Field-Base Exploratory Study of Microbial Activity in Eight Potable Water Storage Tanks in Barbados

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Field-Base Exploratory Study of Microbial Activity in Eight Potable Water Storage Tanks in Barbados

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Dedication

The croton bug.
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Abstract

There have been many initiatives to combat the global phenomenon of water scarcity. Potable water storage tanks have become widely known among water insecure countries and communities to provide adequate water quantity and quality for consumer use. Despite ubiquitous use in many low- and middle-income communities (LMIC), knowledge of microbial activity inside potable water storage tanks is limited. Barbados is a Small Island Developing State (SIDS), labeled as a high income country, and also water scarce. The Barbados Water Authority (BWA) has adapted potable water storage tanks for hundreds of residents as a solution to water quantity and water quality issues. In the summer of 2022, a collection of eight old (>1 year) and new (<1 year) potable water storage tanks across Barbados were sampled throughout the day for chemical compliance of nitrate and total chlorine residual, as well as microbial presence and activity that includes total coliforms, *Escherichia coli* (*E. coli*), and *Legionella pneumophila* (*L. pneumophila*). All tanks sampled had nitrate concentrations below regulated thresholds. Chlorine residuals, however, were often too low in tanks with concentrations below the WHO drinking water guideline of 0.2 mg/L minimum. Many tanks tested positive for total coliforms (5) and *E. coli* (3). There was no statistical significance regarding the time at which samples were collected for total coliforms or *E. coli*, but there was a statistical significance of coliform contamination based on tank age, as total coliform was more prevalent in new tank whereas *E. coli* was more prevalent in old tanks. Conditions of the water inside the tanks were optimal for *L. pneumophila* to grow as temperatures for all sampled water were within the bacteria’s growth range (25–45°C) and exhibited insufficient chlorine residuals. However, despite these optimal conditions, three of the
eight tanks tested positive for *L. pneumophila* suggesting growth unique within each tank. It is recommended that more sampling take place to include old and new tanks to evaluate key drivers for microbial growth within these tanks. Once this is understood, development of operational and maintenance plans to mitigate microbial growth are possible for both the BWA and tank owners.
Chapter 1: Introduction

With the unknown and limited knowledge of water quality inside potable water storage units, especially outside of the United States, this research aims to provide a preliminary understanding of chemical and microbial concerns and spur research questions for future research and management of potable systems. To achieve this goal, this study has the following research objectives:

1. Analyze water quality in a subset of potable water storage tanks in Barbados to determine compliance with disinfectant residual, pH, and nitrate requirements as outlined by WHO guidelines and USEPA regulations.
   
a) Hypothesis: Water in sampled tanks will comply with threshold guidelines and regulations on average, with chlorine decay as water stagnates.

2. Evaluate microbial proliferation inside potable water storage tanks in correlation with tank and sampling characteristics in Barbados using total coliforms and E. coli counts as indicators.
   
b) Hypothesis: Total coliforms will be present in tanks with low total chlorine concentrations, and little to no E. coli will be detected in the sampled water.

3. Evaluate conditions for optimal L. pneumophila growth, including temperature and chlorine residual in correlation to tank and sampling properties, followed by quantification of the concentration of L. pneumophila by the most probable number (MPN) to develop sample recommendation for routine monitoring and a larger study.
c) Hypothesis: Temperature fluctuations will have the greatest impact of *L. pneumophila* presence and growth within the sampled tanks.

The remainder of this thesis is divided into four sections. Chapter 2 presents a literature review which investigates distribution systems, premise plumbing, and the water situation in Barbados. In Chapter 3, the methodology adopted for this study is explained. Chapter 4 discusses the results and, finally, Chapter 5 contains the summary of findings with recommendations for future research.
Chapter 2: Literature Review

2.1 Water Quality of Distribution Systems

When water leaves a conventional drinking water treatment plant, it travels through many miles of pipes, pumps, valves, and fittings before traveling further into the building to reach where the water is used and collected (NRC, 2006). Integrity of the distribution system is heavily influenced by physical, hydraulic, and water quality integrity. The microbial ecology within this system is poorly understood, except that microbial contamination, growth, and survival within distribution systems are heavily influenced by physical and chemical properties. These properties include temperature, pipe material, and concentration of disinfectant added at the water treatment plant that reach the entirety of the distribution system (Berry et al., 2006; Bradley et al., 2020). Additionally, distribution systems that have low water pressure, high water age, and/or long distribution lengths are more susceptible to diminished drinking water quality (Bradley et al., 2020). Water age is the amount of time it takes a water molecule to reach the tap from the effluent point of the water treatment facility and is affected by water pressure and pipe length at any given location and at any given time (Bradley et al., 2020).

2.1.1 US Regulated Contaminants

The United States Environmental Protection Agency (USEPA) has denoted regulated primary and secondary drinking water parameters. Primary drinking water regulations include contaminants that are enforced by federal law and must be at or below the maximum contaminant level (MCL) or follow a specific treatment technique (USEPA, 2022a). Examples of primary drinking water contaminants include microorganisms, disinfectants, disinfection by-products,
inorganic and organic chemicals, and radionuclides. Contaminants within the secondary drinking water regulations are not enforced by federal law as they do not pose harmful health risks, but are encouraged to be managed by water utilities (USEPA, 2022b). These types of contaminants can cause differing cosmetic effects and alter the water’s aesthetic, odor, taste, and color. Examples of secondary drinking water contaminants are total dissolved solids, sulfate, and pH to have a range between 6.5 and 8.5 (USEPA, 2022b).

2.1.2 Indicator Microorganisms

A well-known and robust way to monitor water quality of a given water system is to analyze the presence and concentration of indicator microorganisms in order to estimate potential pathogen exposure of the delivered water which can cause disease (Bradley et al., 2020). Total coliforms, which fall under this category, are a large group of bacteria found in all natural waters and have been found to regrow in distribution systems (Gerba, 2014; Bradley et al., 2020). Even though they do not necessarily cause any health concerns, total coliforms have become the standard for identifying potential risks to water quality as control of total coliforms is often correlated to public health protection (NRC, 2006; Gerba, 2014; USEPA, 2022a). The most probable number (MPN), membrane filter, and presence/absence test are three common methods to identify coliforms in sampled water (Edberg et al., 2000; Gerba, 2014). The World Health Organization (WHO) suggests less than 10 counts of total coliform units for every 100 mL of drinking water sampled (Graham & VanDerslice, 2007). Additionally, a sub-group of total coliforms are fecal coliforms which have concerning health threats (“Coliform bacteria in drinking water samples,” n.d). A sub-group of fecal coliforms is \textit{E. coli} and is found in warm-blooded animal intestines and feces (Edburg et al., 2000; Gerba, 2014; “Coliform bacteria in drinking water samples”, n.d). Since the late 1800s, \textit{E. coli} has been used to assess potential fecal pathogen exposure (Edburg et al.,
2000). Any positive detection of *E. coli* found in a 100 mL drinking water sample is out of compliance with the WHO drinking water guidelines and suggests that the water source has been exposed to fecal contamination (Edburg et al., 2000; Sule et al., 2011). Thus, the water is an immediate health threat and action must be taken to remove or deactivate the bacteria before consumption or use.

### 2.2 Water Quality of Premise Plumbing

Premise plumbing is simply the plumbing within a home or building. The designated premise plumbing begins at the point where pipes are no longer public property, which is typically any pipe beyond the water meter and consists of differing and complex configurations (Wang et al., 2013; Bradley et al., 2020; Cullom et al., 2020). These plumbing systems experience high water age, high surface to volume ratios, often low disinfectant residual, relatively warm water, and irregular usage trends which in return produces prime opportunities for bacterial growth and nitrification to occur (NRC, 2006; Wang et al., 2013; Bradley et al., 2020; Cullom et al., 2020). Major design (distance to taps, building size, hydraulics, pipes exposed to temperature extremes, thermostatic mixing valves, lack of in-building treatment, electric water heaters and copper versus plastic pipe material) and operational factors (low water demand, infrequently used fixtures, high water age, low temperature, high carbon concentrations, decay of disinfectant residual, and inadequate flushing) make water quality hard to manage in premise plumbing (Singh et al., 2020). While there is a lot of research on water quality inside premise plumbing systems and many guidance documents have been published, there are still knowledge gaps and inconsistencies among experts and sources regarding these major factors (Singh et al., 2020).

Opportunistic premise plumbing pathogens (OPPPs) are heterotrophic microbes that grow and multiply in premise plumbing systems because it provides optimal conditions as previously
mentioned (Wang et al., 2013). One reason why these are called opportunistic pathogens is because it affects immuno-compromised individuals (NRC, 2006; Hamilton et al., 2018). Common OPPPs include *Legionella*, non-tuberculous mycobacteria, *Pseudomonas aeruginosa*, and *Acanthamoeba* (Wang et al., 2013; Cullom et al., 2020). Unlike indicator microorganisms, OPPPs can persist in vast aquatic environments and conditions. Also, unlike indicator microorganisms, which are transmitted through ingestion and can cause acute gastrointestinal illness, OPPPs are transmitted through inhalation and cause acute respiratory illness (Wang et al., 2013; Hamilton et al., 2018). Further, Hamilton reports that in 2005, respiratory illness is attributed to a higher occurrence of waterborne disease than gastrointestinal illness in the United States (2018). This heightened public health concern matched with difficult monitoring and controlling measures have caused OPPPs to be an emerging research topic in recent years (Wang et al., 2014; Cullom et al., 2020; Huang et al., 2021).

A major component of premise plumbing uniqueness and cause of pathogen (i.e., OPPP) growth is disinfectant residual in the bulk water. Many studies have concluded that, in general, disinfectant concentration has the strongest effect on bacteria growth within water biofilms and that water chemistry is a major influencer of the water microbiome in distribution and premise plumbing systems (NRC, 2006; Wang et al., 2020; Huang et al., 2021; Hu et al., 2022). This can be translated to OPPP’s activity in drinking water systems specifically, as they are 20 to 600 times more resistant to treatment disinfectant than indicator microorganisms (Cullom et al., 2020). Furthermore, research has shown repetitively that OPPP growth is negatively correlated with disinfectant residual (Wang et al., 2014; Singh et al., 2020; Huan et al., 2021; Hu et al., 2022).

Free chlorine is one type of disinfectant added at drinking water treatment plants with a suggested residual concentration of 0.2 mg/L or more throughout the distribution system at any
point (WHO, 2017). Chlorine is listed as a primary regulated contaminant by the USEPA with a threshold concentration of 4.0 mg/L as Cl$_2$ as it is associated with eye or nose irritation and minor stomach issues at high concentrations (USEPA, 2022a). Many people perceive high concentrations of chlorine at the tap as being associated with bad tasting water. Therefore, most utilities maintain low effluent concentration, but the challenge persists with maintaining the minimum residual concentration in premise plumbing fixtures as extreme changes in temperature, total organic carbon, and pH can alter chlorine decay (Wang et al., 2013; Martin et al., 2020). Failure to manage this chemical parameter all the way to the tap induces OPPP growth, and this has sparked numerous engineering controls and monitoring approaches. Another common disinfectant is monochloramine. This disinfection types plays a key role in facilitating nitrification (NRC, 2006). Monochloramine is less reactive than free chlorine, yet its decay influences other chemical and physical disturbances like corrosion.

Another difficult component that can affect chemical and microbial interactions and induce OPPP growth is the material of the plumbing pieces. Typical plumbing material includes copper, iron, plastic, stainless steel, and cement lining. Each elbow, joint, and/or pipe can be a different material joined together to make up the entire plumbing system inside a building (Wang et al., 2014; Cullom et al., 2020). Metallic materials can catalyze monochloramine and free chlorine degradation as iron and copper oxides form and leach into the bulk water from biofilms (Wang et al., 2013). It has been found that plastic material maintains adequate disinfectant residual, despite chlorine sometimes degrading PEX and polyethylene material (Cullom et al., 2020; Martin et al., 2020; Singh et al., 2020). Two unique characteristics of premise plumbing, stagnation and high water age, can increase the chance of organic matter leaching off the plastic pipes, or metal ions leaching off copper and/or iron pipes.
The combination and interaction of design components (configuration, material, hydraulics, water age) and chemical processes (disinfectant residual, nitrification) make premise plumbing complex and diverse systems that vary among buildings. Thus, these systems are hard to monitor and regulate by the homeowner or utility manager as there is no “single solution” to OPPP prevention by any current engineering control (Wang et al., 2013; Wang et al., 2014; Cullom et al., 2020).

2.2.1 *Legionella* Contamination

*Legionella* are gram-negative, rod-shaped, polar, or lateral, flagellum bacteria which multiply intracellularly (Fields et al., 2002). This bacterium is typically found in protozoan cells, but opportunistically in mammalian cells (Fields et al., 2002). *Legionella* thrive in fresh aquatic environments and often pose a threat in premise plumbing (Fields et al., 2002; Hamilton et al., 2018; USEPA, 2022a). This bacterium is the arbiter of two major respiratory diseases of legionellosis that induce flu-like symptoms: Legionnaire’s disease and Pontiac fever (Fields et al., 2002; Wang et al., 2013; Hamilton et al., 2018; Cullom et al., 2020). Just like any other OPPP, the method of infection is through inhalation and cause fever, cough, headache, and diarrhea. With 48 known species of the *Legionella* bacteria, the *L. pneumophila* species has 15 serogroups (Fields et al., 2002, Boczek et al., 2021). *L. pneumophila* serogroup 1 is the prime source of 79% culture or urine confirmed cases of Legionnaire’s disease and the source of 90% of legionellosis in the United States (Fields et al, 2002; Boczek et al., 2021). When analyzing outbreak occurrence, Hamilton et al. suggested that this high number is due to the detection method (urinary antigen test) used which only identifies *L. pneumophila* serogroup 1 in samples (2018).

It is argued that if *L. pneumophila* is left in the natural environment, it would not pose a human health threat. However, due to human activity, mainly construction, legionellosis outbreaks
are more common since the 1950s (Fields et al., 2002). Specifically, between 2000 and 2014, the number of reported legionellosis cases increased from 0.42 to 1.62 for every 100,000 Americans (Hamilton et al., 2018). It is estimated that 8,000 to 18,000 cases of Legionnaire’s disease occur each year along with millions of dollars of healthcare costs in the United States (Wang et al., 2013; Cullom et al., 2020; Fields et al., 2022). Typical outbreaks of the disease leading to hospitalization or death are sourced from cooling towers, air conditioners, evaporative condensers, potable water and building systems, pools, and spas (Hamilton et al., 2018). Outbreaks are used to identify clusters of *L. pneumophila* contamination in a water system that should be treated and monitored more closely. However, the under-reporting of Legionnaire’s disease to local and state officials is common as the disease may be misdiagnosed as the true cause of respiratory symptoms experienced.

Legionellosis is a major public health concern that can typically be avoided with improved water quality management. Even though *Legionella* bacteria is part of the primary drinking water regulations, it currently does not have a MCL (USEPA, 2022a). Optimal conditions for *L. pneumophila* growth are that of moist aquatic environments with raised temperatures of 25 to 45 °C, extended periods of stagnation or high-water age, and low disinfectant residuals (Fields et al., 2002; Boczek et al., 2021). Total organic carbon can sometimes correlate with *Legionella* growth, but recent research has not been conclusive on this parameter and the role it could play with *Legionella* proliferation (Wang et al., 2013; Cullom et al., 2020; Martin et al., 2020). About 70% of documented Legionnaire’s disease between 2000 and 2014 is affiliated with inadequate disinfectant residual and many studies have since concluded that residual chlorine is one of, if not, the most important water quality parameter of *L. pneumophila* growth specifically (Hamilton et al., 2018; Martin et al., 2022; Hu et al., 2022). Previous studies have concluded, however, that
using chlorine rather than monochloramine as a means of disinfection at treatment plants can increase *Legionella* growth, exposure, and infection (NRC, 2006; Fields et al., 2002; Martin et al., 2020; Huang et al., 2021). This can be understood by free chlorine’s low stability to remain in the water through long distribution systems, especially in premise plumbing, as well as its inability to penetrate biofilms where OPPPs like *L. pneumophila* often reside (Fields et al., 2002; NRC, 2006; Hossain et al., 2020; Boczek et al., 2021; Huang et al., 2021). *Legionella* is particularly difficult to deactivate through disinfectant residual because it can “hide” in amoeba, which are more resilient in nature (Berry et al., 2006; Wang et al., 2013; Singh et al., 2020; Cullom et al., 2020).

Monitoring techniques have adapted since the discovery of *Legionella* in the 1900s and are used for diagnosis approaches and to indicate outbreaks in a water system. Typical testing includes buffered charcoal yeast extract (BCYE) cultures, heterotrophic plate count with R2A agar, and quantitative polymerase chain reaction (qPCR) (Fields et al., 2002, Hamilton et al., 2018; Boczek et al., 2021). Further testing of serogroups upon positive presence results include latex agglutination tests. The BCYE culture method can be costly and time consuming as it takes seven to ten days for incubation before obtaining results. Recently, new techniques have been introduced to the water sector that utilize the MPN analysis. The MPN is a statistical analysis that estimates viable bacteria counts in a sample by ten-fold dilutions of the liquid broth (Hossain et al., 2022). According to Boczek et al., one such method is the Legiolert test (IDEXX®, Westbrook, ME). Legiolert has showed 96.5% specificity and was comparable to BCYE agar method in a modeled home plumbing system (2021). The Legiolert test is relatively cheaper with results obtained faster than the conventional methods mentioned. As an OPPP, *L. pneumophila* monitoring and treatment is of high importance to ensure adequate drinking water quality and to combat a common and under-reported public health concern.
2.2.2 Nitrification Process and Concerns

Another water quality parameter that poses a threat to human health is that of nitrogen, specifically in the form of nitrate. As a result of fertilizer runoff, septic tank leaks, and eroded natural deposits, nitrate is naturally found in many ecosystems, aquatic environments, and from human sources. This chemical is also the product of nitrification which is often a two-step biological process as indicated in the approximate equation below. For this reaction to occur free ammonia first oxidizes to nitrite by ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA), then oxidizes to nitrate by nitrite oxidizing bacteria (NOB) (Bradley et al., 2020; Hossain et al., 2022).

\[
2\text{NH}_3 + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 2\text{H}^+ + 2\text{H}_2\text{O} \rightarrow 2\text{NO}_3^- \quad (1)
\]

If ingested, nitrates pose a significant threat to infants and young children as they can become sick and contract Methemoglobinemia, or blue baby syndrome (Rakshit et al., 2015). Nitrate in the stomach may reduce to nitrite which can then interact with hemoglobin preventing the necessary adsorption of oxygen to the blood cell. This action leads to the depletion of oxygen in the body producing a change in skin color, shortness of breath, and even death. Additionally, excessive ingestion or inhalation of nitrate and nitrite can cause multiple types of cancer, birth defects, heart disease, and respiratory illness (Rakshit et al., 2015; Bradley et al., 2020; Hossain et al., 2022).

Nitrate and nitrite are both primary standard regulated contaminants. Each contaminant has a MCL and a combined MCL; 1 mg/L as N for nitrite, 10 mg/L as N for nitrate and the sum of the nitrogen species must be less than 10 mg/L (Bradley et al. 2020; USEPA, 2022a). Therefore, nitrate is heavily monitored before leaving the water treatment plant to include monitoring microbial and chemical precursors that could indicate a potential problem. Microbiological tests include MPN,
fluorescence in situ hybridization, flow cytometry, polymerase chain reaction, fluorescent antibody, heterotrophic plate count, and cell mass counting (Hossain et al, 2022). Many of these methods are culture-based and take a long time to see any results. Other methods to detect nitrification include using physical and chemical parameters like dissolved oxygen, alkalinity, total organic carbon, and adenosine triphosphate levels, as well as pipe flow. Many of these parameters may not detect nitrification at early stages, and therefore tests and parameter analyses should be jointly used based on the needs of the water conditions and treatment plant flows. Control strategies to mitigate the onset of nitrification both at the treatment plant and within the distribution system include increasing pH, optimizing the chlorine to ammonia ratio, removal of natural organic matter removal, introduction to breakpoint chlorination, and introducing metal nanoparticles. Overall, Hossain et al, argues that nitrification in a distribution system is critical to the quality of the water and can be done by maintaining adequate levels of disinfectant residual (2022).

2.3 Small Island Developing States and Water Security Concerns

All twenty-five island nations that make up the Caribbean are Small Island Developing States (SIDS) (Figure 2.1). With over 40 million residents in the Caribbean and a booming 15% of its economy based on tourism, the twenty-five Caribbean island nations are susceptible to natural and social disruptions and experience many vulnerabilities (Cashman et al., 2008; Global Water Partnership, 2014). These vulnerabilities include climate change, loss of infrastructure caused by sea level rise, changes in hurricane season intensity, food, water and energy insecurity, public health epidemics, and challenges with agriculture (Cashman et al, 2008; Global Water Partnership, 2014; Mycoo, 2018). Despite these overwhelming challenges, the Caribbean islands contribute minimal greenhouse gases emissions compared to the rest of the world.
Committed in 2002 at the Johannesburg World Summit on Sustainable Development, all Caribbean islands have worked towards implementing both an Integrated Water Resources Management (IWRM) plan and water use efficiency (WUE) plan due to increased water stresses (Global Water Partnership, 2014). Specifically, SIDS of the Caribbean are at an increased level of vulnerability because of their limited land mass, high population density, depletion of freshwater resources and demand for economic development (Global Water Partnership, 2014; Mycoo, 2018).

Along with the IWRM and WUE plans set for Caribbean nations in early 2000s, the United Nations Conference on Sustainable Development created seventeen Sustainable Development Goals (SDG) for all the countries of the world to achieve by the year 2030 (Ritchie et al, 2018).
The concern for water security falls into SDG #6, which outlines targets and indicators to achieve global access to clean water and sanitation (Ritchie et al., 2017; Mycoo, 2018). Achieving SDG #6 is crucial to the well-being and livelihood of SIDS residents. Specifically, this goal is weighted heavily on water scarce countries.

2.3.1 Barbados Water Scarcity

Located as the eastern most SIDS of the Caribbean, the independent nation of Barbados has a land coverage of about 430 square kilometers (166 square miles) (FAO, 2015). The nation is divided into eleven parishes (Saint Lucy, St. Peter, St. James, St. Andrew, St. Thomas, St. Joseph, St. Michael, St. George, St. John, St. Philip, Christ Church), or regions, and one capital city, Bridgetown. Data from 2013 shows that Barbados is home to about 285,000 Bajans (FAO, 2015). Barbados has a population density of 663 residents every square kilometer with close to 50% of its residents aggregated to urban areas along the west coast, and in or around the capital city of Bridgetown (Global Water Partnership, 2014; FAO, 2015). With the Caribbean population doubling over the last 50 years, water distribution infrastructure has not had the opportunity to withstand this unexpected growth, much less handle the 0.2% annual population growth in Barbados (Global Water Partnership, 2014). On the Human Development Index, which assesses life expectancy at birth, education, and gross national income per capita, Barbados received a value of 0.71 in 2012 (United Nations Development Programme, 2023). This value lists Barbados progressing better economically and socially compared to its Caribbean island neighbors. Since its independence in 1966 and an influx of travelers annually, its economy has shifted from predominantly agriculture to predominantly tourism as a source of revenue (FAO, 2015).

While Barbados is not labeled as a LMIC, due to its booming tourist economy, the United Nations Commission of Water has labeled it as a water scarce country (FAO, 2015). Water scarcity
relates adequate water quality and quantity with the demand and available resources for livelihoods and ecosystems (Mycoo, 2018). The Barbados Water Authority (BWA) was established in 1981 with a mission to combat water scarcity with goals to treat and dispose wastewater, supply clean potable water, assess, and monitor water resources, and provide legislative protection for water resources (Global Water Partnership, 2014). Since 2012, it has been reported that the BWA provides access to improved drinking water for the entire population, whether residing in rural or urban environments. Although, water security remains a threat as climate change continues, population increases, and tourists travel to the country (FAO, 2015).

Barbados is a relatively flat coralline island experiencing a tropical climate and temperatures of 20 to 32 °C year-around (Global Water Partnership, 2014; FAO, 2015). The wet season, between June and December, is when the island receives approximately 1,422 mm of precipitation and has the highest potential of hurricanes (Global Water Partnership, 2014; FAO, 2015). The dry season, January to May, is when the island receives up to 125 mm (FAO, 2015).

The majority of the water used by consumers is provided by the BWA with only about 120 private wells for agricultural means (FAO, 2015). Nearly 100 percent of the country’s freshwater is used and is sourced through three major water resource types: groundwater, limited surface water, and a reverse osmosis desalination plant that was created in 2000 and is located in Saint Michael parish (Global Water Partnership, 2014; FAO, 2015; Mycoo, 2018). Groundwater was the source of 98.6% of the municipal water in 2000 and, since then, 14% of the water supply is now from the desalination plant instead. In 2005, 81 million cubic meters of water was used, with 67% used for agriculture, 25% for municipality drinking water, and 8% used for industrial purposes (FAO, 2015). The BWA treats its influent water with chlorine and abides by WHO guidelines to monitor its distributed water quality (Environmental Protection Department, 2019;
For this study, the most stringent monitoring values will also be used for analysis and comes from the USEPA’s primary and secondary regulation contaminant lists. While the BWA may currently be able to provide water to the whole country, this might change with projected increases in water use. A depletion of freshwater resources is leading to a shift in water reuse systems, and therefore, access to drinking water is a continual threat that Barbados faces. On top of basic water security, access to good water quality is another challenge Barbados must confront in the coming years.

2.3.2 Potable Water Storage Tank as a Solution

One solution to ensure continual universal access to water year-around is external potable water storage tanks. Many places that experience fluctuating water demands, irregular pressure in distribution systems, and natural disasters have adapted potable and rainwater harvesting storage tanks (AWWA & Economic and Engineering Services Inc, 2002). Water storage tanks are designed with a variety of volumes, installed at different elevations, have different colors, and are covered or uncovered. They provide a household, school, or office building with water when the rest of the water system is faulted or out of commission (AWWA & Economic and Engineering Services Inc, 2002). Just like distribution system and premise plumbing, storage tanks can undergo microbial contamination and physical-chemical problems like nitrification, disinfectant decay, and sediment formation (AWWA & Economic and Engineering Services Inc., 2002). Storage tanks typically have high water age which encourages stratification of the water inside. Maintenance and regular inspections are encouraged, especially within the United States.

The BWA, in collaboration with the Caribbean Community Climate Change Centre (5Cs) under the Green Climate Fun, has an ongoing effort to install potable and rainwater storage tanks for hundreds of residents across the island. The Green Climate Fund, created in 2010 by the United
Nations Framework Convention on Climate Change, aids Water-Energy Nexus projects globally (Isaacs et al., 2017). The 5Cs is the implementing and financing organization of this fund to Caribbean nations with over $10 billion in revenue (Isaacs et al., 2017). For drinking water storage tanks, the water enters from the main line along the street, flows through a sediment filter, stored in the tank, and then delivered upon consumer demand to any of the water outlets in the building by a solar powered pump (Figure 2.2). It is important to note that drinking water undergoes chlorine disinfectant treatment before reaching the tank (Water, 2020).

Figure 2.2 Flow diagram of water from main line through the storage tank.

Among the limited research efforts to understand consumers’ water quality inside storage tanks, studies have found various microorganisms present within the bulk water and biofilm. This growth is indicative of interactions of the water and microbiome occurring inside the tanks and not
the influent water (Graham & VanDerslice, 2007; Sule et al., 2011; Schafer & Mihelcic, 2012; Salehi, 2022; Hu et al., 2022). Graham and VanDerslice found insufficient chlorine residual concentrations and the presence of total coliforms in storage tanks tested in northern Mexico (2007). This left the water inside the tanks to further vulnerability of other pathogen and microbial contamination. Another study found that the microbial and chemical activity inside storage tanks depend on water stagnation, tank material, and ambient temperature (Salehi, 2022). From this conclusion, storage tanks are essentially an extension of building systems. Results from Schafer and Mihelcic show a decrease of indicator microorganisms present in tanks cleaned three or more times annually than those never cleaned, alluding to the effect sanitizing has on the water quality in the tank (2012). Finally, Hu and colleagues concluded that seasonality, which influences temperature fluctuations inside the tanks, was a major driver of microbial shifts (2022). From this, appropriate knowledge of maintenance is required of the owner. However, there needs to be even more research depicting the water chemistry and microbial activity occurring in these tanks in various contexts and locations globally to strengthen and enhance any conclusions made.

2.4 Research Gaps and Study Objectives

As mentioned, there is a plethora of research pertaining to premise plumbing systems, yet little is known of microbial activity inside storage tanks whether in or out of service. Additionally, there is minimal understanding of how water storage tanks act as an extension of premise plumbing and support of OPPP growth. While many studies from within the United States have detailed the presence of harmful bacteria and OPPPs when stagnation times, chlorine disinfectant residual, and temperature are favorable, there are few studies that take this approach for household potable water storage tanks. This study aims to gain exploratory understanding of microbial activity within
storage tanks and investigate sample strategies for future monitoring campaigns. The research objectives include:

1. Analyze water quality in a subset of potable water storage tanks in Barbados to determine compliance with disinfectant residual, pH, and nitrate requirements as outlined by WHO guidelines and USEPA regulations.

   a) Hypothesis: Water in sampled tanks will comply with threshold guidelines and regulations on average with chlorine decay as water stagnates.

2. Evaluate microbial proliferation inside potable water storage tanks of Barbados in correlation with tank and sampling characteristics using total coliforms and \( E. coli \) counts as indicators.

   b) Hypothesis: Total coliforms will be present in tanks with low total chlorine concentrations, and little to no \( E. coli \) will be detected in the sampled water.

3. Evaluate conditions for optimal \( L. pneumophila \) growth, including temperature and chlorine residual, in correlation to tank and sampling properties, followed by quantification of the concentration of \( L. pneumophila \) by MPN to develop sample recommendation for routine monitoring and a larger study.

   c) Hypothesis: Temperature fluctuations will have the greatest impact of \( L. pneumophila \) presence and growth within the sampled tanks.
Chapter 3: Materials and Methods

3.1 Selection of Sites

For this exploratory study, all collaborators decided to focus on specific communities currently receiving and installing storage tanks as those most vulnerable to interruptions in water supply and experience power outages often. The majority of the sample sites were in the northern parishes of Saint Lucy and Saint Peter, located north of Speightstown. One site in Saint Michael parish was tested for comparison of the northern parish tanks. All tanks were installed by the BWA except tank G which was installed by another entrepreneurial group.

In the summer of 2022, a total of eight potable water storage tanks were sampled and are labeled from A to H as indicated in Figure 3.1.

Figure 3.1 Location of potable storage tanks sampled in Barbados. Image adapted from Google Maps. Circled tank names indicate newly installed tanks since the start of 2022, and boxed tank names indicate old tanks installed up to four years prior to sampling time of this study.
All tanks included in the study were closed to the elements and were made from black polypropylene material with PVC pipe leading to and away from the tank system. Beyond these design features, the tanks varied in several key ways including size, use type, age, elevation and number of tanks within the system, detailed in Table 3.1. For tanks B and F, only one tank within the system was sampled with the assumption that the physical-chemical properties found in one tank was representative of all tanks in series at the site. Of the eight tanks, five tanks (B, C, D, F, H) are considered new with installation in the beginning of 2022. Three tanks (A, E, G) are considered old with installation up to four years prior to the study. Tanks A and E were raised about 10 feet into the air and sat on cement slabs. Only the tank G was continuously filled from the main water line throughout the sampling time. Examples of the selected tanks are compiled in Figure 3.2. Details of sun exposure will be further discussed in Section 3.2.

Table 3.1 Summary of tank characteristics.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Parish</th>
<th>Size</th>
<th>Type</th>
<th>Age</th>
<th>Elevated</th>
<th>Number of Tanks in System</th>
<th>Sun Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Saint Peter</td>
<td>450</td>
<td>Public</td>
<td>Old</td>
<td>Yes</td>
<td>1</td>
<td>Full</td>
</tr>
<tr>
<td>B</td>
<td>Saint Peter</td>
<td>1,000</td>
<td>Public</td>
<td>New</td>
<td>No</td>
<td>2</td>
<td>Full</td>
</tr>
<tr>
<td>C</td>
<td>Saint Lucy</td>
<td>450</td>
<td>Residential</td>
<td>New</td>
<td>No</td>
<td>1</td>
<td>Partial</td>
</tr>
<tr>
<td>D</td>
<td>Saint Lucy</td>
<td>450</td>
<td>Residential</td>
<td>New</td>
<td>No</td>
<td>1</td>
<td>Full</td>
</tr>
<tr>
<td>E</td>
<td>Saint Peter</td>
<td>450</td>
<td>Public</td>
<td>Old</td>
<td>Yes</td>
<td>1</td>
<td>Partial</td>
</tr>
<tr>
<td>F</td>
<td>Saint Peter</td>
<td>1,000</td>
<td>Public</td>
<td>New</td>
<td>No</td>
<td>4</td>
<td>Full</td>
</tr>
<tr>
<td>G</td>
<td>Saint Michael</td>
<td>400</td>
<td>Residential</td>
<td>Old</td>
<td>No</td>
<td>1</td>
<td>Partial</td>
</tr>
<tr>
<td>H</td>
<td>Saint Lucy</td>
<td>450</td>
<td>Public</td>
<td>New</td>
<td>No</td>
<td>1</td>
<td>Full</td>
</tr>
</tbody>
</table>
Figure 3.2 Example pictures of sampled tanks. A) Tank E as a single, elevated, old, 450-gallon, public tank with partial sun exposure. B) Tank F as a series of four new 1,000-gallon tanks with full sun exposure. C) Tank D as a single, new 450-gallon residential tank with full sun exposure. D) Tank B as a series of two new 1,000-gallon tanks with full sun exposure. E) Tank C as a single, new 450-gallon residential tank with partial sun exposure.

3.2 Field Sampling Protocol

A preliminary site visit concluded that tank water temperatures were the highest at the beginning of the day, therefore field sampling occurred hourly so that grab samples were collected at 10:30 am for every half hour until 12:30 pm and continued hourly until 3:30 pm for majority of sites. Before every sample time, sampling spouts were sanitized and about 1 L of water was wasted to ensure the water samples was from the storage tank itself and not from the pipes. For tanks A and E, which were elevated, about 2 L of water was wasted. Careful observation of location of tanks in relation to buildings or fauna, and cloud cover were noted as sun exposure percentage (full or partial) detailed in Table 3.1 as it could cause potential differences in analysis.
3.3 Physical-Chemical Analysis

A 50 mL grab sample was used for all physical and chemical procedures. Temperature and pH were collected using ULTRAPEN™ PT1 and PT2, respectively, with temperature being averaged between the two pens’ values. Both pens were calibrated in the morning of all sampling days. Total chlorine and nitrate values were collected for all sampling times and sites in accordance with Hach DR890 Portable Colorimeter.

Total chlorine was analyzed using the DPD method with Hach Powder Pillow of chemical reactants. Total chlorine concentrations were uncertain prior to this sampling effort, therefore the ultra-high range (0.0 mg/L Cl₂ to 10.0 mg/L Cl₂) under program 12 was selected for preliminary data collection of tanks A and B. After the analysis of these tanks showed less than 1.0 mg/L Cl₂ for all samples, the range was reduced to 0.0 mg/L Cl₂ to 2.00 mg/L Cl₂ and changed to program 9. The colorimeter was prepared with a blank of 10 mL in the plastic vial for every sample.

Nitrate sampling used a similar Hach Powder Pillow procedure for a cadmium reduction analysis method. Similar to chlorine, the range of nitrate was unknown in the tank water, so a high range was used (0.0 mg/L NO₃⁻N to 30.0 mg/L NO₃⁻N) under program 51 and was found sufficient for all samples. A glass vial was filled up to the 10 mL line and the colorimeter was prepared with a blank for every sample.

3.4 Microbial Analysis

All microbial handling and analysis were performed at The University of West Indies in the Department of Biological and Chemical Sciences laboratory spaces. Total coliform, E. coli, and L. pneumophila were analyzed using IDEXX® trays and sealed with the Quanti-Tray Sealer Plus (IDEXX®, Westbrook, ME).
A 100 mL grab sample was collected for both the Colilert (total coliform and *E. coli*) and Legiolert (*L. pneumophila*) potable microbial tests. Colilert samples were collected at each grab sample time stated previously. Legiolert samples were collected at or shortly after the highest temperature of the day and again, a few hours later to attempt to reflect peak *L. pneumophila* activity as ambient temperature varied throughout the day. Therefore, two Legiolert samples were collected for every tank besides tank A, B and G. Tanks A and B were only sampled once at noon of that sampling day. Tanks C, D, E and H were sampled for *L. pneumophila* at 11:30 am and 2:30 pm based on preliminary temperature results. Tank F was sampled at 12:00 pm and 2:30 pm, and tank G was sampled at 12:30 pm. This time for tank G was selected because temperature increased steadily throughout the day and the Legiolert sample was taken at the highest expected temperature of the day.

Each sterile vessel contained sodium thiosulfate to neutralize chlorine at a maximum range of 15 mg/L. All microbial samples were stored on ice for transportation up to 8 hours before acclimation to room temperature prior to the addition of the respective reagents. After the Colilert and Legiolert reagent were added, samples were transferred to designated trays, tapped gently to remove air bubbles, sealed by Quanti-Tray Sealer PLUS, and incubated at the appropriate temperatures detailed by IDEXX®. Results were quantified using the MPN statistical method, counting positive large and small wells for each tray type.

3.4.1 Colilert Test Specifics

Colilert reagent was added to each sample and shaken until dissolved. Then, the sample was added to a 51 Well Quanti-Tray/2000 and sealed before being incubated at 35 °C. After 24 hours of incubation, each well was counted as positive for total coliforms if it showed a yellow color darker than the Colilert Quanti-Tray Comparator provided by IDEXX®. For quantification
of *E. coli*, the Quanti-Tray was exposed to UV light and the wells that were both yellow in color and fluoresce were considered positive. Figure 3.3 gives an example of a positive Quanti-Tray for tank A.

![Figure 3.3 Comparator and positive Colilert trays. Comparator tray of color change for positive indication of total coliform provided by IDEXX® (left). Positive tray for tank A with one line on a well suggesting positive for total coliform presence and an “X” on a well suggesting positive for *E. Coli* presence when placed under UV light (right).](image)

3.4.2 Legiolert Test Specifics

Samples were tested for hardness with the hardness dip strip which determines corresponding volume of Legiolert Supplement to add. If two of the four pads turned purple, the sample was considered to have low hardness and 0.33 mL of the Legiolert Supplement was added. If more than two pads turned purple, the sample was considered to have high hardness and 1.0 mL of the Legiolert Supplement was added. Then, Legiolert reagent was added to each sample and shaken until all was dissolved. The sample was added to a Quanti-Tray/Legiolert and sealed before
being incubated at 39 °C. After 7 days, results were read where a brown turbid color indicated a positive well for the presence of *L. pneumophila*. Figure 3.4 gives a negative result for tank A and positive result for tank B.

Figure 3.4 Quanti-tray/Legiolert for tank A and tank B. Tank A (left) water sample did not have any brown-turning wells indicating no *L. pneumophila* in the sample. Tank B (right) had multiple positive wells indicating a positive result.
Chapter 4: Results and Discussion

4.1 RO1: Physical and Chemical Water Quality

Ambient temperatures were not recorded during the study, so values were obtained from records online afterwards. Temperatures during the sampling days and times had a small range of 29.4 °C to 31.7 °C with peak temperatures between noon and 1 pm (Water Spark, n.d.). Between 10:30 am and 4:00 pm, the temperature of the water inside the tanks fluctuated and as expected, the tanks with partial sun exposure contain cooler water than those with full sun exposure. No consistent time of day was associated with peak temperatures as this varied daily by site, ambient temperature, and moving cloud cover. Categorizing all eight tanks based on age (old or new) and sun exposure (full or partial) creates four groups: old and full sun, old and partial sun, new and full sun, new and partial sun, plotted in Figure 4.1. No consistent profile is apparent from these plots; however, all temperatures are well within the optimal growth range for Legionella. Tank water temperatures ranged from at 28.3 °C to 32.8 °C with an average of 29.8 °C across all water samples.

Total chlorine concentrations were below WHO guideline compliance threshold of 0.2 mg/L for all tanks except tank H; however, the total chlorine concentrations were not consistent throughout the day. Tanks A and B had chlorine concentrations that fluctuated between 0.1 mg/L and 0.2 mg/L Cl₂ throughout the day. The remaining five tanks never exceeded the minimum total chlorine concentration throughout the sampling time, with maximums of 0.16 mg/L Cl₂. Figure 4.2A shows the total chlorine found in all eight tanks with the red solid line indicating the minimum
disinfectant residual guideline. Figure 4.2B zooms in on the lower concentrations of the five other tanks and demonstrates that chlorine steadily decreases throughout the day for most of the tanks.

Unexpectedly, pH, plotted in Figure 4.3, varied widely for samples collected throughout the day at any single tank. For example, tank A had the greatest variation with a minimum pH of 5.55 in the morning and 7.91 in the afternoon, whereas tank G was almost consistent with an average pH of 7.76. Majority of the water samples were between 7.0 and 8.0, with an average pH for all samples collected at 7.29. These values tend to be within the range suggested by the USEPA’s secondary drinking water contamination regulation (USEPA, 2022b).

Nitrate concentration across all tanks was below the compliance threshold of 10 mg/L NO₃-N, with an average of 3 mg/L NO₃-N across all tanks, a minimum of 2.3 mg/L NO₃-N in tank G, and maximum of 3.8 mg/L NO₃-N in tank H. There is no apparent trend of nitrate concentrations in each tank as plotted in Figure 4.4. Finally, all averages and one standard deviation for temperature, pH, total chlorine, and nitrate concentrations can be found in Table 4.1.
Figure 4.1 Temperature time series of the sampled tanks. Average temperature from pH and conductivity probes were calculated for all tanks A-H. The red line depicts tanks with full sun exposure and the blue line depicts tanks with partial sun exposure. The dotted line depicts old tank age of more than a year and solid line depicts new tank age of less than a year.

A) B)

Figure 4.2 Total chlorine time series for the eight sampled tanks. Red solid line depicts minimal disinfectant residual that suggested by the WHO guideline.
Figure 4.3 pH time series for the eight sampled tanks.

Figure 4.4 Nitrate time series for the eight sampled tanks. All tanks are in compliance with the USEPA regulation of maximum concentration of 10 mg/L NO$_3$-N.
Table 4.1 Tank averages of physical and chemical water quality characteristics.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Total Chlorine (mg/L Cl₂)</th>
<th>Nitrate (mg/L NO₃-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30.2 ± 0.4</td>
<td>6.96 ± 0.65</td>
<td>0.12 ± 0.04</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>B</td>
<td>29.5 ± 0.7</td>
<td>6.91 ± 0.61</td>
<td>0.12 ± 0.07</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>C</td>
<td>29.8 ± 0.3</td>
<td>7.31 ± 0.08</td>
<td>0.07 ± 0.03</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>D</td>
<td>30.4 ± 0.5</td>
<td>7.41 ± 0.08</td>
<td>0.06 ± 0.04</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>E</td>
<td>28.5 ± 0.3</td>
<td>7.47 ± 0.17</td>
<td>0.04 ± 0.02</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>F</td>
<td>30.5 ± 0.4</td>
<td>7.50 ± 0.13</td>
<td>0.04 ± 0.03</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>G</td>
<td>28.7 ± 0.2</td>
<td>7.76 ± 0.07</td>
<td>0.10 ± 0.03</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>H</td>
<td>31.9 ± 0.9</td>
<td>7.12 ± 0.16</td>
<td>0.29 ± 0.11</td>
<td>3.5 ± 0.2</td>
</tr>
</tbody>
</table>

4.2 RO2: Microbial Activity Related to Indicator Microorganisms

4.2.1 Total Coliform Quantification

There are several factors to consider when analyzing the total coliform data. The objectives of this study are not only to quantify concentration of water in each tank, but also to use the data to develop sampling plans for the BWA. The minimum, maximum, and average MPN counts for each tank are tabulated in Table 4.2. Water in tanks A through E were positive for total coliforms and ranged from the study’s minimum of 3.1 MPN in 450-gallon, old, single, public tank A to the study’s maximum depiction of 2,419.6 MPN in 450-gallon, single, residential tank C. For every sample between 10:30 am and 2:30 pm, tank C exceeded 2,000 MPN indicating proliferation within the tank. Analyzing the data based on tank age (old and new), there is a statistical significance (p < 0.05) of more total coliforms in the newer tanks (Figure 4.5). Since this analysis only tests total coliforms present in planktonic form, there are two primary causes for these values. First, is due to the older tanks being cleaned more frequently as recommended by the BWA. The
other cause could be due to immature, loosely bound biofilms. More sampling of both old and new tanks is required to confirm these results and to determine exact cause. However, it is important to note that the total chlorine residual and total coliform positives of this study confirms the results obtained by Graham and VanDerslice which suggest total coliform presence in tanks with little disinfectant residual (2007).

An analysis of positive results of total coliform in relation to sampling time depicts no statistical difference (Figure 4.6). Even with the maximum concentrations of 2,419.6 MPN detected in tank C were consistent throughout the sampling day, this suggests no correlation of detection of total coliform and time of day that was sampled. Therefore, it is concluded that a sampling campaign does not need to adhere to strict sampling times to provide a representative sample of total coliforms within a given tank.

4.2.2 E. Coli Quantification

Only water samples collected at tanks A, C and E were positive for E. coli. While this is a limited data set, there were 18 total positive samples for these three locations, detailed in Table 4.3. Tank A was positive throughout the sampling day with a minimum of 1 MPN and maximum of 6.3 MPN. Tanks C and E had a minimum of 1 MPN and maximum of 3.1 MPN that were sporadic with intermittent negative samples throughout the day. Tank A and E are characterized as old, and tank C is characterized as new.

Comparing the positive samples in relation to old and new tanks (Figure 4.7) does not result in a statistical significant difference (p < 0.005). However, old tanks are more likely to contain samples with E. coli positives rather than new tanks. Again, given the limited number of samples within this study, a larger study is required to fully understand the correlations between tank age
and microbial activity. What can be concluded is that any monitoring campaign designed by the BWA must include a representative number of old and new tanks.

Similar to the total coliform results, there is no statistical difference in the time of day a sample is collected (Figure 4.8). The majority of the samples had a microbial count of less than 2 MPN in both the morning and afternoon. Therefore, a sampling campaign will not need to adhere to a strict time of day like testing for total coliform.

Table 4.2 Total coliform minimum, maximum, and average values. Nd means no total coliforms were detected.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Minimum (MPN)</th>
<th>Maximum (MPN)</th>
<th>Average (MPN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20.3</td>
<td>32.7</td>
<td>25.4</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>71.7</td>
<td>360.8</td>
<td>162.2</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2419.6</td>
<td>2419.6</td>
<td>2419.6</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>238.2</td>
<td>410.6</td>
<td>330.0</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>6.3</td>
<td>14.8</td>
<td>10.0</td>
</tr>
<tr>
<td>n=8</td>
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<td></td>
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</tr>
<tr>
<td>H</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>n=6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.5 Total coliform quantification in relation to tank age. The edges of the box plot depict total coliform concentrations at 25th and 75th percentile. The line inside the box depicts the 50th percentile. The whiskers depict the 10th and 90th percentile. Any data markers are outliers. Statistical significance: p < 0.05.

Figure 4.6 Total coliform quantification in relation to sampling time. The edges of the box plot depict total coliform concentrations at 25th and 75th percentile. The line inside the box depicts the 50th percentile. The whiskers depict the 10th and 90th percentile. No statistical significance.
Table 4.3 *E. Coli* minimum, maximum, and average values. Nd means *E. coli* was not detected.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Minimum (MPN)</th>
<th>Maximum (MPN)</th>
<th>Average (MPN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>6.3</td>
<td>4.0</td>
</tr>
<tr>
<td>B</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>D</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>3.1</td>
<td>1.8</td>
</tr>
<tr>
<td>F</td>
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<td>Nd</td>
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</tr>
<tr>
<td>G</td>
<td>Nd</td>
<td>Nd</td>
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</tr>
<tr>
<td>H</td>
<td>Nd</td>
<td>Nd</td>
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</tbody>
</table>
Figure 4.7 *E. Coli* quantification in relation to tank age. The edges of the box plot depict total coliform concentrations at 25\(^{th}\) and 75\(^{th}\) percentile. The line inside the box depicts the 50\(^{th}\) percentile. The whiskers depict the 10\(^{th}\) and 90\(^{th}\) percentile. Statistical significance: $p < 0.001$.

Figure 4.8 *E. Coli* quantification in relation to sampling time. The edges of the box plot depict total coliform concentrations at 25\(^{th}\) and 75\(^{th}\) percentile. The line inside the box depicts the 50\(^{th}\) percentile. The whiskers depict the 10\(^{th}\) and 90\(^{th}\) percentile. No statistical significance.
4.3 RO3: Legionella Quantification

Risk is a function of hazard and exposure. *L. pneumophila* bacteria can cause disease when aerosolized. Aqueous aerosols in the home occur when heated, typically from stoves or water heaters. Recently, Barbados has been utilizing solar water heaters as a solution to the high energy demand and as a renewable energy source. Ranked fourth worldwide for the most installed solar water heaters per capita and the only SIDS in the Caribbean to do so until 2013, Barbados has surpassed 50,000 installations (Rogers, 2019; Smart Energy Barbados, n.d.). Additionally, hot water is commonly used in cooking and cleaning, and in hotels (Rogers, 2019). The storage tank effluent feeds into the hot water heater. Therefore, there is a potential exposure and hazardous route if *L. pneumophila* is found in water samples of the storage tanks.

A review of Table 4.1 reveals that all tanks contain water within the temperature range for *L. pneumophila* proliferation and there are very low disinfectant residual present in the stored tank water. Despite optimal conditions, only three of the eight tanks contained water samples with *L. pneumophila* (Table 4.4). Tanks C and D were both single residential tanks assumed to be filled from the same water main and source. Therefore, it can be assumed, while both tanks are seeded from the same source, the growth of *L. pneumophila* is unique in each tank system. It is important to note that tanks B, C and D are all new tanks of less than a year old.

While only three tanks testing positive for *L. pneumophila* might seem low, it is important to consider the MPN concentrations. For all tested tanks, the MPN numbers are high enough to suggest growth within the tanks. This is especially true for tanks C and D with repeated positive results in the morning and afternoon. There is no MPN threshold for *L. pneumophila*, however, repeated testing of tanks is the best method of detecting a growth issue.
Table 4.4 *L. pneumophila* quantification for each tank and sampling time. N/A means no sample taken; Nd means *L. pneumophila* was not detected.

<table>
<thead>
<tr>
<th>Tank</th>
<th>AM Sample (MPN)</th>
<th>PM Sample (MPN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nd</td>
<td>N/A</td>
</tr>
<tr>
<td>B</td>
<td>126.9</td>
<td>N/A</td>
</tr>
<tr>
<td>C</td>
<td>239.6</td>
<td>196.4</td>
</tr>
<tr>
<td>D</td>
<td>1459.8</td>
<td>1717.8</td>
</tr>
<tr>
<td>E</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>F</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>G</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>H</td>
<td>Nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

### 4.4 Cross Examination of Tank Characteristics and Water Quality Quantification

All the chemical and microbial results are compiled in Table 4.5 along with tank characteristics. Proper analysis of water stagnation was not performed in this study as only Tank G was consistently filled throughout the sample time in the morning. No single tank characteristic is correlated with diminished physical-chemical or microbial water quality. The sample size of this study is not large enough to permit a regression analysis with adequate substance. However, despite these limitations, there are key site-specific data necessary to collect for future studies. In Table 4.5, only the clarification of general sun-exposure is captured, but weather and ambient temperature conditions at the time of sampling are important for a more detailed understanding of the impact of individual tank specifics on water quality.
Table 4.5 Summary of chemical and microbial results.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Tank Age</th>
<th>Sun Exposure</th>
<th>Total Chlorine Compliance</th>
<th>Nitrate Compliance</th>
<th>Total Coliform Positive</th>
<th>E. Coli Positive</th>
<th>Legionella Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Old</td>
<td>Full</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>New</td>
<td>Full</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C</td>
<td>New</td>
<td>Partial</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
<td>New</td>
<td>Full</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>E</td>
<td>Old</td>
<td>Partial</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>F</td>
<td>New</td>
<td>Full</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>G</td>
<td>Old</td>
<td>Partial</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>H</td>
<td>New</td>
<td>Full</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Chapter 5: Conclusions and Recommendations

The purpose of this study was to understand basic delivered water quality for a subset of potable water tanks installed throughout Barbados as a water security measure. The first research objective was to analyze water quality parameters including disinfectant residual, pH, and nitrate concentrations for compliance with WHO guidelines and USEPA regulations. This preliminary study demonstrates that nitrate concentrations for the sampled tanks were always in compliance with the USEPA regulations and upheld the hypothesis that water would be in compliance with threshold values. However, only one tank was regularly in compliance with the total chlorine residual minimum regulation from the USEPA throughout the day. Additionally, besides a few sample times from Tank A and G, pH values also varied throughout the day with an average of 7.29 for all sample tanks and times. Temperature of the water inside the tanks ranged, on average, between 28.2 °C to 32.8 °C.

The second research objective was to evaluate microbial proliferation inside the potable water tanks using total coliforms and E. coli as indicators. Total coliforms were detected in several (5) tank water samples as hypothesized, but E. coli was also detected in three tanks, which was unexpected. Samples positive for total coliform were more likely detected in new tanks whereas samples positive for E. coli were more prevalent in old tanks. There was no correlation of positive water samples for total coliform or E. coli based on morning or afternoon sample time.

The final research objective was broken into two parts; evaluate two conditions (temperature and disinfectant residual) for optimal L. pneumophila growth and quantify microbial counts in water samples. It was hypothesized that L. pneumophila would be present in potable
water tanks and that temperature would be a high determining factor. Results show that temperatures of sampled water, along with the relatively low chlorine residuals, which were determined in research objective one, are conducive of microbial presence and growth. *L. pneumophila*, was detected in only new tanks that were installed since the beginning of 2022. The relatively high MPN counts in three of the eight study tanks suggest growth unique to each tank. This conclusion is confirmed by morning and afternoon positive samples, and with a large MPN difference in two tanks despite having the same influent water source.

A major limitation of the study was the small number of tanks sampled. This was a component of this study’s design to focus more on water quality fluctuations throughout the day to adequately plan future sampling campaigns. Additionally, only potential sun exposure of the tanks, relating to shadows of flora and buildings were noted. Daily cloud cover also has the potential to impact water temperatures inside the tanks.

Based on the conclusions of this study, it is recommended that a similar sampling campaign be performed. For a study to determine if correlations of tank age, sun exposure, seasonality, filling rates, water temperature, and/or chlorine concentrations can predict microbial contamination in the potable tanks, approximately 100 tanks are required (power = 0.80, effect size $f^2 = 0.15$, $\alpha = 0.05$). A widespread sampling initiative in more parishes across the island could also address the water quality in the potable tanks with respect to the vulnerability of a community. Additionally, a future study should include tank biofilm samples to better determine the influence of tank age on microbial water quality since only the bulk water inside the tanks were tested for this analysis. With Hu et al., alluding to seasonality as one of the biggest drivers of microbial ecology shifts in water storage tanks, the sampling initiative should also occur in Barbados’ dry season (2022). Both Sule et al. (2007), and Schafer and Mihelcic (2012) concluded that frequent flushing and
appropriate maintenance of water storage tanks notably decreased microbial presence and growth. Thus, current maintenance is an important factor to consider for the next study in Barbados. Since the age of the tank emerged as a potential determination of microbial water quality, residents should be surveyed to understand current tank maintenance practices, as well as the social perception of water quality they receive from their tanks.

It is recommended that the BWA explore potential contamination mitigation strategies. These include chemical measures, such as shock chlorination disinfection, and non-chemical approaches aimed at reducing water stagnation, such as frequent flushing. The BWA should continue to monitor the water quality of these potable water storage tanks around the island and continue to recommend flushing when needed after installation. Flushing is often done in water rich countries as it can be costly and energy intensive for tank owners. This is why solar panels are part of the installation of these tanks to offset the high costs of pumping water continuously (Isaacs et al., 2017). If a tank owner has concern about their water quality, they bring it to the BWA for further investigation.
References


