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Assessment of behavioral contributors to microbial water quality in public swimming pools in Atlanta, GA

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

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Background: There has been a large increase in recreational water illness outbreaks over the last twenty years. Several studies show a need for improved maintenance of public swimming pools and a need for improved swimmer hygiene.

Objective: 1) To compare microbial water quality indicators from pool surface samples to those in pool bulk water samples; 2) To identify pool staff practices that influence pool water quality; and 3) To identify swimmer behaviors that influence pool water quality.

Methods: Data was used from a pilot study conducted between July 2002 and August 2002 at 26 public swimming pools in Atlanta, Georgia. Surveys were administered to pool staff to collect information on operation practices and swimmer behaviors. Pool surface and bulk water samples were collected to analyze for total coliforms, heterotrophic bacteria (HPC) on high-nutrient agar (PCA) and low-nutrient agar (R2A), *Staphylococcus* spp., and *P. aeruginosa*. Correlation analysis was used to assess the association between surface microbial levels and bulk water microbial levels. Multiple linear regression was used to assess the association between pool staff practices and pool microbial levels. Multiple linear regression was used to assess the association between swimmer behaviors and pool microbial levels.

Results: A significant association was found between bulk water *Staphylococcus* spp. and surface *Staphylococcus* and between bulk water *Staphylococcus* spp. and surface HPC-PCA ($p<0.05$). A significant positive association was found between pool vacuuming rate (days between vacuuming events) and bulk water HPC-PCA ($p=0.048$). A significant positive association was found between pool staff often seeing swim diapers in the pool and surface *P. aeruginosa* ($p=0.045$).

Conclusion: The correlation between bacteria from the pool surface and bulk water suggests that pool surface sampling may be a suitable practice for testing pool water quality. The positive association between pool vacuuming rate and pool microbial levels indicates that pool staff should increase pool vacuuming. The positive association between observed swimmer behaviors and pool microbial levels indicates that pool water can be improved by reducing contamination from swimmers. Pool operators should require pre-swim showers to reduce pool contamination and provide foot sprays to minimize transfer of dirt into the pool.

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Chapter I. Literature Review

Swimming is a popular form of recreation in the United States that can provide many health benefits. However, swimming pools can cause illness if not properly maintained. Reports from the Centers for Disease Control and Prevention (CDC) show that there has been a large increase in recreational water illness (RWI) outbreaks in the last twenty years (Centers for Disease Control and Prevention, 2015b). Additionally, a number of studies show that there is a need for improved water quality in public swimming pools, specifically improved pool operation and swimmer hygiene. The assessment in the present study focuses on behavioral contributors to microbial quality in metro-Atlanta, Georgia public pools involving the following bacteria: *Staphylococcus* spp., *Pseudomonas aeruginosa,* heterotrophic plate count (HPC) bacteria, and total coliforms. Therefore, this literature review will provide an overview of studies, regulations, and maintenance procedures related to microbial water quality in public swimming pools.

The review will first discuss research related to pool safety such as recreational water illnesses, water quality in public swimming pools, water testing methods, and regulations/guidelines governing water quality of public pools overall and in Georgia. Second, the review will provide an overview of swimmer behaviors associated with pool water quality, as a variable of interest in the study. Third, the review will provide an overview of pool operator practices associated with pool water quality, as another variable of interest in the study. Last, the review will describe further research needs and demonstrate why it is important to explore the role that swimmer hygiene and pool operator practices play in water quality.

1. Pool Safety

i. Recreational Water Illness

According to the CDC, a large increase in RWI outbreaks has occurred in the last twenty years with some of the top ten causes of treated recreational water outbreaks including *Cryptosporidium*, *Pseudomonas,* disinfection byproducts (DBPs), *E. coli*, *Staphylococcus*, and *Streptococcus* (Centers for Disease Control and Prevention, 2014a, 2015b). An RWI outbreak is defined as occurring when two or more people have similar illnesses that derived from the same location and within similar exposure timeframes (Hlavsa et al., 2015).

The 2011-2012 CDC *Morbidity and Mortality Weekly Report* (*MMWR*) on recreational water illness outbreaks in the United States is the latest surveillance data available to date. During 2011-2012 there were a total of 90 outbreaks linked to recreational water that caused at least 1,788 cases. Of the 90 outbreaks 69 (77%) were from treated recreational water (e.g., pools and hot tubs or spas) that caused at least 1,309 cases. There were 21 (23%) outbreaks from untreated recreational water (e.g. lakes) causing 479 cases. The cases were reported to the CDC's Waterborne Disease and Outbreak Surveillance System (WBDOSS) (Hlavsa et al., 2015).

Cryptosporidium caused the most outbreaks 36 of the 69 (52%) and 874 illnesses. *E. coli* O157:H7 and *P. aeruginosa* each caused 2 (2.9%) of the treated water outbreaks resulting in 21 and 16 cases, respectively. Of the 69 outbreaks 2 occurred at pools in Georgia due to bacteria, resulting in 7 cases (Centers for Disease Control and Prevention, 2015a). Acute respiratory illness (ARI) was associated with 18% (13/69) of the outbreaks and skin illness was associated with 12% (8/69) of the outbreaks. These illnesses were also the top reported in 2009-2010 and 2007-2008 surveillance data (CDC, 2014b; Hlavsa et al., 2011).

RWI trends from 2001-2010

In examining surveillance reports from 2001-2010 notable trends in RWI outbreaks can be seen. First, the total number of waterborne disease outbreaks (WBDOs) has increased over this time period. From 2001-2002 there were 65 outbreaks causing 2,536 cases. Treated water caused 44 (67.7%) of these outbreaks. From 2009-2010 there were 81 reported outbreaks causing 1,326 cases with 57 outbreaks (70%) from treated water causing 1,030 cases (78%). The second trend is that over half of WBDOs are associated with treated water venues for each reporting period (CDC, 2015f).

Another trend seen is that acute gastrointestinal illness (AGI) outbreaks show an increase often occurring in the summer months. Acute gastrointestinal illness (AGI) remains the most commonly reported recreational water illness (Craun, Calderon, & Craun, 2005; Hlavsa et al., 2015). AGI was associated with 64% (44/69) of the treated water outbreaks from 2011-2012 (Hlavsa et al., 2015). Further, *Cryptosporidium* is often a top cause of AGI and is the leading cause of RWI outbreaks with the number of annual outbreaks reported increasing each year. This can be related to its high resistance to chlorine and ineffectiveness of pool filters in removing oocysts (Castor & Beach, 2004; Craun et al., 2005; Hlavsa et al., 2015). *Cryptosporidium* caused 50% of the treated water outbreaks from 2001-2002 and 42% of the treated water outbreaks from 2009-2010 (CDC, 2015f).

Craun, Calderon, and Craun (2005) conducted a review of recreational water outbreak causes from 1971-2000. The review found that *Cryptosporidium* and *Pseudomonas* caused the majority of treated water outbreaks. *Cryptosporidium* caused 32%, *Pseudomonas* 31%, and *E. coli* caused 3% of the treated water outbreaks. Frequently reported factors that led to swimming pool outbreaks were poor maintenance, disinfection, and filtration procedures causing 52% of the outbreaks. Additionally, swimmers were found to be significant sources of contamination for treated venues due to fecal accidents, ill bathers, and diaper-age children (Craun et al., 2005). Overall, these reports highlight the need for improved pool operator training and maintenance procedures. The reports also show a need for continued public education regarding healthy swimming behaviors in order to reduce the risk of future outbreaks.

Pool maintenance

Regarding pool maintenance, it appears that management of pool chemicals and other pool staff procedures are suboptimal. A report published in the CDC's *MMWR* found that 1 in 8 (12.1%) routine public pool inspections conducted in 2008 found critical code violations such as unacceptable chlorine levels that led to immediate closure of the pools. The report includes data for over 121,000 routine pool inspections. Over 10,000 pools (8.9%) had pH level violations while circulation and filtration system violations were seen in over 35,000 pools (35.9%). Improper maintenance of pool logs was reported in over 12,600 (10.9%) inspections and over 1,500 (18.3%) inspections reported that required operator training documentation was not provided and/or posted (Centers for Disease Control and Prevention, 2010). For inspection records that included pool setting, child-care pools had the greatest percent of immediate closures at 17.2% and pH level violations at 11.8%. Kiddie/wading pools had the greatest percent of disinfectant level violations at 13.5%.

Fecal contaminants and other organic material can quickly diminish the chlorine available (free chlorine) to disinfect the water. Since outbreaks of bacterial etiology have been largely associated with inadequate residual disinfectant levels, it is important that pool operators regularly monitor and maintain chlorine levels (Barna & Kadar, 2012; Castor & Beach, 2004; Craun et al., 2005; Friedman et al., 1999). Greater attention is needed for children's pools given their higher percentage of disinfectant violations and the higher risk for children to develop serious illness. Elderly, pregnant, and immunocompromised individuals are also at higher risk of developing serious or life threatening illness from waterborne infections, therefore proper pool maintenance is especially important for these groups (Castor & Beach, 2004).

ii. Water Quality

There are various factors that can affect the quality of water in swimming pools from microbial pathogens to chemical products. Various studies have conducted assessments of public swimming pools in non-outbreak settings and detected fecally-derived pathogens (e.g. *E. coli*, enterococci, and total coliforms) and non-fecally-derived pathogens (e.g. *Pseudomonas*, *S. aureus*, and HPC) (Centers for Disease Control and Prevention, 2013; Davis, Standridge, & Degnan, 2009; Martins et al., 1995; Sawabe, Suda, Ohshima, Hattori, & Sawabe, 2015; Shields, Gleim, & Beach, 2008). The presence of these microbes in swimming pools can be indicators of poor water quality due to suboptimal maintenance or frequent pool contamination by swimmers. In 2012 county and state environmental health specialists along with the CDC collected 161 pool filter backwash samples from public pools in metro-Atlanta. *P. aeruginosa* was detected in 95 (59%) samples, *E. coli* was detected in 93 (58%) samples, and both microbes were detected in 67 (42%) samples. Since contaminants gather in filters, the concentration in filters are likely to be greater than in the pool water. Nevertheless, detection of microbes in filters can indicate source of pool contamination (Centers for Disease Control and Prevention, 2013).

Non-fecal microorganisms

P. aeruginosa is widely found in the environment and its detection in filters and pool water can indicate contamination from the environment (e.g. dirt) or contamination from swimmers (e.g. skin) (World Health Organization, 2006). While *P. aeruginosa* is considered to have moderate resistance to chlorine, it has been observed that biofilms can physically protect bacteria from disinfectants and contribute to their growth (Barna & Kadar, 2012; Centers for Disease Control and Prevention, 2013; Davis et al., 2009; Rice, van den Akker, Pomati, & Roser, 2012; World Health Organization, 2006, 2008).

P. aeruginosa is the second most prevalent pathogen found in swimming pools and spas following *Cryptosporidium* (Barna & Kadar, 2012). It is a common opportunistic pathogen. The infectious dose for healthy individuals is thought to be greater than 1,000 organisms per ml (World Health Organization, 2006). It can cause eye infections, ear infections such as otitis externa (“swimmer’s ear”), and skin infections such as folliculitis (“hot tub rash”) (Barna & Kadar, 2012). Other potential sources of non-fecal microorganisms include vomitous, mucus, and saliva. *S. aureus*, a species of the genus *Staphylococcus*, is another bacterium found on the skin and in the nasal mucosa of the human body of healthy individuals that can be dispersed in pool water. Staphylococci are slightly more resistant to chlorine than *E. coli*, which has low chlorine resistance (World Health Organization, 2008). The infectious dose is at least 100,000 organisms (Schmid-Hempel & Frank, 2007). It can cause rashes, otitis externa, wound infections, and conjunctivitis (Barna & Kadar, 2012).

Fecal microorganisms

E. coli is found in fecal material of humans and other warm-blooded animals. It has low resistance to chlorine and is a common water quality indicator when testing pools (Centers for Disease Control and Prevention, 2013; World Health Organization, 2008)**.** Most *E. coli* are harmless, but some can cause diarrheal illness when swimmers ingest contaminated water. *E. coli* O157 particularly can cause bloody diarrhea and hemolytic uremic syndrome (HUS) along with vomiting and fever. HUS, which is associated with hemolytic anemia and acute renal failure, is more likely to develop in young children and the elderly. The infectious dose is suggested to be low and is estimated to be between 10 and 100 organisms (World Health Organization, 2006). Fecal contamination can occur in pools when residual fecal matter is washed off swimmers' bodies or through Accidental Fecal Releases (AFR) of formed stool or diarrhea (Centers for Disease Control and Prevention, 2013). Birds and rodents can also be a source of fecal contamination in outdoor pools (World Health Organization, 2006).

Public pool assessments

While assessing for bacteria in public pools, studies have found that the microbial makeup of pools vary by pool characteristics (children's pools versus adult pools). Shields et al. (2008) found that the prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* in public pool filter backwash samples varied by pool age characteristics. The study was conducted in Atlanta, Georgia and used a convenience sample of 160 public swimming pools. One liter samples of filter backwash were collected from August to October of 2006. The samples were analyzed by conducting DNA extraction and real-time PCR. The results of the study show that children's pools had the highest prevalence of the parasites at 10.7% (3/28), pools mixed with children and adults had a prevalence of 10.1% (9/89), and adult pools had the smallest prevalence of 2.3% (1/43).

A study conducted by Sawabe et al. (2015) in Japan found that the microbial composition of children's paddling pools differed by the ages of swimmers in the pools. Microbiota changes in the pools were examined by conducting 16S rRNA gene-based metagenome analysis. Indicator bacteria were analyzed using standard protocols and *E. coli* was enumerated using a sheet medium with results reported in colony-forming units (CFU)/ml. Ten water samples were collected from paddling pools at two nursery facilities from July to August 2014. The paddling pools were filled with tap water that had 1.0 ppm of chlorine. The age of the children ranged from zero to six years. The study found that after the children used the pool bacterial counts exceeded the public health limits in Japan of standard plate counts (SPC) <200 CFU/ml and *E. coli* non-detectable.

After metagenome analysis, the major class of bacteria found were *Gammaproteobacteria* and *Betaproteobacteria*. These bacteria which include intestinal, skin, and soil bacteria were used as indicators of contamination from the children and the environment. The study found that pools used by children ages 3-5 years had higher levels of *Betaproteobacteria*, which are found in soil, compared to the pools used by 1-2 year olds. This was consistent with the observed behavior of the children ages 3-5 years who often got in and out of the pool after playing on the ground surrounding the pool.

A study by Davis et al. (2009) found that play features used by infants and toddlers at a water park in Wisconsin averaged a significantly larger number of bacteria compared to play features used by older children and adults. The study tested for bacteria such as *E. coli* and total coliforms on 31 different pad materials, play features, and pools at 10 water parks. Pool water samples were collected from activity pools (water depth >2 feet), plunge pools, and wading pools (water depth <2 feet). Swab samples from submerged and unsubmerged play features were also collected. Standard methods were used and results were reported in CFU/100 ml for water samples and CFU/100 cm^2 for swab samples. The study found that toddler play features had up to 1,000 times more bacteria in some instances. Further, *E. coli* and total coliforms were detected on toddler swings, but not on adult play features.

Although these studies involved different methods such as study location, pool characteristics, and microbes analyzed they indicate that the age of swimmers is one factor that effects pool contamination. It is shown that children largely contribute fecal contamination to pool water, which may be due to incontinence or having greater perianal fecal contamination (Davis et al., 2009; Shields et al., 2008). Maintaining pool water quality is especially important for children due to their higher risk of experiencing severe effects of diarrheal illness (Shields et al., 2008).

The present study focuses on the microbial quality of public swimming pools, however, chemical quality is also an important factor. Proper disinfectant and pH levels are needed to prevent accumulation of microbes in pools. While pool operators may maintain a proper balance of pool chemicals, swimmers introduce contaminants into pool water such as lotions, urine, and sweat that can make the disinfectants ineffective. Additionally, the contaminants can react with chlorine in the water and create disinfection by-products (DBPs) such as trihalomethanes and chloramines. DBPs can cause skin, eye, and respiratory irritation particularly in individuals with asthma (Dang, 2010; Teo, Coleman, & Khan, 2015; Villanueva, Cordier, Font-Ribera, Salas, & Levallois, 2015).

Although these various studies on public pool water quality have some limitations such as small sample size and not measuring the viability of the detected microbes, they still demonstrate the need for improved pool maintenance. Properly maintained pools should prevent bacteria from reaching concentrations that can be hazardous to swimmer health. The detection of the fecal indicators shows that swimmers commonly contaminate public pools with fecal matter. It is estimated that each swimmer sheds an average of 0.14 grams of fecal material in pools with an estimated range for children of 0.01 to 10 grams (Gerba, 2000; Rose, Sun, Gerba, & Sinclair, 1991). This is important because the risk of transmitting infectious pathogens and causing recreational water illness (RWI) increases when fecal material is introduced into pools (Centers for Disease Control and Prevention, 2013). The risk also increases when pools are inadequately chlorinated (Friedman et al., 1999). Microbial contamination and pool water quality can be improved by maintaining proper disinfectant levels, encouraging pre-swim showers, and controlling bather loads (World Health Organization, 2006).

iii. Water Testing Methods

The CDC’s Model Aquatic Health Code (MAHC) states that microbiological testing equipment and methods shall be EPA-approved. They should also conform to the American Public Health Association’s (APHA) *Standard Methods* or be listed under the National Sanitation Foundation Standard 50 (NSF 50) (Centers for Disease Control and Prevention, 2014c). Commonly tested microorganisms include *E. coli*, total coliforms, heterotrophic plate count bacteria, *P. aeruginosa,* and *S. aureus* (Environmental Protection Agency, 2016; World Health Organization, 2006). The two generally used methods for microbiological testing of water are the Most Probable Number (MPN) technique also referred to as Multiple Tube Fermentation (MTF) and the Membrane Filtration (MF) technique (World Health Organization, 1996).

Most Probable Number method

The MPN method involves preparing a series of 10-fold dilutions from a water sample. For example, 1 ml of a sample is added to a bottle with 9 ml of dilution water (World Health Organization, 1996). Each dilution is then used to inoculate separate test tubes containing a culture medium. Next, a presumptive test and a confirmation test is conducted to examine gas production or acid formation in the test tubes to determine presence of coliforms. Each of these tests has a separate selection of media that is suitable for use. The presumptive test uses media such as lactose broth or MacConkey broth to enumerate coliforms (World Health Organization, 1996). The confirmation test uses a more selective medium such as brilliant green lactose bile (BGLB) to confirm total coliforms or *E. coli* medium to confirm fecal coliforms. Incubation for the MPN method can range from 24-48 hours with a temperature range of 35˚C- 44.5˚C depending on the microorganism of interest. The results are obtained by matching the observed positive results to a statistical table. The results are stated as MPN per 100 ml of sample (World Health Organization, 1996).

The MPN method is considered more sensitive compared to the MF method. The advantages of MPN is that it allows flexible sample volume range, it can recover damaged organisms, and the results are simple to interpret (World Health Organization, 2003). The disadvantages of MPN is that it is a slower testing method that requires 48 hours for a positive result, it requires more labor and culture medium, and it produces low precision results through statistical approximation. Additionally, the results of this method are a most probable number index and do not provide an actual count of the indicator bacteria that are in the sample (World Health Organization, 1996).

Membrane Filtration

The MF method involves filtering a certain volume of water through a cellulose acetate membrane that typically has 0.45 µm pores (World Health Organization, 1996). The membrane is then placed on a selective medium. A range of media can be used such as lactose agar or Endo medium. Incubation ranges from 18-24 hours with a temperature range of 35˚C-44.5˚C. If the water sample contains coliforms, then colonies will grow on the medium. The results are obtained by counting the colonies and is stated as number of colony forming units (CFU) per 100 ml (World Health Organization, 1996).

The MF method is considered less sensitive than the MPN method. The advantage of MF is that it provides results in about 18 hours, which is faster than the MPN method. Additionally, MF requires less labor and glassware and the results are high precision retrieved by direct count of coliforms present in a water sample. The disadvantages are that the quality of membranes vary, particles and chemicals transferred onto the membrane from the sample can inhibit microbial growth, and it can be difficult to score colonies (World Health Organization, 1996).

Although microbial testing methods can provide useful information for pool water quality, it is subject to some limitations. First, colony forming units only provide an estimate of the number of cells on a plate and the type of cells that grow depend on the test conditions such as incubation media, temperature, and time. Cell clumping can make it difficult to distinguish and count cells. Additionally, the number of cells that can be counted on a plate is limited to a range of 30-300 and 25-250 (Sutton, 2011).

Another limitation deals with the relatively small sample sizes and small volumes that are collected from swimming pools compared to the overall volume of the pool. Further, pool quality can change rapidly given the amount of contaminants that are frequently introduced. Microbiological testing may require up to 48 hours before results are available. Therefore, microbial samples will only be an indication of past water quality in a pool and may not represent current quality (American National Standards Institute, 2009).

Another important note is that microbial testing of pools does not ensure that the water is safe because although indicator organisms may be absent, other pathogens may be present that are more resistant to disinfectants. While there is no perfect indicator organism, testing does provide information about the effectiveness of pool operation and maintenance practices (World Health Organization, 2006).

iv. Regulations and Guidelines

Presently in the United States, there are no federal regulations or minimum federal standards regarding operation, disinfection, or filtration of public swimming pools. Each state and local government manages and implements their own public pool codes, which has resulted in varied regulation, compliance, and enforcement standards across the country (Castor & Beach, 2004; Hlavsa et al., 2011). Additionally, there is no requirement in the U.S. for routine microbial testing of public pools. The World Health Organization (WHO), however, provides recommendations for routine microbial testing in its guidelines for safe recreational water (see Table 1 below). The document states, “Samples of pool water from public and semi-public pools should be monitored at appropriate intervals for microbial parameters”. Further, both fecal and non-fecal microorganisms should be tested for given that they both present health risks (World Health Organization, 2006).

Table 1.1. WHO recommended routine microbiological sampling frequencies for swimming pools

Pool type	**HPC**	**Thermotolerant coliforms/*E. coli***	***P. aeruginosa***
Disinfected pools, public (e.g. municipal) and heavily used	Weekly	Weekly	When situation demands (e.g. health problems)
Disinfected pools, semi-public (e.g. health club, hotel, school, housing complex)	Monthly	Monthly	When situation demands (e.g. health problems)

Source: WHO, 2006.
Note: HPC=heterotrophic plate count.

Microbial quality guidelines

According to WHO recommendations, HPC should be monitored along with either thermotolerant coliforms or *E. coli*. It is also recommended that *P. aeruginosa* is monitored routinely, particularly when problems exist related to disinfection, filters, water quality, or health. Routine monitoring of *S. aureus* is not recommended, but may be conducted when investigating pools with known health problems (World Health Organization, 2006). Chapter 511-3-5 of Georgia’s rules and regulations regarding microbial quality is to superchlorinate and follow proper maintenance procedures if bacterial counts exceed the maximum allowed level (Chapter 511-3-5 Georgia Department of Public Health).

In regard to sample collection for water quality testing, it is recommended that samples are taken when the pool is heavily loaded. Also, frequency should be increased if operational parameters (e.g. turbidity, pH, residual disinfectant concentration) are not maintained within target ranges. Samples should be taken from multiple sites where the water velocity is low and away from any inlets to ensure that the collected water has already circulated through the pool (World Health Organization, 2006).

The CDC's MAHC states that samples should be taken at least 18 inches below the water surface with a water depth between 3 to 4 feet (Centers for Disease Control and Prevention, 2014c). Sodium thiosulfate (18-20 mg/l) should be used to neutralize chlorine disinfectants (World Health Organization, 2006).

Table 1.2. Microbiological criteria for swimming pools

Bacteria	**Criterion**	**Source**
Fecal coliforms	<1 CFU/50 ml	ANSI, 2009
Total coliforms	<1 CFU/50 ml	ANSI, 2009
E. coli	<1 CFU/100 ml	WHO, 2006
Heterotrophic Plate Count (HPC)	< 200 CFU/1 ml	WHO, 2006
P. aeruginosa	<1 CFU/100 ml	WHO, 2006
S. aureus	<100 CFU/100 ml	WHO, 2006

CFU=colony forming units.

Chemical quality guidelines

The recommended levels for pH are 7.2-7.8. The recommended levels for free available chlorine (FAC) is a minimum of 1 ppm (mg/l) and maximum of 10 ppm (mg/l). For bromine the minimum is 3 ppm (mg/l) (Centers for Disease Control and Prevention, 2014c; World Health Organization, 2006). Georgia's rules and regulations provide the same minimum and maximum pH range in addition to an ideal pH range of 7.4-7.6. The requirement for free chlorine is 1.5-5.0 ppm with an ideal range of 1.5-3.0 ppm. The range for bromine is 2.0-8.0 with an ideal range of 3.0-5.0 (Chapter 511-3-5 Georgia Department of Public Health). The CDC MAHC guidelines state that FAC and pH should be tested prior to the pool opening each day and daily bulk water samples should be taken at least once per day (Centers for Disease Control and Prevention, 2014c). The American National Standards Institute (ANSI) guidelines state testing frequency for sanitizer should be every 2 hours and for pH twice per day (American National Standards Institute, 2009).

Equipment guidelines

Public pools should have recirculation and treatment system equipment such as filters, recessed automatic surface skimmers, disinfection feeders, and chlorine generators that meet the requirements of the NSF 50 (Centers for Disease Control and Prevention, 2014c; Chapter 511-3-5 Georgia Department of Public Health). Filter options include cartridge, sand, and diatomaceous earth/ultrafine filters (American National Standards Institute, 2009; World Health Organization, 2006). Filter backwashing frequency should be based on the manufacturer’s recommendations or when the turbidity value has exceeded the allowable limit (Centers for Disease Control and Prevention, 2014c; World Health Organization, 2006).

Additional guidelines include actions related to accidental release of feces or vomit into pools. If solid stool is released, then it should be collected and discarded. No further action is required if the pool is operating properly. In the case of diarrhea or vomit, the pool should be evacuated of swimmers. The material should be collected and discarded and disinfectant levels kept at the maximum range value. The water should be filtered for six turnover values and the filters backwashed (World Health Organization, 2006). According to the GDPH rules, fecal incidents should be reported to the local health department and the swimming pool closed and properly disinfected (Chapter 511-3-5 Georgia Department of Public Health).

2. Swimmer Hygiene and Pool Water Quality

Swimmer hygiene studies

The previously discussed studies on pool water quality and recreational water illnesses demonstrate the significant role that swimmer hygiene plays in maintaining a healthy swimming pool environment. In addition to these studies, various studies and surveys have been conducted that examine swimmer hygiene behaviors and the implications of these behaviors on pool water quality. Keuten, Schets, Schijven, Verberk, and van Dijk (2012) conducted a study on initial anthropogenic pollutant release in swimming pools. The study utilized a standardized shower cabin in a laboratory and on-site swimming pools to measure anthropogenic pollutant release. Initial anthropogenic pollutant release consisted of chemicals and microorganisms released by bathers in the first 60 seconds of showering, the timeframe in which the most pollutants were released. The microorganisms tested included *E. coli*, enterococci, and staphylococci.

The study found that staphylococci were the highest microorganisms detected in the shower water with a range of $1.0 \times 10^4 – 2.2 \times 10^9$ per 100 ml. *E. coli* had a range of $2.5 \times 10^1 – 1.9 \times 10^6$ per 100 ml of shower water and intestinal enterococci had a range of $1.7 \times 10^1 – 7.0 \times 10^5$ per 100 ml of shower water. The detection of staphylococci in greater numbers indicated that pre-swim showering was more effective at dislodging skin pathogens than fecal pathogens. As explained in the study, this could have been due to the short showering time of 2 minutes and swimmers wearing full swimwear. This studied demonstrated that pre-swim showering reduces the amount of pollutants that swimmers introduce into pool water, thereby leading to a decrease in DBP formation and chlorine demand. Another study done by this group regarding anthropogenic pollutants released in swimming pools found that unhygienic behaviors such as not showering before swimming and urinating in the pool causes the largest amount, 63%, of total pollutants released (Keuten et al., 2014).

In examining swimmer hygienic behaviors Pasquarella C., Veronesi, Napoli, Castaldi, Pasquarella M.L., Saccani, Colucci, Auxilia, Gallè, Di Onofrio, Tafuri, Signorelli, & Liguori, (2014) found a significant association between swimmers not reading pool rules and unhygienic behaviors. The association between not reading the pool rules and behaviors such as not showering had an odds ratio of 1.44, 95% CI 1.25-1.65 and urinating in the pool had an odds ratio of 1.70, 95% CI 1.41-2.03. Questionnaires (4,315 total analyzed) administered to swimmers ages 6 to 86 years on swimmer hygiene habits revealed that 70.9% of swimmers answered that they always shower before entering a pool, 64.7% always use the footbath, 13.5% have urinated in a pool at least once, and 36.8% have blown their nose in a pool. In regard to reading the pool rules, 58.3% of the respondents stated they read the rules. Further, when asked about the reason for pre-swim showers, 47.2% of respondents answered “To wash oneself” while 48% of respondents believed it is done to get used to the water temperature.

The study also found differences between age groups related to behaviors and swimmer hygiene knowledge. Having an age of 18 years or less was a risk factor for not reading the pool rules (OR: 2.32 95% CI 2.05-2.63). Additionally, participants in the 14 to 17 year age group had the lowest use of pre-swim showering and footbaths and were most likely to urinate and blow their nose in the pool. Overall, the study which was conducted in Italy found low compliance with basic swimming hygiene rules and knowledge about swimming health risks (Pasquarella et al., 2014).

Swimmer hygiene surveys

A 2012 swimmer hygiene survey conducted by the Water Quality & Health Council (WQHC) in the United States found a great percentage of Americans practice unhygienic behaviors in public pools (Water Quality and Health Council, 2012). The results of the survey suggests that Americans display less compliance with basic swimming hygiene compared to what was observed in the Pasquarella et al., (2014) study. The survey found that from a sample of 1,000 American adults, 18 years and older, only 32% always shower before entering a public pool while 22% never shower before entering a public pool. Forty-four percent believe showering before entering a pool is unnecessary. Additionally, 19% said they have urinated in a public pool, 11% have swum with a runny nose, and 15% have brought drinks into a public pool.

The survey also revealed a lack of knowledge regarding the effects of chlorine. Eighty-seven percent of respondents believed that eye irritation is directly caused by chlorine and 38% believed strong chemical smells are directly caused by chlorine (Water Quality and Health Council, 2012). However, a properly maintained pool should have little odor. A strong odor is caused by DBPs such as chloramines and indicates a maintenance problem (Centers for Disease Control and Prevention, 2015e; Water Quality and Health Council, 2016). The highlights from the survey indicate that most Americans do not always shower before swimming in a public pool and many believe it is unnecessary. However, some Americans state that they would more likely shower if cleaner, more accessible, and private shower facilities were available (Water Quality and Health Council, 2012).

As reported in the CDC *MMWR*, after a 2007 cryptosporidiosis outbreak linked to recreational water venues occurred in Utah, public health agencies in the state prepared a multimedia healthy swimming campaign (Centers for Disease Control and Prevention, 2012). In 2009, a national HealthStyles survey was conducted. In the survey, 100% of Utah residents and 78.4% of residents of other states correctly answered that "not swimming while ill with diarrhea protects others from RWIs". Additionally, 96.4% of Utah residents and 85.7% of residents of others states correctly answered that "not swallowing water you swim in" is a healthy swimming behavior. Further, 85.8% of Utah residents and 65.9% of residents of other states correctly answered that "chlorine does not kill germs instantly". The CDC report states that no recreational water outbreaks were detected in Utah from 2008 to 2011 and this might have resulted from the healthy swimming campaign in Utah.

The report contains some limitations such as a cross-sectional design that does not indicate cause and effect between the education campaign and respondent knowledge. Additionally, no baseline measurements were conducted regarding healthy swimming knowledge of Utah residents prior to the outbreak or education campaign. However, the lower percentages of correct survey answers from residents in other states compared to Utah residents demonstrates that greater efforts are needed to promote healthy swimming behaviors, particularly with *Cryptosporidium* being the leading cause of treated recreational water outbreaks (Centers for Disease Control and Prevention, 2012).

Recommendations for improving swimmer hygiene

It has been seen that swimmers that have attended a swimming course demonstrate healthier behaviors such as pre-swim showers and not urinating in the pool (Pasquarella et al., 2014). Interventions should emphasize healthy swimming habits particularly for adolescents who have been seen to show less compliance (Pasquarella et al., 2014). The CDC emphasizes healthy swimming habits such as showering before swimming, not swimming while ill with diarrhea, not urinating in the water, taking children on bathroom breaks every hour and checking diapers in changing areas away from the pool, and not swallowing the water (Centers for Disease Control and Prevention, 2015d). While showering reduces the amount of skin pathogens (e.g. staphylococci and *S. aureus*) introduced in pools, nude showering is suggested to be more effective for removing fecal pathogens (Keuten, Schets, Schijven, Verberk, & van Dijk, 2012; Robinton & Mood, 1966). Additionally, WHO suggests using foot sprays to control transfer of dirt into pools rather than footbaths since they have caused concerns regarding foot infections (World Health Organization, 2006).

3. Pool Staff Practices and Pool Water Quality

Currently, there is no nationwide requirement for pool operator training and certification. However, Georgia’s rules and regulations do require public pool operators to be trained (Chapter 511-3-5 Georgia Department of Public Health). Overall, there are about 20 states that require verifiable training (Lachocki, 2006). Various studies and reports have demonstrated the importance of requiring pool operator training in order to prevent the increasing number of RWI outbreaks that are often a result of inadequate pool maintenance (Bilajac, Vukic Lusic, Doko Jelinic, & Rukavina, 2012; Buss et al., 2009; LaKind, Richardson, & Blount, 2010).

Buss, et al. (2009) looked at the association between swimming pool operator certification and reduced pool chemistry violations in Nebraska from 2005 to 2006. The study found that public pools that were not required to have trained certified operators were twice as likely to have free chlorine and pH violations as public pools that were required to have certified pool operators. Of non-municipal pools 30.9% (167 of 541) had free chlorine violations and of municipal pools 13% (60 of 460) had free chlorine violations. When examining just Sarpy County, 27% (34 of 126) of non-municipal pools had pH violations compared to 6.5% (2 of 31) of municipal pools. Simultaneous pH and free chlorine violations were seen in 12.7% (16 of 126) of non-municipal pools and 3.2% (1 of 31) municipal pools in Sarpy County. The results from this study indicate that requiring pool operator training and certification for public pools can help prevent RWIs (Buss et al., 2009).

A dissertation by Bush (2002) involved a study that measured knowledge and behavior change of pool staff at treated aquatic facilities in Tennessee before and after an intervention training session aimed at preventing waterborne disease. The training session involved topics such as RWI prevention, facility maintenance, pool chemicals and water quality, pool policies, and diaper policies. The aquatic staff demonstrated a 35% increase in knowledge when comparing their pre-test and post-test. Overall, the results showed that the intervention was a successful and effective way to train aquatic staff in waterborne disease prevention (Bush, 2002).

4. Research Needs

As discussed in this review swimmers play an important role in public pool water quality. Swimmers frequently introduce considerable amounts of contaminants into pool water. Additionally, surveys show a large percentage of Americans practice unhygienic swimming behaviors in public pools and have a lack of knowledge related to healthy swimming. In regard to pool operators, studies show there is a lack of adequate training and knowledge related to proper pool maintenance and RWI prevention. With the incidence of RWIs increasing, there is a need for increased efforts to promote healthy public swimming pool environments and behaviors.

Furthermore, there are three areas where more research is needed. First, the methods used in the previously described pool water quality studies involved testing bulk water samples or filter backwash samples. However, the studies did not assess the microbial quality of bulk water compared to surface water. It can be beneficial to assess the microbial quality of surface water to determine if it is a good indicator of overall pool water quality. Second, there is no federal policy or state policy in Georgia that requires routine microbiological testing for public swimming pools. However, the WHO provides guidelines for routine microbial testing. More research in this area is needed to determine if routine microbiological testing would be a feasible and beneficial practice to minimize the risk of recreational water illnesses.

Third, while the papers discussed in this review examined swimmer hygiene behavior and pool operator behavior, these studies did not quantitatively assessed the association between these behaviors and pool microbial quality. This gap in research prevents a comprehensive assessment of behavioral contributors to swimming pool water quality. More research on this association can provide evidence to inform better decision-making and policies for pool safety practices. The present study will aim to elucidate these gaps in knowledge by assessing the correlation between microbes in pool water surface samples and pool water bulk samples. Additionally, the present study will examine the association between pool staff behaviors and pool microbial levels and swimmer behaviors and pool microbial levels, using survey data and water samples collected from public swimming pools in Atlanta, Georgia

Chapter II. Manuscript

1. Introduction

Context of Project

Swimming is a popular recreational activity in the United States. Based on the most recent data from the United States Census Bureau, in 2009 swimming was the fourth most participated in recreational activity (U.S. Census Bureau, 2015). According to the Centers for Disease Control and Prevention (CDC) "in the United States during 2009, there were approximately 301 million swimming visits each year by persons over the age of six" (Centers for Disease Control and Prevention, 2015c). Although swimming is a popular activity that can provide many benefits to physical and mental health, swimming pools can cause illness if not properly maintained. Given the large number of visits to community pools and nature of the pool environment, it is important for pool operators and staff to practice good maintenance procedures and for swimmers to practice healthy swimming behaviors in order to maintain pool quality and prevent illness.

According to the CDC, in the last twenty years there has been a large increase in Recreational Water Illness (RWI) outbreaks (Centers for Disease Control and Prevention, 2015b). RWIs are caused by microbes and chemicals present in the water and include diarrheal illness, the most commonly reported as well as skin, ear, eye, and respiratory infections (Centers for Disease Control and Prevention, 2015b). Some of the top ten causes of treated recreational water outbreaks are *Pseudomonas, Escherichia coli* (*E. coli*), *Staphylococcus, Streptococcus, Cryptosporidium, Giardia,* and disinfection byproducts (DBPs) such as trihalomethanes and chloramines (Centers for Disease Control and Prevention, 2014a).

The CDC's *Morbidity and Mortality Weekly Report* (*MMWR*) on outbreaks of illness associated with recreational water in the United States for 2011-2012 is the latest finalized surveillance data available to date. The report states there were 69 RWI outbreaks from treated recreational water (e.g., pools and hot tubs or spas) that caused at least 1,309 cases reported to the CDC's Waterborne Disease and Outbreak Surveillance System (WBDOSS) for 2011-2012. *Cryptosporidium* caused 36 (52%) of the 69 outbreaks and it remains the leading cause of RWI outbreaks with the number of annual outbreaks reported increasing each year. *E. coli* O157:H7 and *Pseudomonas aeruginosa* (*P. aeruginosa*) each caused 2 (2.9%) of the treated water outbreaks (Hlavsa et al., 2015). Of the 69 outbreaks, 2 occurred in Georgia resulting in 7 cases (Centers for Disease Control and Prevention, 2015a).

In a 2012 study conducted by the CDC in Atlanta, Georgia 161 samples of pool filter backwash was collected from metro-Atlanta public pools. *P. aeruginosa* was detected in 95 (59%) of the samples, *E. coli* was detected in 93 (58%) samples, *Giardia* was detected in 2 (1.2%) samples, and *Cryptosporidium* was detected in 1 (0.6%) sample (Centers for Disease Control and Prevention, 2013). Another study conducted by Shields, Gleim, & Beach (2006) in Atlanta, Georgia showed the prevalence of *Cryptosporidium* and *Giardia* from filter backwash samples collected from 160 public swimming pools. *Cryptosporidium* was detected in 2 (1.2%) samples and *Giardia* was detected in 10 (6.2%) samples (Shields et al., 2008). These two studies present some limitations such as small sample size. Nonetheless, they highlight the need for improved water quality in community swimming pools.

A key factor that remains vital to reducing the risk of outbreaks from swimming pools is maintaining proper balance of pool disinfectants along with proper pH levels to aid in the effectiveness of the disinfectants and limit DBP formation (Hansen, Willach, Mosbaek, & Andersen, 2012).

Regarding pool maintenance, it appears that management of pool chemicals and other pool staff procedures are suboptimal. A study published in the CDC's *MMWR* found that 1 in 8 (12.1%) routine public pool inspections conducted in 2008 found critical code violations such as unacceptable chlorine levels that led to immediate closure of the pools. The highest percentage of violations (35.9%) was for the circulation and filtration system. Improper maintenance of the pool log was reported in 10.9% of the inspections and 18.3% of the inspections reported that required operator training documentation was not provided and/or posted (Centers for Disease Control and Prevention, 2010). Even if swimming pools are properly maintained by staff, swimmers can introduce many contaminants to the water through sunscreen, hair products, sweat, urine, and fecal matter creating demand on pool disinfectants and reducing their effectiveness (Teo et al., 2015). Therefore, swimmer behavior also plays a role in pool water quality.

The 2009 HealthStyles national survey reported that more than 1 in 5 (21.6%) adults in the United States excluding Utah, Alaska, and Hawaii, do not know that swimming while ill with diarrhea can contaminate the water and make other swimmers sick (Centers for Disease Control and Prevention, 2012). A 2012 survey given by the Water Quality & Health Council found that 68% of Americans don't always shower before getting into a swimming pool and 44% think showering before entering a pool is unnecessary (Wiant, 2012).

Problem Statement

There a various reports on RWI outbreaks, swimming pool inspection data, and swimmer hygiene attitudes and practices in the United States that establish the need for improved pool quality. Limited research however, has quantitatively assessed the association between swimmer hygiene and swimming pool water quality and pool staff behavior and swimming pool water quality. Buss, Safranek, Magri, Török, Beach, & Foley (2009) looked at the association between swimming pool operator certification and reduced pool chemistry violations in Nebraska from 2005 to 2006. The study found that compared to locations that required certified operators, locations without certification were twice as likely to have violations for free chlorine and simultaneous pH and free chlorine violations. The results from this study indicate that requiring pool operator certification can help prevent RWIs (Buss et al., 2009). This study however, does not provide a quantitative assessment of pool operator behaviors associated with water quality.

The overall lack of research prevents a conclusive assessment of behavioral contributors to swimming pool water quality. More research on this association can provide evidence to inform better decision-making and policy for pool safety practices. Furthermore, there is no federal policy in the U.S. that requires routine microbiological testing for public swimming pools. More research is needed to determine if routine microbiological testing would be a feasible and beneficial practice to minimize the risk of RWI caused by pathogens. The research done on this project will aim to reduce the knowledge gap.

Purpose of Project

Therefore, there are three overall aims of this current project: 1) to compare microbial water quality indicators from pool water surface samples to those in pool water bulk samples; 2) to identify pool staff practices that influence pool water quality; and 3) to identify swimmer behaviors that influence pool water quality, using data from a pilot study conducted at public swimming pools in Atlanta, Georgia.

The three hypotheses of this study are:

1. Surface microbial levels are correlated with bulk microbial levels.
    1.a. *Staphylococcus* spp. and *P. aeruginosa* are correlated with each other.
    1.b. *Staphylococcus spp.* and *P. aeruginosa* are correlated with heterotrophic plate count (HPC) bacteria.
2. Pool staff practices are associated with pool microbial levels.
3. Swimmer behaviors are associated with pool microbial levels.

2. Methods

Data Collection

Setting and Participants

This study used data from a pilot study that surveyed 26 public swimming pools in Atlanta, Georgia between July 2002 and August 2002. The pilot study collected information on microbiological and chemical water quality in public swimming pools, routine pool maintenance practices, and information on public health-related policies and response to fecal accidents. Public and private swimming pool management organizations in Atlanta, Georgia were recruited to participate in the pilot study. Out of six organizations that were recruited, three agreed to participate in the study. Two of the participating organizations were public and one was private. A meeting was held with a representative from each of the participating organizations to explain the purpose and methods of the study and to obtain permission to make unannounced visits to swimming pool facilities. Swimming pools were limited to those with a minimum of two lifeguards present.

A total of 26 pools outdoor pools at 23 facility visits were made between July 2002 and August 2002. Most of the pools were categorized as “mixed adult/child pool”. Three pools were wading pools, two pools were large 50 meter lap pools, one pool was an adult pool, and one pool was a combined adult/child pool with a wading pool. Regarding facility type, 18 were community facilities (municipal or public), 3 were neighborhood homeowner’s association facilities, and 2 were private club facilities. Daily site visits were randomly selected from the list of available facilities. One to two facilities were visited per day.

Surveys

Upon arrival at the facility, a pool manager or lifeguard was contacted and given a consent form that explained the purpose of the study, the types of questions being asked, their rights as a research subject, and the voluntary nature of their participation. A survey was developed and administered to a lifeguard or pool manager. At larger facilities, a pool manager was usually interviewed, whereas at smaller pools, a lifeguard was usually interviewed. A total of 26 individual swimming pools were included in the survey. At one facility personnel did not consent to answering any questions therefore, 22 of 23 facilities responded to questions.

The surveys included questions on operation and maintenance of the pool and observations of swimmer behaviors. Survey questions regarding operation and maintenance procedures included questions on monitoring frequency of free chlorine and pH, filter backwash frequency, and pool vacuuming frequency. For the survey questions regarding swimmer behaviors, subjects were asked to rate how often they observed certain behaviors or events in the swimming pool area. The survey questions were based on a 0 to 5 Likert scale with 0 being ‘never’, 1 being ‘hardly ever’, and 5 being ‘all the time’.

Microbial Water Quality

Microbial water quality was evaluated by sampling from each of the 26 swimming pools. Both bulk water samples and water surface samples were taken one time at each pool. Water surface samples were taken by laying a membrane filter on the surface of the pool for 20 to 30 seconds and then placing it immediately on a plate for the appropriate analysis.

Surface samples were performed on all 26 swimming pools for heterotrophic plate count (HPC) bacteria for both low-nutrient agar (R2A) and high-nutrient agar (PCA). HPC is non-specific and includes a wide range of bacteria. R2A and PCA media select for different bacteria, therefore both were used to enumerate different bacteria within the HPC range. Surface samples were also taken for *Staphylococcus* spp. at 24 pools and for *Pseudomonas aeruginosa* at 15 pools.

Bulk Water Samples. Each bulk water sample was collected in a sterile collection vessel containing sodium thiosulfate to neutralize residual chlorine, according to “Standard Methods”. The bacteria were analyzed using the membrane filter method from “Standard Methods” (*Standard Methods for the Examination of Water and Wastewater*, 1998). Method 9215 B was used to quantify heterotrophic plate count bacteria on PCA and R2A agar. Method 9213 B was used to quantify *Staphylococcus* spp. on Baird-Parker agar and method 9213 E used to quantify *P. aeruginosa* on M-PA agar. Total coliforms were quantified using method 9222 B on m-Endo agar. The bacteria were incubated at 35°C for 24-48 hours.

Microbiological analysis

For surface and bulk water samples, the bacteria were presumptively identified based on growth and colony morphology. Counts were reported as colony-forming units (CFU) per 100 ml for pool bulk water samples and CFU for pool surface samples.

Statistical Analysis

All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

Correlation analysis

A Pearson and Spearman correlation analysis was conducted to assess the association between pool surface microbial levels and pool bulk water microbial levels. Correlation analysis was also conducted to assess the relationship among the different organisms. The significance level used was $\alpha=0.05$. The bacteria used included surface and bulk water HPC-PCA, surface and bulk water HPC-R2A, surface and bulk water *Staphylococcus* spp., surface and bulk water *P. aeruginosa*, and bulk water total coliforms. All of the microbial variables were log-transformed to achieve a normal distribution. Microbial values below the limit of detection, LOD <1 CFU/100 ml, were substituted with 0.05 in order for log-transformation to work.

Multiple linear regression analysis

The correlation results were used to guide the initial outcome variables used for the multiple linear regression models. Out of eleven outcome variables seven were initially used: 1) $\log_{10}$ bulk water HPC-PCA, 2) $\log_{10}$ surface HPC-PCA, 3) $\log_{10}$ surface HPC-R2A, 4) $\log_{10}$ bulk water *Staphylococcus* spp., 5) $\log_{10}$ surface *Staphylococcus* spp., 6) $\log_{10}$ surface *P. aeruginosa,* and 7) $\log_{10}$ bulk water total coliforms. The bacteria were categorized by contamination source with HPC representing overall water quality, *Staphylococcus* spp. and *P. aeruginosa* indicating non-fecal contamination, and total coliforms indicating fecal contamination. When microorganisms within one category showed correlation, such as $\log_{10}$ bulk water HPC-PCA and $\log_{10}$ bulk water HPC-R2A, one was chosen as an outcome variable.

When microorganisms within a category (e.g. skin indicators) did not show correlation both were used as outcome variables, such as $\log_{10}$ surface *Staphylococcus* spp. and $\log_{10}$ surface *P. aeruginosa*.

The remaining four outcome variables were later used since few statistically significant models were produced from the initial six outcome variables. The variables include: 8) $\log_{10}$ bulk water HPC-R2A, 9) $\log_{10}$ bulk water *P. aeruginosa*, 10) $\log_{10}$ surface *P. aeruginosa* + $\log_{10}$ *Staphylococcus* spp. (microbial sum), and 11) $\log_{10}$ bulk water *P. aeruginosa* + $\log_{10}$ *Staphylococcus* spp. (microbial sum). A microbial sum of $\log_{10}$ *Staphylococcus* spp. and $\log_{10}$ *P. aeruginosa* was created as an outcome variable since these bacteria represent a common source of contamination (e.g. swimmers and the environment).

In order to choose variables from the pilot study data, a literature review was conducted to identify swimmer behaviors and pool staff behaviors that are associated with pool water quality. Backward elimination method was used to select potential predictors for the models. Given the small sample size, models were selected using a significance level of $p<0.10$ for the overall F-test. Variables were evaluated using a significance level of $p<0.05$.

Multiple linear regression analysis

Multiple linear regression analysis was used to examine the relationship between pool operator practices and pool microbial counts. Models were run for each of the previously listed 11 outcome variables. Six starting predictors were used in each model including continuous variables: 1) filter backwash rate (days between filter backwashing), 2) last filter backwash in days, 3) vacuum rate (days between vacuuming), 4) last vacuumed in days, and dichotomous variables: 5) pH measurement rate (every 1 hr. or 2 hrs.), and 6) chlorine measurement rate (every 1 hr. or 2hrs.). The predictors were chosen considering that routine swimming pool inspections commonly identify violations related to disinfectant and pH level and circulation and filtration system. A bivariate analysis was conducted to assess the association between these predictors and the outcome variables. All continuous variables were log-transformed for normality except for the variable ‘last vacuumed’ since log transformation did not yield a normal distribution.

Multiple linear regression analysis

Multiple linear regression analysis was used to examine the relationship between pool staff observations of swimmer behaviors and microbial counts. Models were run for each of the previously listed 11 outcome variables. Five starting predictor variables were used in each model including dichotomous variables: 1) pre-swim shower (‘How often do you think swimmers shower before entering the pool?’), 2) diaper changing (‘How often do you see parents changing diapers near the pool deck area?’), 3) swim diapers (‘How often do you see children wearing swim diapers in the pool?’), 4) eating near the pool (‘How often do you see people eating or drinking near the pool’?), and 5) dirt in the pool (‘How often do you see dirt in the pool’?). The predictors were chosen considering that many studies show swimmers often introduce contamination into pools such as dirt and fecal matter. A bivariate analysis was conducted to assess the association between these predictors and the outcome variables.

The predictor variables were created by collapsing the survey data which consisted of a 6-point Likert scale where 0=“Never” and 5=“All the time”. This was done in order to create dichotomous variables for the analysis. The responses that were given a

0-3 were recoded as 0 (not often) and the responses that were given a 4-5 were recoded as 1 (often).

3. Results

The data used in the statistical analyses came from a pilot study that was conducted between July 2002 and August 2002 at 26 public swimming pools in Atlanta, Georgia. Samples were collected from pool surface water and bulk water to test the microbial quality of the pools. Pool surface samples are reported in colony-forming units (CFU) and bulk water samples are reported in CFU/100 ml. The microorganisms tested include heterotrophic plate count bacteria on high-nutrient agar (HPC-PCA) and low-nutrient agar (HPC-R2A), total coliforms, *Pseudomonas aeruginosa*, and *Staphylococcus* spp. PCA agar and R2A agar were both used to measure HPC because it includes a wide range of bacteria and the two media enumerate different bacteria.

Data was collected through surveys on pool staff operation and maintenance procedures and pool staff observations of swimmer behaviors. Pool staff was asked about chemicals added to the pool and responses to fecal, diarrheal, and vomitous accidents. The response categories included actions related to cleaning the pool, chlorinating the pool, and closing the pool. The data on pool staff practices also include filter backwash rate (days between filter backwashing), last filter backwash in days, vacuum rate (days between vacuuming), last vacuumed in days, pH measurement rate (every 1 hr. or 2 hrs.), and chlorine measurement rate (every 1 hr. or 2hrs.).

The data on swimmer behaviors include pre-swim shower (‘How often do you think swimmers shower before entering the pool?’), diaper changing (‘How often do you see parents changing diapers near the pool deck area?’), swim diapers (‘How often do you see children wearing swim diapers in the pool?'), eating near the pool ('How often do you see people eating or drinking near the pool'?), and dirt in the pool ('How often do you see dirt in the pool'?).

Descriptive statistics

Descriptive statistics for all of the continuous variables used in the analyses are shown in Table 2.1 below. All continuous variables were log-transformed for the correlation and multiple linear regression analyses except 'last filter backwash' since log transformation did not yield a normal distribution. Samples from all 26 pools were analyzed for most of the microorganisms. Surface *P. aeruginosa* has the smallest sample size (n=15) followed by surface *Staphylococcus* spp. (n=24). The highest mean concentrations were bulk water HPC-PCA (mean: 139.42 CFU/100 ml; range: 4 - 601 CFU/100 ml) and bulk water HPC-R2A (mean: 136.23 CFU/100 ml; range: 3 - 705 CFU/100 ml). The lowest mean concentration was surface *P. aeruginosa* (mean: 0.93 CFU/100 ml; range: 0 - 13 CFU/100 ml) and bulk water total coliforms (mean: 3.01 CFU/100 ml; range: 0 - 73 CFU/100 ml). For pool staff practices, the average number of days between pool vacuuming was 14.22 days (range: 1 - 90 days). The average time since the last pool vacuuming was 9.31 days (range: 0 - 46 days). The average number of days between filter backwashing was 9.15 (range: 1 - 60 days). The average time since the last filter backwash was 4.80 days (range: 0 - 30 days).

Descriptive statistics for the categorical variables are shown in Table 2.2 below. The majority of the pools measured chlorine every 1 hour (72%) and pH every 1 hour (68%) versus every 2 hours. For the observed swimmer behaviors, 36% of the pools reported seeing children wearing swim diapers in the pool all the time. Pre-swim showering and seeing dirt in the pool were each reported by 20% of the pools as occurring all the time. Seeing people eating or drinking near the pool was reported by 16% of the pools as occurring all the time. Diaper changing near the pool was reported by 0% of the pools as occurring all the time.

Table 2.1. Descriptive statistics for all continuous variables used in analyses

Variables	**N**	**Mean**	**SD**	**Min**	**Max**
Outcomes[*]					
HPC-high nutrient agar (PCA)					
Bulk	26	139.42	154.48	4	601
Surface	26	12.38	20.79	1	100
HPC-low nutrient agar (R2A)					
Bulk	26	136.23	166.87	3	705
Surface	26	21.16	21.84	0	64
P. aeruginosa					
Bulk	26	3.53	13.88	0	71
Surface	15	0.98	3.34	0	13
Staphylococcus spp.					
Bulk	26	4.49	15.06	0	74
Surface	24	16.31	30.03	0	109
P. aeruginosa + Staphylococcus spp.					
Bulk	26	8.02	28.51	0	145
Surface	24	16.92	30.07	0	109
Total Coliforms					
Bulk	26	3.01	14.29	0	73
Predictors					
Filter backwash rate (days)‡	23	9.15	13.28	1	60
Last filter backwash (days)	24	4.80	7.52	0	30
Vacuum rate (days)‡	24	14.22	19.90	1	90
Last vacuumed (days)	23	9.31	13.09	0	46

Bulk water values are reported as CFU/100 ml.
Surface values are reported as CFU (colony forming unit).
Abbreviations: HPC=heterotrophic plate count; SD=standard deviation.
[*] samples below the limit of detection (LOD), <1 CFU/100 ml, were substituted with 0.05 for calculation of mean and SD.
‡Number of days between events.

Table 2.2. Descriptive statistics for all categorical variables used in analyses

Variables	**Frequency N (%)**	
	1 hour	**2 hour**
Chlorine measurement rate	18 (72)	7 (28)
pH measurement rate	17 (68)	8 (32)

	0 **Never**	1 **Hardly Ever**	2	3 **Sometimes**	4	5 **All the time**
Pre-swim shower	2 (8)	4 (16)	4 (16)	7 (28)	3 (12)	5 (20)
Diaper changing near pool	9 (36)	9 (36)	1 (4)	5 (20)	1 (4)	0 (0)
Swim diaper in pool	0 (0)	3 (12)	1(4)	6 (24)	6 (24)	9 (36)
Eating near pool	6 (24)	2 (8)	6 (24)	6 (24)	1 (4)	4 (16)
Dirt in pool	1 (4)	1 (4)	1 (4)	13 (52)	4 (16)	5 (20)

Note: The swimmer behavior variables are based on a Likert scale where 0="Never" and 5="All the time".

Correlation analysis

To determine whether surface microbial counts were associated with bulk water microbial counts, correlation analysis was performed. The relationship between different types of bacteria was also assessed. Table 2.3 below presents the Pearson correlation coefficients (log$_{10}$) and Table 2.4 below presents the Spearman rank correlation coefficients (log$_{10}$). Both tests are presented because they show some similarities and differences in significant correlations. The differences in correlations indicate non-normality of the data.

The results from the Pearson correlation show 7 significant positive correlations ($p<0.05$). For surface and bulk water samples for the same organism there is a significant correlation between log$_{10}$ surface *Staphylococcus* spp. and log$_{10}$ bulk water

Staphylococcus spp. ($p=0.008$). For surface and bulk water samples comparing different bacteria a significant correlation is shown between $\log_{10}$ surface HPC-R2A and $\log_{10}$ bulk water *Staphylococcus* spp. ($p=0.008$) and between $\log_{10}$ surface HPC-PCA and $\log_{10}$ bulk water *Staphylococcus* spp. ($p=0.02$). Among bacteria in the bulk water samples a significant positive correlation is shown between $\log_{10}$ bulk water *Staphylococcus* spp. and $\log_{10}$ bulk water *P. aeruginosa* ($p=0.02$), $\log_{10}$ bulk water *Staphylococcus* spp. and $\log_{10}$ bulk water total coliforms ($p=0.005$), $\log_{10}$ bulk water *P. aeruginosa* and $\log_{10}$ bulk water total coliforms ($p=0.002$), and between $\log_{10}$ bulk water HPC-R2A and $\log_{10}$ bulk water HPC-PCA ($p<0.0001$).

The results from the Spearman correlation show 6 significant positive correlations ($p<0.05$) (Table 2.4). Four correlations that are significant in the Spearman correlation are also significant in the Pearson correlation and have similar coefficient values. The similar correlations are between $\log_{10}$ surface *Staphylococcus* spp. and $\log_{10}$ bulk water *Staphylococcus* spp., $\log_{10}$ surface HPC-R2A and $\log_{10}$ bulk water *Staphylococcus* spp., $\log_{10}$ bulk water total coliforms and $\log_{10}$ bulk water *Staphylococcus* spp., and between $\log_{10}$ bulk water HPC-R2A and $\log_{10}$ bulk water HPC-PCA. The significant correlations seen in the Spearman correlation that are different from the Pearson correlation are between $\log_{10}$ surface HPC-R2A and $\log_{10}$ surface HPC-PCA, ($p=0.03$) and between $\log_{10}$ bulk water total coliforms and $\log_{10}$ bulk water HPC-PCA ($p=0.049$).

Table 2.3. Pearson correlation coefficients of microbial indicators from pool surface and bulk water samples

Microbial Parameters	**Correlation‡ coefficients**								
	TC	Staph	Pa	HPC-R2A	HPC-PCA	Surface Staph	Surface Pa	Surface HPC-R2A	Surface HPC-PCA
TC		**0.532**[*]	**0.582**[*]	0.143	0.209	0.333	-0.088	0.208	0.355
Staph	-		**0.449**[*]	0.295	0.181	**0.527**[*]	0.142	**0.509**[*]	**0.466**[*]
Pa	-	-		0.036	-0.043	0.072	-0.144	0.255	0.059
HPC-R2A	-	-	-		**0.710**[*]	-0.018	0.233	0.119	-0.038
HPC-PCA	-	-	-	-		0.216	0.372	0.120	-0.048
Surface Staph	-	-	-	-	-		0.367	0.267	0.384
Surface Pa	-	-	-	-	-	-		-0.117	-0.177
Surface HPC-R2A	-	-	-	-	-	-	-		0.334
Surface HPC-PCA	-	-	-	-	-	-	-	-	

Note: 26 pools sampled.

Abbreviations: TC=total coliforms; Staph=*Staphylococcus* spp.; Pa=*P. aeruginosa*.

[*] p<0.05.

[‡] coefficients represent log-transformed values.

Table 2.4. Spearman rank correlation coefficients of microbial indicators from pool surface and bulk water samples

Microbial Parameters					**Correlation‡ coefficients**				
	TC	Staph	Pa	HPC-R2A	HPC-PCA	Surface Staph	Surface Pa	Surface HPC-R2A	Surface HPC-PCA
TC		**0.587***	0.356	0.373	**0.390***	0.380	0.418	0.304	0.229
Staph	-		0.299	0.360	0.071	**0.515***	0.157	**0.509***	0.349
Pa	-	-		-0.067	-0.118	0.024	-0.153	0.362	-0.004
HPC-R2A	-	-	-		**0.730***	0.080	0.121	0.022	-0.025
HPC-PCA	-	-	-	-		0.243	0.333	-0.061	-0.115
Surface Staph	-	-	-	-	-		0.420	0.299	0.336
Surface Pa	-	-	-	-	-	-		-0.155	-0.244
Surface HPC-R2A	-	-	-	-	-	-	-		**0.415***
Surface HPC-PCA	-	-	-	-	-	-	-	-	

Note: 26 pools sampled.

Abbreviations: TC=total coliforms; Staph=*Staphylococcus* spp.; Pa=*P. aeruginosa*.

*p<0.05.

‡coefficients represent log-transformed values.

Multiple linear regression analysis

The correlation results were used to guide the initial outcome variables used for the multiple linear regression models. Initially, seven outcome variables were used from Table 2.1 including $\log_{10}$ bulk water HPC-PCA, $\log_{10}$ surface HPC-PCA and $\log_{10}$ surface HPC-R2A, $\log_{10}$ bulk water *Staphylococcus* spp. , $\log_{10}$ surface *Staphylococcus* spp. and $\log_{10}$ surface *P. aeruginosa*, and $\log_{10}$ bulk water total coliforms. The bacteria were categorized by contamination source with HPC representing overall water quality, *Staphylococcus* spp. and *P. aeruginosa* indicating non-fecal contamination, and total coliforms indicating fecal contamination. When microorganisms within one category showed correlation, such as $\log_{10}$ bulk water HPC-PCA and $\log_{10}$ bulk water HPC-R2A, one was selected. When microorganisms within a category (e.g. skin indicators) did not show correlation both were used as outcome variables, such as $\log_{10}$ surface *Staphylococcus* spp. and $\log_{10}$ surface *P. aeruginosa*. However, this approach yielded few significant models, so the rest of the outcome variables from Table 2.1 were later used for additional models. A microbial sum of $\log_{10}$ *Staphylococcus* spp. and $\log_{10}$ *P. aeruginosa* was created as an outcome variable because these bacteria represent a common source of contamination, swimmers and the environment.

Multiple linear regression analysis
Pool operator practices

Multiple linear regression analysis was used to examine the relationship between pool operator practices and pool microbial counts. Models were run for each of the 11 outcome variables listed in Table 2.1. Due to small sample size, the models were selected using a significance level of $p<0.10$ for the overall F-test. Four linear regression models produced statistically significant F-tests ($p<0.10$) while one model (Model 5) had a borderline significant F-test ($p=0.109$). The starting predictors in each model included $\log_{10}$ filter backwash rate (days between filter backwashing), last filter backwash in days, pH measurement rate (every 1 hr. or 2 hrs.), chlorine measurement rate (every 1 hr. or 2hrs.), $\log_{10}$ vacuum rate (days between vacuuming), and $\log_{10}$ last vacuumed in days. The predictors were chosen considering that routine swimming pool inspections commonly identify violations related to disinfectant and pH level and circulation and filtration system. A bivariate analysis showed $\log_{10}$ vacuum rate as the only predictor significantly associated with any outcome variable ($p<0.05$, Spearman Correlation). The results of the five models are shown in Table 2.5 below. The outcome variables include $\log_{10}$ bulk water HPC-PCA (Model 1), $\log_{10}$ surface HPC-R2A (Model 2), $\log_{10}$ bulk water total coliforms (Model 3), $\log_{10}$ bulk water *P. aeruginosa* + $\log_{10}$ *Staphylococcus* spp. microbial sum (Model 4), and $\log_{10}$ bulk water *P. aeruginosa* (Model 5). Chlorine measurement rate was the only variable not remaining as a predictor in any model.

Model 1 uses $\log_{10}$ bulk water HPC-PCA as the outcome variable. After backward elimination, $\log_{10}$ vacuum rate was left in the model as a significant predictor ($p=0.048$). The positive regression coefficient ($\beta=0.46$) indicates that as days between vacuuming increase, HPC-PCA levels increase in bulk water. The final model explains 16.7% of the variation in $\log_{10}$ bulk water HPC-PCA.

Model 2 uses $\log_{10}$ surface HPC-R2A as the outcome variable. After backward elimination $\log_{10}$ filter backwash rate and pH measurement rate were left in the model. The results show pH measurement rate as a significant predictor ($p=0.03$). The regression coefficient ($\beta=1.561$) indicates that measuring pH every 2 hours compared to every 1 hour is associated with an increase in HPC-R2A on the pool surface. The final model explains 29.8% of the variation in $\log_{10}$ surface HPC-R2A.

Model 3 uses bulk water total coliforms as the outcome variable. Last filter backwash, $\log_{10}$ vacuum rate, and $\log_{10}$ last vacuumed were selected as predictors in the model. The predictor variable $\log_{10}$ last vacuumed ($\beta=0.65$) is significant at $p=0.03$. The results indicate that as the number of days since the last vacuuming increase bulk water total coliforms increases. The variable $\log_{10}$ vacuum rate ($\beta=-1.10$) shows borderline significance at $p=0.055$ and indicates that an increase in the number of days between vacuuming is associated with a decrease in bulk water total coliforms. The final model explains 30% of the variation in $\log_{10}$ bulk water total coliforms.

Model 4 uses a microbial sum of bulk water *P. aeruginosa* and bulk water *Staphylococcus* spp. as the outcome variable. The variable $\log_{10}$ vacuum rate was left in the model as a significant predictor ($p=0.04$; $\beta=-0.71$). The results show that an increase in days between vacuuming is associated with a decrease in the outcome variable. The final model explains 17.2% of the outcome variable.

Model 5 uses bulk water *P. aeruginosa* as the outcome variable. The overall F-test for the model has a p-value just above the significance level at $p=0.109$. The variables last filter backwash, pH measurement rate, $\log_{10}$ vacuum rate, and $\log_{10}$ last vacuumed were left in the model. The predictor variables $\log_{10}$ vacuum rate ($\beta=-1.33$) and $\log_{10}$ last vacuumed ($\beta=0.68$) are significant at $p=0.03$ and $p=0.04$, respectively. The results show that an increase in days between vacuuming is associated with a decrease in bulk water *P. aeruginosa*. Conversely, an increase in the number of days since the last vacuuming is associated with an increase in bulk water *P. aeruginosa*. The final model explains 34.4% of the variation in $\log_{10}$ bulk water *P. aeruginosa*.

Table 2.5. Multivariable linear regression models assessing the association between pool operator practices and pool microbial levels

Parameter	**Estimate** (β)	**Std. Error**	**p-value**	**95% CL**
Model 1. Outcome variable: $\log_{10}$ bulk water HPC-PCA Pr>F=0.0478; R^2=0.167				
Intercept	3.407	0.494	<0.0001	2.381, 4.432
Vacuum rate	0.455	0.217	0.048	0.005, 0.905
Model 2. Outcome variable: $\log_{10}$ surface HPC-R2A Pr>F=0.0292; R^2=0.298				
Intercept	-0.690	1.043	0.516	-2.867, 1.486
Filter backwash rate	0.489	0.283	0.0995	-0.102, 1.080
pH measurement rate	1.561	0.654	0.0271	0.196, 2.926
Model 3. Outcome variable: $\log_{10}$ bulk water total coliforms Pr>F=0.086; R^2=0.300				
Intercept	-0.933	0.960	0.344	-2.949, 1.083
Last filter backwash	0.072	0.050	0.163	-0.032, 0.178
Vacuum rate	-1.103	0.538	0.055	-2.23, 0.027
Last vacuumed	0.646	0.269	0.028	0.080, 1.211
Model 4. Outcome variable: $\log_{10}$ bulk water Pa + Staph microbial sum Pr>F=0.044; R^2=0.172				
Intercept	1.124	0.759	0.153	-0.450, 2.698
Vacuum rate	-0.712	0.333	0.044	-1.40, -0.021
Model 5. Outcome variable: $\log_{10}$ bulk water Pa Pr>F=0.109; R^2=0.344				
Intercept	-1.925	1.750	0.287	-5.618, 1.769
Last filter backwash	0.089	0.054	0.122	-0.026, 0.203
pH measurement rate	1.430	0.938	0.146	-0.550, 3.409
Vacuum rate	-1.334	0.579	0.034	-2.555, -0.112
Last vacuumed	0.676	0.297	0.036	0.049, 1.302

Abbreviations: CL=confidence limits; Pa=*P. aeruginosa*; Staph=*Staphylococcus* spp.
Note: Vacuum rate and filter backwashing rate=days between events; last vacuumed and last filter backwash=days since last event; pH measurement rate=hours between events.

Multiple linear regression analysis

Swimmer behaviors

Multiple linear regression analysis was used to examine the relationship between pool staff observations of swimmer behaviors and microbial counts. Models were run for each of the 11 outcome variables listed in Table 2.1. The models were selected using a significance level of $p<0.10$ for the overall F-test. Two linear regression models produced statistically significant F-tests ($p<0.10$) while two models (Models 3 & 4) had borderline significant F-tests ($p=0.10$). The results of the four models are presented in Table 2.6 below.

The outcome variables in the models shown below include surface *Staphylococcus* spp. (Model 1), bulk water total coliforms (Model 2), bulk water *P. aeruginosa* + *Staphylococcus* spp. (Model 3), and surface *P. aeruginosa* (Model 4). The predictor variables were created by collapsing data from a 6-point Likert scale where 0="Never" and 5="All the time" (Table 2.2). Responses given a 0-3 were recoded as 0 (not often) and responses given a 4-5 were recoded as 1 (often). The models each produced similar results with the predictor variables selected from backward elimination including pre-swim shower, diaper changing, swim diapers, and dirt in the pool. The variable 'eating near the pool' was not selected as a predictor. A bivariate analysis showed visible dirt in the pool as the only predictor significantly associated with any outcome variable ($p<0.05$, Spearman Correlation).

The results of all four models show a negative parameter estimate for pre-swim shower. However, pre-swim showering is only statistically significant in Model 2 ($p=0.03$; $\beta=-1.44$) with $\log_{10}$ bulk water total coliforms as the outcome variable. This indicates that when swim staff rate swimmers as showering often compared to not often there is a decrease in bulk water total coliforms. Visible dirt in the pool is a significant predictor of $\log_{10}$ bulk water total coliforms ($p=0.003$; $\beta=2.16$; Model 2) and $\log_{10}$ surface *P. aeruginosa* ($p=0.03$; $\beta=1.96$; Model 4). The positive parameter estimate indicates that when pool staff rate seeing dirt in the pool as ‘often’ there is an increase in the microbial count of interest. Use of swim diapers is a significant predictor of $\log_{10}$ surface *P. aeruginosa* ($p=0.045$; $\beta=2.08$; Model 4). The results show that when pool staff often see children wearing swim diapers in the pool surface *P. aeruginosa* increases.

Surprisingly, diaper changing near the pool shows a negative association with $\log_{10}$ surface *P. aeruginosa* ($p=0.048$; $\beta=-3.97$; Model 4); however, the variable only has 15 observations. This indicates that when pool staff often see parents changing diapers near the pool there is a decrease in surface *P. aeruginosa*. Diaper changing also has a negative parameter estimate in Model 2 with $\log_{10}$ bulk water total coliforms as the outcome variable, but it is not statistically significant.

Table 2.6. Multivariable linear regression models assessing the association between swimmer behaviors and pool microbial levels

Parameter	**Estimate** (β)	**Std. Error**	**p-value**	**95% CL**
Model 1. Outcome variable: $\log_{10}$ surface *Staphylococcus* spp. Pr>F=0.098; R^2=0.208				
Intercept	0.264	0.791	0.742	-1.386, 1.914
Pre-swim shower	-2.501	1.295	0.068	-5.203, 0.201
Dirt in pool	2.032	1.221	0.112	-0.515, 4.579
Model 2. Outcome variable: $\log_{10}$ bulk water total coliforms Pr>F=0.013; R^2=0.395				
Intercept	-2.636	0.390	<0.0001	-3.448, -1.825
Pre-swim shower	-1.438	0.637	0.0345	-2.763, -0.112
Diaper changing near pool	-2.516	1.545	0.118	-5.729, 0.697
Dirt in pool	2.156	0.637	0.003	0.831, 3.482
Model 3. Outcome variable: $\log_{10}$ bulk water Pa + *Staphylococcus* spp. microbial sum Pr>F=0.104; R^2=0.186				
Intercept	-0.078	0.510	0.879	-1.136, 0.979
Pre-swim shower	-1.624	0.817	0.060	-3.319, 0.071
Dirt in pool	1.126	0.794	0.170	-0.521, 2.774
Model 4. Outcome variable: $\log_{10}$ surface Pa Pr>F=0.100; R^2=0.545				
Intercept	-3.071	0.628	0.0009	-4.492, -1.649
Pre-swim shower	-1.214	0.772	0.150	-2.960, 0.531
Diaper changing near pool	-3.972	1.734	0.048	-7.895, -0.049
Swim diapers in pool	2.084	0.896	0.045	0.058, 4.109
Dirt in pool	1.963	0.772	0.032	0.218, 3.709

Abbreviations: CL=confidence limits; Pa=*P. aeruginosa*; Staph=*Staphylococcus* spp.

Note: Vacuum rate and filter backwashing rate=days between events; last vacuumed and last filter backwash=days since last event; pH measurement rate=hours between events.

4. Discussion

Major findings

The purpose of this study was to assess the association between pool surface microbial levels and bulk water microbial levels. This study was also conducted to identify pool staff and swimmer behaviors that influence pool water quality. A Pearson correlation and Spearman correlation was conducted to test the first hypothesis that surface microbial levels are correlated with bulk water microbial levels. However, the tests showed some differences in statistically significant correlations (Tables 2.3 & 2.4). The differences indicate non-normality in the data, a result of the small sample size. This shows that there is limited support that some of the correlations indicate a true association. The correlations would need to be assessed using a larger sample size in order to present a clearer picture. One correlation that was seen in the Pearson test, but not the Spearman test was between $\log_{10}$ bulk water *Staphylococcus* spp. and $\log_{10}$ bulk water *Pseudomonas aeruginosa* (Tables 2.3 & 2.4). Sub-hypothesis 1a of this study is that *Staphylococcus* spp. and *Pseudomonas aeruginosa* are correlated. Since the tests were different, there is limited support for this hypothesis. However, a study by Martins et al. (1995) found a statistically significant correlation between bulk water samples of *S. aureus* and *P. aeruginosa* spp. using 1,345 pool samples ($p<0.0001$, Spearman correlation). Correlations between these bacteria suggest a common source of contamination. Both bacteria can be found on human skin and their presence in the water indicates swimmers as a source of pool contamination.

Although, the Pearson and Spearman correlations showed some differences, four statistically significant correlations ($p<0.05$) were the same in both tests (Tables 2.3 & 2.4). In comparing surface and bulk water samples, $\log_{10}$ *Staphylococcus* spp. in bulk water samples was correlated with $\log_{10}$ *Staphylococcus* spp. from surface water samples and $\log_{10}$ heterotrophic plate count bacteria on low-nutrient agar (HPC-R2A) from surface water samples ($p<0.05$, Pearson & Spearman correlations). This supports the first hypothesis of the present study that surface microbial levels are correlated with bulk water microbial levels. This suggests that the microbial quality of the pool surface may be an indicator for overall pool water quality. This also suggests that the bacteria in these different compartments of the pool come from the same source and the levels are influenced by the same factors.

Sub-hypothesis 1b was also supported by the results showing a statistically significant correlation between $\log_{10}$ bulk water *Staphylococcus* spp. and $\log_{10}$ surface HPC-R2A ($p<0.05$, Pearson & Spearman correlations, Tables 2.3 & 2.4). HPC consists of a variety of bacteria including *Staphylococcus* spp. and is used an indicator of overall water quality. The correlation between these bacteria suggests that skin indicators are significant drivers of the overall water quality. This further suggests that one way to improve pool water quality would be to reduce pool contamination from swimmers. The finding that *Staphylococcus* spp. and HPC are correlated is consistent with the results from the study conducted by Martins et al. (1995). The study found statistically significant correlations between *S. aureus* and HPC ($p<0.0001$, Spearman correlation).

The second hypothesis of this study was that pool staff practices are associated with pool microbial levels. Initial bivariate analysis of the outcome and predictor variables showed $\log_{10}$ vacuum rate as the only predictor significantly associated with any outcome variable. This is indicated by the small sample size. However, in the final models three predictors showed statistically significant associations with at least one outcome variable: days between vacuuming ($\log_{10}$), last time the pooled was vacuumed ($\log_{10}$), and time between pH measurements (Table 2.5). Vacuum rate (days) showed a positive association with $\log_{10}$ bulk water HPC-PCA ($p<0.05$). This indicated that as the number of days between pool vacuuming increase $\log_{10}$ bulk water HPC-PCA levels increase. Conversely, it showed a negative association $\log_{10}$ bulk water *P. aeruginosa* ($p=0.03$) and a microbial sum of $\log_{10}$ bulk water *P. aeruginosa* and $\log_{10}$ *Staphylococcus* spp. ($p=0.04$). This indicated that increasing days between vacuuming was associated with a decrease in these bacteria. Pool vacuuming is a maintenance practice used to remove sediment and debris from pools such as leaves and twigs. The frequency of pool vacuuming may be influenced by the surrounding environment of the pool. For example, pools that are surrounded by a lot a trees may have to conduct more frequent vacuuming compared to pools not surrounded by trees. The different associations between the vacuuming frequency and the various microbes may be related to this factor and indicate different sources of microbial contamination.

The results showed that measuring pH every two hours compared to every hour resulted in an increase in $\log_{10}$ surface HPC-R2A ($p=0.03$) (Table 2.5). Reports show that pool pH levels are common violations seen in inspection reports (Centers for Disease

Control and Prevention, 2010). This finding indicates there may be existing problems affecting the water quality of the pool that should be further addressed.

The linear regression models for pool operator behaviors indicated a positive association between $\log_{10}$ filter backwash rate and $\log_{10}$ surface HPC-R2A (Table 2.5). A positive association was also seen between last filter backwash and $\log_{10}$ bulk water total coliforms. Neither of these parameters were statistically significant. Filter backwashing is important in maintaining pool water quality. Further, filtration violations are often identified in pool inspections (Centers for Disease Control and Prevention, 2010). The non-significance of the filter backwashing variables may be due to small sample size.

It is important to note that the two measures of HPC, high-nutrient agar (PCA) and low-nutrient agar (R2A) had no shared predictors. HPC includes a wide range of bacteria and the two media enumerate different bacteria within the range. The different predictors associated with HPC-PCA and HPC-R2A are an indication of the differences in the two media. Further, HPC-PCA and HPC-R2A can represent different sources of contamination related to the predictor variables. Another important note is that all of the models had R^2 values less than 50%. This indicates there is still a lot of variability not accounted for in the models. An assessment of additional predictors is needed to gain a better understanding of pool staff behaviors that affect pool microbial quality.

The third hypothesis of this study was that swimmer behaviors are associated with pool microbial levels. An initial bivariate analysis showed visible dirt in the pool as the only predictor significantly associated with any outcome variable ($p<0.05$, Spearman Correlation). This was a limitation of small sample size. In the final models pre-swim showering, dirt in the pool, swim diapers, and diaper changing were statistically significant ($p<0.05$) predictors of microbial levels ($\log_{10}$). Pool staff indicating that swimmers often use pre-swim showers showed a negative association $\log_{10}$ bulk water total coliforms ($p=0.03$) (Table 2.6). This is consistent with findings from studies showing that pre-swim showers decrease the amount of contamination that swimmers introduce into pools (Keuten et al., 2014; Keuten et al., 2012).

The results showed a positive association between pool staff often seeing dirt in the pool and $\log_{10}$ bulk water total coliforms ($p=0.003$). Additionally, a positive association was seen between pool staff often seeing children wearing swim diapers in the pool and surface *P. aeruginosa* levels ($p=0.045$) (Table 2.6). These results support the hypothesis that swimmers behaviors are associated with pool microbial levels. A number of studies show that swimmers often introduce contaminants into pools such as dirt and fecal matter that can reduce the effectiveness of pool disinfectants (Centers for Disease Control and Prevention, 2013; Keuten et al., 2014; Keuten et al., 2012). The findings from this study are consistent with these studies.

In looking at diaper changing behaviors near the pool, a negative association was seen with $\log_{10}$ surface *P. aeruginosa* ($p=0.05$) (Table 2.6). This finding may be due to the small sample size of this bacteria ($n=14$) and pool staff rating this as an uncommonly observed behavior. Although the analysis of pool staff and swimmer behaviors was limited by small sample size, the detection of skin indicators and HPC in this study reaffirms the importance of swimmer hygiene and using pre-swim showers in improving pool water quality.

Limitations

This study was subject to at least four limitations. First, the study involved a small convenience sample of 26 public swimming pools in Atlanta, Georgia. Due to this, the study may not be generalizable outside of the study area. Further, the small sample size may have reduced the detection of significant associations. For example, although filter backwashing is considered an important procedure for maintaining pool water quality, it was not shown to be a significant predictor in the models. Another limitation related to the small sample size is that model validation was not performed on the multiple linear regression models. The small sample size produced low power results and reduced the ability to produce strong fitting models.

The second limitation was related to the use of non-specific microbial indicators such as HPC as an outcome variable. HPC consists of a variety of bacteria that could be fecally derived or non-fecally derived. Therefore, we were not able to determine which type of bacteria the predictor variables were associated with when using HPC as an outcome variable. However, this is a common indicator used in water quality studies and system operations.

The third limitation was that pool staff may have presented recall bias in their response to survey questions. For example, when asked how often the pool is vacuumed or when the last time the pool was vacuumed, respondents may not have provided accurate responses.

The fourth limitation was that swimmer behavior data were based on pool staff observations and were not directly collected from swimmers. As a result, pool staff observations of swimmer behaviors may have been over or under underestimated. Some behaviors can be easily seen by pool staff, such as changing diapers near the pool or eating near the pool. Other behaviors are not as easily observable such as showering or using swim diapers. Therefore, while some of these variables have some misclassification bias, it is much higher for some than other.

Future steps

A number of studies have assessed the quality of public swimming pools (Centers for Disease Control and Prevention, 2013; Davis et al., 2009; Martins et al., 1995; Sawabe et al., 2015; Shields et al., 2008). However, these studies do not quantitatively assess behavioral predictors of pool microbial levels. Considering the limitations of this current study, future studies should be conducted using larger sample sizes in order to gain a better understanding of behaviors that effect pool microbial levels. Another important component in conducting this type of study would be to directly survey swimmer behaviors. Further research is also needed to determine if routine microbial testing would be beneficial to minimize the risk of recreational water illnesses.

Chapter III. Conclusions and Recommendations

Swimming is a top recreational activity in the U.S. that can provide many health benefits. However, reports show that the occurrence of recreational water illness (RWI) outbreaks are increasing. Taking this issue into account, the first goal of this study was to determine if microbial samples from pool water surfaces are correlated with microbial samples from pool bulk water. The significant correlation found between surface *Staphylococcus* spp. and bulk water *Staphylococcus* spp. and between surface heterotrophic plate count bacteria and bulk water *Staphylococcus* spp. suggests that pool surface water can be used as a measure for overall pool water quality. The second and third goal of this study was to assess pool staff practices and swimmer behaviors that contribute to microbial quality of public swimming pools. A positive association was seen between pool vacuuming and HPC bacteria while a negative association was seen with total coliforms and *P. aeruginosa*. Regarding swimmer behaviors, swim diapers were positively associated with *P. aeruginosa* while dirt in the pool was positively associated with *P. aeruginosa* and total coliforms. The study, however, was limited by small size with the findings representing a limited view of behavioral contributors to pool microbial quality. Further studies are needed to gain a better understanding of this relationship.

Recommendations

The results of this study along with evidence from a broad body of literature demonstrate the need for improved pool staff practices and swimmer hygiene behaviors. Considering this information, there are four recommendations that pool operators should consider in order to improve pool water quality and prevent recreational water illnesses. First, while pool chemicals are monitored daily, microbial testing is not conducted as a part of routine pool maintenance. In finding that bacteria in pool surface samples are correlated with bacteria in pool bulk water samples, pool surface sampling may be suitable for testing pool water quality. Surface sampling is a simple and feasible alternative to bulk water testing because it does not require a lot of equipment. The only material needed is a membrane and medium, whereas bulk water testing requires various glassware for collecting and filtering water. To date, there are no U.S. regulations that require routine microbial testing of public swimming pools. However, the World Health Organization recommends weekly testing for heterotrophic plate count and *E. coli* in public, heavily used pools. Testing for *P. aeruginosa* is recommended when pools have been associated with health problems (World Health Organization, 2006).

This study found that pool staff maintenance practices such as increasing time between pool vacuuming is associated with increased heterotrophic plate count bacteria. The average time between pool vacuuming for the pools in this study was 2 weeks. The range of time between pool vacuuming for the pools was every day to every 3 months (Table 2.1). Taking this into consideration, pool operators should improve pool maintenance practices such as vacuuming more frequently. The swimming pool industry recommends vacuuming pools weekly. This can prevent dirt and debris from reaching high levels that reduce the effectiveness of pool disinfectants. Further, the study found that reducing the frequency of pH measurements is associated with increased heterotrophic plate count bacteria. Reports from the Centers for Disease Control & Preventions (CDC) show that some of the top reported violations from public pool inspections include disinfectant and pH violations (Centers for Disease Control and Prevention, 2010). Pool operators should monitor pH levels every 1 to 2 hours. The CDC recommends monitoring every hour when the pool is in heavy use.

Currently, there is no nationwide requirement for pool operator training and certification in the U.S. Various studies show that pools with certified operators present fewer pool chemistry violations and closures upon inspection (Buss et al., 2009; Lachocki, 2006). Following this, it is recommended that pool operators ensure that staff are trained on proper pool maintenance in addition to RWI prevention.

Even when conducting proper pool maintenance procedures, swimmer hygiene still plays a significant role in pool water quality. Swimmers often introduce contaminants into pools such as dirt and fecal matter. These contaminants reduce the effectiveness of pool disinfectants and are associated with increased pool microbial counts. Similar to other studies, this study found that visible dirt in the pool and swimmers not showering before entering the pool are associated with increased pool microbial counts. In addition to improving maintenance procedures, pool operators should require swimmers to take pre-swim showers in order to reduce pool contamination. The World Health Organization also recommends providing foot sprays to minimize the transfer of dirt into the pool (World Health Organization, 2006).Taking these recommended steps for public pool maintenance can lead to improved outcomes for pool microbial quality and recreational water illness.

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