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Baseline

Detection of *Helicobacter pylori* in the coastal waters of Georgia, Puerto Rico and Trinidad

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ABSTRACT

Fecal pollution in the coastal marine environments was assessed at eleven sampling locations along the Georgia coast and Trinidad, and nine sites from Puerto-Rico. Membrane filtration (EPA method 1604 and method 1600) was utilized for *Escherichia coli* and enterococci enumeration at each location. Quantitative polymerase chain reaction (qPCR) amplification of the 16S ribosomal RNA gene was used to determine the presence of the *Helicobacter pylori* in marine samples. There was no significant correlation between the levels of *E. coli*, enterococci and *H. pylori* in these water samples. *H. pylori* was detected at four of the 31 locations sampled; Oak Grove Island and Village Creek Landing in Georgia, Maracas river in Trinidad, and Ceiba Creek in Puerto Rico. The study confirms the potential public health risk to humans due to the widespread distribution of *H. pylori* in subtropical and tropical costal marine waters.

Fecal contamination of recreational water can have a detrimental effect on the environment, ecology and economy of a region and increase the risk for transmission of human pathogens (Staley et al., 2012; Savichtcheva and Okabe 2006). Fecal indicator bacteria (FIB), such as *Escherichia coli* and *Enterococcus* spp. are often used as a proxy to predict the presence of human pathogens in waterbodies (Savichtcheva and Okabe 2006). The acceptable levels of these fecal indicators have been determined from epidemiological studies (USEPA, 1986). Recent studies have shown unacceptably high levels of fecal pollution in several coastal regions of the Georgia, Puerto-Rico and Trinidad impacted by human fecal pollution (Bachoon et al., 2010). Therefore, it is likely that these marine waters containing human fecal pollution may harbor pathogenic microorganisms. However the dangers of pathogenic bacteria in marine waters of the Caribbean Islands is currently unknown.

A common human pathogen is *Helicobacter pylori* which infects more than 50% of the world's population and as much as 70% of the population in some developing countries (Kim et al., 2011; Janzon et al., 2009; Giao et al., 2008). This gram-negative spiral shaped bacterium commonly infects the gastric mucosa of humans (Vale and Vitor 2010). The vast majority of *H. pylori* infections are asymptomatic and only a small percentage of cases progress to cancer. However, it is estimated that 70% of gastric cancers are directly caused by this organism (Kim et al., 2011). It is suspected that *H. pylori* is transmitted person to person through contaminated food or water (Vale and Vitor 2010; Janzon et al., 2009; Giao et al., 2008; Sen et al., 2007; Gomes and De Martinis, 2004). Several studies have indicated that drinking fecal contaminated water might aid the transmission of *H. pylori* (Ghosh and Bodhankar 2012; Vale and Vitor 2010; Linke et al., 2010) and aquatic environments may be a potential natural reservoir for these bacteria (Twing et al., 2011; Sen et al., 2007).

Traditionally, *H. pylori* was difficult to detect in natural water samples by culture-based methods. Today, there are numerous PCR-based methods available for detecting the presence of *H. pylori* in water samples that have used to demonstrate the widespread presence of this bacterium in marine water from Italy and Delaware, as well as freshwater samples from the United States, Mexico, Japan, England, Sweden, and Germany (Twing et al., 2011; Agustí et al., 2010; Sen et al., 2007; Carbone et al., 2005; Gomes and De Martinis, 2004; Maxzari-Hiriart et al., 2001; Mackay et al., 1998; Hulten et al., 1996). However it appears that the detection of this bacterium in water samples does not always correlate with high levels of fecal indicator bacteria (*E. coli* or enterococci) or the presence of human fecal bacteria (Twing et al., 2011; Gomes and De Martinis, 2004). This can be very problematic for public health officials that often attempt to predict the safety of waterbodies based on fecal indicator bacteria levels. Additional studies are needed to assess the distribution and relationship of *H. pylori* to common fecal indicator bacteria levels in other marine systems over broader geographical scales.

In this study, *H. pylori* 16S ribosomal RNA gene fragment was used as a molecular marker for detection of the bacterium in

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quantitative polymerase chain analysis assay (Kobayashi et al., 2002). The aim of this study was to determine the presence of *H. pylori* in marine water samples from Georgia, Puerto Rico and Trinidad in addition to determining whether traditional FIB enumeration can be used to predict the presence of *H. pylori* in subtropical to tropical marine waters.

Between 2009 and 2010 water samples were collected in triplicate in sterile whirlpack bags or polypropylene bottles from 31 coastal sites from U.S. Georgia coast, Puerto Rico and Trinidad (Fig. 1) kept on ice and processed within 6 h. Sampling sites were classified as Urban/Suburban and Rural based on arbitrary knowledge of existing population density.

The samples were filtered using USEPA method 1604 (USEPA 2000) and USEPA method 1600 (USEPA 2000). Triplicates of 100 ml volume of water from each sample site were filtered through a 0.45-μm GN-6 gridded membrane filter (Pall Corporation, Ann Arbor, MI) and then incubated on MI plates of the fluorogen, 4-methylumbelliferyl-β-D-galactopyranoside (MUGal), and chromogen, indoxyl-β-D-glucuronide (IBDG), at 35 ± 0.5 °C for 24 h and on m-EI plates, membrane-enterococcus indoxyl-β-D-glucoside agar at 41 ± 0.5 °C for 24 h. The MI plates were displayed under ultra-violet light (366 nm), the fluorescent colonies were counted, and the data was recorded as colony forming units (CFU) per 100 ml (Zimbro and Power 2003).

Water samples were filtered through, 0.22-μm-pore nitrocellulose membrane filter (Type GS, Millipore, Billerica, MA, USA). The filters were frozen and shipped on dry ice by overnight courier to Milledgeville, GA. Filters were processed with the MoBio Ultraclean™ Soil DNA Kit (Carlsbad, CA, USA) using a modified protocol (Bachoon et al., 2010; Sherchan and Bachoon 2011). Extracted DNA was quantified using a nanodrop ND-1000 spectrophotometer (Wilmington, DE).

H. pylori genomic DNA from ATCC® 700392D-5 was used as positive controls in 16S ribosomal RNA gene qPCR assays. The primers used were HP-F, 5′-CTCATTGCGAAGGCGACCT-3′, and HP-R, 5′-TCTAATCCTGTTTGCTCCCCA-3′ (Kobayashi et al., 2002) and produced a amplicon of 85-bp. The specificity of the qPCR assay was optimized against common fecal bacteria (*E. coli* strain B genomic DNA Sigma® D4889, Enterococcus faecalis ATCC 29212, Bacteroides dorei DSM 17855 (DSMZ, Germany) and *Bifidobacterium adolescentis* genomic DNA ATCC® number 15703D™). After which, we used *B. adolescentis* genomic DNA ATCC® number 15703D™ was used as a negative control. DNA extracts were amplified using the Bio-Rad CFX96 (Hercules, CA). Samples were run using optimized qPCR assays. Each qPCR contained a 25 μl volume with 12.5 μl of iQ™ SYBR® Green 2x Supermix, 12 μl deionized H_2O, 250 nM of primer, 1 μl (~10 ng) of template DNA and 0.04 μl of magnesium under the following conditions: initial denaturing at

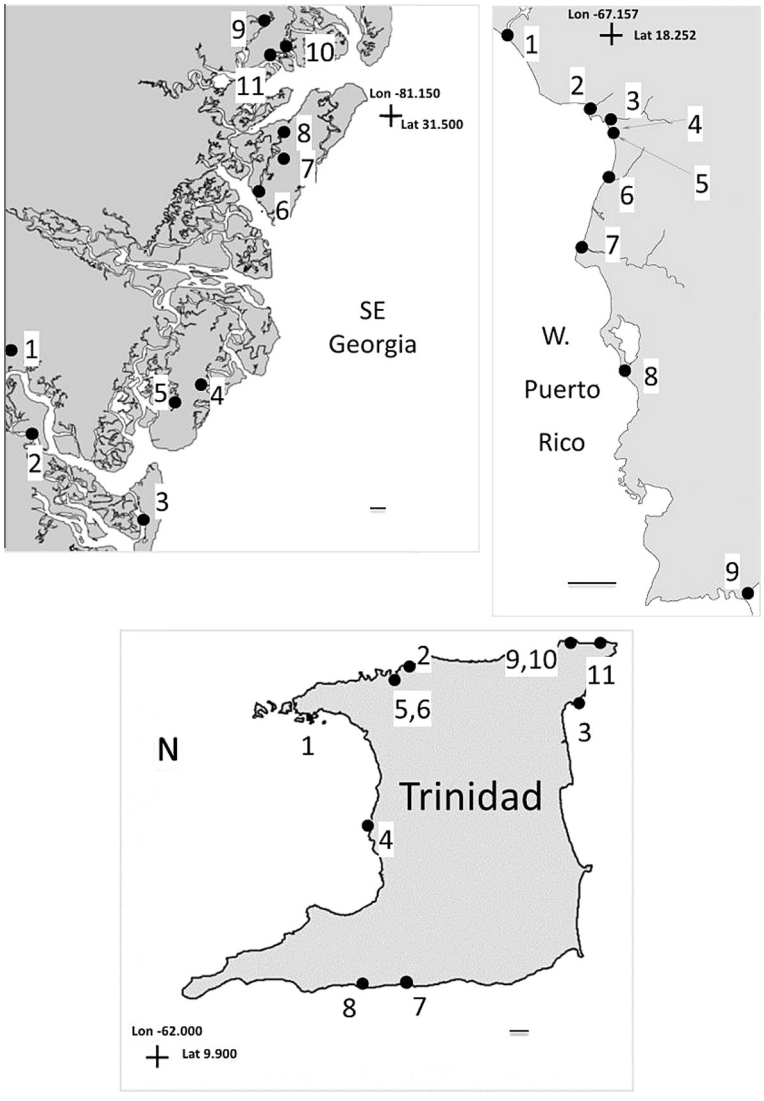

Fig. 1. Sampling locations in Georgia, Puerto Rico and Trinidad. Sampled locations are referenced in Tables 1–3 respectively. Horizontal scale bar = 2 km.