Escherichia coli Virulence Genes Profile of Aquatic Environments with Different Characteristics

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Summary

We investigated the number and presence of 58 virulence genes (VGs) of pathogenic *E. coli* strains in 3 estuarine , 4 brackish and 13 freshwater sites in SE Qld during dry (n=20) and storm events (n=20). The numbers of *E. coli* varied during the dry season (2cfu/100mL to >5000cfu/100mL) and high rainfall events (28cfu/100mL and >5000cfu/100mL). Eighteen (90%) samples collected during the dry period were positive for multiple VGs ranging from 4 to 22 genes per sample. During episodes of high rainfall events all sites were positive for multiple VGs ranging from 7 to 30 VGs per sample. Six of the VGs (*iutA*, *cdtB*, *focG*, *kpsMT*K5, *eaeA* and *paa*) were over 50% more prevalent during the storm events. We also found that higher *E. coli* numbers didn't necessarily potentiate a higher presence of VGs.

Introduction

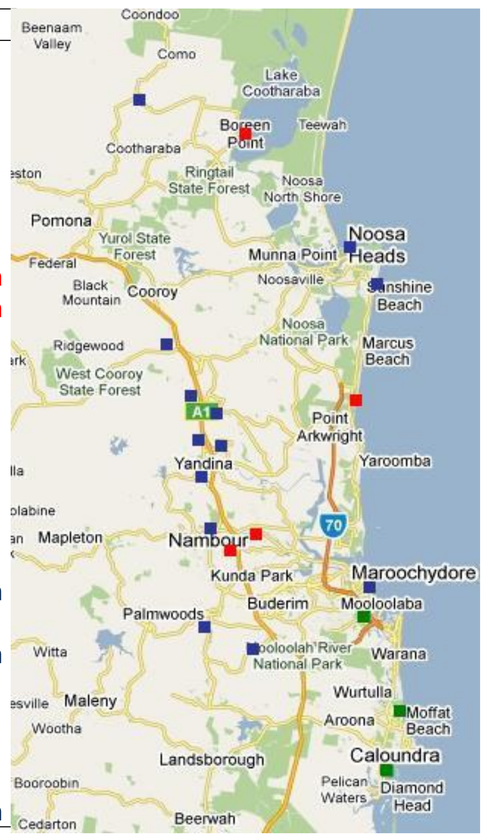
E. coli strains are commonly used as indicator bacteria for measuring the level of faecal pollution in water bodies. Whilst most *E. coli* strains are non-pathogenic commensals, certain strains may carry a combination of virulence genes (VGs) which enable them to cause intestinal or extraintestinal infections. However, the possession of a single or multiple VGs does not necessarily indicate a strain is pathogenic unless that strain has the appropriate combination of VGs to cause disease in a specific host[1]. Faeces from domestic and wild animals, and humans potentially contain high numbers of *E. coli* strains harbouring one or more VGs[2]. Runoff from agricultural lands and sewer overflows may also contribute pathogenic *E. coli* strains containing these VGs in environmental waters. Whilst some studies have investigated the presence of *E. coli* strains carrying VGs in environmental waters[3,4,5,6,7] they have not investigated VGs to the extent reported in this study and not in waters of various characteristics.

Materials and Methods

Samples were collected and processed within 6 hrs, 1lt of sample was filtered and incubated on Chromocult® agar. Samples were enriched in tryptic soy broth (TSB). 2 ml of broth culture was used for DNA extraction (DNeasy blood and tissue kits, QIAGEN).

Sampling sites: 20 sites with different characteristics were chosen for this study (see below).

Sampling sites	Location	Land use
Estuarine water		
EW1	Caloundra	Urban
EW2	Currimundi	Urban
EW3	Mooloolaba	Urban
Brackish water		
BW1	Coolum	Urban
BW2	Bli Bli	Peri-urban
BW3	Nambour/Bli Bli	Peri-urban
BW4	Boreen Point	Urban
Freshwater		
FW1	Alexandra Beach	Urban
FW2	Eumundi	Pasture
FW3	Noosa	Urban
FW4	Sunshine Beach	Urban
FW5	Mooloolah	Pasture
FW6	Eudlo	Peri-urban
FW7	Nambour	Urban
FW8	Yandina	Peri-urban
FW9	Yandina	Pasture
FW10	North Arm (east)	Pasture
FW11	North Arm (west)	Pasture
FW12	N. Arm/Eumundi	Pasture
FW13	Kin Kin	Peri-urban



58 virulence genes associated with intestinal and extra-intestinal *E. coli*: are outlined below, protocol for VG screening as previously described in Chapman et al[8].

E. coli pathotypes	Associated virulence genes					
	Adhesins	Toxins	Capsule Synthesis	Siderophores	Invasins	Additional virulence genes
ExPEC	*PapAH*	*hlyA*	*kpsMT*III	*fyuA*	*ibeA*	PAI
	fimH	*cvaC*	*kpsMT* K1	*iutA*		*traT*
	papEF	*cdtB*	*rfc*	*iroN*$_{E.coli}$		*ompT*
	bmaE	CNF1	*kpsMT* II			*iss*
	sfa/focDE	Univcnf	*kpsMT* K5			*yjaA*
	papG allele I	*cdt*	*ireA*			TSPE4C2
	nfaE					
	papC					
	focG					
	papG allele II					
	papG allele III					
	Afa/draBC					
	sfaS					
	papG allele *I'*					
DAEC	*aah*		*aidA* AIDA			
	aidA AIDA-I (*orfB*)					
	(*orfB*^{C})					
EHEC	*Iha*	*exhA*				*chuA*
	eaeA	*stx*$_2$				
	saa	*stx*$_1$				
ETEC	*fasA*	LT				
	faeG	STa				
	fanC	STb				
	fedA					
	F41					
EPEC	*Paa*					
	bfpA					
Eagg		*east1*				
EIEC					*ipaH*	

Results

The numbers of *E. coli* in the estuarine, brackish and fresh waters differed between sites and during the dry and storm events with the highest numbers found in fresh water during the storm water episodes (Figure 1 & 2).

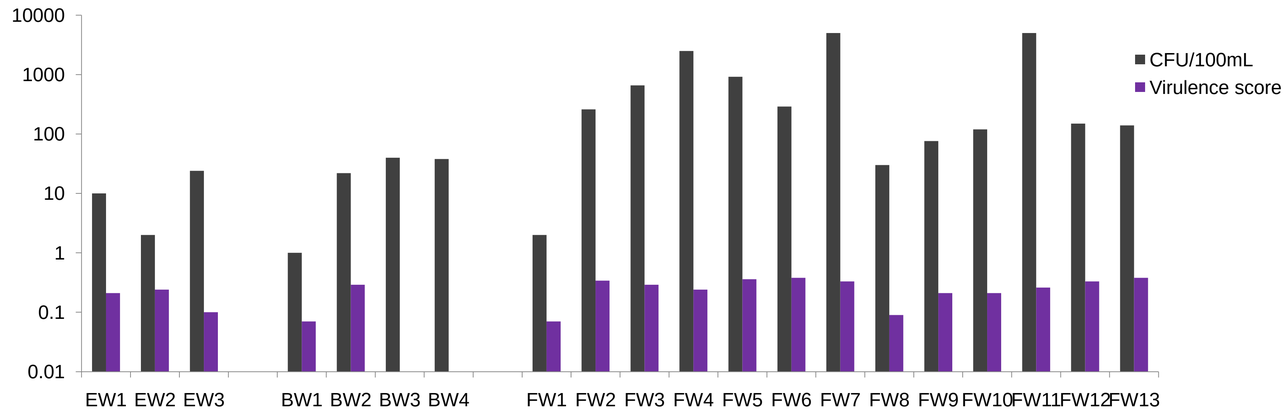


Figure 1. Number of *E. coli* (cfu/100mL) at sampling sites during the dry season, and the number of VGs present divided by the number of virulence genes tested (virulence score).

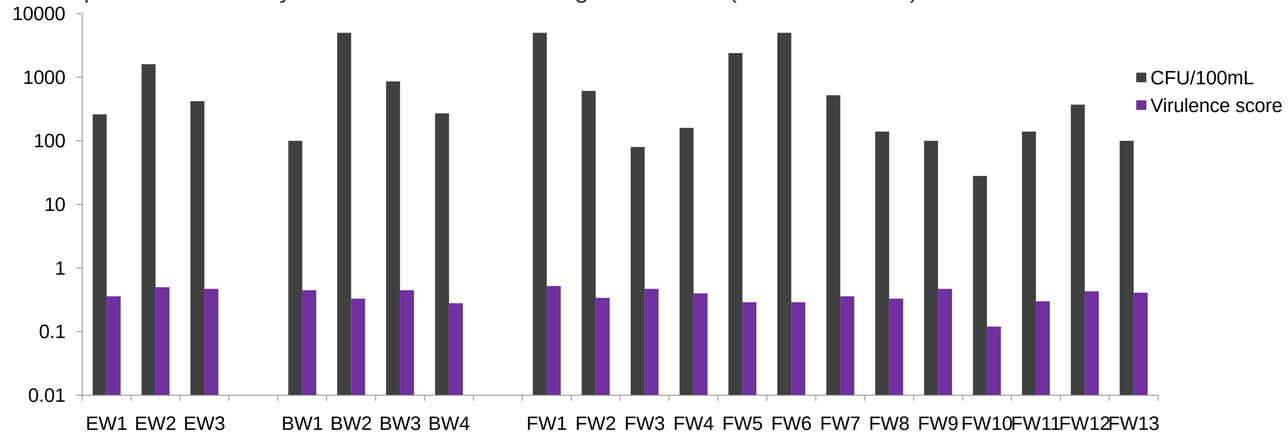


Figure 2. Number of *E. coli* (cfu/100mL) at sampling sites during the high rainfall events, and the number of VGs present divided by the number of virulence genes tested (virulence score).

18 (90%) samples were positive for between 4 to 22 genes per water sample during the dry season, all samples collected during high rainfall events were positive for multiple VGs ranging from 7 to 30 genes per water sample. Nineteen out of 58 tested VGs were never found in any water samples. The most commonly detected genes (>40%) are highlighted in table 3 and the prevalence of *E. coli* associated toxin genes is illustrated in table 4.

Table 3. The most commonly detected virulence genes (VG) during the dry season or high rainfall events (>40%).

VG	Dry Season (%)	Wet Season (%)	VG	Dry Season (%)	Wet Season (%)	VG	Dry Season (%)	Wet Season (%)
PAI	55	60	*fimH*	85	100	*ibeA*	60	90
fyuA	65	90	*sfa/focDE*	20	45	*iutA*	35	90
*kps*MTII	50	10	*cdtB*	0	90	*focG*	10	95
traT	80	55	*kps*MTk5	35	80	iroN $_{E.coli}$	45	10
ompT	70	30	*iss*	50	90	*ireA*	5	45
eaeA	10	100	F41	5	55	*chuA*	85	95
yjaA	55	85	TSPE4.C2	85	90			

Table 4. The number (%) of sites positive for toxin genes associated with various virulent *E. coli* pathotypes.

Toxin VG	Dry Season (%)	Wet Season (%)	Toxin VG	Dry Season (%)	Wet Season (%)	Toxin VG	Dry Season (%)	Wet Season (%)
hlyA	0	0	*cvaC*	35	20	*cdtB*	0	90
cnf1	0	0	univCNF	0	35	*ehxA*	0	45
stx$_2$	25	60	*stx*$_1$	25	5	*eltA*	0	10
estII	0	0	*estI*	5	15	*east1*	70	95
cdt	40	60						

Conclusion

- Numbers of *E. coli* in 7 and 13 samples collected during the dry and wet season respectively exceeded the Australian and New Zealand Environment and Conservation Council (ANZECC) recreational water quality guidelines for fresh and marine waters.
- 18 sites during dry period and all sites during the rainy episodes were positive for multiple *E. coli* VGs some of which were significantly more prevalent in the wet season samples indicating a potential association with the storm water run-off.
- The virulence scores established for each water sample, indicated that higher *E. coli* numbers didn't necessarily potentiate a higher presence of virulence genes and that a water sample with low *E. coli* number could contain strains with high and potential VGs.

References

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