Original Research

Coliphage as an indicator of the quality of beach water to protect the health of swimmers in coastal Georgia

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ABSTRACT

Background: Gastrointestinal disease affects millions of people in the United States and places a substantial economic burden upon healthcare systems. Recreational waters polluted with fecal material are a main source for transmission of gastrointestinal disease. Georgia beaches are monitored for the presence of fecal indicator bacteria, but these bacteria are not well associated with enteric viruses. The United States Environmental Protection Agency (US EPA) has recently proposed coliphage (a virus of *Escherichia coli*) as an alternative indicator of fecal contamination in recreational waters. The present study compares fecal indicator bacteria and coliphage concentrations at two Georgia beaches with adjacent creeks that have a history of pollution.

Methods: For one year, samples and environmental data were collected from four sites on Jekyll Island, GA, during the peak swimming season and the off-season. Samples were processed using US EPA-approved methods for membrane filtration and plaque formation. Statistical analyses were performed using t-tests and Spearman correlations.

Results: The highest numbers of enterococci and significant differences with coliphage were found at Saint Andrews Creek during the swimming season and the off-season. The enterococci concentrations at Clam Creek sites did not exceed recommended recreational water criteria. During the off-season, concentrations of enterococci and coliphages were different at Clam Creek sites, indicating a potential risk for presence of enteric virus when enterococci could not be detected.

Conclusions: The US EPA has proposed to adapt coliphage concentrations as an alternative indicator of water pollution for routine beach monitoring nationally. The present study provides a background for adoption of this method in Georgia. Measures of enterococci do not provide sufficient information about the associated human health risk. Inclusion of these viral indicators will improve decision making for beach closures and for protection of the health of swimmers.

Keywords: coliphage, enterococci, enteric viruses, beach water quality, health risk, Georgia

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INTRODUCTION

As described in the latest report from the CDC, the United States and its territories reported 90 recreational water disease outbreaks between 2011 and 2012, and the incidence rates of recreational water-related outbreaks have increased since the 1970's (Hlavsa et al., 2015). Recreational water illnesses occur by exposure to contaminated waters in lakes, streams, pools, and oceans through activities such as fishing, swimming, or boating (Colford et al., 2007). These water bodies are vulnerable to contamination with pathogens from a variety of sources that can include septic tank malfunctions, the introduction of wastewater, storm drain contamination, and fecal discharge from animals and humans directly into the water (Withers et al., 2014; Bambic et al., 2015). Upon contact with contaminated recreational waters, children are at greater risk of gastroenteritis (Oh et al., 2003; Jones et al., 2014; Kotwal and Cannon, 2014). In recreational waters, enteric viruses, such as adenovirus, norovirus, and rotavirus, are the most common agents of disease (Tran et al., 2013; Karst, 2016). For hospitals in the United States, the financial impact of illnesses attributed to human enteric pathogenic viruses is nearly $1 billion dollars annually (Bernstein, 2009; Bartsch et al., 2016). Estimating the healthcare cost of enteric viruses is difficult because healthcare providers underreport infections, infected people do not visit a healthcare provider, and the surveillance systems for reporting enteric viral illnesses cannot obtain accurate data. It is important, then, to identify with accuracy what pathogens are causing infections so that healthcare providers and public health entities can mitigate water-related infections that are causing the highest burden on the healthcare system (Given et al., 2006; Gibbons et al., 2014).

The United States Environmental Protection Agency (US EPA) recommends criteria for detecting and minimizing the number of pathogens found in recreational waters. These recommendations can be used by local governments to set criteria for use of recreational waters under their jurisdiction. Current recreational water quality criteria (RWQC) recommend monitoring of enterococci for pollution at marine beaches (US EPA, 2012). Under these guidelines, Georgia beaches are reported as under advisory when enterococci concentrations are above the beach action value (BAV) of 70 CFU/100 ml (GADNR, 2015). Enterococcus, a bacterium living in the gastrointestinal system of humans and many animals, is commonly secreted along with fecal waste (Scott et al., 2002). Its concentration has a high correlation with gastrointestinal illnesses for people exposed to contaminated waters (Wade et al, 2008).

Enteric viruses are poorly correlated with fecal indicator bacteria due to their variability in survival, persistence, and sources (Byappanahalli et al., 2003; Boehm et al., 2009; Fujioka et al., 2015). To provide a more accurate assessment of pollution, the US EPA is currently developing criteria for coliphage, a bacteriophage that is a more accurate indicator for waterborne viruses in recreational waters (Nappier, 2015). Coliphages are viruses that target *E. coli* for replication (USEPA, 2006). Two coliphage groups, somatic and male-specific phages, have been well studied in laboratory settings because of their similarity to human enteric viruses such as rotavirus and norovirus, their survivability in the environment, and their capacity to be detected when fecal indicator bacteria are not present in a sample (Borrego et al., 1990; Savichtcheva and Okabe, 2006; Dutka et al., 1987; Griffin et al., 2000). Measurements of these viruses is relatively easy in a non-academic (i.e., health department) laboratory, require minimum laboratory space, and have short processing times (<30 min). Results can be reported within 24 hr of sample collection as opposed to other waterborne viruses, which require substantial expertise, resources, and time to culture (>14 days). Coliphages have been measured in waters polluted with sewage, but there is limited information on their distribution in polluted recreational waters.

The purpose of this study was to provide a comparison between enterococci and coliphage occurrence at two beaches and adjacent creeks with histories of fecal pollution and analyze the effect of seasonality (swimming season and off-season) on the occurrence of somatic and male-specific phage.

METHODS

Study area

Field studies were conducted at Saint Andrews and Clam Creek Beaches on Jekyll Island, Georgia (Figures 1a & 1b). Jekyll Island is located on the southeastern border of Georgia. St. Andrews beach is on the north side of the island, and Clam Creek is on the south side, almost directly opposite. These sites were chosen due to their historic levels of pollution (GADNR, 2015). At these two beaches, water samples were collected from four sites twice a month during the swimming season and once a month during the off-season.

Figure 1a. Location of the sampling points at St. Andrews Beach

Figure 1b. Location of the sampling points at Clam Creek Beach

Sample collection

Grab samples were collected in sterile bottles and placed on ice until transported to the laboratory. All samples were analyzed within 24 hr of collection. Environmental data (water temperature, salinity, dissolved oxygen, and pH) were collected by use of a multi-probe. For one year in 2016-2017, samples were collected twice monthly during the swimming season, the months in which beach use was heaviest (May to September), and once a month during the off-season (November to February).

Coliphage enumeration

Somatic and male-specific coliphage were enumerated in water samples as described in US EPA Method 1602 (USEPA, 2001). Host bacteria (*Escherichia coli*) were incubated overnight and mixed with 100 ml of the sample treated with $MgCl_2$. These cell suspensions and samples were then plated on double-strength tryptic soy agar treated with antibiotics (nalidixic acid for somatic and streptomycin/ampicillin for male-specific phage). All plates were incubated for 16-24 hr at 37 °C. At the end of the incubation, coliphages were quantified by counting plaques and reported as plaque-forming units per 100 ml (PFU/100 ml).

Enterococci enumeration

US EPA Method 1600 was used to enumerate and quantify the presence of enterococci in the environmental samples (USEPA, 2006). Each sample was filtered in triplicate using the volumes of 10, 10, and 100 ml of sample. Filters then were placed on membrane-enterococcus indoxyl-B-D-glucoside agar plates and incubated for 24 hr at 41 °C. At the end of the incubation, all colonies greater than or equal to 0.5 mm in diameter (regardless of color) with a blue halo were recorded as enterococci colonies, and results were reported as colony-forming units (CFU)/100 ml.

Statistical analyses

All data were log-transformed to ensure normality. A Spearman correlation analysis was conducted for environmental factors, enterococci, and bacteriophage present during the swimming season and the off-season. Box-and-whisker plots were constructed showing the mean and standard deviation of log concentrations of enterococci and bacteriophage at individual sites during the swimming and off-seasons. To determine differences between mean log concentrations of bacteriophage and enterococci, a t-test assuming unequal variance was performed for samples from all four sites between the swimming and off-seasons. All statistical analyses were performed with Microsoft Excel 2016.

RESULTS

A total of 52 samples were collected from four sites (JK1, JK3, JK5, and JK6) between January 2016 and January 2017. During the swimming season (May to September), enterococci concentrations were above the beach action value (BAV, 70 CFU/100 ml) in 63% of the samples collected from JK1 (St. Andrews Creek). The percent exceedance was 13% for JK3 (St. Andrews Beach). For samples collected at the Clam Creek sampling points (JK5 and JK6), none of the samples exceeded the BAV. The highest levels of enterococci were detected at JK1, with a maximum concentration of 2333 CFU/100 ml (Figure 2a). Both male-specific and somatic coliphage were present at all four sites. The highest concentrations of somatic coliphage (89 PFU/100 ml) and male-specific coliphage (393 PFU/100 ml) were found at JK1.

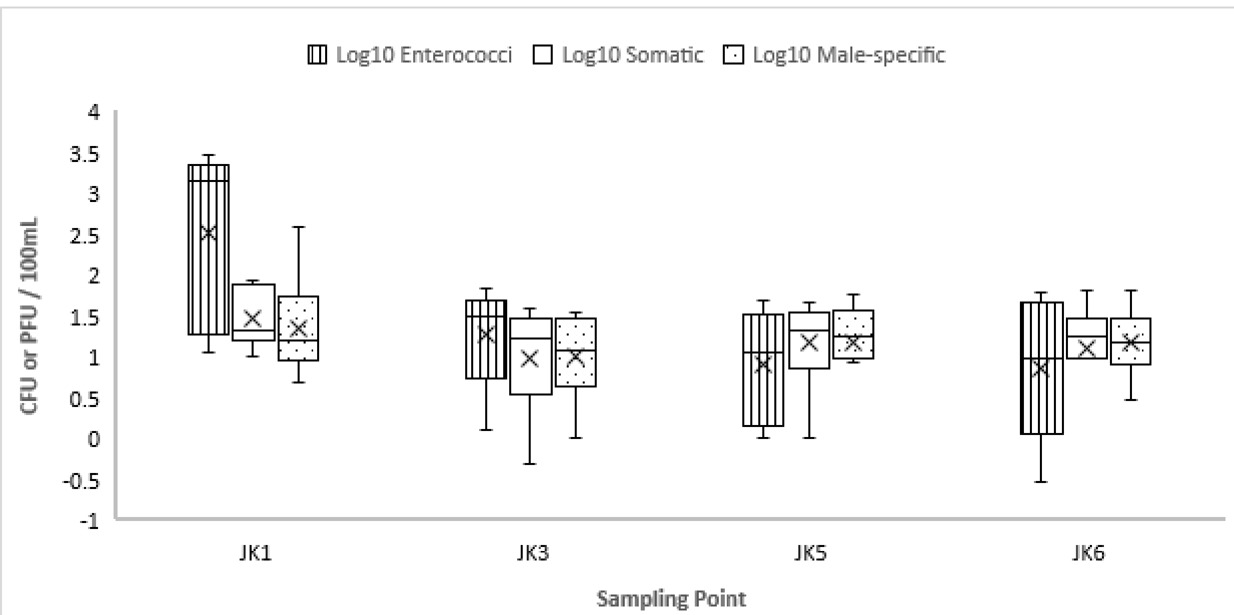

Figure 2a. Box-and-whisker plots of enterococci and somatic and male-specific coliphage at Jekyll Island during the swimming season (n=32)

Throughout the off-season, enterococci concentrations exceeded BAV for 56% of the samples collected from JK1. The maximum concentration of enterococci detected was 2900 CFU/100 ml. During this period, results for enterococci at JK3 exceeded the BAV in 13% of the samples. None of the samples collected from Clam Creek (JK5 and JK6) exceeded the BAV. Both somatic and male-specific coliphage were highest at JK1 (76 and 79 PFU/100 ml, respectively) (Figure 2b).

Figure 2b. Box-and-whisker plots of concentrations of enterococci and somatic and male-specific coliphage at Jekyll Island during the off-season (n=36)

During the swimming season, there were, at JK1, significant differences between concentrations of enterococci and somatic coliphage ($t = 2.1$, $p < 0.05$) and between enterococci and male-specific coliphage ($t = 3.02$, $p < 0.05$). In addition, at JK6 concentrations of enterococci and somatic coliphage means were significantly different ($t = -2.4$, $p < 0.05$). During the non-swimming season, there was also a significant difference during off-season between concentrations of enterococci and somatic coliphage ($t = 2.7$, $p < 0.05$) and between concentrations of enterococci and male-specific coliphage ($t = 2.7$, $p < 0.05$). For JK1, concentrations of enterococci and somatic coliphage were moderately correlated during the swimming season (0.58). For JK3, there was a moderate correlation ($r = 0.62$) between enterococci and male-specific coliphage during both the swimming and the off-season, and a correlation between somatic and male-specific coliphage during the swimming season ($r = 0.50$). During the swimming season, results for JK5 showed a moderate correlation ($r=0.59$) between somatic coliphage and male-specific coliphage. For JK6, there was no correlation between any of the results ($p>0.05$).

DISCUSSION

In the present study, we investigated the concentrations of fecal indicator bacteria and bacteriophages at two creeks and beaches on Jekyll Island under permanent advisory. The relationships among the bacteria and viruses as well as the environmental parameters were compared during the swimming and off-seasons. In the presence of fecal pollutants, concentrations of coliphages and traditional fecal indicator bacteria correlated well. Therefore, these viral indicators can be used for monitoring water that receives fecal pollution. For example, the small creek at St. Andrews Beach (JK1) presented the highest levels of enterococci and coliphages, indicating a potential source of contamination. This relation was weaker at sites that did not show any point sources of pollution. Coliphages were close to the detection limit, and yet enterococci were detected at these sites. In addition to sewage leaks, sources from animals can contribute to the levels of enterococci in recreational waters (Roll, 1997; Layton, Walters, Lam, & Boehm, 2010). In the environment, there are more than 35 species of enterococci; only a small portion of these are of human origin and cause a public health concern (Byappanahalli et al., 2012). Other enterococci species present in water are an indication of sources other than sewage. Reporting these high numbers of enterococci during regular monitoring may cause false-positive results and overestimations of potential health risk, which produces an economic burden with unnecessary beach closures (Kim and Grant, 2004).

Both somatic and male-specific coliphages presented variability, depending on the season and location. Persistence of these bacteriophages varies depending on the environmental conditions (Ibarlueza et al., 2007). Although the enterococci concentration decreased at St. Andrews Beach (JK3) compared to the St. Andrews Creek (JK1), somatic and male-specific coliphages were present during the swimming season and the off-season, indicating that these bacteriophages survive for a long period in the environment. Concentrations of enterococci, on the other hand, were diluted during their travel from the creek to the beach caused by hydrological movements such as currents and tidal fluctuations. These findings are supported by previous studies in which the survival rate of enterococci is shown to be shorter than that for coliphages. Temperature is a factor in bacteriophage occurrence and viability (Jończyk et al., 2011; Napier et al., 2015). Future studies on the ecology of these viral indicators will provide further information on these relationships.

CONCLUSIONS

In routine monitoring, measurements of enterococci alone do not deliver the most accurate information about the quality of beach water. From a public health perspective, it is necessary to provide the most accurate and current information to the public in a manner in which communities are informed about the potential risks associated with use of polluted waters. Although measurements of enterococci have been sufficient as an indicator at beaches affected by sewage, measures of other indicators provide more accurate information for beaches receiving mixed sources of pollution. The present study provides preliminary information on the usefulness of coliphage in routine monitoring. To mitigate pollution and deliver the most accurate information about beach water quality to the public, further studies are needed to identify point and non-point sources of pollution at Georgia beaches.

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