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Precipitation influences pathogenic bacteria and antibiotic resistance gene abundance in storm drain outfalls in coastal sub-tropical waters

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ABSTRACT

Stormwater contamination can threaten the health of aquatic ecosystems and human exposed to runoff via nutrient and pathogen influxes. In this study, the concentrations of 11 bacterial pathogens and 47 antibiotic resistance genes (ARGs) were determined by using high-throughput microfluidic qPCR (MFQPCR) in several storm drain outfalls (SDOs) during dry and wet weather in Tampa Bay, Florida, USA. Data generated in this study were also compared with the levels of fecal indicator bacteria (FIB) and sewage-associated molecular markers (i.e., *Bacteroides* HF183 and crAssphage markers) in same SDOs collected in a recent study (Ahmed et al., 2018). Concentration of FIB, sewage-associated markers, bacterial pathogens and many ARGs in water samples were relatively high and SDOs may be potentially hotspots for microbial contamination in Tampa Bay. Mean concentrations of culturable *E. coli* and *Enterococcus* spp. were tenfold higher in wet compared to dry weather. The majority of microbiological contaminants followed this trend. *E. coli eaeA*, encoding the virulence factor intimin, was correlated with levels of 20 ARGs, and was more frequently detected in wet weather than dry weather samples. The bla_{KPC} gene associated with carbapenem resistant *Enterobacteriaceae* and the beta-lactam resistant gene (bla_{NPS}) were only detected in wet weather samples. Frequency of integron genes *Intl2* and *Intl3* detection increased by 42% in wet weather samples. Culturable *E. coli* and *Enterococcus* spp. significantly correlated with 19 of 47 (40%) ARG tested. Sewage-associated markers crAssphage and HF183 significantly correlated ($p < 0.05$) with the following ARGs: *intl1*, *sul1*, *tet*(M), *ampC*, *mexB*, and *tet*(W). The presence of sewage-associated marker genes along with ARGs associated with sewage suggested that aging sewage infrastructure contributed to contaminant loading in the Bay. Further research should focus on collecting spatial and temporal data on the microbiological contaminants especially viruses in SDOs.

1. Introduction

Authorities worldwide are exploring alternative water sources to meet increasing demands for potable (drinking) and non-potable (gardening, landscaping and irrigation) water. Stormwater has been considered as an alternative water source for both potable and non-potable uses (Guizani, 2016). Stormwater capture and reuse can reduce pressure on using a town's water supply and provide an alternative source of water during times of restrictions. Despite these advantages, stormwater has not been widely used as a non-potable source in urban settings, perhaps due to a lack of knowledge about the presence and risks associated with microbiological and chemical contaminants. Public perception of health risks associated with microbiological contaminants, in particular, remains a key barrier to the expansion of stormwater reuse (Higgins et al., 2002). Therefore, there is a need to assess potential public health significant pathogens in stormwater, which can cause acute illnesses in humans, especially among elderly, and immunocompromised populations.

Point and nonpoint contaminant sources such as untreated and treated sewage, defective septic systems, agricultural runoff, and defecation from wild animals are known to degrade microbiological quality of stormwater (Ahmed et al., 2005; Noble et al., 2006; Rajal

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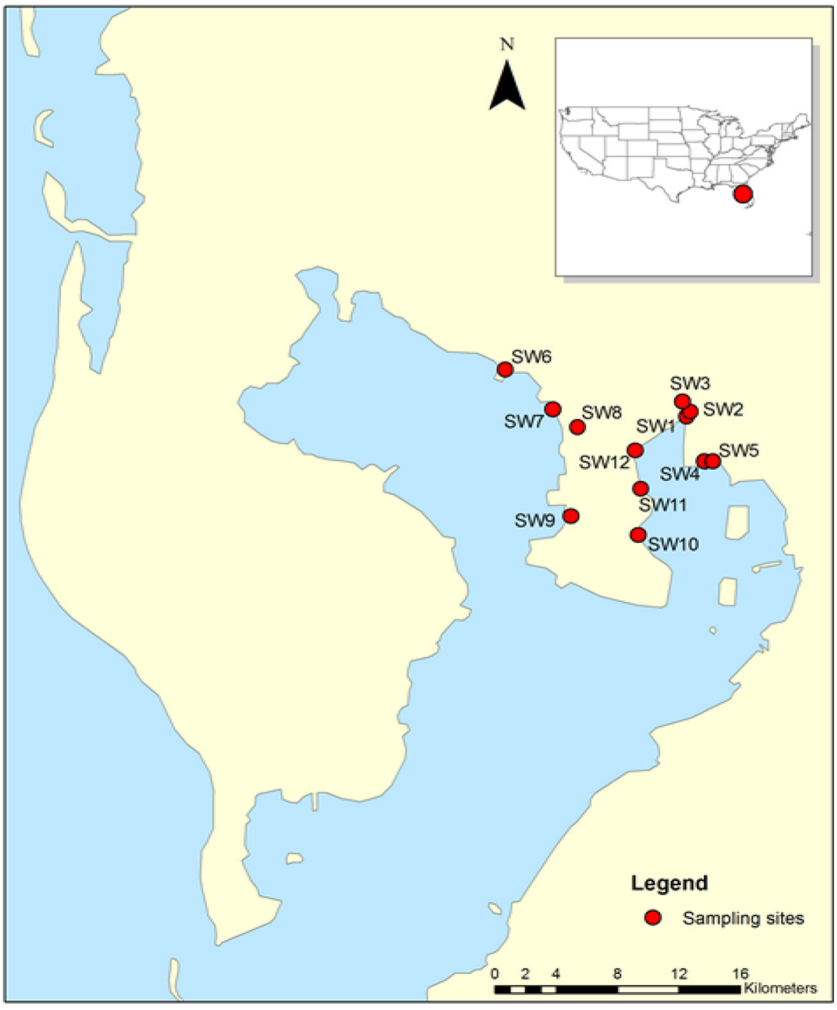

Fig. 1. A map showing the storm drain outfall (SDO) sampling sites in Tampa Bay, Florida.

et al., 2007). Several studies have reported the high prevalence of fecal indicator bacteria (FIB) and enteric pathogens in stormwater (Noble et al., 2006; Rajal et al., 2007; Sidhu et al., 2012; Cizek et al., 2008). One major limitation of FIB is their inability to predict the presence of pathogens in environmental waters, especially protozoa and enteric viruses (Hörman et al., 2004; McQuaig et al., 2009; Selvakumar and Borst, 2006). Another limitation of FIB is that they cannot provide information regarding the sources of fecal pollution (Field and Samadpour, 2007; Stoeckel and Harwood, 2007).

Remediation strategies for microbial pollution from stormwater can be more effectively implemented if the abundance and potential sources of pathogens are known (Sidhu et al., 2012; Harwood et al., 2014). Since the monitoring of FIB in water resources does not provide information on the contamination source(s), e.g. human or animal feces, researchers have developed microbial source tracking (MST) tools. These tools provide information on whether the fecal pollution in water originates from human or animal wastewater or a combination of both (Harwood et al., 2014). Several studies have reported the presence of sewage contamination in stormwater samples by testing bacterial and viral MST markers (Sidhu et al., 2012; Marsalek and Rochfort, 2004; Sauer et al., 2011; Guérineau et al., 2014).

The rapid emergence of antibiotic-resistant bacteria (ARB) is increasing throughout the world (Tripathi and Cytryn, 2017). New resistance genes are evolving and spreading globally, threatening our ability to treat common diseases (Munita and Arias, 2016). Antibiotic resistance genes (ARGs) are currently considered as emerging contaminants (Pruden et al., 2006; Engemann et al., 2008). Several studies have reported the presence of ARGs in ambient environmental waters (Pruden et al., 2006; Zhang et al., 2016). These studies have focused on PCR/qPCR analysis to detect or quantify a small number of ARGs in either viable bacteria or total DNA extracted from water samples. A recent study used qPCR and next-generation sequencing to characterize the abundance of five ARGs in stormwater runoff and investigated the breadth of the resistome detectable during storm events relative to baseline levels (Garner et al., 2017). The results indicated that storm-driven transport contributed significant concentrations of ARGs to surface waters. However, qPCR based methods allow quantification of only a few pathogens or ARGs because the qPCR platform is equipped with a small number of fluorophores.

Ishii et al. (2013) reported the development of a microfluidic qPCR (MFQPCR) method for the simultaneous quantification of various waterborne pathogens in environmental samples. In the MFQPCR system, multiple qPCR assay reagents are mixed with sample DNA in nanoliter-volume chambers that are present in high density on a chip, providing high-throughput qPCR reactions (up to 9216 qPCRs from 96 assays and 96 DNA samples). Therefore, the MFQPCR system can reduce sample analysis time and reagent requirements when compared to conventional qPCR assay. This system has been used to monitor bacterial pathogens in beach water, lake water, sewage effluent, and animal fecal samples from the USA and Japan (Ishii et al., 2013; Ishii et al., 2014; Zhang et al., 2016). This method, however, has not been used to monitor stormwater samples, which likely contain more pathogens than ambient surface water samples. Ishii and colleagues also developed a MFQPCR for simultaneous quantification of dozens of ARGs in sewage and water samples (Sandberg et al., 2018).