of drinking water and sanitation amenities demonstrate inequalities of a region with small social economic disparities. Most of these disparities occur in the rural areas, still overall drinking-water coverage increased from 83% to 91% between 1999

and 2004 (Schneider *et al*., 2011). Yet, regardless of the plentiful water resources available, there are still some regions which are plagued by the water crisis.

LAC countries as a whole have had the amount people without access to improved drinking water sources currently reduced by about a third (UNICEF, 2006). Sanitation coverage increased from 68 % to 77 % between 1990 and 2004 and the region has also improved hygiene practices (Mitra & Rodriguez-Fernandez, 2010). While urban drinking-water coverage in LAC is relatively high (96%), rural coverage lags behind at 73% (UNICEF, 2006).

While the global targets regarding improved water status in MDG have been met well in advance, these achievements are relative, considering the gaps within regions and tools used to analyze these goals (JMP, 2012). The MDG compares water safety using two classifications – improved versus unimproved; however, these descriptions do not always reflect the need for better understanding and management of drinking water safety.

Onda *et al*., (2012) took a closer at the implications behind the MDG target achievements, suggesting that the current system of analysis needs to take a more granular approach to represent the information more accurately (2012). During their analysis, Onda *et al*. noted that some of the assumptions made by the MDG targets skew the representation of the water contamination and sanitary risk progress globally (e.g. even distribution across the world) [2012]. They found that performing calculations by their models increased the 1990 MDG baseline by 15%, ultimately affecting projections for access to safe water sources for 2010 and 2015 by 10 and 8 percentage points, respectively (Onda *et al*., 2012). If more concise definitions were applied to the MDG's perception of safe water access and decrease sanitary risk, there is potential for even larger discrepancies in estimations.

In 2011, a study was performed examining the relationship between the Joint Monitoring

Program criterion and their association with the progress of the MDGs for 2015. One major point of contention expressed by Bain *et al*. was the need for water quality data (2012). They noted that without information regarding the quality of water received from these sources, access to improved water did not accurately portray a reduction in exposure to contamination (Bain et. al, 2012). A significant revision would be made to the MDG targets if water quality was taken into consideration in comparison to reducing the amount of individuals with safe water access. In turn, this would cause the target values that need to be met to increase as well as the baselines each target year. While access to safe drinking water is important, other environmental factors such as microbial compliance should also be considered when determining improved quality of water to truly represent target progress (JMP, 2012). Microbial indicators provide a more accurate depiction of water quality and can identify areas that have improved water sources, but are maintained poorly (Bain et. al, 2012).

2.3 Microbial indicators and Their Impact on Health

Within the scope of public health, microbial indicators can be utilized in many different ways. They are vital for various risk assessment frameworks, including potential hazard and exposure assessment, identification of contaminants and their source, and assessing efficiency of risk and burden reduction (National Academy of Sciences, 2006). Instead of directly measuring the pathogen, microbial indicators predict the presence of the pathogen and help identify the possibility of human health risks. They have been utilized since to late 1800s to identify fecal contamination to prevent waterborne and foodborne illness (Sobsey and Pfaender, 2002). A number of the microbial indicators used to detect fecal contamination have both fecal and non-fecal environmental sources. These enteric organisms have environmental origins besides feces

and serve as environmental reservoirs. Some fecal indicator microbe tests also detect similar non-fecal microbes. Standard indicators and methods are unable to identify specific fecal sources impacting water and other environmental media (WHO, 2003). For those microbes which have fecal origins, these tests are not always ideal for detection and can be a major limitation (Sobsey, 2008). Microbial indicators are vital for overall healthcare, but most importantly, to enhance surveillance activities for control of infectious diseases. They assist in analyzing relationships between levels of contamination and the risk of illness (WHO, 2003).

2.4 Bacterial indicators

Ideal characteristics

There are many contributing factors that affect decisions about water consumption ranging from taste, smell, color, safety, cost, and convenience (Theron & Cloete, 2002). Of these contributing factors, safety is at the forefront of the mission on providing safe water to its consumers. With that, it is essential to have testing that is accurate to ensure proper water quality. Over the years, fecal indicator tests have been developed to do just that.

From the start of the 20th century, fecal indicator bacteria have been used to detect levels of contamination in drinking water and determine the presence or absence of pathogenic microorganisms (WHO, 2002). Waterborne enteric pathogens present in water can pose a potentially substantial risk to the health of an individual (Theron & Cloete, 2002). Evidence supports that while waterborne outbreaks are rare, they are often associated with these enteric pathogens and the occurrence of waterborne microbial disease (Theron & Cloete, 2002). Although for most of the population in developed countries minor gastroenteritis may simply

mean several hours of discomfort; however, in developing countries, many people die every year as a result of the consumption of contaminated water (Theron & Cloete, 2002).

The WHO classified microbial indicators into three categories to reduce the ambiguity associated with the term (Ashbolt, Grabow, & Snozzi, 2001).

Group	Definition
Process Indicator	A group of organisms that demonstrates the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection.
Fecal Indicator	A group of organisms that indicates the presence of fecal contamination, such as the bacterial groups thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present.
Index and model indicator	A group/or species indicative of pathogen presence and behavior respectively, such as *E. coli* as an index for *Salmonella* and F-RNA coliphages as models of human enteric viruses.

Table 1. Definitions for indicator and index micro-organisms of public health concern (Ashbolt, Grabow, & Snozzi, 2001)

Ideally, both the pathogen and indicator should be absent or present in the sample simultaneously. The life span of each indicator should be similar to that of the pathogen of concern. Most importantly, the indicator should be present in large numbers, readily detectable by simple and cost efficient methods, and should not proliferate in the environment once shed by the host. Using an indicator to test a water sample which possesses these characteristics, can most accurately infer the presence or absence of pathogenic organisms (Figueras & Borrego, 2010). However, with the complexities surrounding the use of microbial indicators (cost, time necessary to monitor pathogens), simpler, inexpensive techniques are needed to encourage more

sample testing. Ascertaining a better overall picture of the water quality of developing areas allows for better protection of public health (Ashbolt, Grabow, & Snozzi, 2001).

2.5 Traditional Indicator Methods

Turbidity

There have been some associations with turbidity and various health outcomes epidemiologically. Water samples, which are unfiltered, have evidence reflecting fecally prepared water solely disinfected with chlorine permits GI and outbreaks (Allen, Brecher, Copes, Hrudey, & Payment, 2008). Turbidity can provide information regarding what type and amount of treatment is needed for the drinking water treatment processes (Ponk, Goldscheider, & Zopfi, 2007). In water sources subjected to significant levels of fecal pollution, some correlation can be expected between turbidity and fecal indicators or pathogens. Turbidity levels are a simple but efficient parameter to assess source water variations as well as filtration efficiency during conventional treatment of drinking water (Mann, Tam, Higgins, & Rodrigues, 2007). Turbidity is also a useful indicator of groundwater quality; however, does not indicate pathogen presence but provides information on general water quality.

Total Coliforms

Coliform bacteria have the capability to maturate in the presence of bile salts and other surface agents. These microorganisms are comprised of a genera of bacteria found in the intestinal tract of warm blooded animals. Traditionally, these bacteria were used to test water and determine if it was contaminated. Total coliforms are the most basic test for fecal contamination.

Using this method indicates presence of pathogens; however, there has been found to be a lack of association between the number of coliforms and those of pathogenic microorganisms (Ashbolt, Grabow, & Snozzi, 2001).

Within the genera of total coliforms, fecal and environmental species are included. Since these organisms reside and may proliferate in water, they are not a suitable indicator of the presence of all fecal pathogens; however, can detect the possibility of biofilms within the water source or inadequate disinfection (WHO, 2011). The presence of total coliforms after disinfection indicates inadequate treatment. In distribution systems and stored water sources, detection of total coliforms within a water source shows the possibility of regrowth and development of biofilm contamination through an introduction of unknown materials (WHO, 2011).

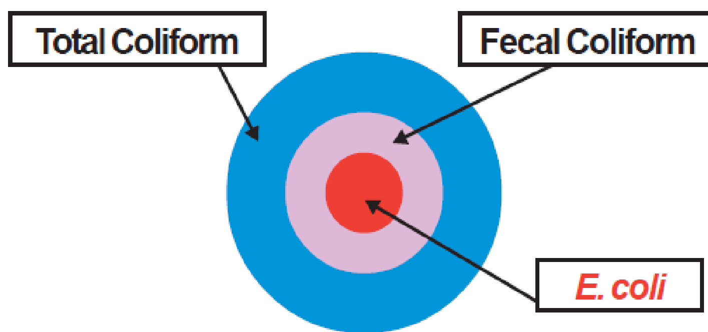

Figure 2. Bacterial Indicators

Once the presence of total coliforms has been detected, there is a possibility of fecal contamination and increasing the necessity of testing for fecal coliforms and E. coli.

Fecal Coliforms

Fecal coliforms are a subset of total coliforms that exist within in the gut and fecal matter of warm-blooded animals (New York State Department of Health, 2011). These coliforms have a more fecal specific origin than those within the total coliform genera; making them a more accurate indication of the presence of animal or human waste (New York State Department of Health, 2011). Literature notes fecal coliform presence as most reliable as an indicator of the bacterial pathogens, particularly *Salmonella sp* (Ashbolt, Grabow, & Snozzi, 2001). However, concerning the existence within the environment, enumeration of these coliforms should be analyzed with apprehension.

Fecal coliform testing can be considered a good indicator of bacterial pathogen regrowth. Other organisms such as viruses and parasites are not able to proliferate with a host that is warm blooded. Bacteria are the only group of pathogens that can reproduce in the environment, and in turn, makes fecal coliforms a reliable good indicator of pathogenic bacteria. While there are potential limitations, fecal coliform analysis is the most practical indicator of the presence and/or absence of pathogenic organisms and an effective tool for evaluating potential public health or environmental impacts (New Hampshire Department of Environmental Health, 2003).

Escherichia coli

Escherichia coli are a subset of the coliform genera. According to the WHO Guidelines for Drinking-water Quality, it is the fecal indicator of choice when determining fecal pollution (2011). While *E. coli* can cause disease and illness in humans, the bacteria naturally occurs in the

lower section of the gut in warm-blooded animals (Figueras & Borrego, 2010).

Of the contaminants present in drinking water, those found in human and animal feces pose the greatest danger to public health. This supports the necessity to detect fecal contamination in drinking water to ensure public safety. It was identified as the only species in the coliform group found exclusively in the intestinal tract of humans and other warm-blooded animals and subsequently excreted in large numbers in feces (Department of National Health and Welfare, 1977). In addition to being fecal specific, *E. coli* do not usually multiply in the environment and have a life span on the same order of magnitude as those of other enteric bacterial pathogens, both of which are qualities of an ideal indicator. As mentioned previously, they are also excreted in the feces in high numbers, making detection possible even when diluted.

A study conducted in Ontario, analyzing quality of rural well water found that the occurrence of *E. coli* in the well was statistically associated with gastrointestinal illness (GI) in an individual (Raina *et al.*, 1999). It is important to note that the no bacteriological analysis can replace knowledge of the water source quality, during treatment, and throughout a distribution system. Contamination is often intermittent and is not always identified in water samples. Bacteriological water analysis is a not always a reliable indication of fecal contamination; however, it provides information that will be protect and educate the user.

Hydrogen Sulfide Producing Bacteria

Some enteric pathogens have origins in the environment other than feces, and can be found in reservoirs in the environment. There are microbial indicators which have fecal and non-

fecal sources within the environment. To mitigate this problem, hydrogen sulfide (H_2S) producing enteric organisms, such as *Salmonella*, *Citrobacter*, and *Proteus*, have been used to identify fecal contamination in water (Pathak & Gopal, 2005).

In 1982, Manja et al. developed the H_2S test under the construct of hydrogen sulfide producing bacteria that are usually associated with fecal impurity (Hirulkar & Tambekar, 2006). It operates on the basis that enteric bacteria are responsible for a reduction of sulfur to hydrogen sulfide producing an odor and black precipitate (Gupta *et al*., 2008).

There are many advantages using hydrogen sulfide producing bacteria as a determination of fecal contamination. As a whole, the H_2S testing has broad applicability, suitable sensitivity, and measures viability or infectivity (McMahan, 2011). This simple one step presence/absence test can recognize heavy to moderate contamination within 24 hours and lower levels of impurities in 48 hours of testing. It is suitable for field, has a long shelf-life, and is easily transported and operated (Pathak &. Gopal, 2005). Hirulkar & Tambekar performed a study to determine the sustainability of H_2S testing in detecting fecal contamination in drinking water. They found that in a field setting, where laboratory materials were not as readily available, hydrogen sulfide producing bacteria was an adequate test of pathogenic organisms in water (2006). As with most developing nations, water sources and testing facilities are sparse – translating to a need for cost efficient and simple testing methods to measure pollution and to identify ways to decrease pollution leading to diarrheal disease.

In such a scenario, a dependable and simple field test is essential in effective monitoring of source and drinking water. Through a concerted effort, Manja et al. developed H_2S test utilizing those bacteria which produce hydrogen sulfide and are always associated with fecal contamination (1982). The simplicity of this test affords the user to minimize cost, have no need

for technical knowledge, and have a conventional bacteriological test for detection of fecal contaminant in water (Manja *et al*., 2001).

Relatively, the H_2S test is new; only being developed and utilized for the last 30 years. However, with numerous means already established to detect adulteration, this test has not been utilized as frequently and needs further scientific examination (Sobsey and Pfaender, 2002). Several studies have evaluated the suitability of the H_2S test as an indicator of fecal contamination. Tambekar et al. performed a study to evaluate hydrogen sulfide producing bacteria to detect contamination in several water sources. Their study concluded that the H_2S test is a simplistic field test that is suitable for detection of fecal contamination in potable water quality and routine monitoring of water in both tropical and subtropical potable waters (2007). They also indicated that this method was an appropriate alternative to conventional MPN method for detection, especially at the village level (Tambekar et al., 2007).

Ratto et al. (1989) assessed the hydrogen sulfide test compared to total and fecal coliform presence/absence test as well as MPN testing. In most instances where the fecal and total coliform tests were compared against the H_2S test, they yielded the same results. There was no instance where the presence/absence test yielded a positive result and the H_2S test was negative. Therefore, Ratto et al. concluded that the hydrogen sulfide producing bacteria test was just a sensitive in detecting contamination as the total and fecal coliform test (1989).

In a study performed by Nair et al. (2001) which examined that appropriateness of the H_2S test to analyze adulterants in untreated and treated water. Using the sensitivity and specificity method to determine the efficacy of the test, they found that it was sufficient to detect contamination.   Nair et al. (2001) determined that the test would an adequate screening method

in other regions and in developing countries, would be a suitable method to detect contamination microbiologically (2001). They also highlighted that areas that did not have proper testing facilities available would be able to utilize this test to monitor their drinking water (Nair et al., 2001).

2.6 Burden of diarrheal disease in Dominican Republic

Access in the Dominican Republic

The Dominican Republic is often viewed as a more developed country and yearly, over 4.2 million people visit the area (ACS, 2007). However, while the tourists contribute greatly to its economy, the gross national income per capita of the Dominican Republic is still only $4,550, ranking it as an upper middle income society and developing country (World Bank, 2009). Of those residing in the Dominican Republic, 70% reside in urban locations and 49% live below the national poverty line (World Bank, 2009). According to the WHO, 87% of the urban population has access to improved water and 87% to improved sanitation (2010). Within the rural population, 84% have access to improved water and 74% to improved sanitation facilities (WHO, 2010). While, those living in these populations have the ability to utilize piped water, these sources deliver erratic flows and are known to be of poor quality (Stauber *et al*., 2009).

The Demographic Health Survey (DHS) determined that 75% of those residing in urbanized areas had access to water from a house connection compared to 53% of those living in rural areas (WHO/UNICEF, 2010). However, unlike most developing countries, the inhabitants of the Dominican Republic chose to use bottled water as their main improved water source; an overwhelming 65% of urban dwellers compared to 35% of rural (WHO/UNICEF, 2010).

Bottled water, while promoted as pure and natural, is less regulated than communal water (WHO, 2010). Similar to tap water, bottled water has been found to have contamination levels in excessive to that of quality standards used by the Environmental Protection Agency (EPA) for public water systems (Huerta-Saenz, Irigoyen, Benavides, Mendoza, 2012). Also, with bottled water being transported from one source to being bottled, there are more instances where contamination may occur (Holt, 2009).

According to the Joint Monitoring Program for water supply and sanitation (JMP), almost 20% of the Dominican Republic inhabitants do not have access to improved sanitation, rural and urban combined (WHO/UNICEF, 2010). Compared to other regions this statistic is relatively low; however, that still does not account for the 9% of people which have no access to facilities and use bushes or fields to defecate (WHO/UNICEF, 2010). Overwhelmingly, it is more common that urban areas use private toilets compared to those in the rural areas, 74% to 31.4%, respectively. However, in the rural areas, improved covered latrines seem to be the more prevalent sanitation facility utilized 54% to 18% in urban places (WHO/UNICEF, 2010).

Latrines are the cheapest, most basic method of improved sanitation and are essential in reducing human feces exposure (Golovaty *et al.*, 2009; Fact Sheet 3.4: Simple pit latrines).

It is estimated that the 64.4% of the total population residing in urban areas will increase improvements in basic sanitation and nutrition, and growing access to health services has reduced morbidity and mortality from communicable diseases, and reproductive events, while increasing prevalence of non-communicable diseases and injuries in the Dominican Republic (Rathe & Moliné, 2011). Poverty stricken communities often suffer from pre-transitional diseases such as communicable diseases, maternal causes, perinatal conditions, and nutritional deficiencies (Rathe & Moliné, 2011). Overall, childhood mortality is decreasing with the

exception of LAC and other regions with comparable economic situations. The DHS determined that 15% of children under the age of 5 died with at least one diarrheal episode (WHO/UNICEF, 2010). These numbers are slightly higher in rural areas (16%) and poorer regions (Rathe & Moliné, 2011).

Environmental burdens weigh heavily on developing countries, and while the Dominican Republic is a more developed region, there are still environmental and societal factors that cause hardship on the area. Estimates were compiled by the Comparative Risk Assessment and the WHO determining that 19% of the total burden is contributed to environmental factors (WHO, 2009). Generally, environmental burdens of disease contribute 40 DALYs per 1,000 capita in the Dominican Republic, resulting in 15,000 deaths (WHO, 2009). Issues with water, sanitation, and hygiene, more specifically diarrheal disease, account for 1,300 deaths per year and 5 DALYs per 1,000 capita per year, having the highest impact compared to any other environmental factors in the area (WHO, 2009). While these statistics are not the highest globally, there is still a significant impact on LAC and undeniably the Dominican Republic.

Due to the fact the Dominican Republic is boarded by the Atlantic Ocean and the Caribbean Sea, climatologic fluctuations can affect water quality. These meteorological changes potentially contribute to increases in waterborne illnesses from their wide-ranging effects. With inconsistencies in infrastructure, the impact on unimproved water and sanitation sources and effects on health are even more troubling (Fricas & Martz, 2007). These events have potential to cause severe public health and financial concerns. Infrastructure that is destroyed or overburdened during natural disasters leads to major health consequences whether from drought or excessive rainfall. During these times, it is likely that households utilize rainwater as their source and chose to store it over a period of time. However, elongated storage time of any water

can result in degradation of its microbial quality and ultimately put households as risk for waterborne illness (Stauber, Ortiz, Loomis, & Sobsey, 2009).

Chapter III: Methodology

3.1 Data Sources

All data used in this investigation was supplied by Dr. Christine Stauber. The analysis performed was secondary data analysis on data collected between 2005 and 2007 from two larger studies performed and described in Stauber et al, 2009 and Aiken et al., 2011. IRB approval (for secondary data analysis) was granted through Georgia State University Institutional Review Board Protocol H10061.

3.2 Study Population

Briefly, households in six communities in the city of Bonao were recruited to participate in the two larger studies described previously. These households resided in the following six communities: Jayaco Central, KM 100, Brisas del Yuna, Jayaco Arriba, KM 101, KM 103, Majaguay, The main purpose of this study was to compare how well the test for H_2S detected drinking water contamination as detected by tests for more traditional indicators such as total coliforms and E. coli. Various types of water samples were also included.

The data set (previously collected) consisted of data from a prospective cohort studies that focused on examining the relationship between water quality and household diarrheal disease (Stauber et al., 2009, Aiken et al., 2011). Participants were interviewed weekly and had water samples collected from their storage containers biweekly. At the time of water sample collection, data were collected on type of water source, description of their storage container, and any drinking water treatment performed at each household per visit.

At five different occasions, water samples were collected from each household and

analyzed for hydrogen sulfide (H_2S) producing bacteria in addition to those tests previously mentioned. Water samples were taken with what was available at the time of each visit, regardless of what type of water household's reported drinking (e.g. treated).

3.3 Study Measures

The three main water quality measures that were examined in this analysis were the detection of total coliforms, *E. coli*, and H_2S-production. Water quality data for *E. coli* and total coliforms were log-transformed to obtain more normal distributions. Data on H_2S production was collected as both presence/absence in 10mL and 90mL portions at time periods for ambient temperature incubation at 24 and 48 hours. In addition, the H_2S-producing bacteria test was also scored for intensity of black precipitate formation. For the purpose of this study, a scoring system was applied to analyze the information gathered. The scoring had designations of zero to three and represented values of opaqueness (due to formation of black precipitate); zero signifying no or negative cloudiness ranging to three with complete opaqueness (Table 2). While this was a subjective scoring system, it best represented and described the levels of contamination.

Score	Description
0	Negative
1	Any drop of opaqueness
2	Slightly opaque
3	Completely opaque

Table 2. H_2S Scoring Designations

For the purpose of the analysis, data were analyzed to assess the following: H_2S testing presence/absence compared to presence/absence of total coliforms and E. coli. These conditions were compared using sensitivity, specificity, positive predictive value, negative predictive value and accuracy (as described below in Table 3). Also, the associations of water quality variables with diarrheal disease for untreated water sources were analyzed. The health outcome assessed in this study was the occurrence of diarrheal disease at the household level for children <5 years of age and for adults. Main exposures assessed for diarrheal disease was the presence of H_2S production in household drinking water samples (stored but untreated).

In addition to analysis of diarrheal disease and ability of the test to predict the presence of other more well-known bacterial indicators, different types of water treatment was considered. Since the original study consisted of households enrolled in a study on household water treatment, the water samples underwent various types of reported water treatment. Water samples were classified as untreated, treated, Biosand filter treated (BSF), BSF treated and stored, and other treated (chlorination, boiling, etc.). Presence/absence variables were created to analyze the H_2S-producing bacteria data at the various time intervals and measure their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The linear relationship between the H_2S testing scores and *E. coli* concentrations were also examined.

3.4 Study Analysis

Type of Statistical Test	Equation	Values used for Association	Comparisons Made
Sensitivity	$\frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$	CI, P-value	p/a for total coliforms and E. coli via Colilert IDEXX Quantitray 2000 sample vol. - 10 & 90mL and 24 & 48 hours (for H_2S) test at ambient temperatures
Specificity	$\frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$		
Positive Predictive Value	$\frac{\text{True positives}}{\text{True positives} + \text{False positives}}$		
Negative Predictive Value	$\frac{\text{True negatives}}{\text{True negatives} + \text{False negatives}}$		
Mean Estimation		CI, P-value	$\log_{10}$ concentration of E. coli and total coliforms variation over H_2S scores (0 – 3)
Linear Regression	Y = B0 + B1X + e	Coefficient, P-value, CI, R^2	score of H_2S in 10mL sample at 24 hours to predict $\log_{10}$ E. coli and total coliform concentration in same sample
Logistic Regression	$\ln\left(\frac{\hat{P}_i}{1-\hat{P}_i}\right) = \alpha + \beta_1 X_i + \varepsilon_i$	OR, P-value, CI	H_2S test scoring and associations with diarrheal disease

Table 3. Summary of Study Analysis

Chapter IV: Results

4.1 Demographics

Within the dataset, there were 816 observations from participants among the 6 communities involved in the study. Participants residing in Brisas, Brisas del Yuna, Jayaco Arriba, KM 100, and KM 101 utilized tap water; however, Majaguay primarily used well water (Table 4). There were six main sources of water analyzed in this study. Overall, tap water was the source most utilized and river was the least utilized in 384 and 25 observations of biweekly houeshold visits, respectively. Only two out of the six communities used river water as a source of drinking water. Well water was the second most common source of drinking water across the communities involved in this study, with the exception of KM 103. In the community of KM 103, most of the respondents used rain water as their main source of drinking water.

Community	Tap (%)	Well (%)	Rain (%)	Spring (%)	Bottled (%)	River (%)	Total (%)
Brisas	54 (60.00)	17 (18.89)	2 (2.22)	12 (13.33)	5 (5.56)	0 (0)	90 (100.00)
Brisas del Yuna	77 (51.68)	44 (29.53)	0 (0)	19 (12.75)	6 (4.03)	3 (2.01)	149 (100.00)
Jayaco Arriba	122 (73.49)	24 (14.46)	2 (1.20)	2 (1.20)	16 (9.64)	0 (0)	166 (100.00)
KM 100	62 (69.66)	23 (25.84)	0 (0)	0 (0)	4 (4.49)	0 (0)	89 (100.00)
KM 101	61 (74.39)	3 (3.66)	0 (0)	0 (0)	18 (21.95)	0 (0)	82 (100.00)
KM 103	6 (6.25)	26 (27.08)	32 (33.33)	0 (0)	10 (10.42)	22 (22.92)	96 (100.00)
Majaguay	2 (3.85)	49 (94.23)	0 (0)	1 (1.92)	0 (0)	0 (0)	52 (100.00)
Total	384 (53.04)	186 (25.63)	36 (4.97)	34 (4.70)	59 (8.15)	25 (3.45)	724 (100.00)

Table 4. Drinking water source stratified by community (N = 816).

4.2 Analysis of Sensitivity and Specificity of H2S Test compared to IDEXX Colilert Quantitray

In order to determine how the H_2S test compared to tests for *E. coli* and total coliforms, an analysis of sensitivity and specificity were both measured and analyzed for all of the conditions of the H_2S Test. To examine sensitivity and specificity for H_2S-producing bacteria, the presence/absence of H_2S production was compared to the presence/absence for E. coli and for total coliforms for the following conditions for two volumes and two time periods: 10 and 90mL and 24 and 48 hours (for H_2S) test at ambient temperatures. These were compared to the presence/absence results for total coliforms and *E. coli* via Colilert IDEXX Quantitray 2000 after 24 hours incubation. The results were stratified by type of water sample tested: untreated water, BSF-treated water, BSF-treated and stored, and other treated and stored.

Untreated Water:

Untreated water samples represented the largest number of water samples submitted for analysis and available for statistical analysis. The analysis of sensitivity, specificity, et for untreated water is presented in Table 5. The sensitivity for total coliforms (under the four conditions (temperature/volume combinations)) ranged from 82.4 – 94.3%. The specificity ranged from 16.3-36.1%. The sensitivity for *E. coli* (under the four conditions) ranged from 88.7-97.8%. The specificity for *E. coli* ranged from 52.5-78.7%. The H_2S test condition that had the highest sensitivity and specificity (94.23% and 36.07%, respectively) for total coliforms was 90mL volume incubated at room temperature for 48 hours. This test condition also produced the highest sensitivity and specificity for *E. coli* (97.82% and 78.67%, respectively). However, the test condition that produced the highest positive predictive value and negative predictive value for both total coliforms and *E. coli* was 10mL at 24 hours.

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
10mL								
E. coli	88.74	52.48	**95.48**	**74.75**	92.76	59.81	93.89	64
Total coliforms	82.44	16.31	**100**	**100**	87.52	21.50	99.83	95.83
90mL								
E. coli	93.81	61.46	92.88	57.84	**97.82**	**78.67**	91.54	47.06
Total coliforms	89.36	25	**100**	**100**	**94.23**	**36.07**	99.69	91.67

Table 5. Sensitivity and specificity for H_2S-producing bacteria compared total coliform and E. coli detection for untreated water samples after 24and 48 hours of incubation.* conditions that produced the highest results in bold

BSF treated water samples were analyzed to determine whether the H_2S test is a good indicator of fecal contamination for BSF-treated water. The sensitivity for total coliforms (under the four conditions) ranged from 41.7 – 79.4% and the specificity ranged from 11.1-24.5%. The sensitivity for *E. coli* (under the four conditions) ranged from 49.0-88.4%. The specificity ranged from 37.8-66.04%. The H_2S test condition that had the highest sensitivity and specificity (79.4% and 24.5%, respectively) for total coliforms was 90mL at 48 hours. This test condition also produced the highest sensitivity and specificity for *E. coli* (88.4% and 66.0%, respectively). However, the test condition that produced the highest positive predictive value and negative predictive value for both total coliforms and *E. coli* was 10mL at 24 hours.

While the test conditions that produced the best results for the various types of analyses

were similar between untreated and BSF-treated water, there was a decrease in all comparison values for both total coliforms and *E. coli* for BSF-treated waters compared to untreated waters.

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
10mL								
E. coli	49.03	37.80	**92.68**	**88.89**	56.77	40.18	90.72	83.33
Total coliforms	41.75	11.02	98.78	**93.33**	48.97	11.61	97.94	86.67
90mL								
E. coli	77.42	53.33	89.55	74.07	**88.39**	**66.04**	87.82	64.81
Total coliforms	68.56	18.67	99.25	**93.33**	**79.38**	**24.53**	**98.72**	86.67

Table 6. Sensitivity and specificity for H_2S-producing bacteria compared predict log_{10}/100mL total coliform and E. coli concentrations in the same sample *BSF treated* water sources after 24and 48 hours of incubation. *conditions that produced the highest results in bold

BSF treated and stored water samples were also analyzed to determine whether the H_2S test is a good indicator of fecal contamination for BSF-treated and stored water. The sensitivity for total coliforms (under the four conditions) ranged from 62 – 92% and the specificity ranged from 50 – 100%. The sensitivity for *E. coli* (under the four conditions) ranged from 69.3 – 96.3%, while specificity ranged from 33.3 – 69.7%. The H_2S test condition that had the highest sensitivity and specificity (92% and 100%, respectively) for total coliforms was 90mL at 48 hours. This test condition also produced the highest sensitivity and specificity for *E. coli* (96% and 61%, respectively) for sample volumes of 90mL at an incubation period of 48hours. However, the test condition that produced the highest positive predictive value and negative

predictive value for both total coliforms and *E. coli* was 10mL at 24 hours and 90mL at 48 hours, respectively.

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
10mL								
E. coli	69.27	**69.70**	**93.01**	28.05	75.52	54.55	90.63	27.70
Total coliforms	62.71	**100**	**100**	2.94	71.75	50	99.23	1.96
90mL								
E. coli	92.71	39.40	89.90	48.15	**96.35**	33.33	89.37	**61.11**
Total coliforms	87.57	**100**	**100**	8.33	**92.09**	**100**	**100**	**12.50**

Table 7. Sensitivity and specificity for H_2S-producing bacteria compared predict log_{10}/100mL total coliform and E. coli concentrations in the same sample *BSF treated and stored* water sources after 24and 48 hours of incubation. *conditions that produced the highest results in bold

Analysis was performed on other treated water samples (i.e. boiled, chlorinated, filtered, etc.) to determine whether fecal contamination could be detected by the H_2S test for this type of water (n=1708). The sensitivity for total coliforms (under the four conditions) ranged from 53.6 – 81.2% and the specificity ranged from 13.5 – 28.9%. The sensitivity for *E. coli* (under the four conditions) ranged from 74.2 – 92.6%. The specificity ranged from 70.2 – 82.7%. The H_2S test condition that had the highest sensitivity and specificity (81.2% and 28.9%, respectively) for total coliforms was 90mL at 48 hours. This test condition also produced the highest sensitivity and specificity for *E. coli* (92.6 and 82.7%, respectively). The test condition that produced the highest positive predictive value and negative predictive value for both total coliforms was 90mL at 24 hours; however, the highest test results yielded for *E. coli* was 10mL at 24 hours.

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
10mL								
E. coli	74.17	70.19	**80.91**	**77.64**	82.50	76.40	79.84	73.12
Total coliforms	53.61	13.46	97.20	82.35	61.14	15.73	97.52	82.35
90mL								
E. coli	84.30	76.83	76.12	66.32	**92.56**	**82.70**	67.89	44.79
Total coliforms	66.33	19.51	**99.24**	**94.18**	**81.22**	**28.85**	98.77	88.24

Table 8. Sensitivity and specificity for H_2S-producing bacteria compared predict log_{10}/100mL total coliform and E. coli concentrations in the same sample *other treated* water sources after 24and 48 hours of incubation. *conditions that produced the highest results in bold

Results for sensitivity, specificity, positive and negative predictive value varied when comparing the H_2S test across the different types of water samples and their treatment. Overall, the test that produced the highest sensitivity for total coliforms was untreated water (94%) for 90mL at 48 hours. BSF treated and stored water samples resulted in the highest specificity and PPV (100%) with sample volumes of 10mL at 24 hours and 90mL for both 24 and 48 hours of incubation for the test. In addition to the BSF treated and stored water, untreated water also yielded the same PPV result under a different condition of 90mL at 24 hours. The test condition which yielded the highest NPV was found in untreated water after 24 hours of incubation in both 10 and 90mL sample volumes.

In general, the H_2S test yielded the highest sensitivity and positive predictive value for *E. coli* concentrations in untreated water (97.82 and 95.48, respectively) for 90mL at 48 hours of incubation. While the H_2S test produced the highest specificity for other treated water, it was still

most specific in predicting *E. coli* concentrations in sample volumes of 90mL at 48 hours. However, the test condition that resulted in the highest negative predictive value was samples from BSF treated water for volumes of 10mL at an incubation period of 24 hours.

Comparisons of H_2S Scores and Total Coliform and E. coli Concentrations

Data from total coliforms and *E. coli* were log transformed to analyze how these values corresponded to the semi-quantitative scoring system for H_2S conditions. Mean concentrations for total coliform and *E. coli* were calculated for each score and for each volume (range of score 0-3). These were also compared across between 24 and 48 hours incubation times. As shown in Table 9, *E. coli* concentrations increased for each increase in H_2S score for both 24 and 48 hours and for both volumes. For 10mL volumes held at 24 hours at ambient temperatures, H_2S samples scored 0 averaged 0.3 $\log_{10}$ *E. coli* MPN/100mL and H_2S samples scored 3 averaged 1.7 $\log_{10}$ *E. coli* MPN/100mL. Similar results were seen at 48 hours and for 90mL.

		24 hours Incubation		48 hours Incubation	
H_2S-producing bacteria sample volume in *untreated* household drinking water	**H_2S Score**	**Mean log_{10} E. coli /100mL**	**CI (95%)**	**Mean**	**CI (95%)**
10mL		*N = 695*		*N = 697*	
	0	0.35	0.24 – 0.45	0.22	0.12 – 0.32
	1	0.88	0.61 – 1.14	0.63	0.28 – 0.98
	2	1.55	1.43 – 1.66	1.53	1.41 – 1.65
	3	1.73	1.61 – 1.83	1.62	1.51 – 1.71
90mL		*N = 701*		*N = 700*	
	0	0.2	0.10 – 0.30	0.06	-0.02 – 0.14
	1	0.55	0.32 – 0.78	0.36	-0.17 – 0.88
	2	1.31	1.17 – 1.45	1.18	0.99 – 1.37
	3	1.65	1.57 – 1.74	1.53	1.45 – 1.61

Table 9. Mean estimations of the log_{10} E. coli /100mL E. coli concentration over the H_2S-producing bacteria scoring for *untreated* water samples collected after 24 and 48 hours of incubation.

Unlike the *E. coli* concentrations, the H_2S scores were not as predictive of fecal contamination compared to the log_{10} transformation for total coliforms. As shown in Table 10, total coliform concentrations increased over the H_2S score but the mean concentration of total coliforms did not vary as significantly as did *E. coli* over the range of H_2S scores as shown in Table 9. For 10mL volumes held at 24 hours at ambient temperatures, H_2S samples scored 0 averaged 2.1 log_{10} total coliforms MPN/100mL and H_2S samples scored 3 averaged 3.3 log_{10} total coliforms MPN/100mL. Similar results were seen at 48 hours. For 90mL, the ranged of total coliform was slightly larger and varied more between 24 and 48 hours.

		24 hour Incubation		48 hours Incubation	
H_2S-producing bacteria sample volume in *untreated* household drinking water	**H_2S Score**	**Mean**	**CI (95%)**	**Mean**	**CI (95%)**
10mL		*N = 695*		*N = 697*	
	0	2.07	1.86 – 2.29	1.78	1.52 – 2.02
	1	2.99	2.77 – 3.20	2.71	2.33 – 3.08
	2	3.30	3.27 – 3.33	3.28	3.24 – 3.32
	3	3.28	3.25 – 3.31	3.25	3.22 – 3.29
90mL		*N = 701*		*N = 700*	
	0	1.69	1.42 – 1.96	1.34	0.99 – 1.68
	1	2.84	2.52 – 3.15	1.85	1.03 – 2.66
	2	3.18	3.11 – 3.25	3.05	2.91 – 3.19
	3	3.27	3.24 – 3.30	3.22	3.19 – 3.26

Table 10. Mean estimations of the $\log_{10}$/100mL total coliform concentration over the H_2S-producing bacteria scoring for *untreated* water samples collected after 24 and 48 hours of incubation.

Linear regression was performed to examine the ability of the H_2S scoring system to predict *E. coli* and total coliform concentrations. As shown in Table 11, all linear regression models demonstrated that the H_2S scoring system was able to predict both total coliform and *E. coli* concentrations. For *E. coli*, the highest R^2 value was found for 24 hours ambient temperature incubation and 10mL. This model predicated a 0.48 $\log_{10}$ E. coli increase for each one unit increase in the H_2S score (10mL). Similar results were found for 48 hours although the R^2 value is slightly lower (0.22). Additionally, linear regression for the 90mL H_2S score suggested a similar increase in *E. coli* concentration per unit change in H_2S score but the models had slightly lower R^2 values (0.25 and 0.18 for 24 and 48 hours, respectively).

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume in *untreated* household drinking water	**Coefficient**	**P – value**	**CI (95%)**	**R^2**	**Coefficient**	**P – value**	**CI (95%)**	**R^2**
10mL	*N = 695*				*N = 697*			
	0.46	0.00	0.40 – 0.52	0.27	0.45	0.00	0.38 – 0.51	0.22
90mL	*N = 701*				*N = 700*			
	0.48	0.00	0.42 – 0.54	0.25	0.48	0.00	0.40 – 0.56	0.18

Table 11. Comparison of H_2S-producing bacteria scores to predict log_{10}/100mL E. coli concentrations in the same sample for *untreated* water after 24and 48 hours of incubation.

Linear regression was also performed for the log-transformed total coliform concentrations in the same sample for untreated water for incubations periods of 24 and 48 hours. The linear regression suggested similar results for H_2S score's ability to predict total coliforms (shown in Table 12). The 90mL scoring system for H_2S- had the greatest increase for log_{10}/100mL total coliform concentration for each one point increase of H_2S-producing bacteria at 0.59. It also had the highest R^2 value (0.41).

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria sample volume in *untreated* household drinking water	**Coefficient**	**P – value**	**CI (95%)**	**R^2**	**Coefficient**	**P – value**	**CI (95%)**	**R^2**
10mL	*N = 695*				*N = 697*			
	0.39	0.00	0.34 – 0.43	0.30	0.46	0.00	0.41 – 0.50	0.35
90mL	*N = 701*				*N = 700*			
	0.48	0.00	0.43 – 0.52	0.38	0.59	0.00	0.54 – 0.64	0.41

Table 12. Comparison of H_2S-producing bacteria scores to predict log_{10}/100mL total coliform concentration in the same sample for *untreated* water after 24and 48 hours of incubation.

Linear regression was also performed for the log-transformed total coliform concentrations across the various water types collected for incubations periods of 24 and 48 hours. As shown in Table 13, the linear regression models revealed that the H_2S scoring system was able to predict total coliform concentrations in the different water sources. For total coliforms, the highest R^2 value was found for 48 hours ambient temperature incubation in untreated water (0.45). This model predicated a 0.30 log_{10} total coliform increase for each one unit increase in the H_2S score (100mL). Although slightly lower, similar results were seen in BSF treated and other treated water for 48 hours, with R^2 values of 0.37. These models projected a 0.29 log_{10} total coliform increase for each one unit increase in the H_2S score (100mL). Additionally, linear regression for the untreated water H_2S score identified a similar R^2 value (0.37); however, the model has suggested a smaller increase in total coliform concentration per unit change in H_2S score (0.24 for 24 hours). While variance was still present among the different water types during 48 hours ambient temperatures for each one unit change (0.21 – 0.30), a greater increase per unit change was seen during the 48 hour incubation period compared to the 24 hour period for each water type analyzed (0.17 – 0.28).

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria in household drinking water	**Coefficient**	**P – value**	**CI (95%)**	**R^2**	**Coefficient**	**P – value**	**CI (95%)**	**R^2**
	N = 695				*N = 696*			
Untreated	0.24	0.00	0.21 – 0.26	0.37	0.30	0.00	0.28 – 0.33	0.45
	N = 209				*N = 209*			
BSF Treated	0.28	0.00	0.22 – 0.33	0.33	0.29	0.00	0.24 – 0.34	0.37
	N = 225				*N =225*			
BSF Treated & Stored	0.17	0.00	0.12 – 0.21	0.20	0.21	0.00	0.15 – 0.26	0.22
	N = 213				*N = 213*			
Other Treated	0.21	0.00	0.16 – 0.27	0.23	0.29	0.00	0.24 – 0.34	0.37

Table 13. Comparison of H_2S-producing bacteria scores to predict log_{10}/100mL total coliform concentrations in the same sample for various water sources after 24and 48 hours of incubation.

Comparison of H_2S-producing bacteria scores to predict log10/100mL *E. coli* concentrations in the same sample for various water sources after 24and 48 hours of incubation was performed using linear regression. The linear regression models demonstrated that the H_2S scoring system was able to predict *E. coli* concentrations in the different water sources; there were larger differences in the R^2 values than found in the prediction of total coliform concentrations. For *E. coli*, the highest R^2 value was found for 24 hours ambient temperature incubation in other treated water (0.29). While this model predicated a 0.22 log_{10} *E. coli* increase for each one unit increase in the H_2S score (100mL), the highest increase per unit change was found in untreated water at the same incubation period. The model identified a 0.46 log_{10} *E. coli* increase for each one unit in the H_2S score with a R^2 value of 0.27. While BSF treated water (24 hours) and other treated water (48 hours) shared the same R^2 values as untreated water (24 hours)

of 0.27, each had a significantly lower increase per unit of *E. coli* (0.20 and 0.22, respectively).

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria in household drinking water	**Coefficient**	**P – value**	**CI (95%)**	**R^2**	**Coefficient**	**P – value**	**CI (95%)**	**R^2**
	N = 695				N = 696			
Untreated	0.46	0.00	0.40 – 0.52	0.27	0.27	0.00	0.24 – 0.31	0.23
	N = 209				N = 209			
BSF Treated	0.20	0.00	0.15 – 0.24	0.27	0.17	0.00	0.12 – 0.22	0.20
	N = 225				N =225			
BSF Treated & Stored	0.16	0.00	0.11 – 0.22	0.13	0.17	0.00	0.11 – 0.24	0.11
	N = 213				N = 213			
Other Treated	0.22	0.00	0.17 – 0.27	0.29	0.22	0.00	0.17 – 0.27	0.27

Table 14. Comparison of H_2S-producing bacteria scores to predict log_{10}/100mL *E. coli* concentrations in the same sample for various water sources after 24and 48 hours of incubation.

4.3 Diarrheal Disease Analysis

As the basis of this study, data collected from households was used to categorize households into households that reported diarrheal disease during the week of interview or households that did not report diarrheal disease at the week of interview. For the purposes of the analysis, diarrheal disease was classified by the WHO standard, which is defined as three or more watery or loose stools per day (WHO, 2012). Over the time period of the analysis, a total of 88 households had at least one member that had diarrheal disease during the week of the visit.

This was approximately 10% of all observations used in the analysis. When classified into age groups, those households that reported diarrheal disease in children constituted 46 observations and those that reported at least one case of diarrheal disease in an adult member of the household consisted of 42 observations as shown in Table 15.

Diarrheal Disease	Household reported at least one participant with diarrheal disease(%)	Household did not report any participant with diarrheal disease (%)	Total Responses (%)
Adult	42 (5.15)	774 (94.85)	816 (100.00)
Children	46 (5.64)	770 (94.36)	816 (100.00)
Total	88 (10.29)	732 (89.71)	816 (100.00)

Table 15. Total diarrheal disease in all communities

In addition, logistic regression was performed to analyze household diarrhea and its association with the presence and absence of H_2S-producing bacteria in untreated water for 10mL and 90mL samples. Water samples for BSF treated, BSF treated and stored, and other treated were limited in comparison to untreated water samples collected from households. During the 24 hour time period, only H_2S-producing bacteria found in untreated water was significant in indicating diarrheal disease with an OR of 1.18 (p=0.03; 1.02 – 1.35) (Table 16). As the bacteria increases by one unit, the odds of occurrence of diarrheal disease increases by a factor of 1.18, suggesting a positive association between the two. There was no significant association for the other water types: BSF treated, BSF treated and stored, and other treated water.

For the 48 hour incubation period, both BSF treat and stored water along with untreated

water were statistically significant for their association with household diarrheal disease. Those who consumed untreated water had a 1.42 increase in occurrence of diarrheal disease ($p<0.001$; 1.14 – 1.78) for an increase in one unit of H_2S-producing bacteria in the sample (Table 16). Participants that use BSF treated and stored water had a 1.60 increased odd of experiencing an episode of diarrhea with every one unit change in H_2S score ($p=0.04$; 1.03 – 2.47). H_2S-producing bacteria found in BSF treated and other treated water samples were statistically non-significant as an indicator for diarrheal disease.

	24 hours Incubation				48 hours Incubation			
H_2S-producing bacteria in household drinking water	**N**	**OR**	**P – value**	**CI (95%)**	**N**	**OR**	**P – value**	**CI (95%)**
Untreated	696	1.18	0.03	1.02 – 1.35	697	1.42	<0.001	1.14 – 1.78
BSF Treated	209	1.11	0.47	0.83 – 1.49	209	1.04	0.81	0.77 – 1.40
BSF Treated & Stored	225	1.34	0.08	0.96 – 1.86	225	1.60	0.04	1.03 – 2.47
Other Treated	214	1.01	0.90	0.84 – 1.22	214	1.18	0.13	0.95 – 1.46

Table 16. Binary logistic regression of any reported household diarrhea and its association with presence/absence of H_sS-producing bacteria in household drinking water.

Chapter V: Discussion and Conclusion

5.1 Discussion

Reducing the burden throughout the world of people without access to safe drinking water is one of the key millennium development goals. In addition, these MDG's also aim to enhance sanitation in developing nations. While there has been considerable advancements made in the improvement of water access, there are still many improvements necessary to achieve this as well as improve sanitation in developing nations (Hunter, Zmirou-Navier, & Hartemann, 2009). However, with improved methods of intervention and testing method for detection fecal contamination, the burden of disease can be reduced (Pruss-Ustun & Corvalan, 2007).

Analysis of Sensitivity and Specificity of H_2S Test compared to E. coli

Validity of a test is often measured by sensitivity and specificity (Lalkhen & McCluskey, 2008). Both are independent of the population being measured in the test and are often associated with clinical testing for diseases. In this case, sensitivity and specificity were used to identify whether or not H_2S results predicted the presence of E. coli and total coliforms. Sensitivity measured the likelihood of the occurrence of H_2S producing bacteria in the presence of *E. coli* and total coliforms; while specificity focused on the absence of H_2S when these indicator organisms were not present. Ideally, results of this test should have both a high sensitivity and specificity. However, in some cases test will have a higher sensitivity, resulting in a lower specificity (Lalkhen & McCluskey, 2008).

When the H_2S test was compared with traditional tests to identify *E. coli* and total

coliforms, the analysis showed that the H_2S test was a very good surrogate (>90% sentivitiy and specificity) for the standard test to identify E. coli contamination.(Hirulkar & Tambekar, 2006). Analysis showed H_2S test had high specificity and sensitivity for E. coli under a wide range of conditions.

Mack and Hewison (1988) suggested for a test to be useful, the sensitivity and specificity should be 80% or better. If the water samples were going to be screened for PPV and NPV accurately, the results should be 100%. When testing the drinking water samples, they received a sensitivity and specificity of 61.5 and 62.9% respectively with the H_2S method (Mack and Hewison, 1988). In the present study, better results were obtained when analyzing sensitivity when detecting *E. coli* under the four conditions. However, the specificity results of this study were not as high under some conditions. Similar results were seen in the comparisons of PPV and NPV. In most circumstances, the results reflected a PPV and NPV of 100%; however in some situations the results were significantly lower.

The incubation period had significant effect on the efficiency of H_2S test. As the time period of incubation period increased from 24 hours to 48 hours, the efficiency of the test increased as well as also shown in Tambekar et al., 2007. However, the predictive nature of the test, both positive and negative, decreased over time and volume generally. Previous studies agreed that the H_2S test was a more sensitive indicator than other fecal coliform tests (Hirulkar & Tambekar, 2006). These results suggest that in the presence of hydrogen sulfide producing bacteria, this test will likely identify the presence of *E. coli* or total coliforms. However, the H_2S testing method is more likely to overestimate the presence of *E. coli* and fecal coliforms than total coliforms. This could be attributed to the greater specificity of the fecal coliform and E. coli indicator grouping (Tambekar et al., 2007).

This study also revealed that the H_2S test was not as predictive for water sources treated in the home but is more suitable for examining untreated drinking water. McMahan et al., 2012 found that overall, the results of their experiments showed that when a water sample tested positive for H_2S-producing bacteria in a quantitative H_2S bacteria culture test for fecal contamination, there are detectable fecal bacteria in the water sample. Roser et al., 2005 also found the H_2S to be effective in distinguishing between water sources with difference levels of fecal contamination. However, it is important to note that when the H_2S test was negative, very few fecal organisms or known pathogens were identified.

Associations with Diarrheal Disease

The H_2S test was only able to detect risk of diarrheal disease for untreated water sources. The increase in both bacteria per unit and the odds ratio suggested that there was a positive association between the two. However, there was no significant association for the other water types: BSF treated, BSF treated and stored, and other treated water. This may have been due to the limited samples available for analysis from each household. With increased incubation periods, significance of H_2S test predicting diarrheal disease increased. Only at the increased incubation period, was the logistic regression was able to predict odds of diarrheal disease from sample of BSF treated and stored water. In most cases, although H_2S water tests for water samples treated in the home were not significant predictors of diarrheal disease. Increased incubations times however, did increase the significance of the results.

5.2 Study Limitations

There were clear limitations to the quality of the data available for analysis in this study. Most importantly, even after thorough surveying of households, there was no way to say with

certainty which of the water samples collected (untreated, treated, BSF treated, BSF treated and stored, and other treated.) was actually being used by the household. Water samples taken from the homes depended solely on what was available during the time of the visit. Regardless of what type of water the household reported routinely using, interviews may have collected other source types from the household. Moreover, there was limited availability of samples for water types other than those classified as untreated, creating a smaller sample size to be analyzed.

In addition, water source may have impacted the test performance. Other H_2S producing bacteria can be found in the environment and may have been detected resulting in false positive results. Although this was truer for our treated samples, this may suggest that if there is an association with water source causing a specific effect on test performance, it is a less important factor of test performance than *E. coli* contamination (Gupta et al, 2007).

The method used within this study may have been considered a limitation. The hydrogen sulfide producing bacteria test is not currently standardized leading to subjectivity of H_2S scoring designations. Also, this method was likely to overestimate the presence of *E. coli* and fecal coliforms than total coliforms. This could be attributed to the greater specificity of fecal coliform and *E. coli* indicator organisms (Hirulkar & Tambekar, 2006).

5.3 Recommendations

The datasets used in this study need to be further investigated to address any additional unanswered questions and further clarify the results of this study. Future studies should focus on the standardization of the H_2S test and its test protocols. With the refinement of these protocols, the test can be more reliable and a more accurate indicator of diarrheal disease (Hirulkar & Tambekar, 2006).

Further testing also needs to be performed to ensure that the H_2S tests meet the requirements as a fecal indicator and can be used more universally. The association of the H_2S test compared to the presence/absence of total coliforms in predicting diarrheal disease need to be further explored.

5.4 Conclusions

Microbiological testing is often preformed to detect contamination of drinking water and their sources. Yet, many nations do not have the infrastructure needed to adequately monitor water quality or it is inadequate (Izadi *et al*. (2010). Most chemical testing is costly and requires a high level of maintenance (e.g. refrigeration, various apparatuses, and certain reagents). There are various tests currently available to evaluate indicator and enteric organisms; however, each technique has its set of limitations. Utilizing the H_2S test is an alternative, low cost method for detection of fecal contamination in household drinking water and other water sources (Stauber et al, 2009). Many studies have been performed to evaluate the efficiency and informative nature of the test in determining the presence of adulteration within the water. These studies analyzed H_2S testing and any modifications necessary due to different tropic and temperate regions, as well as comparing other traditional bacterial indicator testing methods. The results of their studies generally indicated that using the hydrogen sulfide producing bacteria test produced similar results when compared to the more traditional fecal contamination indicator bacteria, and in some instances were more predictive than traditional testing methods for detection (Izadi *et al*. (2010).

The initial results here suggest that the use of the test for H_2S producing bacteria has potential with high sensitivity (>90%) for *E. coli* and total coliforms. The application of the

semi-quantitative scoring system may also have applications in predicting concentration *of E. coli* and total coliforms and well as possibly predicting diarrheal disease. However, more work needs to be completed to standardize the semi-quantitative approach to reduce subjectivity of scoring as well as examine the role of the test in additional epidemiologic studies.

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