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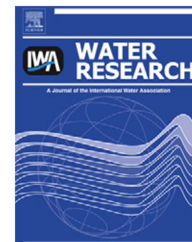

Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia

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ABSTRACT

Elevated numbers of enteric pathogens in the receiving waters following a storm event can be a serious public health concern. The purpose of this study was to conduct a preliminary investigation into the presence of human pathogens of concern in urban stormwater runoff. The involvement of a human sewage as a potential source of contamination was also investigated by using microbial source tracking methods. Water samples (20 L) were collected after storm events and during the dry weather from six sites in Brisbane, Australia. Collected samples were analyzed for fecal indicator bacteria (FIB), and then concentrated using hollow fiber ultrafiltration followed by molecular detection of selected enteric pathogens. The levels of FIB were found to frequently exceed the upper limit of Australian guidelines for managing risks in recreational water, during the dry periods and by further several orders of magnitude in the stormwater runoff. *Enterococcus* spp. numbers as high as 3×10^4 100 mL^{-1} were detected in the stormwater runoff at the Fitzgibbon site. Human adenovirus and polyomavirus were frequently detected from all six sampling sites during wet and dry weather conditions suggesting their wide spread presence in the urban aquatic environments. *Campylobacter jejuni*, *Campylobacter coli* and *Salmonella enterica* were also detected during both dry and wet weather conditions. Presence of human-specific HF183 *Bacteroides* marker in most of the samples tested suggests ubiquitous sewage contamination in the urban environment. Since stormwater runoff routinely contains high numbers of FIB and other enteric pathogens, some degree of treatment of captured stormwater would be required if it were to be used for non-potable purposes.

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1. Introduction

Urban stormwater is a relatively untapped alternate water source in Australia (Hatt et al., 2006), which can be used to augment non-potable and potable water supplies within cities and other urban areas. Potential presence of fecal contamination in stormwater runoff (Parker et al., 2010; Sauer et al., 2011) however, can negatively impact public health if polluted surface water bodies are used for recreational purposes or captured stormwater is used for non-potable purposes such as gardening and landscape irrigation. Concerns over this issue are heightened by the reported links between the occurrence of storm events, microbial inputs from non-point sources and increase in the incidence of waterborne diseases (Curriero et al., 2001; Gaffield et al., 2003). Thus, it is important to understand the presence and

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environmental loadings of pathogens in the receiving waters especially during and after storm events.

Human enteric pathogens can find their way into stormwater and subsequently surface water through leaking sewer systems, sewer pumping station overflows, seepage from septic systems, agricultural runoff and discharge of treated wastewater into aquatic environments (Gaffield et al., 2003; Noble et al., 2006; Rajal et al., 2007). High numbers of enteric viruses, bacteria and protozoa have been reported in stormwater runoff indicating the presence of sewage pollution (Noble et al., 2006; Rajal et al., 2007; Sercu et al., 2009; Cizek et al., 2008). Hence, monitoring for fecal pollution in stormwater is required to make an assessment of health risks from exposure to harvested stormwater and the extent of treatment required prior to its use as alternative water. Although the detection of fecal pollution indicators, *Escherichia coli* and *Enterococcus* spp. in water is relatively straight forward, the presence of these indicator bacteria does not necessarily correlate with the presence of viral and protozoan pathogens (Hörman et al., 2004; Selvakumar and Borst, 2006). The adequacy of monitoring for FIB has also been questioned in recent years as viral and protozoan pathogens are known to be more persistent in the environment than indicator bacteria (Tree et al., 2003; Robertson and Nicholson, 2005). In addition, monitoring for the presence of FIB in the stormwater does not provide definitive information on the possible origin of contamination. Human health risk assessment and remediation strategies for microbial pollution from stormwater can be more effectively implemented if the potential source of pathogenic microorganisms is known in addition to FIB numbers.

Under the updated Australian guidelines for managing risks in recreational water, *Enterococcus* spp., *E. coli*, *Salmonella enterica*, *Campylobacter* spp. and enteroviruses have been identified as microorganisms of public health significance (NHMRC, 2008). However, interpretation of risk is solely based on *Enterococcus* spp. numbers, there are four microbial assessment categories ($A \leq 40$, B 41–200, C 201–500 and $D > 501$ cfu 100 mL^{-1}). The category D, presents highly significant health risks of >10% gastrointestinal illness with a single exposure. At present, very little information is available on the microbiological quality of stormwater runoff in Australia and most of the available data is limited to FIB (Page et al., 2010; McCarthy et al., 2008).

Recent advances in molecular techniques have made it possible to rapidly and easily detect enteric pathogens in the surface water (Rajal et al., 2007; Muscillo et al., 2008; Dyke et al., 2009; Mull and Hill, 2009). Thus direct surveillance for pathogens such as enteric viruses in addition to indicator bacteria may be a better approach for risk identification and protection of public health. Human adenovirus and polyomavirus were reported to be present in higher numbers (10^5 to 10^6 L^{-1}) in the wastewater and also found to be highly prevalent in rivers and coastal waters (Muscillo et al., 2008; Bofill-Mas et al., 2006; Jiang, 2006). Thus by inference may be suitable indicators of human-specific enteric viral pollution in the stormwater runoff. However, very limited information is available on the presence of adenovirus, polyomavirus and bacterial pathogens of concern such as *Campylobacter* spp. and *S. enterica* in stormwater runoff. Recently, microbial source tracking (MST) methods have been developed to identify potential sources of stormwater contamination (Parker et al., 2010; Sauer et al., 2011). Use of MST techniques in conjunction with monitoring for pathogens and indicators has the potential to provide information on the extent of fecal pollution and potential sources of contamination.

Since viral and protozoan pathogens are often present in low numbers in stormwater (Rajal et al., 2007; Cizek et al., 2008), an efficient concentration method coupled with sensitive detection is required for successful detection and quantification of these pathogens. Due to high suspended solids content along with complex physical and chemical properties of surface waters, pathogen concentration techniques may yield variable results (Loge et al., 2002). In addition, existing methodologies for extraction and purification of nucleic acid from these sample types are limited to very small starting volumes (Fuhrman et al., 2005) which may also lead to false negative results due to potential polymerase chain reaction (PCR) inhibition. Previously, hollow fiber ultrafiltration system (HFUFS) combined with PCR have been used to detect enteric viruses in drinking, estuarine and stormwater with some degree of success (Hill et al., 2005; Hernandez-Morga et al., 2009; Rajal et al., 2007; Mull and Hill, 2009). In this study, we have used HFUFS for the concentration of stormwater samples followed by PCR detection of pathogens and MST markers.

The aims of this study were to (i) conduct a preliminary survey of the presence of enteric bacterial and viral pathogens in the stormwater runoff in Brisbane, Australia, (ii) develop an understanding of the extent of increase in pathogens and indicator numbers in surface water bodies after the storm event, (iii) determine the involvement of a human sewage as potential source of contamination by using human-specific MST techniques. .

2. Materials and methods

2.1. Study sites

Six sites in Brisbane (Brisbane River, Oxley Creek, Cabbage Tree Creek, Fitzgibbon site, Enoggera Creek and Pine River in Samford) were selected to determine microbial contaminant numbers in the base-flow and in stormwater runoff from November 2010 to May 2011. The sites were selected to cover a broad range of land use and urbanization and site description along with GPS coordinates are provided in Table 1. In this study, two samples were collected from each site during the dry period (no rain within 48 h prior to sampling) and two samples were collected after >10 mm precipitation.

2.2. Quantification of fecal indicator bacteria (FIB)

Quantification of FIB (*E. coli* and *Enterococcus* spp.) was performed by the membrane filtration technique. Briefly, 1 and 10 mL samples were filtered through 0.45 μm nitrocellulose (Millipore) filters (47 mm) and placed on respective selective agar plates in triplicate. *E. coli* was enumerated on Chromocult™ coliform agar (Merck) and *Enterococcus* spp. on Chromocult™ enterococci agar (Merck). Plates were incubated

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Table 1 – Sampling sites description and location around Brisbane.

Site name	Description	GPS coordinates
Cabbage Tree Creek	Medium density urban residential and industrial developments, serviced by a wastewater treatment plant.	27°20′59.7″S; 153°02′06.6″E
Fitzgibbon site	Low density urban areas, some animal fecal input from Cattle, horses and other domestic animals.	27°20′08.7″S; 153°01′14.5″E
Oxley Creek	Major tributary of Brisbane River, industrial area close by, medium density Urban population, serviced by a wastewater treatment plant.	27°32′07.8″S; 152°59′31.4″E
Enoggera Creek	Moderately populated urban area, some animal fecal input from cattle, horses and other domestic animals.	27°26′41.96″S; 152°57′16.90″E
Samford site	Rural area with large block size, animal fecal input such as cattle, horses and sheep	27°22′37.45″S; 152°59′54.1″E
Brisbane River	Stormwater drain outlet form urban area bring, dilution effect due to tidal influence	27°28′50.05″S; 152°59′53.84″E

at 37 °C overnight and then typical colonies were counted to determine the average number of colony forming units (cfu) per 100 mL.

2.3. *Stormwater sample collection and concentration*

Approximately 20 L water samples were collected in sterile carboy containers (Nalgene) and transferred to the laboratory for storage at 4 °C prior to processing. Collected samples were concentrated for the detection of enteric pathogens which are often found in low numbers within 6 h of collection by using Hemoflow HF80S dialysis filters (Fresenius Medical Care, Lexington, MA, USA) as previously described by Hill et al. (2005). Briefly, the water sample to be concentrated was pumped with a peristaltic pump (Masterflex: Cole Parment Instrument Co, USA) in a closed loop with high-performance, platinum-cured L/S 36 silicone tubing (Masterflex; Cole Parmer Instrument Co., USA). In between sampling events, tubings were cleaned and disinfected by soaking in 1% bleach followed by washing and then sterilized by autoclaving. At the end of the concentration process, pressurized air was passed through the filter cartridge from the top to recover as much water as possible. The samples were concentrated to approximately 100 mL and further concentration of sample was carried out by JumboSep with 100 K MWCO filters (Pall, Australia) to a final concentration of approximately 10 mL.

2.4. *Molecular detection of pathogens and indicators*

Nucleic acid was extracted from 200 μL of each concentrated sample using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) as per manufacturer instructions and stored at −80 °C prior to analysis. Nested PCR was used for the detection of pathogens from concentrated water samples. Briefly, first amplification was carried out with 5 μL of DNA template extracted from the sample with primers sets followed by detection of target genes with TaqMan probes from 1 μL of amplified product.

Adenovirus, polyomavirus, *S. enterica*, *Campylobacter* spp. and *Bacteroides* HF183 gene were detected with the published primer and probe sets given in Table 2. In order to detect sewage associated *Enterococcus faecium esp* gene, 500 mL water sample was filtered through 0.45 μm membrane filter (PALL, Australia) followed by enrichment in Brain Heart Fusion Broth (Oxoid) at 37 °C for 24 h. PCR was carried out after extraction of DNA from the enriched sample (500 μL) as outlined by Scott et al. (2005).

Fresh primer and probe sets for real time PCR were designed for *Campylobacter jejuni* and *Campylobacter coli* with *mapA* and *ceuE* genes as target areas respectively (GenBank numbers X80135 and X88849) using Primer3 software. A homology search was performed against the GenBank database sequence similarity using BLAST program to check for primer and probe specificity (http://www.ncbi.nlm.nih.gov/BLAST/). The primer pairs were also checked for specificity against a panel of non *Campylobacter* bacteria (*Bacterodies vulgatus* ATCC 8482, *Aeromonas hydrophila* ATCC 7966, *C. coli* ATCC 43478, *Legionella pneumophila* ATCC 33152, and *Salmonella* serovar Typhimurium ATCC 14028. *C. jejuni* NCTC 11168, *Clostridium perfringens* ATCC 13124, *E. coli* ATCC 9637, *Enterococcus faecalis* ATCC 19433, *E. faecium* ATCC 19434, *Pseudomonas aeruginosa* ATCC 27853, *Cryptosporidium* ATCC PRA-67D, *Citrobacter freundii* ATCC 8090, *Shigella sonnei* ATCC 29930 and *Klebsiella pneumoniae* ATCC 31314).

2.5. *PCR standards*

Standards for PCR amplification were prepared from the genomic DNA of standard cultures of *C. jejuni*, *C. coli*, *S. enterica* serovar typhimurium, human adenovirus type 41, and human polyomavirus (JC). Concentration of the standard adenovirus genomic DNA was measured by using a NanoDrop ND-1000 spectrophotometer. After calculation of genomic copy numbers, a serial 10-fold dilution (10^6 to 10^0 DNA copies μL^{-1}) was prepared. The aliquots of these serial dilutions were stored at −80 °C for subsequent use. To determine the potential presence of PCR inhibitory substances in the DNA extracted from water samples, each sample was spiked with 10^3 gene copies of adenovirus. The cycle threshold (C_T) values obtained for stormwater samples spiked with adenovirus were compared to those of MilliQ water (control) spiked with adenovirus DNA.

2.6. *PCR amplification*

PCRs were performed on a Bio-Rad iQ5 (Bio-Rad Laboratories, California, USA), using iQ supermix (Bio-Rad) or Sso Fast™

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Table 2 – Primers, probes and cycling parameters for PCR assays.

Target	Primer sequence (5′–3′)	Cycling parameters	Amplicon size (bp)	Reference
Adenovirus	F: GCC ACG GTG GGG TTT CTA AAC TT R: GCC CCA GTG GTC TTA CAT GCA P: FAM TGC ACC AGA CCC GGG CTC AGG AGG TAC TCC GA BHQ1	10 min at 95 °C, 50 cycles of 15 s at 95 °C and 20 s at 60 °C and 20 s at 72 °C	132	Heim et al., 2003
Polyomavirus	F: SM2 AGT CTT TAG GGT CTT CTA CCT TT R: P6 GGT GCC AAC CTA TGG AAC AG P: KGJ3 (FAM)-TCA TCA CTG GCA AAC AT-(MGBNFQ)	10 min at 95 °C, 50 cycles of 15 s at 95 °C and 20 s at 55 °C and 60 s at 60 °C	176	McQuaig et al., 2009
Campylobacter spp. 16S rRNA	F: CAC GTG CTA CAA TGG CAT AT R: GGC TTC ATG CTC TCG AGT T P: FAM CAG AGAA CAA TCC GAA CTG GGA CA BHQ1	10 min at 95 °C, 50 cycles of 15 s at 95 °C and 20 s at 58 °C and 30 s at 72 °C	108	Lund et al., 2004
Campylobacter coli ceuE gene	F: AGA TAC TTT CCA TGC CCT AAG ACT R: TTG CTT TGG CGT CCG GAA ATG A P: FAM-AAC GAT AAA GTT GCA GGA GTT CCA GCT BHQ1	10 min at 95 °C, 45 cycles of 15 s at 95 °C and 20 s at 60 °C and 20 s at 72 °C	180	This study
Campylobacter jejuni mapA gene	F: GCT AGA GGA ATA GTT GTG CTTGAC AA R: TTA CTC ACA TAA GGT GAA TTT TGA TCG P: FAM GCG ATG TTG GAA TTC AAT GTT GTG CCA BHQ1	10 min at 95 °C, 45 cycles of 15 s at 95 °C and 20 s at 60 °C and 20 s at 72 °C	72	This study
Salmonella invA gene	F: ACA GTG CTC GTT TAC GAC CTGAAT R: AGA CGA CTG GTA CTGATC GAT AAT	10 min at 95 °C, 40 cycles of 30 s at 94 °C, 35 s at 59 °C, and 120 s at 72 °C	244	Chiu and Ou, 1996
E. faecium esp gene	F: TAT GAA AGC AAC AGC ACA AGT T R: ACGTCG AAA GTT CGA TTT CC	10 min at 95 °C, 45 cycles of 45 s at 95 °C, 45 s at 59 °C, and 45 s at 72 °C	870	Scott et al., 2005
Bacteroides HF183	F: ATC ATG AGT TCA CAT GTC CGG R: TAC CCC GCC TAC TAT CTA ATG	95 °C for 10 min, 45 cycles 95 °C for 30 s, 53 °C for 1 min, and 60 °C for 1 min	570	Seurinck et al., 2005

F: Forward primer; R: Reverse primer; P: Probe.

EvaGreen® Supermix (Bio-Rad). For first round PCR, 12.5 μL of iQ Supermix, 200 nM each primer, and 5 μL of template DNA was used for all target microorganisms. Second round of nested PCR was carried out with 10 μL of Sso Fast EvaGreen® Supermix, 200 nM each primer and 1 μL of template DNA for the detection of *Bacteroides* HF183, *esp* gene, and *S. enterica*. Melt curves were also added after the PCR step to eliminate false-positive results. Whereas, for the second round of nested PCR detection of adenovirus, polyomavirus, *Campylobacter* spp., *C. jejuni*, and *C. coli* was performed using 12.5 μL of Supermix, 350 nM each primer, 250–350 nM corresponding TapMan probes and 1 μL of template DNA from the first round of PCR amplification with iQ Supermix. Bovine serum albumin (BSA) was added to each reaction mixture to a final concentration of 0.2 $\mu g \mu L^{-1}$ to reduce PCR inhibition (Kreader, 1996).For each PCR run, a corresponding positive (i.e., target DNA) and negative (sterile water) controls were included. Thermal cycling conditions for PCRs to detect microorganism are presented in Table 2.

2.7. *Statistical analysis*

Prior to the statistical analysis, all determined FIB numbers were Log_{10} transformed. A student's *t*-test was performed to compare *E. coli* and *Enterococcus* spp. numbers during the wet and dry conditions. The critical P-value for the *t*-test was set at 0.05 and all tests were considered significant if the P-value was <0.05. Pearson correlation analysis was carried out on the Log_{10}-transformed FIB numbers to determine the existence of correlation between *E. coli* and *Enterococcus* spp. during dry and wet weather.

3. Results

3.1. *FIB numbers in collected water samples*

Collected samples were analyzed for FIB numbers, and the results were plotted after $\log_{10}$ transformation of raw data (Fig. 1). Mean of $\log_{10}$-transformed numbers from dry and wet weather samples from all sites were compared. Both *E. coli* and *Enterococcus* spp. numbers during the wet period were significantly higher ($p < 0.05$) than the dry period. The combined mean of *E. coli* numbers from all sites was higher (3.54 100 mL^{-1}) during the wet period than the dry period (2.32 100 mL^{-1}). Similarly, the combined mean of *Enterococcus* spp. numbers from all sites was 2.43 and 3.58 log 100 mL^{-1} respectively (Fig. 1). In general, *E. coli* numbers from all sites varied between 8×10^1 and 1×10^3 100 mL^{-1} between dry and wet period whereas, corresponding numbers for *Enterococcus*

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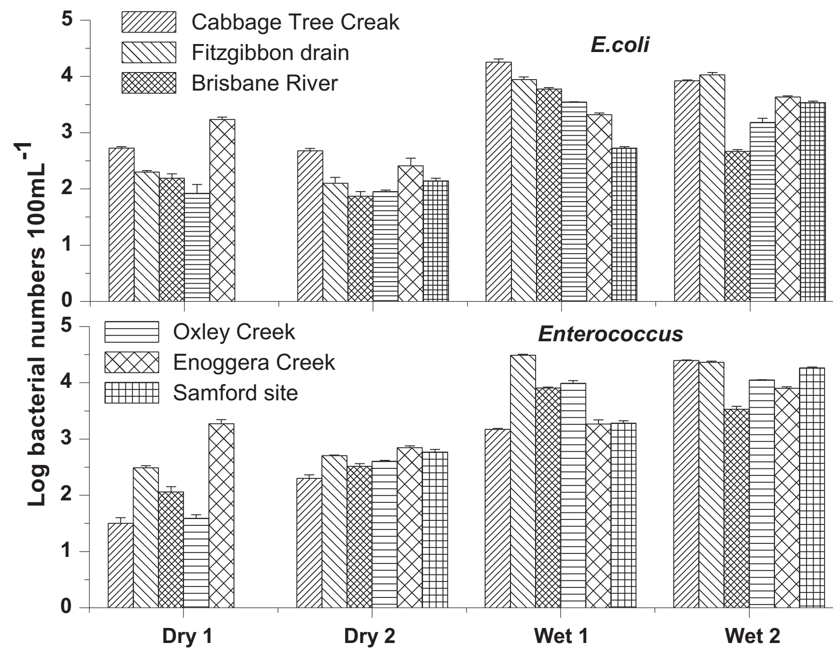

Fig. 1 – *E. coli* and *Enterococcus* spp. numbers during two dry and wet sampling events at six sites across Brisbane.

spp. varied between 4×10^1 and 3×10^4 100 mL^{-1} (Table 3). A good correlation ($R^2 = 0.84$) between *E. coli* and *Enterococcus* spp. numbers was observed during the dry period when data from all six sites was pooled. Whereas, a poor correlation between *E. coli* and *Enterococcus spp*. was observed during the wet period ($R^2 = 0.42$). Samples collected from Cabbage Tree Creek and the Fitzgibbon site have significantly higher ($p < 0.05$) counts of both *E. coli* and *Enterococcus* spp. after the storm events compared to the other four Brisbane sites tested.

3.2. *PCR inhibition assessment*

To test for the PCR inhibitory effect, 10^3 gene copies of adenovirus were seeded into the extracted DNA from the water samples. Variable degree of PCR inhibition was observed in nearly all samples, with samples collected from Brisbane River showing the least inhibition and samples collected from the Enoggera and Fitzgibbon sites showing highest inhibition with no amplification of seeded adenovirus at 100 fold dilution of extracted nucleic acid (data not shown). To improve detection limit in the presence of PCR inhibition, nested PCR amplifications were carried out to detect presence of pathogens in the collected water samples.

3.3. *Pathogen detection in water samples*

All samples tested positive for the presence of *Campylobacter* genus specific primer set. After further investigation the presence of *C. jejuni* or *C. coli* was detected in 17 and 12 samples respectively, out of the 22 samples (Table 3). Some samples also tested for the presence of both *Campylobacter* spp. *Salmonella* spp. was also detected in eight samples primarily after the storm events. Human-specific adenovirus and polyomavirus were also detected in the collected water samples after the storm events. Adenovirus was detected more frequently (15 samples) than polyomavirus (12 samples) during the dry and wet weather.

3.4. *Human sewage specific markers detection in water samples*

Human-specific *Bacteroides* HF183 marker was widely detected in 16 samples out of the 21 tested during both dry and wet weather conditions at all six sites. Whereas, *E. faecium esp* gene was found in only eight samples out of the 23 tested. Unlike HF183, *esp* gene was found to be absent in the water samples, even those containing high FIB count and enteric virus after the storm events (Table 3).

4. Discussion

The urban stormwater runoff can mobilize and transport human pathogens from various non-point sources to surface water bodies such as rivers, lakes and creeks (Noble et al., 2006; Sercu et al., 2009) and exposure to stormwater runoff impacted waters has been linked to increased risk of illness (Gaffield et al., 2003). In this study, we tested surface water samples collected during the dry period and after storm event for the presence of traditional FIB along with selected human pathogens and fecal contamination markers with PCR. This approach could improve our ability to identify and prioritize sources that contribute pathogens to surface waters.

Enterococcus spp. numbers detected in the water samples collected from all sites during the dry period were generally higher than the recommended limits for category D (<501

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Table 3 – PCR analysis of collected water samples for pathogens and human fecal markers.

Site/event	*E. coli* (/100 mL)[a]	*Enterococcus* spp. (/100 mL)[a]	*Campylobacter* spp.	*C. jejuni*	*C. coli*	*Salmonella enterica*	*Adenovirus*	*Polyomavirus*	*esp*	*HF183*	Rainfall (mm)
Enoggera Creek											
Dry	1.73×10^3	1.93×10^3	NT	NT	NT	NT	NT	NT	–	NT	0
Dry	2.83×10^2	7.10×10^2	+	–	–	–	+	NT	–	+	0
Wet	2.10×10^3	1.90×10^3	+	–	–	–	+	–	+	+	21.4
Wet	4.33×10^3	8.00×10^3	+	+	–	+	+	+	+	+	18.4
Cabbage Tree Creek											
Dry	4.83×10^2	2.03×10^2	+	+	+	–	NT	NT	–	+	0
Dry	5.33×10^2	4.57×10^2	+	+	+	+	+	+	–	+	2.6
Wet	8.37×10^3	2.50×10^3	+	+	+	+	+	+	–	+	19.4
Wet	1.82×10^4	1.48×10^3	+	+	–	–	NT	NT	+	NT	134.6
Fitzgibbon site											
Dry	3.60×10^2	5.10×10^2	+	+	–	–	NT	NT	–	+	0.4
Dry	1.33×10^2	1.27×10^2	+	+	–	–	NT	NT	–	–	2.6
Wet	8.93×10^3	2.23×10^3	+	–	+	–	+	–	–	+	19.4
Wet	1.07×10^4	3.11×10^3	+	–	+	+	+	+	+	+	134.8
Brisbane River											
Dry	1.60×10^2	1.20×10^2	+	–	–	–	+	+	–	–	0
Dry	7.67×10^1	3.30×10^2	+	+	–	–	+	+	–	–	0
Wet	6.00×10^3	8.10×10^3	+	+	–	–	+	+	+	+	4.6
Wet	4.67×10^2	3.43×10^3	+	–	+	+	+	–	+	+	10.2
Oxley Creek											
Dry	9.33×10^1	4.00×10^1	+	–	+	–	–	–	–	–	0
Dry	9.00×10^1	4.03×10^2	+	+	–	+	–	–	+	+	0
Wet	3.50×10^3	9.93×10^3	+	+	+	–	+	+	–	+	7
Wet	1.57×10^3	1.12×10^1	+	+	+	–	+	NT	–	+	15
Samford site											
Dry	1.40×10^2	5.93×10^2	+	+	+	–	–	–	–	–	0
Wet	5.33×10^2	1.93×10^3	+	+	+	–	+	–	+	+	40
Wet	3.43×10^3	1.84×10^4	+	+	+	+	+	–	–	+	33.2

NT = sample not tested.
a Mean bacterial counts.

Enterococci per 100 mL) under Australian guidelines for managing risks in recreational water and by further several orders of magnitude after the storm events (NHMRC, 2008). High FIB numbers observed after the rain events were also similar to what had been previously reported in the literature (Brownell et al., 2007; Parker et al., 2010).

The amount of rainfall prior to sampling appeared to have some influence on the FIB numbers at the Cabbage Tree Creek and Fitzgibbon sites as the highest numbers of *E. coli* and *Enterococcus* spp. were observed after the highest rainfall event (135 mm), which was several fold higher than the other wet periods (20 mm). In contrast, Parker et al. (2010) found no link between the FIB numbers and the amount of precipitation. The increase in FIB numbers observed after the storm event could be due to the movement of fresh fecal pollution from sewage leakage and animal sources into the surface water. In addition, the storm event might also have stirred up sediments which could have led to the re-suspension of bacteria bound to particles. A significant proportion of bacteria (15–30%) in the stormwater have been reported to be attached to particulate matter (Cizek et al., 2008; Krometis et al., 2010). The sediments in the stormwater conveyance system may also act as reservoir for enteric pathogens, hence better understanding of pathogen presence and persistence in the sediments is required for a proper health risk assessment. In general, *Enterococcus* spp. numbers observed in this study was higher than the *E. coli* at all sites. This is in agreement with previously reported numbers of *Enterococcus* spp. in stormwater runoff (Parker et al., 2010; Krometis et al., 2010).

Stormwater samples can have a high amount of particulate matter and compounds that interfere with PCR amplification of DNA amplification. In this study, we observed a variable degree of PCR inhibition which appeared to be site specific. Similar PCR inhibition issues in the stormwater water have been reported previously (Rajal et al., 2007). For example, the sample concentrates from the Enoggera Creek and Fitzgibbon site have a dark brown appearance suggesting the presence of humic substances which are known to inhibit PCR (Wintzingerode et al., 1997). The primary source of humic acids at these sites is most likely decomposing plant material as there is more vegetation around these sites. The least PCR inhibition was observed from the concentrated water samples from the Brisbane River which did not have brown appearance on visual inspection. This is not unexpected as Brisbane River has a variety of inputs as well as being more saline which would aid in removing humic substances through dilution and

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cation exchange influences. The results of this study suggest that PCR inhibition may lead to under reporting of pathogen numbers or false negative results hence, internal controls should be used to overcome this problem.

Campylobacter spp. was detected in all water samples collected, showing their wide prevalence at all sites. The *Campylobacter* spp. specific primer set used in this study are reported to primarily detect *C. jejuni*, *C. coli* and *Campylobacter lari* (Lund et al., 2004) which are known to cause campylobacteriosis in humans. There are a number of documented evidences on the extensive presence of *Campylobacter* spp. in surface water (Savill et al., 2001; Dorner et al., 2007; Dyke et al., 2009). This is not unexpected as apart from human input, domestic animals and birds are known reservoirs of *Campylobacter* genus (Dyke et al., 2009). We also detected the specific presence of *C. jejuni* and *C. coli* which are known to cause human infections. The presence of *Campylobacter* spp. in surface water is a cause of concern which needs further investigation to assess their numbers and the infectivity status of this bacterium in stormwater runoff. *S. enterica* was found to be less prevalent than *Campylobacter* with only seven positive samples out of the 22 tested. However, *Salmonella* was detected more frequently in the stormwater runoff than in the samples collected during the dry weather. A similar increase in the prevalence of *Salmonella* after storm events has been previously reported (Krometis et al., 2010). Apart from the human fecal input from the sewage leakages, domestic animals such as cattle, dogs, horses, sheep, poultry and birds may also contribute to bacterial pathogen loads in the stormwater runoff (Dyke et al., 2009; Lemarchand and Lebaron, 2003).

Human adenovirus and polyomavirus were detected in the stormwater runoff and dry weather samples from all sites, suggesting a wide prevalence of human fecal contamination in these waterways. Adenovirus was found to be more prevalent than polyomavirus, which was mostly detected in water samples collected after the storm events. This corroborates with previous findings on the presence of human adenovirus and polyomavirus in the surface water and stormwater (Sauer et al., 2011; Hamza et al., 2009; Muscillo et al., 2008; Rajal et al., 2007). The presence of these viruses in the environment is not unexpected as they are known to be present in high numbers (10^5 to 10^6 L^{-1}) in wastewater matrices (Bofill-Mas et al., 2006). Both human adenovirus and polyomavirus numbers in surface water and stormwater have been reported in the range of 1×10^2 to 10^5 L^{-1} (Sauer et al., 2011; Hamza et al., 2009; Muscillo et al., 2008). Thus, if there is sewage contamination then there is a high probability of adenovirus and polyomavirus being present in the stormwater runoff. The detection of adenovirus and polyomavirus is also an indication that other human pathogens such as other viruses and protozoa could be in the water, thus further increasing the potential health risks. The potential use of adenovirus and polyomavirus as indicators of human fecal contamination needs to be further investigated as they could provide a better health risk assessment than fecal indicators due to their direct relevance to human health.

E. faecium surface protein gene (*esp*) and human-specific *Bacteroides* HF183 gene, have been previously used to detect the presence of human fecal pollution in aquatic environment with a high degree of accuracy (Scott et al., 2005; Seurinck et al., 2005). In this study, human-specific *Bacteroides* HF183 genetic marker was detected in all stormwater runoff samples and frequently in the water samples collected during the dry period, which suggested the ubiquitous presence of sewage pollution in the urban environment. Prevalence of fecal pollution in surface water from urban catchments is reported to be wide spread in the literature (Sauer et al., 2011; Brownell et al., 2007). The relatively low prevalence of the *E. faecium esp* gene compared to the *Bacteroides* HF183 gene in the water samples could potentially be due to its low prevalence in sewage in the environment (Lund et al., 2006; Scott et al., 2005).

The wide spread prevalence of human-specific fecal markers along with human adenovirus and polyomavirus in the water samples collected during the dry period suggests that sources other than stormwater runoff such as point sources and sediments may act as reservoir for pathogens. Sediment carried by stormwater has been shown to contain pathogens and FIB which reported to survive for longer period of time in the sediments (Cizek et al., 2008; Haller et al., 2009). The results of this study also suggested that the presence of high numbers of FIB in surface water after the storm events cannot be attributed to non-human sources alone as human enteric pathogens were also found at these sites. This observation has important ramifications for stormwater runoff management, as testing for pathogens such as human adenovirus at known "FIB hot spots" may be a more effective approach for proper health risk assessment and also for identification and abatement of sources of contamination.

Molecular methods offer the opportunity for accurate and rapid detection of a wide range of pathogens which could be used as alternative indicators for risk assessment from stormwater runoff. However, concentration of water samples is often required to enable detection of pathogens which are intermittently present in low numbers. This approach also results in the co-purification of PCR inhibitory compounds which then impact on the accurate detection and quantification of pathogens with PCR. The approach used in this study appears to provide good results for the first tier assessment of pathogens of concern in the stormwater. However, its suitability for quantitative PCR detection of pathogens needs to be validated. Some techniques such as virus adsorption and elusion from charged membranes have been reported to perform better than concentration approach using ultrafiltration for the detection of enteric virus due to very limited presence of PCR inhibitors (Katayama et al., 2002; Hamza et al., 2009) but this approach is not suitable for the detection of bacterial and protozoan pathogens. Hence, a careful consideration is required in determining the volume of water to be concentrated as well as methodologies used for the detection of pathogens of interest.

5. Conclusions

Presence of human sourced bacterial and viral pathogens in the stormwater suggests that aging sewage infrastructure combined with numerous non-point pollution sources may present a significant risk of fecal contamination of

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stormwater. The results of this study suggest that in addition to monitoring for FIB, testing for specific pathogens and fecal pollution markers with sensitive and specific approaches would improve our ability to detect pathogens and improved health risk assessments which are based on pathogen prevalence. The wide presence of adenovirus and polyomavirus in the urban stormwater runoff indicates that there is potentially significant human fecal contamination, as opposed to contamination from animals, and thus has much higher public health implications. Consequently, some degree of treatment of captured stormwater prior to its reuse for potable and non-potable purposes would be required for public health risk mitigation. This study has shown that the use of simple and rapid molecular detection methods can provide additional information on health risks of urban stormwater runoff. Further research is needed to determine the prevalence of other enteric pathogens in stormwater runoff, especially enteric viruses and protozoa and any correlation to alternative indicators along with a quantitative assessment of human health risks.

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