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Microbial source tracking markers associated with domestic rainwater harvesting systems: Correlation to indicator organisms

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ABSTRACT

Domestic rainwater harvesting (tank water) systems were screened for the presence of a panel of microbial source tracking (MST) markers and traditional indicator organisms. The indicator organisms were enumerated utilizing traditional culture-based methods, while the MST markers were quantified by quantitative PCR (qPCR). The indicators *Escherichia coli* (*E. coli*) and enterococci were also quantified using qPCR. Correlations and concurrence between these parameters were then investigated to determine which markers could be utilized to supplement traditional indicator analysis. Quantitative PCR analysis indicated that *Bacteroides* HF183, adenovirus, Lachnospiraceae and *E. coli* were detected and quantifiable in 100% of the tank water samples collected throughout the sampling period, while human mitochondrial DNA (mtDNA) was quantifiable in 90% of the tank water samples and *Bifidobacterium adolescentis* (*B. adolescentis*) and enterococci were quantifiable in 67% of the tank water samples, respectively. Significant positive correlations were recorded for Lachnospiraceae versus heterotrophic bacteria ($p = 0.000$), adenovirus versus *E. coli* (culturing) ($p = 0.000$) and heterotrophic bacteria ($p = 0.024$), the HF183 marker versus *E. coli* (qPCR) ($p = 0.024$) and *B. adolescentis* versus fecal coliforms ($p = 0.037$). In addition, 100% concurrence was observed for the HF183 marker, adenovirus and Lachnospiraceae versus *E. coli* (qPCR), enterococci (qPCR) and heterotrophic bacteria, amongst others. Based on the correlations and the concurrence analysis, the HF183 marker, Lachnospiraceae and adenovirus may be utilized to supplement indicator organism analysis for the monitoring of harvested rainwater quality.

1. Introduction

Indicator organisms are utilized globally to monitor water quality and predict the presence of pathogens in contaminated environmental waters. However, there is growing evidence that most indicator organisms are capable of proliferating in water sources (Field and Samadpour, 2007) and certain strains have been shown to survive, grow and establish populations in other natural environments such as plant cavities, algal mats, beach sands, soils and sediments (Fujioka et al., 1998; Solo-Gabriele et al., 2000; Whitman et al., 2003, 2005; Byappanahalli and Fujioka, 2004; Anderson et al., 2005; Byappanahalli et al., 2006b, 2006a; Ishii et al., 2006; Olapade et al., 2006; Field and Samadpour, 2007). Moreover, research has indicated that the presence of indicator organisms generally exhibits a poor correlation with the presence of pathogens in contaminated water and the detection of these organisms does not provide information on the specific sources of fecal contamination in water bodies (Harwood et al., 2005, 2014; Field and Samadpour, 2007). Therefore the detection of elevated levels of indicator organisms in water sources may not necessarily signify a corresponding increase in the concentration of pathogens (Hughes et al., 2017). The screening of water sources for various pathogens may thus provide a direct measure of the potential health risk. However, the methods employed in these analyses are generally time-consuming and expensive (Harwood et al., 2014; Hughes et al., 2017). In addition, there are numerous pathogenic organisms in contaminated waters and various concentration and detection methods will be required for a detailed analysis (Harwood et al., 2014). Conversely, monitoring for only a small number of pathogenic microorganisms may provide a false impression of the safety of the water source (Harwood et al., 2014). To compensate for these pitfalls, it is essential that supplementary indicators are identified to monitor for fecal contamination and the possible presence of pathogens in environmental waters. This may assist in accurately determining the potential health risk associated with the use of the water source, as contaminated waters may harbor numerous pathogens such as enteric viruses, *Salmonella enterica*, and *Pseudomonas aeruginosa*, amongst other organisms (Scott et al., 2002; Liang et al., 2015).

Microbial source tracking markers are being investigated as

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potential alternative indicators of water quality (Field and Samadpour, 2007; Harwood et al., 2014). The premise of MST is that certain fecal microorganisms may be strongly associated with specific hosts and may therefore be employed to indicate host-specific contamination of environmental waters (Harwood et al., 2014). These markers may subsequently be utilized as a potential proxy for traditional indicator organisms (Harwood et al., 2014). In addition, understanding the sources of fecal contamination influencing the quality of a water source is imperative for risk assessment studies, as it is known that contamination originating from human sources are a greater risk to human health than contamination originating from animal sources (Scott et al., 2002). Identifying the origin of the contamination may also greatly aid in remediation and contamination prevention efforts to improve water quality (Hughes et al., 2017).

A few common MST markers include the human-specific *Bacteroides* HF183 marker, *Methanobrevibacter smithii nif*H (*M. smithii nif*H) (Seurinck et al., 2005; Ufnar et al., 2006; Sercu et al., 2011; Sidhu et al., 2013), human-specific *B. adolescentis* (Gourmelon et al., 2010), the *Enterococcus esp* marker (Ahmed et al., 2008a), human adenovirus and polyomavirus (Muscillo et al., 2008; Sauer et al., 2011; Sidhu et al., 2013) and enterovirus (Wolf et al., 2010). These source tracking markers have diverse applications ranging from monitoring beach water quality to food quality and have potential applications in the legal arena (for example identifying sources of untreated human waste discharge into environmental waters) (Brownell et al., 2007; Abdelzaher et al., 2010; Korajkic et al., 2011; Harwood et al., 2014). In addition, source tracking markers have been applied to rivers (Seurinck et al., 2005; Kobayashi et al., 2013), lakes (Ahmed et al., 2010a), seawater (Muscillo et al., 2008) and stormwater run-off (Sidhu et al., 2013), to identify the dominant sources of contamination. In a study conducted by Staley et al. (2016) the MST markers general *Bacteroides* (GenBactF3), human- (HF183), ruminant- (CF128) and canine- (DG37) specific *Bacteroides* and a gull marker (*Catelicoccus marimammalium*–qGull4), were utilized to monitor the quality of the Humber River watershed in Toronto, Canada. Based on the detection of the human-associated markers in the river water samples, it was concluded that sewage was the major source of pollution of the watershed. In addition, Kirs et al. (2016) investigated the concentrations of human polyomavirus and the *Bacteroides* HF183 marker in streams and marine water in Oahu, Hawaii. The results obtained again indicated that sewage was the main source of the HF183 marker and human polyomavirus detected in the marine water and that the streams and beaches may thus be impacted by anthropogenic activities. Villemur et al. (2015) investigated the robustness of using mtDNA markers to assess surface water from different watersheds (natural, urban and agricultural areas) for fecal contamination. It was concluded by the authors that human contamination was eminent across all the watersheds analyzed as the human mtDNA marker and the HF183 marker were the most frequently detected markers. In addition, it was deduced that mtDNA markers may be utilized to assess the extent of fecal pollution from different sources in various watersheds. In another study, Liang et al. (2015) screened urban surface water for alternative fecal indicators (MST markers) and correlated their concentrations with those of indicator organisms, enteric viruses, *Salmonella enterica* and *Pseudomonas aeruginosa*. The authors concluded that the correlations observed between the human-specific MST markers, *E. coli* and enterococci indicated recent fecal contamination of the water sources analyzed (Liang et al., 2015). In addition, the correlations observed between the human MST markers and the pathogens detected, indicated that sewage contamination may be the source of the pathogens detected in the surface water. Lastly, it was concluded by Liang et al. (2015) that by utilizing MST markers in conjunction with traditional indicator organisms to monitor water quality would provide improved methods to predict the presence of pathogens in environmental waters. Furthermore, Hughes et al. (2017) investigated the potential use of various MST markers to monitor recreational beach water quality. It was concluded that a combination of the human-specific *Bacteroides* HF183 marker and a viral marker such as pepper mild mottle virus may be the most accurate measurement of human fecal contamination in recreational waters and these markers may then be valuable assets in public health risk assessments.

Therefore, identifying source tracking markers that correlate well with both waterborne pathogens and indicator organisms may improve their predictive capability in indicating fecal contamination and the presence of other pathogens in a water source (Harwood et al., 2014; Liang et al., 2015). For example, Bradshaw et al. (2016) applied the general- (GenBac), ruminant- (CowM3 and Rum-2-bac) and human- (HF183) specific *Bacteroides* MST markers to river water and sediment samples collected from the South Fork Broad River in Georgia, United States of America (USA). Correlations between these markers and *E. coli*, *Listeria*, *Campylobacter*, *Salmonella* and the specific virulence gene encoding for the Shiga toxin (stx_2) were subsequently determined. Significant positive correlations between the ruminant MST markers versus the Shiga toxin gene and *Campylobacter*, respectively were detected. It was deduced that the presence of the Shiga toxin gene and *Campylobacter* in the river water could be attributed to agricultural land use as cattle pastures were observed around the river system. In addition, *Listeria* positively correlated with the human-associated HF183 marker and this correlation indicated that the presence of the *Listeria* in the river water could possibly be attributed to sewage contamination. The authors also noted that other MST markers should be included in future screenings of the river water samples in order to fully elucidate all the sources of the pathogens detected in the river water (Bradshaw et al., 2016).

Rainwater harvesting (RWH) is currently being utilized worldwide as an alternative fresh water source, however numerous studies have indicated that the microbial quality of harvested rainwater does not adhere to drinking water guidelines as indicator organisms and various pathogens have been detected in stored rainwater (Crabtree et al., 1996; Verrinder and Keleher, 2001; Handia, 2005; Field and Samadpour, 2007; Ahmed et al., 2008b, 2010b, 2011, 2012; Simmons et al., 2008; Despins et al., 2009; Dobrowsky et al., 2014a, 2014b, 2014c). Limited research on the application of source tracking markers for the screening of RWH systems is however available (Ahmed et al., 2016; Waso et al., 2016). Correlations between source tracking markers and indicator organisms in harvested rainwater have also not been extensively studied. The aim of the current study was thus to investigate the relationship of previously validated MST markers versus traditional indicator organisms in RWH systems in order to identify MST markers that would ideally supplement culture-based traditional indicator analysis of harvested rainwater. This aim was achieved by completing the following objectives: (i) screening harvested rainwater for indicator organisms; *E. coli*, enterococci, total and fecal coliforms and heterotrophic bacteria, utilizing traditional culturing techniques, (ii) optimizing and applying qPCR assays for the quantification of *E. coli* and enterococci in harvested rainwater, (iii) optimizing and applying qPCR assays for the quantification of the MST markers, shown in literature to exhibit host-specific distributions, in harvested rainwater and (iv) performing statistical analysis to identify correlations and concurrence between the MST markers and indicator organisms in the RWH tanks.

2. Materials and methods

2.1. Sampling site

All harvested rainwater samples (henceforth referred to as tank water samples) were collected from ten domestic rainwater harvesting (DRWH) systems connected to ten houses located in the Kleinmond Housing Scheme site in Kleinmond, a peri-urban coastal town situated in the Western Cape, South Africa (GPS co-ordinates: 34°20′11.81"S 19°00′59.74"E). The ten houses were selected from a pool of houses utilized in previous studies conducted by Dobrowsky et al. (2014a),

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