Identifying the primary sources of fecal contamination along the beaches and rivers of Trinidad

Ronell S. H. Bridgemohan, Dave S. Bachoon, Yingfan Wang, Puran Bridgemohan, Christine Mutiti and Adesh Ramsubhag

ABSTRACT

The aim of this study was to identify the main sources of fecal pollution at popular beaches and rivers in the island of Trinidad. *Escherichia coli* enumeration and microbial source tracking (MST) were used to identify the primary sources of fecal bacteria contamination at the sites. Nineteen sites exceeded USEPA water quality standards for safe recreational use. Highest levels of fecal contamination were recorded on the central and west coasts of the island and included Brickfield River (4,839 MPN 100 ml^{-1}), Orange Valley Bay (2,406.6 MPN 100 ml^{-1}) and Chaguaramas Bay (1,921.2 MPN 100 ml^{-1}). MST detected human (HF183) fecal pollution at ~63%, birds at ~67%, chicken at ~36% and cattle (BacCow) at ~34% of the sites. MST is a useful and rapid method for identifying major sources of fecal pollution in rivers and beaches. In Trinidad water bodies, the main sources of fecal pollution were humans and birds. The large number of sites with elevated levels of fecal pollution detected is particularly alarming and represents a serious public health risk.

Key words | *Bacteroidales*, *E. coli*, fecal pollution, source tracking, Trinidad, water

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INTRODUCTION

In the Caribbean, fecal contamination of freshwater and marine environments is a growing threat to human health, tourism and the food industry (Shuval 2003; Walker *et al.* 2013; Institute of Marine Affairs (IMA) 2016). In the island of Trinidad, heavy industrial development and a long period of agricultural development have contributed to the input of fecal bacteria in freshwater, estuarine and marine systems (Alleng 2007; Bachoon *et al.* 2010). Over the past two decades, there have been several reports of high levels of fecal contamination at some of the popular beaches, rivers and swamps (Rampersad *et al.* 1999; Bachoon *et al.* 2010; Environment Management Association (EMA) 2011; Institute of Marine Affairs (IMA) 2016). The Trinidad and Tobago IMA attributed the presence of fecal contamination at beaches and rivers to seepage of sewage from pit latrines situated along river banks and coastlines, nonfunctional sewage treatment plants, poorly constructed septic tanks and run-off from livestock farming operations (EMA 2011; IMA 2016). As such, contamination of the island's marine and freshwater environments with high levels of fecal pollution, and possibly pathogenic bacteria from human and non-human sources, can present a public health concern. However, in Trinidad, routine monitoring of the level of fecal pollution in rivers and beaches is not conducted and watershed management plans are lacking.

In water quality management systems, regulatory agencies generally focus on fecal indicator bacteria (FIB) and identifying the source of the FIB to adequately assess human health risks and develop watershed management plans (Amador *et al.* 2008; Bachoon *et al.* 2010; US EPA

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2012). A good FIB should meet certain criteria: easily testable, must be of either animal or human sources, survival time should be close to or longer than pathogens, ability to indicate the presence of several pathogens, and can be used in different water environments such as marine or freshwater (USEPA 1986; USEPA 2006; Sinigalliano *et al.* 2007; Staley *et al.* 2013). There is no perfect FIB for recreational water, but the majority of regulatory agencies have opted to use either fecal coliforms (e.g. *Escherichia coli*) or enterococci as indicators for pathogens in surface waters (Palmer *et al.* 1993; US EPA 2012; Walker *et al.* 2013). The acceptable levels of these fecal indicators have been determined during epidemiological studies in the United States, but similar studies have not been conducted in the tropics (USEPA 1986). Although no standard for *E. coli* has been adopted for marine waters, the geometric mean standard for freshwater should be <126 CFU 100 ml^{-1} and the statistical threshold value (STV), which is a value that approximates the 90th percentile of the water quality distribution and should not be exceeded by more than 10 percent of the samples taken, fluctuates from 320 to 410 CFU 100 ml^{-1} (USEPA 2012). For marine waters designated as swimming areas, the geometric mean of enterococci should not exceed 35 CFU 100 ml^{-1} (USEPA 2012). Bachoon *et al.* (2010) reported that 7 of 14 sites sampled in Trinidad, including Maracas Bay which is a major public beach, contained unsafe fecal contamination based on USEPA established thresholds. Unfortunately, FIB enumeration cannot be used for fecal source identification; however, establishing the source of fecal pollution is critical to determine the public health risk associated with fecal pollution events.

Recently, several microbial source tracking (MST) methodologies have been developed based on the knowledge that some *Bacteroidales* species exhibit host specificity (Hemandez *et al.* 2013; Symonds *et al.* 2017; Zang *et al.* 2019). In Trinidad, human fecal pollution is often implicated as the main source of elevated levels of fecal pollution in rivers and beaches, but these assertions are often made without the fecal source tracking analysis (IMA 2016). Previous MST at 14 coastal sites linked human fecal bacteria to only one location (Bachoon *et al.* 2010), indicating the possibility of non-point sources of animal fecal pollution, impacting many rivers and beaches on the island. The detection of animal fecal sources is relevant to public health because it is well established that they carry many zoonotic pathogens (Wade *et al.* 2015; Holman *et al.* 2014; Walker *et al.* 2013). For example, *E. coli* O157:H7, a zoonotic pathogen, was detected in 2% of oyster samples tested in Western Trinidad (Rampersad *et al.* 1999) and at two fishing bays (Walker *et al.* 2013). Among the most high-risk and likely sources of fecal pollution in Trinidad are human, livestock (cattle, pigs, goat and chicken) and wildlife (e.g. birds). In 2010, a limited scope MST for human and cattle sources of fecal contamination detected human fecal pollution at only Cali Bay on the central west coast of the island (Bachoon *et al.* 2010). Currently, MST-polymerase chain reaction (PCR) assays have been developed for the detection of human and a wide range of animal fecal sources (Lu *et al.* 2007; Bachoon *et al.* 2010; Boehm *et al.* 2013; Hemandez *et al.* 2013). One of the most often used MST markers for human fecal pollution is HF183 for *Bacteroides dorei* (Haugland *et al.* 2010; Hernandez *et al.* 2013). Today, there are MST markers for many animal sources of fecal contamination, including chicken and other birds (CP1F/R), chicken (CBR-42F) and cattle (BacCow) (Lu *et al.* 2007; Haughland *et al.* 2010). Consequently, it is now possible to conduct a more inclusive study of the likely sources of fecal contamination of water bodies in Trinidad.

The aim of this study was to conduct an island-wide survey of the level and sources of fecal contamination in Trinidad. Thirty-five sampling sites, including 23 marine and 12 freshwater locations were surveyed and for each location, physiochemical parameters were recorded, *E. coli* levels were determined, and MST assays for human, cattle and birds were conducted. Information obtained will be useful for public health risk assessment and for guiding the development of the most appropriate mitigation steps to improve water quality on the island.

MATERIALS AND METHOD

Sampling sites

In June 2017, duplicate water samples were collected in sterile polypropylene bottles from 23 coastal marine sites and 12 either freshwater or brackish sites, kept on ice and processed within 6 h. The majority of the marine and coastal

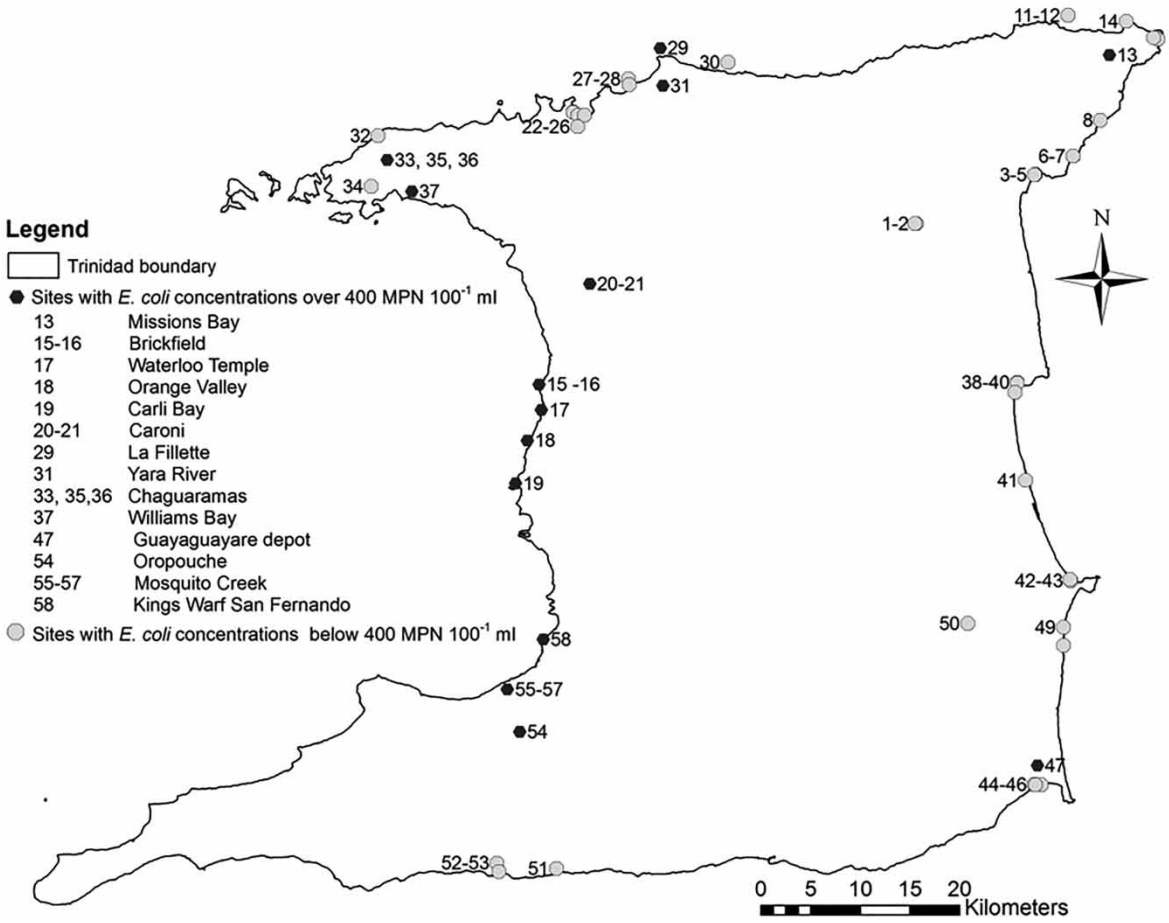

Figure 1 | Map of sampling sites in Trinidad and sites where elevated levels of *E. coli* concentration were recorded.

sites are popular recreational and tourists attractions, and some are used for commercial fishing (Figure 1). Samples were collected from the Caroni Swamp in the area of the popular Bird Sanctuary (bird rookery) and from rivers, including the Valencia, Salybia, Brickfield, Manzanilla 'Le Branche', Nariva, Ortoire, Guayaguayare, Oropouche, Maracas and Yarra. Popular beaches sampled included Maracas Bay, Las Cuevas Bay, Macqueripe Bay, Mayaro beach, Quinam and Chaguaramas Bay. Samples were also collected from fishing villages, including Brickfield Fishing Bay, Carli Bay, Orange Valley Fishing Bay and Waterloo in the Gulf of Paria (West coast) and Toco Bay, Balandra Bay, San Souci Bay, Mission Bay and Rampalanagas Bay in the Northeastern coast of the island. Although situated in rural areas, some sites were generally impacted by human settlements, subsistence farming, livestock and poultry production. Sites were classified as urban, suburban and rural based on knowledge of existing population density. A YSI Pro plus Multiparameter meter (YSI incorporated, USA) was used to collect temperature, salinity, pH and dissolved oxygen values for water column profiles at each site.

E. coli enumeration and DNA extraction

Processing of samples was done at the Mycology and Microbiology Laboratory, Department of Life Sciences, the University of the West Indies, St. Augustine Campus, Trinidad. One hundred milliliters of each sample was analyzed for *E. coli*, using USEPA Standard Method 9223, Colilert™ media sealed using a Quanti-Tray sealer and incubated at 35.5 °C for 18 h (USEPA 2007). Fluorescent cells were considered positive for *E. coli* colonies, and total counts were determined using quantification tables from the manufacturer (IDEXX Laboratories Inc., Westbrook, ME, USA).

For DNA extraction, water samples (100 ml) were filtered through a 0.45-μm-pore nitrocellulose membrane filter (Type GS, Millipore, Billerica, MA, USA), and the filters were frozen (−20 °C) and shipped frozen by overnight courier to Georgia College and State University, Milledgeville Georgia. The filters were processed with the MoBio UltracleanTM Soil DNA Kit (Carlsbad, CA, USA) using a modified protocol (Bachoon *et al.* 2010), and the extracted DNA was quantified using a Nanodrop ND-1000 Spectrophotometer. The $A_{260/280}$ ratio was determined as an indicator of samples' purity (Wilmington, DE). DNA samples were stored at −20 °C until further use.

Microbial source tracking

Quantitative PCR assays were conducted on a CFX 9600 (Bio RAD) Real-Time PCR system for the detection of human and animal fecal bacteria (Table 1). All primers used in this study were based on sensitivity and selectivity cited previously (Table 1), optimized to avoid nonspecific cross reaction against the appropriate non-target fecal DNA from chicken, cattle, goat and human (with local samples), and increased specificity. PCR assays were conducted with 1 μl of sample DNA (approximately 10 ng/μl) and a ten-fold diluted DNA sample with annealing temperatures and primer/probe sequences for each marker gene as listed in Table 1. Standard curves for each quantitative PCR (qPCR) (controls and samples) were linear and had coefficients of determination (r^2) of 0.99. Negative controls without DNA, which were run with each reaction, always exceeded the cycle threshold at a mean C_q of 39.58. Each hydrolysis probe was labeled at the 5′ end with the reporter dye 6-FAM (6-carboxy-fluorescein) and at the 3′ end with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine). For human (HF183), the qPCR assay used a modified protocol of Haugland *et al.* (2010); with *Bacteroides dorei* DSM 17855 (DSMZ) and human sewage used as a positive control and *E. coli* strain B from Sigma® D48890-1UN, cattle and chicken fecal DNA extracts (Trinidad samples) as a negative control. The 25 μl assay contained 0.25 μM of each primer, 0.2 mg of bovine serum albumin (Sigma) and 80 nM of the 6-FAM™ labeled Hydrolysis® probe. Water samples were assessed for possible PCR inhibition by amending with positive control bacteria DNA as described by Bachoon *et al.* (2010). Changes of less than two CT values were observed, which indicates that the extracted DNA did not contain impurities that significantly inhibited the MST assay (Bustin *et al.* 2009; Bachoon *et al.* 2010). In addition, ten-fold dilutions of sample DNA extracts were compared to the undiluted sample DNA extract and a change in the CT shift of <3 CT indicated no significant PCR inhibition (Dick *et al.* 2010).

The samples were run at 95 °C for 15 min, 40 cycles at 95 °C for 10 s, and annealing/extension temperature listed in Table 1 (Wade *et al.* 2015). The qPCR detection limits were determined from the lowest concentration of target

Table 1 | Primers and probes used for MST of fecal contamination

Target	Primer	Sequence	Annealing temp. (°C)	Reference
Human	HF-183-1	ATCATGAGTTCACATGTCCG	60	Haugland *et al.* (2010)
	BtheR1	CGTAGGAGTTTGGACCGTGT		
	PROBE	6FAM-CTGAGGAGAAGGTCCCCCACATTGGA-TAMRA		
Cattle	BacCowF	CCAACYTTCCCGWTACTC	57	Bernhard & Field (2000)
	BacCowR	GGACCGTGTCTCAGTTCCAGTG		
	PROBE	6FAM-TAGGGGTTCTGAGAGGAAGGTCCCCC-TAMRA		
Chicken and other birds	CP1F	GGCAGGCATCAAGTCAACA	64.5	Lu *et al.* (2007)
	CP1R	TGGCAAAAGCAACTGTCATGG		
Chicken	CBR-42F	GACGAGATCTATATTTGCCTCA	60	Lu *et al.* (2007)
	CBR-42R	CGGAGCATATCCTACGATCA		

DNA that could be detected at 95% confidence and gene copies ranged from human 8, cattle 20, chicken and other birds 12 and chicken 25 gene copies.

The hydrolysis assays for cattle (BacCow) were performed as above (Bernard & Field 2000; Bradshaw *et al.* 2016). EvaGreen dye-based assays were used for the detection of chicken and bird fecal pollution with annealing temperatures as specified in Table 1, and following the protocol of Lu *et al.* (2007). For dye-based assays, the melt curve analysis was performed to confirm positive detection.

RESULTS

Physiochemical parameters

The observed temperature, pH, dissolved oxygen (DO) and salinity values at the sampling sites were typical for the island (Khatri & Tyagi 2015; IMA 2016). The mean water temperature ranged from 25.7 to 39.1 °C with an average temperature of 28.6 °C. DO of all sites ranged from 0.32 to 9.62 mg/l and had an average of 6.17 mg/l. The lowest (DO) value was found at the Brickfield River in the Central/West coast and North-East coast sample sites and the highest at Toco Bay sites. The pH ranged from 8.14 to 9.22 at Oropouche/Gordineau River and Yarra River, respectively, and had an average pH of 8.75. Salinity at the freshwater sites ranged from 0.61 to 5.30 ppt and averaged 25.8 ppt at marine sampling sites.

Fecal bacteria enumeration

E. coli were enumerated at each sampling site based on USEPA recommendations STV (320–410 CFU 100 ml^{-1}) (US EPA 2012), and sites exceeding an STV of 400 CFU 100 ml^{-1} were deemed unsafe for recreational purposes. Along the densely populated central western region, *E. coli* counts at all sites exceeded 400 MPN 100 ml^{-1} and ranged from 541.2 MPN 100 ml^{-1} at Carli Bay to 4,839.2 MPN 100 ml^{-1} at Brickfield River (Table 2, Figure 1). *E. coli* levels were relatively low for the less densely populated North-East coast sampling sites (MPN ranged from 43.2 to 416.8 100 ml^{-1}), and 12 sites had *E. coli* concentrations below 200 MPN 100 ml^{-1}. Six sites along the north coast of the island, including Chaguaramas Bay, Site 2 at the Trinidad and Tobago Yacht Sailing Association (TTYSA) boat dock and Williams Bay exceeded 400 MPN 100 ml^{-1} (Table 2). Along the East Coast, *E. coli* counts were the highest at Guayaguayare fishing depot and the lowest at Mayaro public beach. In the southern region, *E. coli* levels were between 62.3 and 1,149.6 MPN 100 ml^{-1}. The Mosquito Creek sites exceeded 400 MPN 100 ml^{-1} (Figure 1, Table 2). Overall, the majority of the 19 sites that had exceedingly high *E. coli* levels were on the western and central regions of the island (Figure 1).

MST: The PCR assay CP1 for *Clostridium tetani* of the avian (chicken and other birds) origin indicated that birds were the most common source (65.52%) of fecal pollution to water systems on the island. The chicken-specific marker (CP29F/R) suggested that over half of the sites (32.38%) impacted by bird fecal pollution contained chicken fecal bacteria (Table 2). The highest concentration of fecal pollution from avian sources was detected along the western coast of Trinidad. Human fecal *Bacteroidales* (HF-183) pollution was detected at 63.8% of the sites and was more predominant along the western region of the island. Similar trends were found for cattle BacCow at (34.48%) sites (Table 2). In addition, over 20% of the sampling areas along the island were impacted by human, avian and ruminant fecal pollution, and at 12% (7 sites), the MST assays used did not detect any human or animal sources of fecal pollution.

DISCUSSION

The concentration of FIB (i.e. *E. coli*) is widely used as an indicator to characterize the degree and potential health risk of fecal pollution found in aquatic environments (Bachoon *et al.* 2010). However, studies in tropical water systems have suggested that FIB may not always originate from fecal sources (Santo-Domingo *et al.* 1989). In spite of these reports, researchers and regulatory agencies in the tropics continue to use FIB concentrations for monitoring or detecting fecal pollution. Previous research in Trinidad has indicated varying levels of marine and freshwater quality with many popular beaches,

Table 2 | *E. coli* concentration and detection of MST markers for human (HF183), chicken and other birds (CP1F/CP1R), chicken (CP29F), ruminant (Rum-2-bac), and cattle (BacCow) in the water samples

Station	Site	*E. coli* (MPN/100 ml)	Human	Avian	Chicken	Cattle	Classification
T1	Valencia Middle Course	56.8	+	+	+	+	R
T2	Valencia Lower Course	76.2	–	+	–	–	R
T3	Salybia river	187.6	+	–	–	–	R
T4	Salybia river mouth	157.8	–	–	–	–	R
T5	Salybia bay	74.2	–	–	–	–	R
T6	Balandra Bay Site One	43.2	+	–	–	–	R
T7	Balandra Bay Site Two	46.6	–	–	–	–	R
T8	Rampalanagas beach	387.4	+	+	–	–	R
T9	Toco Bay Site One	72.6	+	+	–	–	R
T10	Toco Bay Site Two	106.8	–	+	–	–	R
T11	San Souci Bay Site One	110	–	+	–	–	R
T12	San Souci Bay Site Two	105.4	–	–	–	–	R
T13	Missions Bay Site One	416.8	+	–	–	–	R
T14	Missions Bay Site Two	387.2	+	–	–	–	R
T15	Brickfield Fishing Bay	3,972.6	+	+	+	+	R
T16	Brickfield River	4,839.2	+	+	+	+	R
T17	Waterloo Sea	2,599.4	+	+	+	+	R
T18	Orange Valley Fishing Bay	2,406.6	+	+	+	+	R
T19	Carli Bay	541.2	–	+	+	+	R
T20	Caroni Swamp Boat Dock Site One	784.4	+	+	+	+	R
T21	Caroni Swamp Site Two	1,072.8	+	+	+	+	R
T22	Maracas River	240.5	+	+	+	+	R
T23	Maracas River Mouth	312.3	+	+	+	+	R
T24	Maracas Beach Site One	191.8	+	–	–	–	R
T25	Maracas Beach Site Two	180.9	+	–	–	–	R
T26	Tyrico Bay	60.4	–	–	–	–	R
T27	Las Cuevas Bay Site One	277.8	+	+	+	+	R
T28	Las Cuevas Bay Site Two	322.3	+	+	+	+	R
T29	La Fillette Beach	472.1	–	–	–	–	R
T30	Blanchisseuse Beach	243.6	–	+	+	–	R
T31	Yara River	2,419.6	+	+	–	–	R
T32	Macqueripe Bay	170.8	–		–	–	SU
T33	Chaguaramas Bay TTYSA Site One	1,921.2	+	+	–	+	SU
T34	Chaguarama Bay TTYSA Site Two	NA	+	+	–	–	SU SU
T35	Chaguaramas Bay Boardwalk Site One	629.4	+	+	+	+	SU
T36	Chaguaramas Bay Boardwalk Site Two	601.5	+	+	+	–	SU
T37	Williams Bay	550.4	+	+	–	+	SU
T38	Manzanilla 'Le Branche' River Mouth	231	+	+	–	–	R

(*continued*)

Table 2 | continued

Station	Site	*E. coli* (MPN/100 ml)	Human	Avian	Chicken	Cattle	Classification
T39	Manzanilla Fishing Bay	189.4	–	–	–	–	R
T40	Manzanilla Boardwalk	143.7	+	+	–	–	R
T41	Nariva River Mouth	103.1	–	+	+	+	R
T42	Ortoire River Mouth	181.1	–	+	–	+	R
T43	Ortoire River Lower Course	169.1	–	–	+	–	R
T44	Guayaguayare River	281.2	–	+	–	+	R
T45	Guayaguayare Beach/Industrial	150.6	–	–	–	–	R
T46	Guayaguayare Sea Wall	115.9	+	+	–	–	R
T47	Guayaguayare Fishing Depot	689.3	+	+	–	–	R
T48	Mayaro Beach Site One	59.5	–	+	–	–	R
T49	Mayaro Beach Site Two	65.2	–	–	–	–	R
T50	Mafeking Bridge/Ortoire River Middle Course	381.1	–	+	–	–	R
T51	Morne Diablo Beach	62.3	+	–	–	+	R
T52	Quinam Beach Site One	396.8	+	+	+	–	R
T53	Quinam Beach Site Two	NA	+	–	+	–	R
T54	Oropouche/Godineau River	574.8	+	+	–	–	R
T55	Mosquito Creek River Mouth/Godineau Site One	629.4	+	+	–	+	R
T56	Mosquito Creek Site Two	960.6	+	+	+	–	R
T57	Mosquito Creek Site Three	1,011.2	+	+	+	–	R
T58	Kings Warf San Fernando	1,149.6	+	+	–	–	U

R: rural, SU: suburban, U: urban.
A positive (+) detection was based on if any sample or its replicate had a threshold above the limit of detection determined by the standard curve of the assay.

including Maracas Bay, exhibiting unsafe levels of FIB, while other rivers and beaches on the island have relatively low levels of fecal bacteria (Bachoon *et al.* 2010; EMA 2011; IMA 2016). Quantitative *E. coli* results (Table 2) from the current study show that the water samples from most beaches along the north and eastern coast of the island, including Maracas, Las Cuevas and Tyrico Beach, had relatively low (<200 MPN 100 ml^{-1}) or safe levels (USEPA 2012) of fecal pollution for recreational use. This may be attributed to the low density of human development and the lack of large-scale animal farming activity or significant crop production involving the use of animal manure in the northern range of the island. In contrast, high levels of *E. coli* (>400 MPN 100 ml^{-1}) were detected at 22 sites; the majority of these sites were concentrated along the western coast where there is high density of human population along with activities such as fishing and farming of chicken, cattle or goats and field vegetable production with the use of cattle or chicken manure (Figure 1). Furthermore, many of the homes in Waterloo, Brickfield and Orange Valley fishing bays have outhouses and outdated septic systems. Previous FIB monitoring in the central region of Trinidad detected high levels (>1,600 CFU ml^{-1}) of *E. coli* at Cali Bay (Bachoon *et al.* 2010; Walker *et al.* 2013). Another sampling area with high levels of *E. coli* was in the Caroni swamp. This is not surprising because the Caroni swamp is a national bird sanctuary surrounded by agricultural and residential activities. This finding is consistent with previous studies that documented high levels of *E. coli* (> 3,000 CFU ml^{-1}) in this area (Bachoon *et al.* 2010; Walker *et al.* 2013). At sites such as Chaguaramas Bay and Kings Wharf with high levels of *E. coli* but no animal farming or wildlife habitats, human fecal bacteria are the likely source of the pollution. Unfortunately, due to limited sample collection, we were not able to conduct a more

extensive or seasonal sampling regime of fecal pollution on the island.

In marine and freshwater systems, human fecal pollution is considered to carry a higher public health risk than contamination originating from non-human fecal sources (Holman *et al.* 2014; Bradshaw *et al.* 2016). The HF-183 PCR assay for human fecal pollution is widely accepted as a reliable and sensitive PCR assay for the detection of human fecal bacteria contamination (Bernard & Field 2000; Krentz *et al.* 2013). It was alarming that >63% of the sites had detectable levels of human fecal (HF-183) contamination, including North Coast (6), North East (7), Central/West Coast (6), East Coast (4) and all sites in the south of the island (Table 2). Carli Bay was the only site in the Central/West sampling area that was not contaminated with human fecal pollution. This was surprising because Bachoon *et al.* (2010) detected human fecal pollution at Carli Bay using the Hubac marker. It is possible that the detection of human fecal pollution using the *Bacteroidales* Hubac assay in 2010 was caused by non-specific amplification of non-human fecal bacteria (Boehm *et al.* 2013). In addition, a recent study in Costa Rica indicated that the HF-183 assay was less than 100% specific at detecting human fecal pollution (Symonds *et al.* 2017). However, the absence of human fecal bacteria in Carli Bay could also be attributed to the recent addition of a public restroom facility at the site. The other regions of the island (Table 2) where human fecal pollution was detected were close to major cities such as Port of Spain in the north or from rural villages along the southern and eastern coastline. Eighteen of the sites with human fecal contamination exceeded 410 MPN ml^{-1} and would be considered unsafe for recreational use based on USEPA water quality standards (US EPA 2012). Fortunately, these 18 sites did not include any of the popular bathing beaches (Table 2).

Trinidad and Tobago is known for an extraordinary amount of bird species that include both resident and migrant inhabitants (Terborgh 1974; Juman *et al.* 2013). The island's avian population is estimated to be approximately 28.5 million birds mainly domestic chickens (*Gallus domesticus allus domesticus*) and Muscovy ducks (*Carina moschata arina moschata*) (Baboolal *et al.* 2012). Consequently, it was not surprising that approximately 67% of the sampling sites had bird fecal pollution (CP1), especially in the central region of the island where avian fecal bacteria were detected at all sites, including the Caroni swamp (Table 2). The high incident of avian fecal pollution is troubling, even though avian feces contribute less human pathogens than human feces because birds do harbor a wide range of zoonotic pathogens (Baboolal *et al.* 2012). Further analysis using the MST assay developed by Lu *et al.* (2007) indicated that chicken fecal pollution was present at 36% of the sites and was concentrated in the central region. In central Trinidad, many villagers rear poultry for domestic use and we observed several small-scale poultry meat shops in Brickfield village and Waterloo village. Many of the vegetable farmers in these areas use chicken manure to fertilize their crops which may constitute an additional source of pollution. A few of the beaches (Maracas, Las Cuevas and Toco Bay) in the northeastern region of the island were contaminated with avian fecal bacteria; however, the levels of *E. coli* at these beaches were relatively low (<400 MPN ml^{-1}).

Along with human and birds, cattle/ruminants were expected to be a major source of fecal pollution in Trinidad because many people on the island rare domesticated goats, sheep and cattle. In addition, it is common practice to use cattle manure fertilizer for crop farming. The BacCow assay indicated that at least 34% of the sites contained fecal bacteria of cattle origin, and in general the incidence of cattle fecal pollution was concentrated in the central and western sites on the island. Symonds *et al.* (2017) found that the BacCow had high sensitivity (88%) at detecting cattle fecal pollution in Costa Rician samples but is prone to cross-reaction. Surprisingly, cattle fecal pollution was detected at Maracus River, Las Cuevas and Chaguaramas even though there were no noticeable cattle farms in these areas. Therefore, the detection of cattle fecal pollution at these sites could be attributed to run-off of cattle manure that is widely used in food-crop production in the island. It should be noted that the cattle fecal bacteria marker (BacCow) used in this study has been reported to cross-react with fecal bacteria from other ruminant sources, including deer (Boehm *et al.* 2013). However, deer was not present or common at any of the sampling areas used in this study.

CONCLUSION

The high incident of fecal pollution of water bodies in Trinidad associated with human and bird fecal pollution is particularly alarming and represents a serious public health risk on the island. MST is a promising tool that can be applied to water quality surveys in Trinidad and other islands of the Caribbean to develop appropriate water monitoring and management steps on these islands. Future studies should monitor fecal pollution and use MST technology over a longer time scale to improve the evaluation of fecal pollution in Trinidad.

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CONFLICT OF INTEREST

No conflict of interest declared.

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