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Occurrence of Virulence Genes Associated with Diarrheagenic Pathotypes in *Escherichia coli* Isolates from Surface Water

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Escherichia coli **isolates (*n* = 300) collected from six sites in subtropical Brisbane, Australia, prior to and after storm events were tested for the presence of 11 virulence genes (VGs) specific to diarrheagenic pathotypes. The presence of *eaeA*, *stx*$_1$, *stx*$_2$, and *ehxA* genes specific for the enterohemorrhagic *E. coli* (EHEC) pathotype was detected in 56%, 6%, 10%, and 13% of isolates, respectively. The VGs *astA* (69%) and *aggR* (29%), carried by enteroaggregative (EAEC) pathotypes, were frequently detected in *E. coli* isolates. The enteropathogenic *E. coli* (EPEC) gene *bfp* was detected in 24% of isolates. In addition, enteroinvasive *E. coli* (EIEC) VG *ipaH* was also detected in 14% of isolates. During dry periods, isolates belonging to the EAEC pathotype were most commonly detected (23%), followed by EHEC (11%) and EPEC (11%). Conversely, a more uniform prevalence of pathotypes, EPEC (14%), EAEC (12%), EIEC (10%), EHEC (7%), and ETEC (7%), was observed after the storm events. The results of this study highlight the widespread occurrence of potentially diarrheagenic pathotypes in the urban aquatic ecosystems. While the presence of VGs in *E. coli* isolates alone is insufficient to determine pathogenicity, the presence of diarrheagenic *E. coli* pathotypes in high frequency after the storm events could lead to increased health risks if untreated storm water were to be used for nonpotable purposes and recreational activities.**

Storm events can result in mobilization and transport of fecal contaminants from point sources, such as urban wastewater treatment plants, and nonpoint sources, in particular animal fecal material, to receiving water bodies. The presence of fecal contamination in rivers, lakes, and creeks can lead to the degradation of water quality and subsequently result in the water becoming unfit for potable/nonpotable uses, aquaculture, and recreational activities such as swimming and fishing (1–4).

Escherichia coli and *Enterococcus* spp. commonly found in mammalian feces have been traditionally used as indicators of fecal pollution in fresh and marine waters (5, 6). After storm events, a severalfold increase in the fecal indicator bacteria (FIB) numbers occur in the surface waters (7–9). There may be several sources of *E. coli* that contribute to sudden increases in numbers of this bacterium in waterways, including sewage overflows, farm animals, pets, and birds. The elevated microbial contaminants in storm runoff (7, 10) and subsequently in receiving water bodies may pose a serious public health risk. Disease outbreaks related to exposure to contaminated freshwater are well documented (11–14). Exposure to recreational water has been linked to high numbers (21 out of 31) of reported *E. coli* O157:H7 disease outbreaks in the United States from 1982 to 2002 (15). Despite the significant disease burden linked to contaminated water exposure, the prevalence of *E. coli* pathotypes in the urban aquatic environment is not well characterized.

The majority of *E. coli* strains are commensal; however, some strains have acquired specific virulence attributes that allow them to cause a wide spectrum of intestinal and extraintestinal infections, such as diarrhea, urinary tract infection, meningitis, and septicemia (16, 17). Diarrheagenic *E. coli* has been classified into five well-described groups: enterotoxigenic *E. coli* (ETEC) strains, which are associated with traveler's diarrhea and porcine and bovine diarrhea; enteropathogenic *E. coli* (EPEC) strains, which cause diarrhea in children; enterohemorrhagic *E. coli* (EHEC) strains, which are associated with hemorrhagic colitis and hemolytic-uremic syndrome in humans; enteroaggregative *E. coli* (EAEC) strains, which are associated with persistent diarrhea in humans; and enteroinvasive *E. coli* (EIEC) strains, which are involved in invasive intestinal infections, watery diarrhea, and dysentery in humans and animals (16, 17).

Despite increasing evidence that *E. coli* strains originating from human and animal feces contain several VGs (2, 16, 18), only a few studies have determined whether *E. coli* strains isolated from fresh and marine water contain VGs and are potentially pathogenic (3, 19–21). The presence of *E. coli* strains with VG profiles similar to EHEC (21), EPEC (3, 19), and ETEC (21) have been reported previously in the fresh and estuarine waters. However, the distribution of diarrheagenic *E. coli* pathotypes in freshwater, especially after storm events, still remains relatively unknown. In a recent study, an increase in *E. coli* VGs after enrichment of water samples collected after rainfall have been reported (22); however, in the absence of actual isolates, no conclusions could be drawn regarding the relative prevalence of pathotypes carrying VGs or prevalence of potentially pathogenic *E. coli*.

The main aim of this study was to determine the frequency of occurrence of diarrheagenic *E. coli* pathotypes in surface waters and the impact of storm runoff on their prevalence and distribution. The specific objectives of the study were to (i) determine the frequency of occurrence of potentially pathogenic *E. coli* strains in the surface water in subtropical Brisbane, Australia; (ii) characterize the VG profile of *E. coli* isolates to determine the most common pathotypes; and (iii) assess the influence of storm events and run-

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TABLE 1 Sampling site description and location in Brisbane, Australia

Site name	Site description	GPS coordinates
Fitzgibbon Drain	Receives runoff from low-density urban areas, some animal input, such as cattle and horses	27°20′08.7′′S; 153°.01′14.5′′E
Cabbage Tree Creek	Medium-density residential and industrial developments	27°20′59.7′′S; 153°02′06.6′′E
Brisbane River	Storm water drain outlets from urban area, dilution effect due to large vol and tidal influence	27°28′50.05′′S; 152°59′53.84′′E
Pine River	Rural area with large block size, animal input (cattle, horses, and sheep)	27°22′37.45′′S; 152°.59′54.1′′E
Oxley Creek	Tributary of the Brisbane River, industrial area, animal input (cattle, horses, and sheep), medium-density population, and close by wastewater treatment plant	27°32′07.8′′S; 152°59′31.4′′E
Enoggera Creek	Moderately populated area, some animal inputs, such as cattle and horses	27°26′41.96′′S; 152°57′16.90′′E

off on the distribution of potentially pathogenic *E. coli* strains. This was done to determine the extent of potential human health risks posed by the prevalence of diarrheagenic *E. coli* pathotypes in surface waters used for potable, nonpotable, and recreational purposes.

MATERIALS AND METHODS

Water sampling and enumeration of *E. coli*. One-liter grab samples were collected in sterile Nalgene containers from six sites in Brisbane prior to and after storm events, representing diverse fecal pollution sources, ranging from high-density urban areas to a relatively unpolluted site. A brief site description along with GPS coordinates are provided in Table 1. Samples were collected 1 m from the shore and at a depth of about 0.5 m below water surface with a telescopic water sampler following a dry period (no rainfall in 48 h prior to sampling) and 10 to 12 h after a storm event (>20 mm rainfall). Collected samples were transported to the laboratory on ice and processed within 6 to 8 h of collection.

The standard membrane filtration method was used for the quantification of *E. coli* from the collected water samples (8). Briefly, 1- and 10-ml samples were filtered through 0.45-μm nitrocellulose (Millipore) filters (47 mm) and placed on Chromocult coliform agar (Merck). Plates were incubated at 37°C overnight, and typical *E. coli* colonies were counted to determine the average number of CFU per 100 ml.

***E. coli* isolation and extraction of DNA.** Individual well-isolated typical *E. coli* colonies were picked from the Chromocult coliform agar plates and streaked on fresh Chromocult agar plates. During the dry period, from six sampling sites a total of 90 *E. coli* colonies were isolated during two sampling events. The aim was to collect around 10 *E. coli* isolates each time from each site, with no more than 2 to 3 colonies isolated from each plate. Similarly, during two wet-period sampling events from the same six sites, around 15 isolates per site were collected, resulting in 210 *E. coli* isolates. After purification (twice), single colonies were picked from agar plates and inoculated into 2-ml centrifuge tubes containing 1.5 ml nutrient broth (Oxoid). Inoculated tubes were incubated overnight at 37°C in the shaking platform incubator at 100 rpm. All *E. coli* isolates were stored at −80°C in nutrient broth and 15% (vol/vol) glycerol. At the time of DNA extraction, *E. coli* isolates were grown in 5 ml of nutrient broth at 37°C overnight. One milliliter of overnight cell culture from each isolate was centrifuged at 6,000 × *g* for 3 min. The resulting supernatants were removed, and the cell pellets were resuspended in 200 μl of sterile water by vortexing. DNA was extracted from the cell pellets by using the InstaGene matrix according to the manufacturer's instructions (Bio-Rad Laboratories). Presumptive *E. coli* isolates were confirmed by PCR amplification of the *uidA* gene as described previously (23).

PCR-positive controls. *E. coli* ATCC 9637 was used as a positive control (*uidA* gene) in PCR assays to confirm presumptive *E. coli*. *E. coli* O157:H7 (ATCC 35150) was used as a positive control for the *eaeA*, stx_1, and stx_2 genes. *Shigella sonnei* (ATCC 29930) was used as a positive control for the *ipaH* gene. The *E. coli* strain belonging to serotype O138 of porcine origin was used as a positive control for heat-stable (ST) and heat-labile (LT) toxin genes. For the remaining target VGs, pure cultures of clinical *E. coli* isolates containing target genes were used as positive controls.

PCR detection of *E. coli* toxin and other target genes. The list of VGs and the corresponding pathotypes tested in this study are shown in Table 2. Each isolate was screened for a number of adhesion, invasion, and toxin genes to correctly identify them under five main pathotypes. PCR-confirmed *E. coli* isolates ($n = 300$) were screened for the presence of 11 diarrheagenic *E. coli* VGs by using previously published primer sets, stx_1 and stx_2 (24, 39), *eaeA* (25), *ehxA* (18), LT and *bfp* (26), ST (27), *aggR* (28), *ipaH* (29), *astA* (30), *cdtB* (31), and thermal cycling conditions. PCRs were performed on a Bio-Rad iQ5 thermocycler system (Bio-Rad Laboratories, California), using iQ supermix (Bio-Rad). Each 25-μl PCR mixture contained 12.5 μl of supermix, 120 to 200 nM each primer, and 3 μl of template DNA. For each PCR experiment, corresponding positive (i.e., target positive-control DNA) and negative (sterile water) controls were included. A melt curve analysis was performed after each PCR run to differentiate between actual products and primer dimers and to eliminate

TABLE 2 *Escherichia coli* pathotypes and associated virulence genes tested in this study

Pathotype	Adhesion/invasion gene	Function	Toxin gene	Function
EHEC	*eaeA*[a]	Intimin	stx_1	Shiga toxin I
			stx_2	Shiga toxin II
			ehxA[b]	Enterohemolysin
ETEC			LT1	Heat-labile toxin 1
			ST1	Heat-stable toxin 1
EPEC	*eaeA*[a]	Intimin		
	bfp	Type IV bundle-forming pili		
			cdtB[c]	Cytolethal distending toxin
EAEC	*aggR*	Transcriptional regulator for chromosomal gene	EAST1 (*astA*)	EaggEC heat-stable enterotoxin
EIEC	*ipaH*	Invasion plasmid antigen		

[a] Gene carried by both EHEC and EPEC.
[b] Gene also carried by EPEC.
[c] Gene also carried by ExEPC. Typical EHEC strains carry the *eaeA* gene along with stx_1, stx_2, or both; typical EPEC strains carry *eaeA* and *bfp* genes; typical EAEC strains carry *astA* and *agg*R.

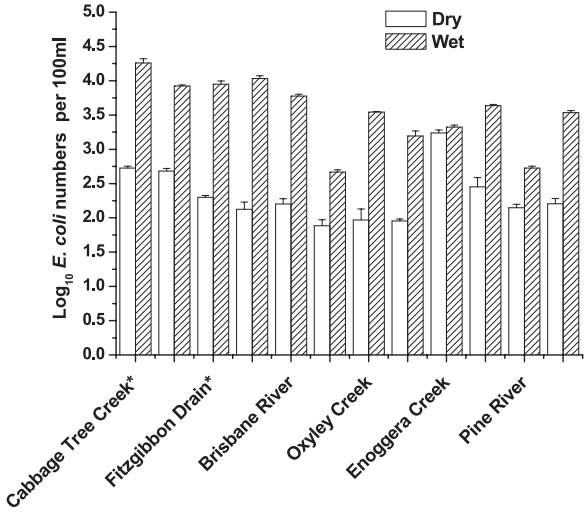

FIG 1 Comparative *E. coli* numbers during the dry and wet weather conditions at the six sampling sites during two wet- and dry-period sampling events. *, *E. coli* counts significantly higher ($P < 0.05$) from other sites after storm events.

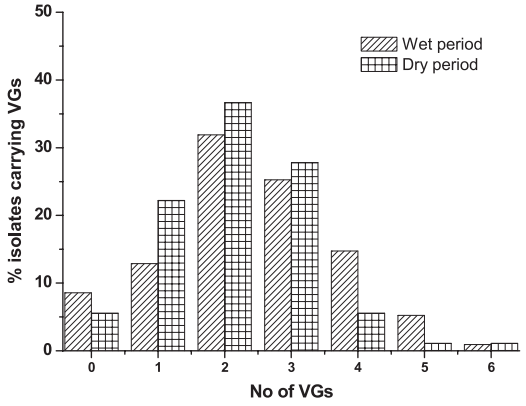

FIG 2 Comparative distribution of the numbers of virulence genes (VGs) carried by individual *E. coli* isolates.

the possibility of false-positive results. The melt curve was generated using 80 cycles of 10 s each starting at 55°C and increasing in 0.5°C intervals to a final temperature of 95°C. The midpoint temperature (T_m) for each amplicon was determined using the iQ5 software (Bio-Rad).

Statistical analysis. The difference in VG distribution among the six sites and existence of variation among VG patterns was determined by analysis of variance (ANOVA) on the pooled *E. coli* data from the dry- and wet-weather isolates, with significance defined as P values of <0.05. All data on *E. coli* numbers from all sites were $\log_{10}$ transformed prior to statistical analysis. The Student t test was performed to compare the significance of difference between *E. coli* numbers across sites and during the dry and wet periods. 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. A linear regression analysis was applied to investigate existence of any correlation between *E. coli* numbers and VGs during the dry and wet weather.

RESULTS

Prevalence of *E. coli* during dry and wet periods. The $\log_{10}$-transformed results for the detection of *E. coli* are shown in Fig. 1. In general, *E. coli* numbers in water samples from all sites varied between 1.8 to 4.2 $\log_{10}$ per 100 ml between dry and wet periods. The mean *E. coli* numbers after the storm events were significantly higher ($P < 0.05$) than the dry period. Samples collected from Cabbage Tree Creek and the Fitzgibbon Drain sites had significantly higher ($P < 0.05$) *E. coli* counts after the storm events than the other four sites tested.

Prevalence of VGs among *E. coli* isolates. Among the 300 *E. coli* isolates tested, 256 (85%) carried at least one VG, and 44 isolates (15%) carried no VGs. During the dry period, 72 out of 90 isolates (80%) carried 1 to 3 VGs, and only 7 isolates (8%) carried >4 VGs (Fig. 2). However, after the storm, 145 isolates (69%) were found to harbor 1 to 3 VGs, and a further 32 (15%) carried >4 VGs. The prevalence of multiple VGs in the *E. coli* isolates was higher after the storm events than during dry periods. However, no correlation was observed between the total number of *E. coli* isolates and *E. coli* isolates that were carrying VGs in water samples during the dry or wet period.

Among the adhesion and invasion VGs, *eaeA*, which codes for intimin protein in both EHEC and EPEC pathotypes, was the single most prevalent gene (56%) (Table 3). Among 300 *E. coli* isolates, only nine (3%) carried only the *eaeA* gene, and these isolates were classified as atypical EPEC. The EAEC transcriptional regulator gene *aggR* was detected in 29% of the isolates, which was followed by the EPEC bundle-forming pili gene *bfp* (24%) and the EIEC invasion plasmid antigen gene *ipaH* (14%). The *eaeA* gene was detected more frequently (61%) in *E. coli* isolates after the storm events than during the dry periods (42%). Similarly, the *bfp* and *ipaH* genes were detected more frequently, 27% and 18%, respectively, after the storm events than during dry periods, where their prevalence was 17% and 6%, respectively. In contrast, the *aggR* gene had a higher prevalence (36%) during the dry periods than during the wet periods (26%).

Among the toxin genes, the enteroaggregative heat-stable enterotoxin 1 (EAST1)-encoding *astA* gene carried primarily by EAEC but also by EHEC was the single most prevalent gene (69%). The *ehxA* gene primarily carried by EHEC was the second most commonly detected toxin gene (13%), with a slight increase in detection from 10 to 14% from the dry to wet periods. The *cdtB* toxin gene was detected in 9% of dry-period isolates and 8% in wet-period isolates. The stx_2 EHEC toxin gene (10%) was more frequently detected than stx_1 (6%), with the stx_2 gene more prevalent in dry-period isolates (13%) than in wet-period isolates (9%). The heat-stable toxin gene (ST gene), generally carried by ETEC, was detected only in *E. coli* isolates during the wet period (6%). In contrast to all the other toxin genes, the heat-labile toxin gene (LT gene) was detected in a small number of isolates (2%) during both the dry and wet periods (Table 3).

In order to further explore the distribution of the 11 VGs among all six sites, an ANOVA was performed on the pooled data of wet and dry periods. There was a highly significant difference ($P < 0.001$) between the occurrence of *eaeA* and ST and LT genes. Similarly a highly significant difference ($P < 0.001$) was observed in the occurrence of the *astA* gene compared to that of the stx_1, ST, LT, and *cdtB* genes. A highly significant difference ($P < 0.001$) was also observed between the occurrence of the *aggR* gene and the ST and LT genes. The difference between the occurrence of *ipaH* and *astA*, ST and *bfp*, stx_2 and *astA*, and *eaeA* and stx_1 genes was significant ($P < 0.01$).

Comparative prevalence of *E. coli* pathotypes. The percentage of *E. coli* isolates with defined pathotypes from the six sam-

TABLE 3 Occurrence of VGs in *Escherichia coli* isolated from surface water samples across six sampling sites in Brisbane, Australia

Period (no. of isolates)	No of *E. coli* strains carrying virulence gene and distribution (%)										
	eaeA[a]	*stx*$_1$	*stx*$_2$	LT1	ST	*bfp*	*cdtB*[a]	*ipaH*	*aggR*	*astA*	*ehxA*[a]
Dry period (90)	38 (42)	4 (5)	12 (13)	2 (2)	0 (0)	15 (17)	8 (9)	5 (6)	32 (36)	67 (75)	9 (10)
Wet period (210)	129 (61)	15 (7)	18 (9)	4 (2)	12 (6)	56 (27)	17 (8)	37 (18)	55 (26)	139 (66)	29 (14)
Total (300)	167 (56)	19 (6)	30 (10)	6 (2)	12 (4)	71 (24)	25 (8)	42 (14)	87 (29)	206 (69)	38 (13)

[a] These genes are shared by more than one *E. coli* pathotype.

pling locations during the dry and wet periods are shown in Fig. 3. On the basis of combinations of VGs, approximately 53% of the *E. coli* isolates could be placed into five main pathotypes (EHEC, EAEC, EIEC, ETEC, and EPEC) during the dry periods, and a further 4% of isolates were observed to have combinations of genes from both EHEC and EAEC pathotypes. During the dry periods, isolates belonging to the EAEC pathotype were the most commonly detected (23%), followed by EHEC (11%) and EPEC (11%). Nearly 40% of the dry-period isolates carrying VGs could not be placed under defined categories due to random distribution of single or multiple genes from different defined pathotypes.

Approximately 50% of the *E. coli* isolates collected after storm events could be placed under five main pathotypes; however, the distribution of pathotypes was more uniform compared to that during the dry periods. The pathotypes EPEC (14%), EAEC (12%), and EIEC (10%) were more commonly detected than EHEC (7%) and ETEC (7%). In addition, due to a more common occurrence of multiple VGs in the *E. coli* isolates collected after the storm events, nearly 20% of *E. coli* isolates could be placed under more than one pathotype. Approximately 9% of the isolates carried a combination of EPEC, EIEC, and EAEC VGs. The remaining 30% of the isolates carrying single or multiple VGs could not be classified into known pathotypes due to a random distribution of genes from more than one pathotype.

Comparison of *E. coli* VG profiles from six sites. A comparative analysis of the distribution of VGs across all six sites during the dry periods and after the storm events is presented in Fig. 4. In general, the frequency of occurrence of VGs in *E. coli* isolates collected after the storm event conditions was higher than that during the dry period. However, at the Oxley Creek site, apart from the *eaeA* gene, all other genes were detected in higher frequency in isolates from the dry periods (Fig. 4E). The Enoggera Creek site has the lowest overall frequency of occurrence of VGs. In contrast, the Fitzgibbon Drain has the highest occurrence of VGs. The VGs

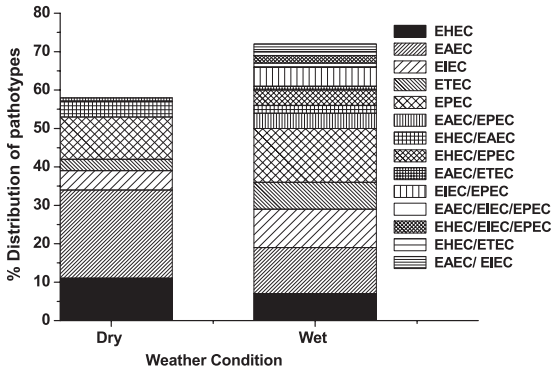

FIG 3 Comparative distribution of *E. coli* pathotypes during dry and wet weather conditions from all six sites in Brisbane, Australia.

astA and *eaeA* were most frequently detected across all the sites, whereas the LT and ST genes were infrequently detected (Fig. 4). Among the toxin genes screened in this study, *ehxA* and *stx*$_2$ genes were the two most commonly detected genes across all sites after the *astA* gene, which was highly prevalent (>60%). Out of the six sites, the toxin gene *cdtB* was detected only in more than 10% of isolates from the Oxley Creek and Enoggera Creek sites.

A comparison between sites was made (ANOVA) to determine if the sites were similar or different on the basis of occurrence of VGs. The Enoggera Creek site was significantly different ($P < 0.05$) from the Brisbane River site, whereas differences between the other sites was found to be statistically nonsignificant. In the *E. coli* isolates from Brisbane River, the *stx*$_1$, *stx*$_2$, ST, and LT toxin genes and *ipah* and *bfp* adhesion genes were detected only after the storm events. In contrast, at the Enoggera Creek site, all these genes were detected during both dry and wet periods, with a slight increase in frequency after rain fall.

DISCUSSION

Storm runoff may lead to an increased prevalence of microbial pathogens, including diarrheagenic *E. coli* pathotypes in the surface water bodies due to transport of fecal contamination from land (8). This study compared the distribution and frequency of occurrence of the potentially diarrheagenic *E. coli* pathotypes in surface water prior to and after storm events to assess if storm runoff could lead to elevated health risks.

The results show a significant increase ($P < 0.05$) in the *E. coli* numbers after rainfall at all sites. A potential cause could be fresh human sewage input from sewage leakage and overflow; other likely sources include input from animal sources (32) and mobilization of *E. coli* surviving in the soil (33), sediments (34), and aquatic environment (35). These findings are in agreement with the previously reported observation of severalfold increase in FIB numbers in the surface water bodies after storm events (7–9). In this study, an increase in the VG distribution among *E. coli* isolates was observed after the storm events; however, no correlation could be found between the *E. coli* numbers and the occurrence of VGs during the dry period or storm events. This demonstrates a potential risk of infection from *E. coli* carrying VGs in storm water even when *E. coli* numbers in water are not high.

The presence of a single or multiple VGs in an *E. coli* strain does not necessarily indicate that a strain is pathogenic unless that strain has the appropriate combination of VGs to cause disease in the host (36). The pathogenic *E. coli* strains use a complex multistep mechanism of pathogenesis involving a number of virulence factors depending upon the pathotype, which consists of attachment, host cell surface modification, invasin, a variety of toxins, and secretion systems which eventually lead toxins to the target host cells (16). Thus, VGs are ideal targets for determining the pathogenic potential of a given *E. coli* isolate (37).

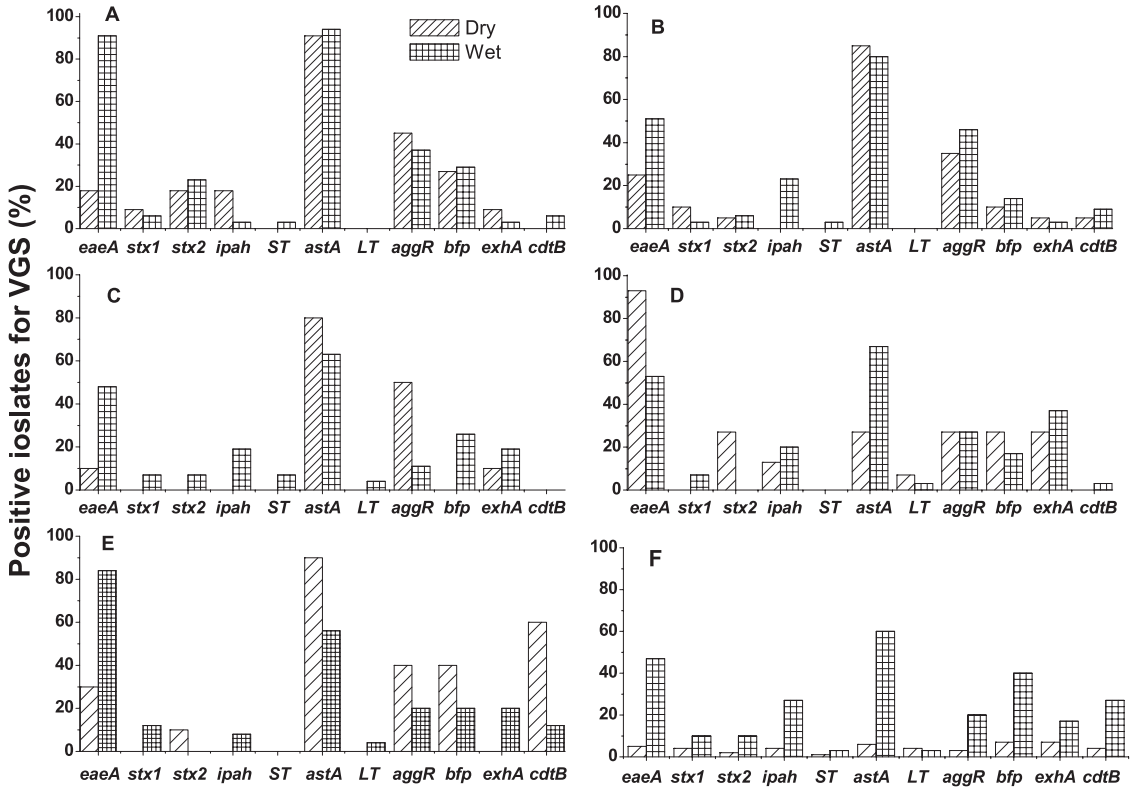

FIG 4 Comparative distribution of VGs in *E. coli* isolates at the six sites during the dry periods and after storm events. (A) Fitzgibbon Drain; (B) Cabbage Tree Creek; (C) Brisbane River; (D) Pine River; (E) Oxley Creek; and (F) Enoggera Creek.

Enteroaggregative *E. coli* (EAEC) strains cause persistent diarrhea in children and adults and are defined by the presence of heat-stable enterotoxin 1 (EAST1) along with *aggR* (16). In this study, the *astA* gene, encoding *E. coli* heat-stable enterotoxin 1 (EAST1), was found to be widely distributed in *E. coli* isolates from all sites (Table 3). The prevalence of the *astA* gene was statistically higher (ANOVA, $P < 0.001$) than that of the stx_1, ST, LT, and *cdtB* genes. The high prevalence of *E. coli* strains carrying the *astA* gene in fresh and estuarine water has also been reported previously (22) and could potentially be due to its reported presence in many commensal *E. coli* isolates (38). The results of this study showing the presence of the *astA* gene in diarrheagenic *E. coli* pathotypes EAEC, EHEC, and EPEC are in agreement with the previously reported wide distribution of this gene among diarrheagenic *E. coli* isolates from humans and animals (30, 39, 40). The high prevalence of the toxin gene *astA* in the *E. coli* isolates from the storm water is a cause of concern, as *E. coli* strains carrying *astA* toxin gene alone have been shown to cause diarrhea in studies from Japan and Spain (41, 42). Since the *astA* gene is also reported to be carried by many commensal *E. coli* strains, the implications of the wide prevalence of this gene in surface water remains an open question (39). All *E. coli* isolates were screened for the presence of the *aggR* gene to determine if they belong to the EAEC pathotype, and *E. coli* strains carrying both the *astA* and *aggR* genes were classified as typical EAEC. The *aggR* gene was detected in isolates from the dry period (36%) at a slightly higher frequency than in those from the wet periods (26%), suggesting a relatively high overall prevalence of this pathotype.

The *eaeA* gene, which codes for intimin protein, was the second most prevalent gene (56%) in the *E. coli* isolates from storm water. This gene is necessary for intimate attachment to host epithelial cells in both the EHEC and EPEC pathotypes. The frequency of occurrence of this gene was statistically higher at all times (ANOVA, $P < 0.001$) that that of ST, LT, and other VGs, which were infrequently detected and had a noticeably higher prevalence in the isolates collected after the storm event (61%) than during dry periods (42%). This observation is in agreement with a previously reported finding of significantly higher prevalence of the *eaeA* gene (up to 96%) in surface water (12, 22). EHEC causes hemorrhagic colitis and hemolytic uremic syndrome in humans, and key virulence factors include intimin (*eaeA* gene) and Shiga toxins (stx_1 and stx_2 genes) (36). The relatively high occurrence of the stx_2 gene (10%) compared to stx_1 (6%) in the storm water *E. coli* isolates suggests that *E. coli* carrying a combination of the *eae*A and stx_2 genes is more common than the combination of *eae*A and stx_1 genes. This observation is of concern, as the former combination of genes is known to cause more severe diarrhea in humans (18, 36). Typical EPEC strains carry the LEE pathogenicity island, which encodes for several virulence factors, including intimin (*eaeA*) and the plasmid-encoded bundle-forming pilus (*bfp*), which mediates adhesion to intestinal epithelial cells (3, 16). Therefore, all isolates were further tested for the presence of the *bfp* gene to determine if they belong to the EPEC pathotype. In this study, a noticeably higher prevalence of the *bfp* gene in isolates from storm water (27%) than from dry-period (17%) isolates suggests that a higher prevalence of the EPEC pathotype could be expected in the surface water bodies after storm events.

In addition to the presence of the *eaeA* gene in both EHEC and EPEC pathotypes, it was also detected in isolates which lacked other typical genes from both groups, and 3% of isolates carried only the *eaeA* gene. This suggests that there is a wide prevalence of this gene in *E. coli* found in aquatic ecosystems. Similarly, high prevalence of the *eaeA* gene in surface water has been reported in other studies (12, 22). This is a cause of concern, as an atypical EPEC pathotype which lacks the *bfp* gene but carries the *eaeA* gene has been found to be a major cause of gastroenteritis worldwide (43), in patients suffering from community-acquired gastroenteritis in Melbourne, Australia (44), and from children with diarrhea in Germany (45).

The *ehxA* toxin gene, which is carried by EHEC and non-Shiga-toxin-producing *E. coli* pathotypes (2, 16, 18, 46), was the second most commonly detected toxin gene (13%), with a slight increase in the detection frequency from 11 to 16% from dry to wet periods. Enteroinvasive *E. coli* (EIEC) strains carry the invasion plasmid antigen H (*ipaH*), which has been used for identification of isolates belonging to this pathotype (27). The frequency of occurrence of this gene was noticeably higher during the wet periods (18%) than during the dry periods (6%), which suggests noticeable movement of this pathotype into surface water from storm runoff.

The results of this study show that diarrheagenic *E. coli* pathotypes occur at each of the sampling sites during both dry and wet periods (Fig. 3). During the dry periods, a high percentage (53%) of isolates could be grouped under five main diarrheagenic *E. coli* pathotypes. EAEC strains, which cause persistent diarrhea in children and adults, were the single most common pathotype (23%). This was expected, as the heat-stable enterotoxin 1 (EAST1) gene (also known as *astA*) and the *aggR* gene which define this pathotype (16) were two of the most frequently detected genes. This is in agreement with the previously reported high prevalence (up to 80%) of the EAEC pathotype in fresh and estuarine samples (22). The high prevalence of this pathotype is of concern, as EAEC strains are the second-most-common agent of traveler's diarrhea after ETEC, with food and water being the most likely means of transmission (47, 48). EHEC and EPEC were the second- and third-most-common pathotypes detected in this study, with each group represented by 11% of isolates. This suggests that the three pathotypes EAEC, EHEC, and EPEC occur widely in the surface water at all sites.

A more uniform distribution of *E. coli* pathotypes was observed in *E. coli* isolates after the storm events, with EPEC (14%), EAEC (12%), and EIEC (10%) strains being the three most commonly detected pathotypes, followed by EHEC (7%) and ETEC (7%). Furthermore, the frequency of occurrence of EAEC pathotypes declined noticeably from 23 to 12% between the dry and wet periods. The observed decline in EAEC pathotypes and more uniform distribution of *E. coli* pathotypes after rainfall could possibly be due to mobilization of *E. coli* from point sources, such as wastewater treatment plant discharge, and nonpoint sources, such as animal sources (17, 18, 49, 50) and *E. coli* surviving in the soil (33), sediments (34), and aquatic environment (35). This could also be a possible explanation of the observed increase (5 to 20%) in the frequency of occurrence of isolates which could be placed under more than one pathotype. The occurrence of unusual combinations of VGs in *E. coli* isolates observed in this study could be explained on the basis of horizontal gene transfer between cells, which enables the exchange of genetic material located on mobile elements (transposons, integrons, or plasmids) among related or unrelated bacterial species (51). Further screening of the *E. coli* isolates with these unusual VG patterns in tissue culture or animal models would be required to demonstrate their pathogenicity.

In this study, we collected water samples from areas with diverse human population density and land use to determine if these factors influence the distribution of VGs (Fig. 4). The results of this study did not show any clear pattern of occurrence of VGs across the sites apart from a noticeable difference of occurrence of the *cdtB* gene (>10% isolates) at Oxley Creek and Enoggera Creek. This suggests that, overall, the contamination sources (point and nonpoint) were potentially similar across sites. There was a difference in the overall occurrence of VGs, with the Fitzgibbon Drain having a high occurrence and the Enoggera Creek site with one of the lowest occurrences of VGs. However, the difference in occurrences was statistically significant (ANOVA, $P < 0.05$) only between the Enoggera Creek and Brisbane River sites, with the latter site showing prevalence of stx_1, stx_2, ST, LT, *ipah*, and *bfp* genes only after rainfall, unlike the former site, which had a prevalence of these genes during both the dry period and after the storm events.

A better understanding of the prevalence and distribution of *E. coli* pathotypes in water sources used for potable, nonpotable, or recreation purposes could be an important tool in the development of public health risk mitigation strategies. Pathotyping of *E. coli* isolates may also provide useful information to identify potential sources of pollution, as the principal reservoirs of EAEC, EIEC, and EPEC pathotypes are humans, whereas the bovine intestinal tract is the main source of the EHEC pathotype (16, 50). The lower prevalence of the EHEC pathotype than other pathotypes suggests that human fecal contamination of the waterways is the main source of diarrheagenic *E. coli* pathotypes in the surface water as opposed to contamination from animals. This underscores the importance of managing municipal wastewater sources, such as sewage leaks and overflows and wastewater treatment plant discharge, in aquatic environments. Although the frequency of occurrence of certain VGs and pathotypes clearly increased after the rainfall, the presence of these genes could not be attributed to storm water runoff alone. The prevalence of VGs in the *E. coli* isolates collected during the dry periods suggests that there is always the presence of pathogenic *E. coli* in the surface water. The results demonstrate that the risk of contracting infection, however, may increase after the storm event.

Since this study was focused on the detection of *E. coli* pathotypes carrying VGs, it is plausible to assume that actual distribution of these VGs in surface water could be higher. While the ability of *E. coli* isolates described in this study to cause human diarrheal diseases was not demonstrated, a high proportion of isolates carried a full set of VGs linked to known pathotypes. Further screening for other VGs along with serotype testing and other assays may provide further information on pathogenicity of these isolates.

In conclusion, we found *E. coli* bacteria with defined pathotypes which originate mainly from human sources, as opposed to contamination from animals, in surface water samples. This underscores the importance of controlling sources of human fecal pollution, such as managing municipal wastewater sources to reduce potential risks to human health. This highlights the need for some degree of treatment of captured storm water prior to its reuse for potable and nonpotable purposes for public health risk

mitigation. This study clearly indicates that there is a need to develop a better understanding of public health implications of occurrence of *E. coli* carrying VGs in water sources used for potable, nonpotable, and recreational purposes.

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