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Baseline

Detection of human-derived fecal contamination in Puerto Rico using carbamazepine, HF183 *Bacteroides*, and fecal indicator bacteria

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ABSTRACT

The level of fecal pollution in 17 sites in Puerto Rico was determined by *Escherichia coli* (*E. coli*) enumeration using an enzyme substrate medium and Quanti-Tray®/2000. Human fecal pollution was identified using an enzyme-linked immunosorbent assay for the detection of carbamazepine (CBZ) and quantitative polymerase chain reaction (qPCR) detection of the human *Bacteroides* marker, HF183. Carbamazepine was detected in 16 out of 17 sites, including Condado Lagoon, a popular recreational area. Elevated *E. coli* levels (>410 CFU 100 mL^{-1}) were detected in 13 sites. Average CBZ concentrations ranged from 0.005 μg L^{-1} to 0.482 μg L^{-1} and 7 sites were positive for HF183. Higher CBZ concentrations were associated with the detection of HF183 (Mann–Whitney test; U = 42.0; df = 7; 1-tailed P value = 0.013). This was the second study to determine surface water concentrations of CBZ in the Caribbean and the first in Puerto Rico.

Many water bodies in the US are contaminated with sewage and are considered impaired due to elevated bacteria levels (US EPA, 2012a). Fecal indicator bacteria are used to monitor recreational waters for fecal contamination. High bacterial densities represent the elevated risk that disease-causing pathogens, such as *Escherichia coli* (*E. coli*), *Salmonella*, and *Cryptosporidium*, may be present in the water. Traditional microbial indicators are not source-specific and cannot be used to identify an origin, which is important to help minimize public health risks and prevent future contamination.

The United States Environmental Protection Agency (US EPA) currently recommends using *E. coli* to detect fecal contamination in recreational fresh water (USEPA, 2012b). Results from epidemiological studies show that *E. coli* correlates well with swimming-associated gastroenteritis and should be used instead of fecal coliforms to provide improved public health protection (USEPA, 1986, 2012b). *E. coli* is a more specific fecal indicator than fecal coliforms, but several limitations still make it an unsuitable wastewater marker. *E. coli* has been detected in pristine tropical environments (Hazen, 1988; Bermúdez and Hazen, 1988), can multiply in sediments (Lopez-Torres et al., 1987; Solo-Gabriele et al., 2000; Desmarais et al., 2002), and is present in the intestinal tract of all warm-blooded animals and thus is not source-specific. The combined use of source-specific indicators and traditional microbial indicators to detect fecal contamination would provide a more detailed and useful water quality assessment.

Traditional microbial indicators cannot be used to discriminate between human and other animal sources (Seidler et al., 1981; Lopez-Torres et al., 1987; Oshiro and Fujioka, 1995). The use of complementary, source-specific indicators would help prevent future contamination and protect public health. Recent technological advances in polymerase chain reaction (PCR) and microbial source tracking analyses have made it possible to rapidly detect bacteria that can identify the source (Green et al., 2014). HF183 *Bacteroides* is specific to sewage and can reliably detect human fecal pollution (Ahmed et al., 2008). Despite technological advances, widespread applicability of PCR is still limited because of the high cost of instrumentation and expertise needed to run the tests. A source-specific, cost- and time-effective method would be convenient to quickly detect the potential for fecal contamination and efficiently allocate resources for confirmation and remediation of the problem.

Chemical detection methods may offer several advantages over microbial methods. Ideal chemical indicators generally require a shorter analysis, are not found naturally in the environment, and are source-specific. Carbamazepine (CBZ), an anti-epileptic, is one of the most ubiquitous and persistent pharmaceuticals in the environment (Lin and Reinhard, 2005; Zhang et al., 2008; Calisto et al., 2011). Over 1000 tons of CBZ is consumed worldwide (Zhang et al., 2008). Metabolites and unchanged forms of the parent compound are excreted in human waste and transported to sewage treatment plants. Carbamazepine is not significantly biodegraded or removed during sewage treatment sludge processes (Clara et al., 2005; Joss et al., 2006), and has been proposed as a cumulative wastewater discharge indicator due to

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its long half-life (Madoux-Humery et al., 2013). Photodegradation is an important removal process in surface waters for compounds like CBZ that are refractory to biodegradation. Many studies have been conducted to estimate the photodegradation rate of CBZ, but differences in methodologies result in a wide range of photochemical half-lives being reported, ranging from hours (Andreozzi et al., 2002; Matamoros et al., 2009) to weeks (Laurentiis et al., 2012). Calisto et al. (2011) reported that CBZ could persist in the environment from 4.5 to 25 days. Thus, CBZ is not significantly degraded or removed during wastewater treatment and enters the environment where it is relatively resistant to photodegradation and persistent.

Because of its resistance to degradation, CBZ has been detected in various environmental matrices from surface water to drinking water wells (Ternes, 1998; Heberer et al., 2002; Kolpin et al. 2002; Clara et al., 2004; Fenz et al., 2005; Hua et al., 2006; Bahlmann et al., 2009). Carbamazepine has been detected in surface water with concentrations ranging from 0.0023 to 1.075 μg L^{-1} (Heberer et al., 2002; Kolpin et al., 2002; Metcalfe et al., 2003; Calisto et al., 2011; Bahlmann et al., 2012). Carbamazepine is not easily degraded or adsorbed and can reach an aquifer (Clara et al., 2004). In Germany, Osenbrück et al. (2007) detected concentrations up to 0.083 μg L^{-1} in groundwater and found that river water infiltration was the main factor leading to CBZ presence in groundwater. Rabiet et al. (2006) detected CBZ concentrations of 0.0432 and 0.0139 μg L^{-1} in 2 out of 7 drinking water wells investigated in the Mediterranean region. The presence and persistence of CBZ in a variety of environments and its sole use as a pharmaceutical make CBZ a suitable human-specific fecal indicator (Clara et al., 2004, 2005; Benotti and Brownawell, 2007; Doummar et al., 2014; Tran et al., 2014a).

The primary objective of this study was to quantify CBZ in tropical surface waters throughout Puerto Rico in order to assess the incidence of human-derived fecal contamination. The secondary objective was to examine the potential relationship between CBZ concentrations and fecal indicator bacteria. Surface water samples were collected in replicates from 17 stations throughout Puerto Rico (Fig. 1) in November and December 2014 (wet season). Rivers, creeks, and lagoons were sampled and the majority of locations (ca. 70%) were in urban or semi-urban areas. Potentially contaminated sites in urban locations were targeted. Rural locations were also sampled to assess the relationship between CBZ concentrations and population density. One stream, located on a mountain summit near Cayey, was the only non-coastal site sampled, and served as a negative control. The Río Guayanilla site was downstream from the Guayanilla Wastewater Treatment Plant sewage outfall. Whirl-Pak® bags attached to a line sampler were used to collect the samples. Care was taken to avoid disturbing the bottom sediment. The bags were sealed, placed into gallon size re-sealable bags, preserved on ice, and transported to the laboratory. Microbiological analysis was carried out within 6 h of the sampling. Colilert® media and Quanti-Tray®/2000 were used to determine most probable numbers (MPNs) of *E. coli* (USEPA, 2007). IDEXX MPN Generator Software Version 3.2 was used to estimate final densities as CFU/100 mL.

A quantitative PCR-based (qPCR) analysis was used to detect HF183. Aliquots (100 mL) were filtered through 0.2 μm 47 mm o.d. polycarbonate filters (Osmonics). Particles on filters were frozen (−80 °C) in plastic tubes and shipped on dry ice to Georgia College and State University, Georgia for further analysis. A MoBio UltracleanTM Soil DNA Kit was used to process the filters, and a modified version of the manufacturer's instructions was followed (Holman et al., 2014). Extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer. Quantitative PCR assays were conducted on a CFX 9600 (Bio RAD). All primers used in this study were optimized to avoid non-specific cross-reaction and increase specificity. The qPCR assay used a modified protocol of Haugland et al. (2010); with *Bacteroidesdorei* DSM 17855 (DSMZ) as a positive control and *E. coli* strain B from Sigma® D48890-1UN as a negative control. The assay contained 1 μM of each primer, 0.2 mg of bovine serum albumin (Sigma), 80 nM Fam™ labeled TaqMan® probe, 9 μL of deionized water, and 1 μL of sample DNA. The samples were run at: 95 °C for 15 min; 40 cycles at 95 °C for 10 s and 66.3 °C for 40 s (Haugland et al., 2010). Standard curves and negative and positive controls were included during each run. The qPCR detection limit was 25 gene copies 100 mL^{-1}.

CBZ concentration was determined using the remaining portion of sample that was not used during the microbiological analysis. Solid phase extraction was used to concentrate CBZ and clean up the samples. Pre-concentration is an important step when targeting compounds that may be significantly diluted in the aquatic environment and close to the

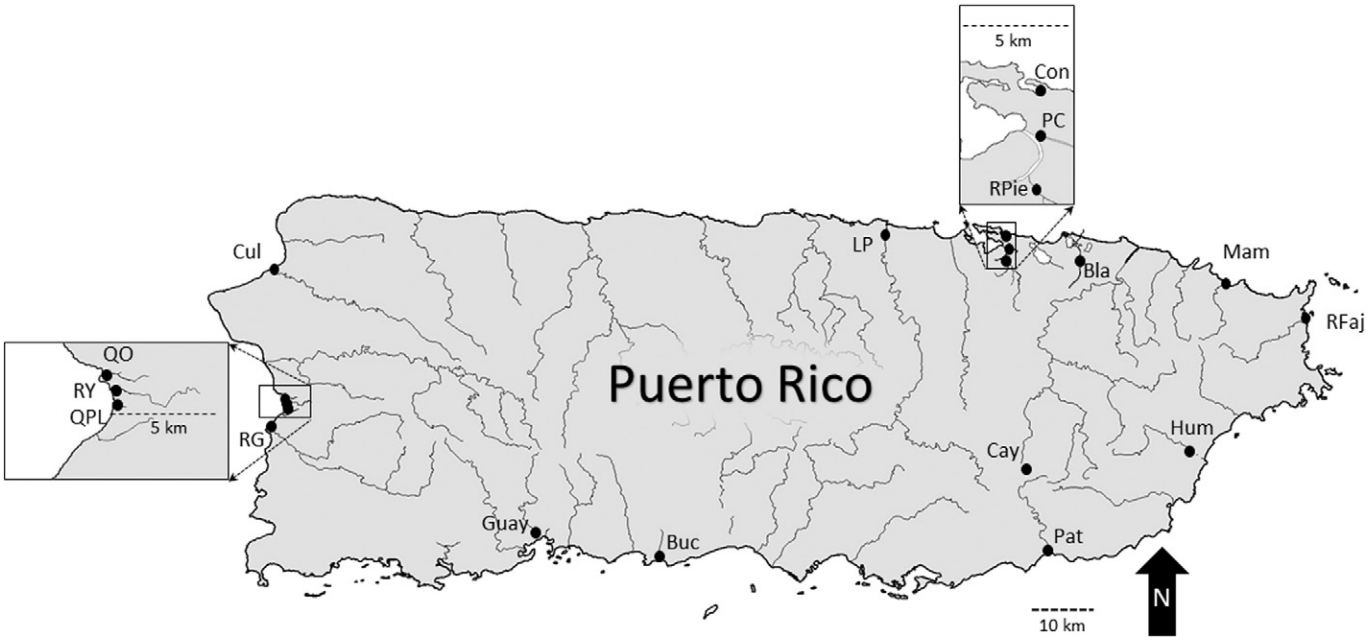

Fig. 1. Sampling Locations: Cay (Cayey Stream), Mam (Mameyes), Cul (Culebrinas), Hum (Río Humacao), Pat (Río Patillas), Con (Condado Lagoon), RY (Río Yaguez), Buc (Río Bucaná), RPie (Río Piedras), Bla (Blasina), PC (Parque Central), RFaj (Río Fajardo), RG (Río Guanajibo), LP (La Plata), QPL (Quebrada Parque Litoral), QO (Quebrada de Oro), Guay (Río Guayanilla). Latitude and longitude for stations are provided in Table 1.