

Supplementary Material

# Characterization of *Escherichia coli* Isolates in Recreational Waters: Implications for Public Health and One Health Approach

Lúcia Gomes<sup>1</sup>, Adriano A. Bordalo <sup>1,2</sup> and Ana Machado <sup>1,2\*</sup>

<sup>1</sup> Laboratory of Hydrobiology and Ecology, Instituto de Ciências Biomédicas Abel Salazar (ICBAS—UP), University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; [bordalo@icbas.up.pt](mailto:bordalo@icbas.up.pt) (A.A.B.); [lcgomes@icbas.up.pt](mailto:lcgomes@icbas.up.pt) (L.G.)

<sup>2</sup> Interdisciplinary Centre of Marine and Environmental Research (CIIMAR—UP), University of Porto, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

\* Correspondence: [ammachado@icbas.up.pt](mailto:ammachado@icbas.up.pt) (A.M.)

**Table S1.** List of primers and PCR conditions used in the present work.

Gene	Primers	Primer Sequence (5' → 3')	bp	Concentration μM	Annealing °C	Reference				
Fecal Detection										
<i>E. coli</i> (23S rRNA)	EC23S857F	GGTAGAGCACTGTTTGGCA	87	1	60	[40]				
	EC23S857R	TGTCTCCCGTGATAACTTTCTC								
<i>uidA</i>	UAL1939b	ATGGAATTTCGCCGATTTTGC	187	0.4		60	[41]			
	UAL2190b	ATTGTTTGCCTCCCTGCTGC								
Phylogenetic Group										
<i>chuA</i>	chuA-F	GACGAACCAACGGTCAGGAT	279	0.4	72	[42]				
	chuA-R	TGCCGCCAGTACCAAAGACA								
<i>yjaA</i>	yjA-F	TGAAGTGTGACGAGACGCTG	211	0.4			72	[42]		
	yjA-R	ATGGAGAATGCGTTCCTCAAC								
tspE4.C2	tspE4C2-F	GAGTAAGTCGGGGCATTCA	152	0.4					72	[42]
	tspE4C2-R	CGCGCCAACAAAGTATTACG								
	ibe10-R	TGGTGCTCCGGCAAACCATGC								

**Table S2.** List of primers and PCR conditions used in the present work (*Cont.*).

Gene	Primers	Primer Sequence (5' → 3')	bp	Concentration μM	Annealing °C	Reference	
DEC Genes							
<i>eltB</i>	LT-F	GGCTGGACATCATGGGAACTGG	322	0.2	72	[77]	
	LT-R	CGTCGGGAACGGGTAGAATCG					
<i>estA</i>	ST-F	TGATTAACCCCGCGACGGGAA	147				
	ST-R	CGCAGTAGGCACGATGTTGTA					
<i>vt1</i>	VT1-F	AAGATGGAGTTTCCTATGCAGGAG	130	0.4		[78]	
	VT1-R	CATTCAGAGTCCTGCCCTCATTATT					
<i>vt2</i>	VT2-F	ACCGTTTTTCAGATTTTGACACATA	298	0.2		[77]	
	VT-R	TACACAGGAGCAGTTTCAGACAGT					
<i>eaeA</i>	eae-F	CACACGAATAAACTGACTAAAATG	376				
	eae-R	AAAAACGCTGACCCGCACCTAAAT					
<i>bfpA</i>	bfpA-F	TTCTTGGTGCTTGCGTGCTTTT	367				
	bfpA-R	TTTGTTTGTTGTATCTTTGTAA					
<i>ipaH</i>	ipaH-F	GCTGGAAAAACTCAGTGCCT	424				[79]
	ipaH-R	CCAGTCCGTAAATTCATTCT					
pCVD	EA-F	CTGGCGAAAGACTGTATCAT	630				[80]
	EA-R	CAATGTATAGAAATCCGCTGTT					
APEC associated genes							
<i>iutA</i>	iutA-F	GGCTGGACATCATGGGAACTGG	302	0.45	68	[81]	
	iutA-R	CGTCGGGAACGGGTAGAATCG					
<i>fyuA</i>	fyuA-F	TGATTAACCCCGCGACGGGAA	880	0.6	63	[82]	
	FyuA-R	CGCAGTAGGCACGATGTTGTA					
<i>cnf1</i>	cnf1-F	AAGATGGAGTTTCCTATGCAGGAG	498	0.4		[83]	
	cnf1-R	CATTCAGAGTCCTGCCCTCATTATT					
<i>cvaC</i>	cvaC-F	CACACACAAACGGGAGCTGTT	680	0.6		[84]	
	cvaC-R	CTTCCCGCAGCATAGTTCCAT					
<i>iss</i>	iss-F	CAGCAACCCGAACCACTTGATG	323	1	60	[84]	
	iss-R	AGCATTGCCAGAGCGGCAGAA					
<i>ompT</i>	ompT-F	TCATCCCGGAAGCCTCCCTCACTACTAT	496			[85]	
	ompT-R	TAGCGTTTGCTGCACTGGCTTCTGATAC					
<i>ibe10</i>	ibe10-F	AGGCAGGTGTGCGCCGCGTAC	170	0.6	63	[82]	
	ibe10-R	TGGTGCTCCGGCAAACCATGC					

**Table S2.** Identification, physico-chemical parameters and fecal contamination indicators for each sampling site evaluated.

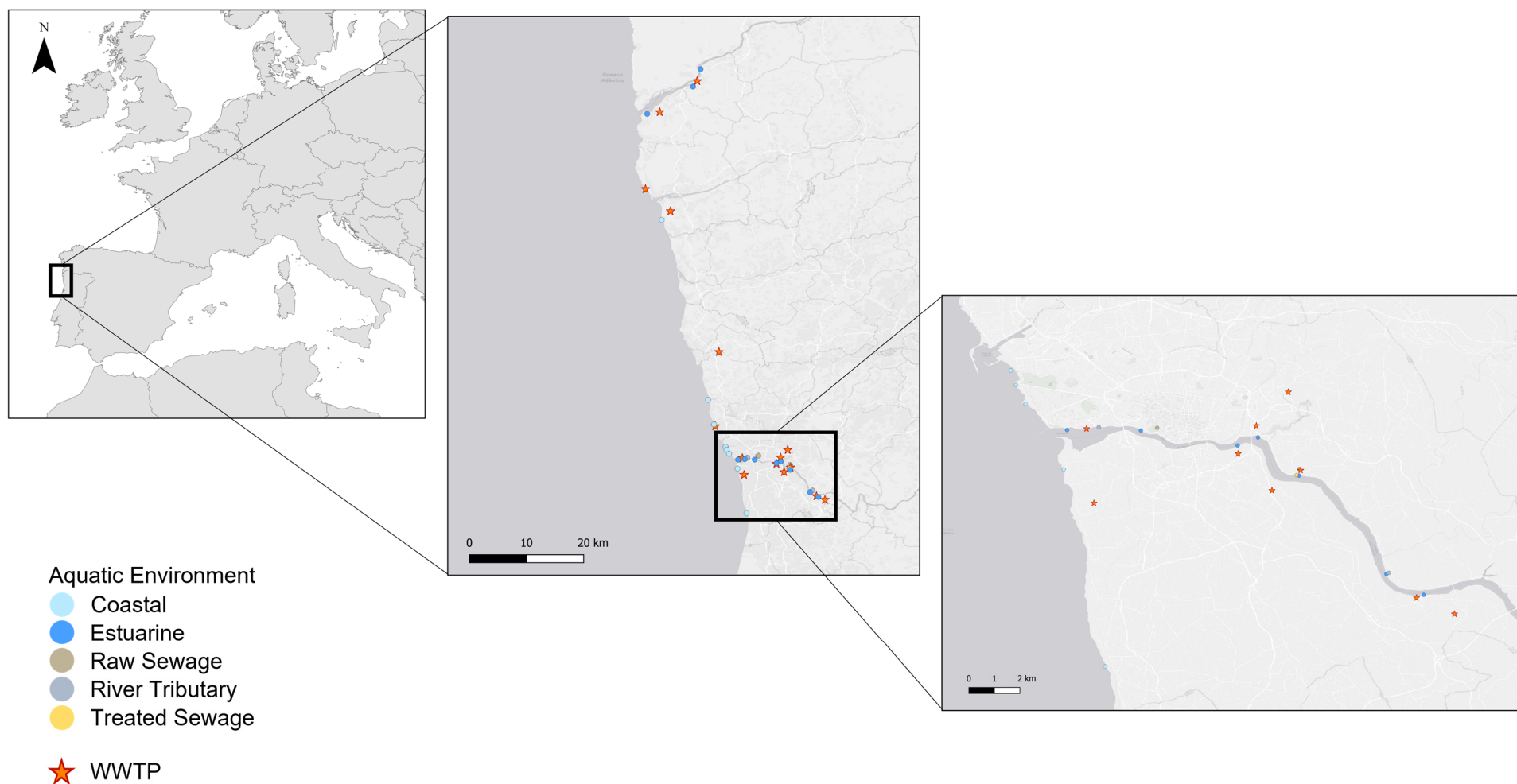
Sampling site	Aquatic environment	Latitude	Longitude	Date	Temperature	Conductivity	Salinity	pH	Turbidity	<i>E. coli</i>		Fecal coliforms	
					°C	mS/cm				CFU/100 mL	SD	CFU/100 mL	SD
Caminha	Estuarine	41.8678	-8.8512	November_22	16.10	29.12	18.10	7.75	2.10	476	351	1250	782
Boega	Estuarine	41.9248	-8.7598	November_22	15.20	0.10	0.10	7.55	3.90	705	960	2230	2120
Morraceira	Estuarine	41.9599	-8.7459	November_22	14.80	0.20	0.10	7.47	2.10	219	289	855	463
Amorosa	Coastal	41.6442	-8.8256	February_22	12.50	52.80	34.80	8.04	0.01	6	1	7	2
Labruga	Coastal	41.2721	-8.7289	December_21	12.30	52.69	34.40	7.40	1.30	20	13	18	6
				January_22	12.20	51.60	33.80	7.45	6.60	45	8	5	3
				June_22	15.40	53.70	35.50	8.06	4.21	62	6	77	15
				August_22	18.50	43.86	30.50	8.16	1.28	22	3	8	3
Cabo do Mundo	Coastal	41.2212	-8.1761	February_22	12.70	41.17	35.50	8.18	0.00	4	2	2	1
Matosinhos	Coastal	41.1733	-8.6918	May_22	17.80	51.20	33.70	8.27	2.20	1430	321	2770	569
Castelo do Queijo	Coastal	41.1674	-8.6904	March_22	13.80	52.80	34.70	8.22	2.60	50	28	143	15
				July_22	17.10	53.20	35.00	8.19	0.99	365	23	380	126
Homem do Leme	Coastal	41.1590	-8.6861	February_22	12.50	53.30	35.00	8.26	0.00	13	6	3	1
				September_21	16.94	27.83	20.64	7.28	1.00	800	200	1900	624
Pilotos	Estuarine	41.1466	-8.6665	November_21	13.66	24.54	19.31	7.40	1.40	1350	71	1900	200
				September_22	16.82	17.35	10.26	7.78	2.70	2400	252	6170	306
Ribeira da Granja	River Tributary	41.1478	-8.6525	October_21	18.14	8.48	4.74	7.18	2.20	28500	4900	56600	3860
Massarelos	Estuarine	41.1463	-8.6322	November_22	13.95	0.50	0.24	6.95	3.30	29400	757	61000	3520
Oliveira de Ouro	Estuarine	41.139	-8.5871	October_21	19.10	10.12	6.50	7.76	4.30	1130	306	4400	872
Freixo	Estuarine	41.1430	-8.5775	November_22	14.15	0.14	0.08	6.92	2.30	3600	651	11700	2000
Gramido - Douro	Estuarine	41.1251	-8.5585	March_22	12.50	0.21	0.10	7.34	33.20	1000	106	2160	536
Zebreiros	Estuarine	41.0794	-8.5178	June_22	21.60	9.41	5.30	8.19	0.38	110	30	340	192
Crestuma	Estuarine	41.1478	-8.6525	November_22	14.53	0.12	0.07	6.70	3.20	650	289	2100	265

SD – standard deviation ( $n=3$ )

**Table S2.** Identification, physico-chemical parameters and fecal contamination indicators for each sampling site evaluated (*Cont.*).

Sampling site	Aquatic environment	Latitude	Longitude	Date	Temperature	Conductivity	Salinity	pH	Turbidity	<i>E. coli</i>		Fecal coliforms	
					°C	mS/cm				CFU/100 mL	SD	CFU/100 mL	SD
Lavadores	Coastal	41.1280	-8.6681	January_22	13.10	51.40	33.70	7.65	3.90	86	9	41	13
S. Félix da Marinha	Coastal	41.0360	-8.6486	February_22	13.40	41.93	35.60	8.23	0.56	39	1	1	-
Tanque de Zebreiros	River Tributary	41.0798	-8.5176	April_22	n.a.	n.a.	n.a.	n.a.	n.a.	1500	707	2500	707
Gramido - WWTP	Raw Sewage	41.1274	-8.5578	July_22	23.98	1.27	0.63	8.00	315	2970000	321000	13700000	8020000
Gramido	Treated Sewage	41.1254	-8.5596	March_22	n.a.	n.a.	n.a.	n.a.	n.a.	115000	26600	177000	-
ICBAS - WWTPin	Raw Sewage	41.1465	-8.6246	August_22	n.a.	n.a.	n.a.	n.a.	n.a.	643000	419000	867000	160000
ICBAS - WWTPout	Raw Sewage	41.1465	-8.6246	August_22	n.a.	n.a.	n.a.	n.a.	n.a.	330000	122000	2260000	243000

SD – standard deviation (*n*=3); n.a. – not analyzed; WWTP – wastewater treatment plant



**Figure S1.** Location of the sampling sites in the NW Portugal identified by aquatic environment. WWTP – wastewater treatment plant.