Supplementary Table 1: List of the four sampling sites, their codes, geographical description and observed anthropogenic activities within the study sites

Site name	Description	Anthropogenic Activities	Coordinates
Maden dam	This dam is about 22km from King William's Town and is well known for regular fishing activities. The dam is surrounded by weed beds which provide food for the fish.	Fishing, tourism and domestic activities.	32° 44' 00" S, 27° 16' 00" E
Rooikrantz dam	It is a dam on the Buffalo River, about 15 km northwest of King William's Town in the Eastern Cape Province. It lies due east of the larger and newer Sandile Dam.	Fishing, animal rearing, irrigation, tourism and domestic purposes	32° 45' 2" S, 27° 19' 07" E
King William's Town	King William's Town is along the banks of the Buffalo River. The area's economy depends on cattle and sheep ranching, and the town itself has a large industrial base producing textiles, soap, candles, sweets, cartons and clothing.	Deposition of dirt and chemical pollutants, dumping site, WWTP effluent discharge	32° 53' S, 27° 24' E
Eluxolzweni	Eluxolzweni is an informal settlement situated next to the N2 freeway just north of Mdantsane. It falls under Buffalo City Metropolitan Municipality in the Eastern Cape Province of South Africa.	Fishing, irrigation, domestic purposes, WWTP effluent discharge	32° 91' 36" S, 27° 75' 69" E

WWTP: Wastewater Treatment Plant (Report highlights impacts on river health, 2005).

Supplementary Table 2: Primer sequence, PCR cycling conditions and expected amplicon sizes for the confirmation and delineation of *E. coli* into its pathotypes.

Target strains	Gene	Primer sequence (5′-3′)	PCR cycling conditions	Amplicon size	Reference
E. coli	*uidA* housekeeping gene	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTAACAGTCTTGCG	94 °C, 5 min; 35[95 °C, 30 sec; 58 °C, 1 min; 72 °C, 1 min] 72 °C, 8 min	147	(Moyo et al., 2007)
EAEC	*eagg* adhesion gene	F: AGACTCTGGCGAAAGACTGTATC R: ATGGCTGTCTGTAATAGATGAGAAC	95 °C, 15 min; 35 cycles [94 °C, 45 s; 55 °C, 45 s; 68 °C, 2 min]; 72 °C, 5 min	194	(Kong et al., 2002)
EIEC	*ipaH* invasion gene	F: CTC GGCACGTTTTAATAGTCTGG R: GTGGAGAGCTGAAGTTTCTCTGC	95 °C, 5 min; 30 cycles [95 °C, 45 sec; 55 °C, 45 sec; 72 °C, 45 sec]; 72 °C, 10 min	320	(Vidal et al., 2005)
DAEC	*daa*E adhesion gene	F: GAACGTTGGTTAATGTGGGGTAA R: TATTCACCGGTCGGTTATCAGT	94 °C, 2 min; 40 cycles [92°C, 30 s; 59 °C, 30 s; 72 °C, 30 s]; 72 °C, 5 min	542	(Vidal et al., 2005)
ETEC	*lt* toxin gene	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	94 °C, 2 min; 35 cycles [94 °C, 1 min; 55 °C, 1 min, 72 °C, 1 min]; 72 °C, 5 min	450	(López-Saucedo et al., 2003)
EPEC	*eae* adhesion gene	F: TCAATGCAGTTCCGTTATCAGTT R: GTAAAGTCCGTTACCCCAACCTG	95 °C, 15 min; 35 cycles [94 °C, 45 s; 55 °C, 45 s; 68 °C, 2 min]; 72 °C, 5 min	482	(Stacy-Phipps et al., 1995)
	bfp adhesion gene	F: AGACTCTGGCGAAAACTGTATC R: ATGGCTGTCTGTAATAGATGAGAAC	94 °C, 2 min; 40 cycles [94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min]; 72 °C, 5 min	300	(Stacy-Phipps et al., 1995)
EHEC	*stx1* toxin gene	F: ATAAATCGCCATTCGTTGACTAC R: AGAACGCCCACTGAGATCATC	94 °C, 3 min; 35 cycles [93 °C, 60 s; 55 °C, 60 s; 72 °C, 60 sec]; 72 °C, 7 min	180	(Paton and Paton, 1998)
	stx2 toxin gene	F: GGCACTGTCTGAAACTGCTCC R: TCGCCAGTTATCTGACATTCTG	94 °C, 3 min; 35 cycles [93 °C, 60 s; 55 °C, 60 s; 72 °C, 60 sec]; 72 °C, 7 min	255	(Paton and Paton, 1998)

Supplementary Table 3: Data inputted for exposure and diarrheal disease risk modelling in each study site

	Maden dam	**Rooikrantz dam**	**King William's Town dam**	**Eluxolzweni dam**	**Data source**
Concentration (C) of *E. coli* (CFU/100 mls × 10^2)	Min: 9.20 Mean: 9.53 Max: 9.80	Min: 10.80 Mean: 11.00 Max: 11.20	Min: 15.00 Mean: 16.00 Max: 17.00	Min: 7.00 Mean: 8.17 Max: 9.50	This study
Recovery efficiency (R) (%)	16.00	12.00	4.00	8.00	This study
Proportion (I) of *E. coli* capable of causing diarrheal disease (%)	29.00	27.00	27.00	28.00	This study
Daily amount (M) of water directly or indirectly ingested (ml/day)	10.00	10.00	10.00	10.00	(Shuval et al., 1997)

Min: Minimum, Max: Maximum

Supplementary Table 4: list of antimicrobials used to carry out antimicrobial susceptibility testing of the DEC

SN	Antimicrobial	Code/concentration	Class
1	Doxycycline	DXT/30 µg	Tetracyclines
2	Tetracycline	T/30 µg	
3	Norfloxacin	NOR/30 µg	Fluoroquinolones
4	Ciprofloxacin	CIP/5 µg	
5	Chloramphenicol	C/30 µg	Phenicols
6	Nalidixic acid	NA/30 µg	Quinolones
7	Cefuroxime	CXM/30 µg	Cephems
8	Cefotaxime	CTX/30 µg	
9	Trimethoprim/sulphamethoxazole	TS/25 µg:25 µg	Sulfonamides
10	Amikacin	AK/30 µg,	Aminoglycosides
11	Gentamycin	GM/10 µg	
12	Nitrofurantoin	NI/300 µg	Nitrofurans
13	Ampicillin	AP/10 µg	β-lactams
14	Amoxicillin-clavulanic acid	AUG/30 µg	
15	Meropenem	MEM/10 µg	Carbapenems
16	Imipenem	IMI/10 µg	

Supplementary Table 5: The primer sequence and expected amplicon size used for the screening of resistance genes in the pathogenic strains of *E. coli*

Antibiotic class	**Genes**	**PCR primer sequence (5′–3′)**	**PCR cycling condition**	**Amplicon size (bp)**	**Reference**
Tetracyclines	*tet*A	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	210	(Mendez et al., 1980)
	*tet*B	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	659	(Marshall et al., 1983)
	*tet*C	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	418	(Marshall et al., 1983)
	*tet*D	F: AAACCATTACGGCATTCTGC R: GACCGGATACACCATCCATC	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	787	(Marshall et al., 1983)
	*tet*E	F: AAACCACATCCTCCATACGC R: AAATAGGCCACAACCGTCAG	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	278	(Marshall et al., 1986)
	*tet*G	F: GCTCGGTGGTATCTCTGCTC R: AGCAACAGAATCGGGAACAC	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	468	(Zhao and Aoki, 1992)
	*tet*K	F: TCGATAGGAACAGCAGTA R: CAGCAGATCCTACTCCTT	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	169	(Warsa et al., 1996)
	*tet*L	F: TCGTTAGCGTGCTGTCATTC R: GTATCCCACCAATGTAGCCG	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	267	(Burdett et al., 1982)
	*tet*M	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC	94 °C,5m; 35[94 °C,1m; 55 °C,1m; 72 °C,1.5m]; 72 °C,5m	406	(Warsa et al., 1996)

Sulfonamides	*sulI*	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	94 °C,5m; 30[94 °C,30s; 65 °C,30s; 72 °C,2m]; 72 °C,10m	433	(Kerrn et al., 2002)
	sulII	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	94 °C,5m; 30[94 °C,30s; 65 °C,30s; 72 °C,2m]; 72 °C,10m	293	(Kerrn et al., 2002)
Phenicols	*cmlA1*	F: CACCAATCATGACCAAG R: GGCATCACTCGGCATGGACATG	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	115	(Post and Hall, 2009)
	catI	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	320	(Maynard et al., 2004)
	catII	F: ACACTTTGCCCTTTATCGTC R: TGAAAGCCATCACATACTGC	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	543	(Maynard et al., 2004)
Aminoglycosides	*strA*	F CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	94 °C,4m; 30[94 °C,45s; 50 °C,45s; 72 °C,45s]; 72 °C,5m	348	(Velusamy et al., 2007)
	aadA	F: GTGGATGGCGGCCTGAAGCC R: AATGCCCAGTCGGCAGCG	94 °C,4m; 30[94 °C,45s; 50 °C,45s; 72 °C,45s]; 72 °C,5m	525	(Velusamy et al., 2007)
	aac(3)-IIa (aacC2)[a]	F: CGGAAGGCAATAACGGAG R: TCGAACAGGTAGCACTGAG	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	428	(Maynard et al., 2004)
	aph(3)-Ia (aphA1)[a]	F: ATGGGCTCGCGATAATGTC R: CTCACCGAGGCAGTTCCAT	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	600	(Maynard et al., 2004)

	aph(3)-IIa (aphA2)[a]	F: GAACAAGATGGATTGCACGC R: GCTCTTCAGCAATATCACGG	94 °C,5m; 30[94 °C,30s; 50 °C,30s; 72 °C,1.5m]; 72 °C,5m	510	(Maynard et al., 2004)

Supplementary Table 6: The primer sequence and expected amplicon size used for the screening of *AmpC* β-lactamase (Velusamy et al., 2007) and ESBLs in pathogenic strains of *E. coli* (Dallenne et al., 2010).

PCR name	Targeted β-Lactamase(s)	Primer name	Primer sequence (5' –3')	PCR cycling condition	Amplicon size (bp)
Simplex AmpC[a]	*AmpC*	AmpC_for AmpC_rev	TTCTATCAAMACTGGCARCC CCYTTTTATGTACCCAYGA	94 °C, 4m; 30[94 °C, 45 s; 60 °C, 45s; 72 °C, 45s]; 72 °C, 7m	550
Multiplex I TEM, SHV and OXA-1-like	TEM variants including TEM-1 and TEM-2	MultiTSO-T_for MultiTSO-T_rev	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	94 °C, 10m; 30[94 °C, 40 s; 60 °C, 40s; 72 °C, 1m]; 72 °C, 7m	800
	SHV variants including SHV-1	MultiTSO-S_for MultiTSO-S_rev	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC		713
	OXA-1, OXA-4 and OXA-30	MultiTSO-O_for MultiTSO-O_rev	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG		564
Multiplex II CTX-M group 1, group 2 and group 9	Variants of CTX-M group 1 including CTX-M-1, CTX-M-3 and CTX-M-15	MultiCTXMGp1_for MultiCTXMGp1-2_rev	TTAGGAARTGTGCCGCTGYA[b] CGATATCGTTGGTGGTRCCAT[b]	94 °C, 10m; 30[94 °C, 40 s; 60 °C, 40s; 72 °C, 1m]; 72 °C, 7m	688
	variants of CTX-M group 2 including CTXM-2	MultiCTXMGp2_for MultiCTXMGp1-2_rev	CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT[b]		404
	Variants of CTX-M group 9 including CTX-M-9 and CTX-M-14	MultiCTXMGp9_for MultiCTXMGp9_rev	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG		561
CTX-M group 8/25	CTX-M-8, CTX-M-25, CTX-M-26 and CTX-M-39 to CTX-M-41	CTX-Mg8/25_for CTX-Mg8/25_rev	AACRCRCAGACGCTCTAC[b] TCGAGCCGGAASGTGTYAT[b]		326

Multiplex III ACC, FOX, MOX, DHA, CIT and EBC (plasmid-mediated AmpC)	ACC-1 and ACC-2	MultiCaseACC_for MultiCaseACC_rev	CACCTCCAGCGACTTGTTAC GTTAGCCAGCATCACGATCC	94 °C, 10m; 30[94 °C, 40 s; 60 °C, 40s; 72 °C, 1m]; 72 °C, 7m	346
	FOX-1 to FOX-5	MultiCaseFOX_for MultiCaseFOX_rev	CTACAGTGCGGGTGGTTT CTATTTGCGGCCAGGTGA		162
	MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11 and CMY-19	MultiCaseMOX_for MultiCaseMOX_rev	GCAACAACGACAATCCATCCT GGGATAGGCGTAACTCTCCCAA		895
	DHA-1 and DHA-2	MultiCaseDHA_for MultiCaseDHA_rev	TGATGGCACAGCAGGATATTC GCTTTGACTCTTTCGGTATTCG		997
	LAT-1 to LAT-3, BIL-1, CMY-2 to MY-7, CMY-12 to CMY-18 and CMY-21 to CMY-23	MultiCaseCIT_for MultiCaseCIT_rev	CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY[b]		538
	ACT-1 and MIR-1	MultiCaseEBC_for MultiCaseEBC_rev	CGGTAAAGCCGATGTTGCG AGCCTAACCCCTGATACA		683
Multiplex IV VEB, PER and GES	GES-1 to GES-9 and GES-11	MultiGES_for MultiGES_rev	AGTCGGCTAGACCGGAAAG TTTGTCCGTGCTCAGGAT	94 °C, 10m; 30[94 °C, 40 s; 57 °C, 40s; 72 °C, 1m]; 72 °C, 7m	399
	PER-1 and PER-3	MultiPER_for MultiPER_rev	GCTCCGATAATGAAAGCGT TTCGGCTTGACTCGGCTGA		520
	VEB-1 to VEB-6	MultiVEB_for MultiVEB_rev	CATTTCCCGATGCAAAGCGT CGAAGTTTCTTTGGACTCTG		648

Multiplex V GES and OXA-48-like	GES-1 to GES-9 and GES-11	MultiGES_for MultiGES_rev	AGTCGGCTAGACCGGAAAG TTTGTCCGTGCTCAGGAT	94 °C, 10m; 30[94 °C, 40 s; 60 °C, 40s; 72 °C, 1m]; 72 °C, 7m	399
	OXA-48-like	MultiOXA-48_for MultiOXA-48_rev	GCTTGATCGCCCTCGATT GATTTGCTCCGTGGCCGAAA		281
Multiplex VI IMP, VIM and KPC	IMP variants except IMP-9, IMP-16, IMP-18, IMP-22 and IMP-25	MultiIMP_for MultiIMP_rev	TTGACACTCCATTTACDG[b] GATYGAGAATTAAGCCACYCT[b]	94 °C, 10m; 30[94 °C, 40 s; 55 °C, 40s; 72 °C, 1m]; 72 °C, 7m	139
	VIM variants including VIM-1 and VIM-2	MultiVIM_for[c] MultiVIM_rev[c]	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG		390
	KPC-1 to KPC-5	MultiKPC_for MultiKPC_rev	CATTCAAGGGCTTTCTTGCTGC ACGACGGCATAGTCATTTGC		538

[a] This primer pair was previously described by (Velusamy et al., 2007).
[b]Y=T or C; R=A or G; S=G or C; D=A or G or T.
[c]This primer pair was previously described by (Ellington et al., 2007).

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