

Supplementary Materials:

Table S1 - Water systems sampled and sampling dates.

Country	Water system	Samples	Dates
Portugal (PT)	WWTP	RWW 11	13.06.2017
		sTWW 11	
		RWW 14	26.07.2017
		sTWW 14	
United States (US)	Simulated reclaimed water distribution system	SRWDS 1	05.09.2017
		SRWDS 2	13.09.2017
		SRWDS 3	18.09.2017
	River	River 1	13.09.2017
		River 2	15.09.2017
		River 3	19.09.2017
	Tap water with GAC system	TW 1	05.09.2017
		TW 2	14.09.2017
		TW 3	18.09.2017
	WWTP	RWW 1	13.09.2017
		sTWW 1	
		tTWW 1	
		RWW 2	15.09.2017
sTWW 2			
tTWW 2			
RWW 3		19.09.2017	
sTWW 3			
tTWW 3			

Table S2- Quantitative-PCR conditions used in this study. US refers to protocols used in the United States and PT to protocols used in Portugal.

Target Gene	Primers	Primers sequence	Conditions	Limit of quantification (no. of copies)	Primers Reference
16S rRNA	1369 FW	CGGTGAATACGTTTCYCGG	US: 98°C 2 min (1 cycle), 98°C 5 s - 55°C 5 s (40 cycles) Other: 3b	100	[56]
	1492 RV	GGWTACCTTGTTACGACTT			
16S rRNA	1114F	CGGCAACGAGCGCAACCC	PT: 95°C for 10 min (1 cycle); 95°C for 15 s, 55°C for 20 s and 72°C for 10 s (35 cycles) Other: 1a	402	[57]
	1275R	CCATTGTAGCACGTGTGTAGCC			
23S rRNA	entero FW	AGAAATTCCAAACGAACTTG	US: 50°C 2 min (1 cycle), 95°C 10 min - 95°C 15 s - 60°C 60 s (40 cycles) Other: 4a	1	[35]
	entero RV Probe	CAGTGCTCTACCTCCATCATT TGGTTCTCTCCGAAATAGCTTTAGGGCTA			
<i>gadAB</i>	gadrt-1	GCGTTGCGTAAATATGGTTGCCGA	US: 95°C 2 min (1 cycle), 95°C 5 s - 69°C 5 s (40 cycles) Other: 3b / PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 1b	US: 10 / PT: 43	[36]
	gadrt-2	CGTCACAGGCTTCAATCATGCGTT			
<i>sul1</i>	sul1 FW	CGCACCGGAAACATCGCTGCAC	US: 98°C 2 min (1 cycle), 98°C 5 s - 69°C 5 s (40 cycles) Other: 3b / PT: 95°C 5 min (1 cycle), 95°C 10 s - 60°C 30 s (35 cycles) Other: 5d	US: 100 / PT: 96	[40]
	sul1 RV	TGAAGTCCGCCGCAAGGCTCG			
<i>sul2</i>	sul2 FW	TCCGGTGGAGGCCGGTATCTGG	US: 98°C 2 min (1 cycle), 98°C 5 s - 67.5°C 5 s (40 cycles) Other: 3b / PT: 95°C 5 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 1a	US: 100 / PT: 47	[40]
	sul2 RV	CGGGAATGCCATCTGCCTTGAG			
<i>tet(A)</i>	tetA FW	GTAATTCTGAGCACTGTCGC	US: 98°C 2 min (1 cycle), 98°C 5 s - 57.2°C 10 s - 72°C 15 s (40 cycles) Other: 3b / PT: 95°C 10 min (1 cycle), 95°C 15 s -	US: 100 / PT: 51	[43]
	tetA RV	CATAGATCGCCGTGAAGAGG			

		60°C 1 min (40 cycles) Other: 1c			
<i>tet(O)</i>	tetO FW	ACGGARAGTTTATTGTATAACC	US: 98°C 2 min (1 cycle), 98°C 5 s - 50.3°C 5 s (40 cycles) Other: 3b / PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 1a	US: 100 / PT: 41	[42]
	tetO RV	TGGCGTATCTATAATGTTGAC			
<i>bla_{oxa-1}</i>	oxa1 FW	TATCTACAGCAGCGCCAGTG	US: 98°C 2 min (1 cycle), 98°C 5 s - 62°C 5 s (40 cycles) Other: 3b / PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 2a	US: 10 / PT: 38	[38]
	oxa1 RV	CGCATCAAATGCCATAAGTG			
<i>uidA</i>	uidA-FW	CAACGAACTGAACTGGCAGA	PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 2a	79	[37]
	uidA-RV	CATTACGCTGCGATGGAT			
<i>bla_{CTX-M}</i>	CTXM-FW	CTATGGCACCACCAACGATA	PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 2a	29	[39]
	CTXM-RV	ACGGCTTTCTGCCTTAGGTT			
<i>intI1</i>	intI1-LC1	GCCTTGATGTTACCCGAGAG	PT: 95°C 10 min (1 cycle), 95°C 15 s - 60°C 1 min (40 cycles) Other: 2a	54	[38]
	intI1-LC5	GATCGGTCGAATGCGTGT			

1) KAPA SYBR® FAST ABI Prism® qPCR Master Mix; 2) SYBR® Select Master Mix; 3) EvaGreen Supermix; 4) Probes Supermix; 5) FAST SYBR; a) 200 nM of primer; b) 400 nM of primer; c) 600 nM of primer; d) 300nM of primer.

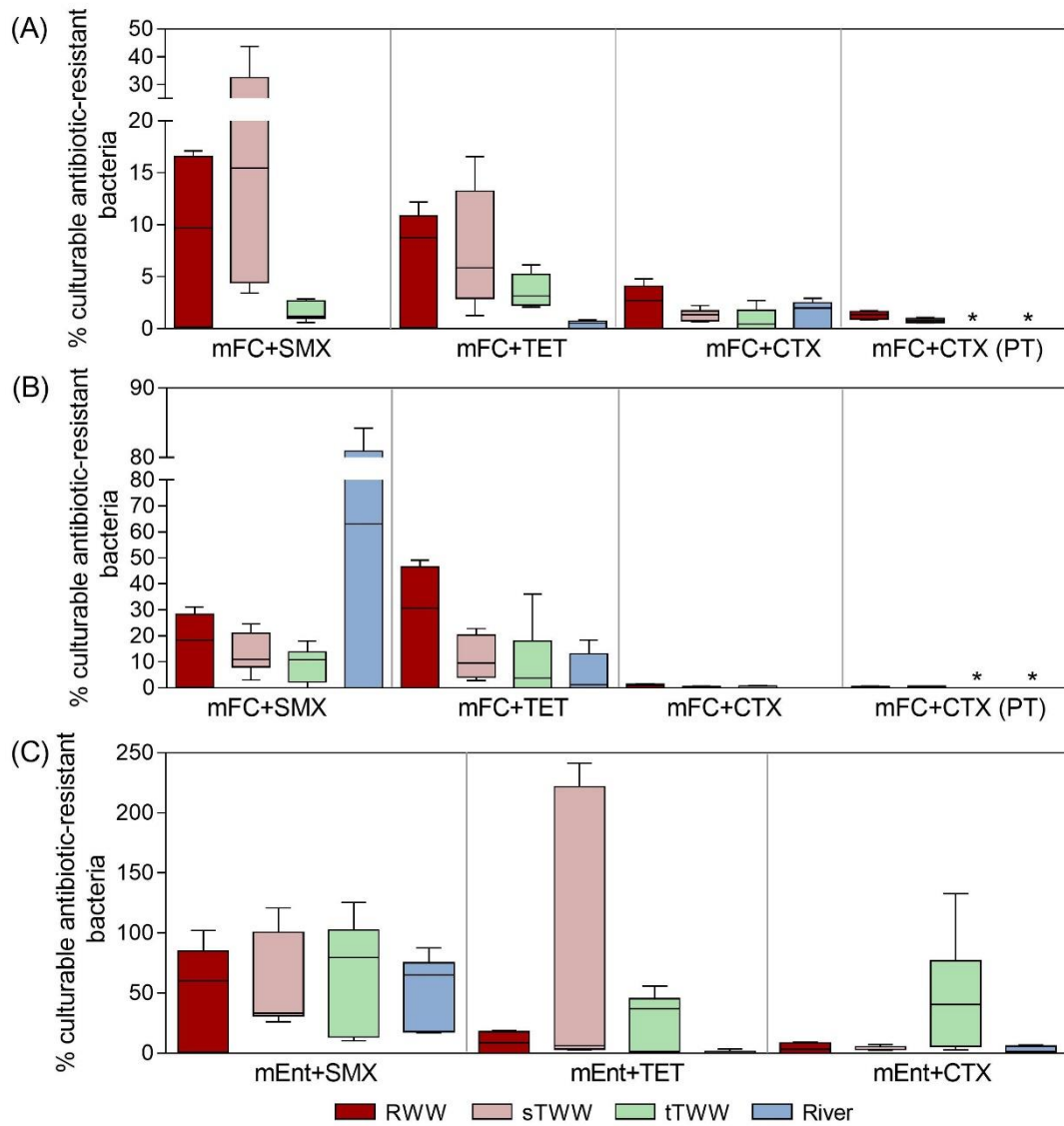


Figure S1 – Percentage of “resistant” bacteria (i.e., growing in presence of target antibiotic at select concentration): (A) presumptive total coliforms, (B) presumptive faecal coliforms and (C) presumptive enterococci in the respective samples, defined as colony forming units (CFU) on cultures with antibiotics relative to CFU on cultures without antibiotics. RWW: wastewater treatment plant influent; sTWW: wastewater collected after secondary wastewater treatment; tTWW: wastewater collected after UV disinfection wastewater treatment. PT refers to data obtained of samples collected in Portugal, the other data presented refer to data obtained of samples collected in United States. * - not determined. SMX: sulfamethoxazole (350 mg/L); CTX: cefotaxime (4 mg/L); TET: tetracycline (16 mg/L).

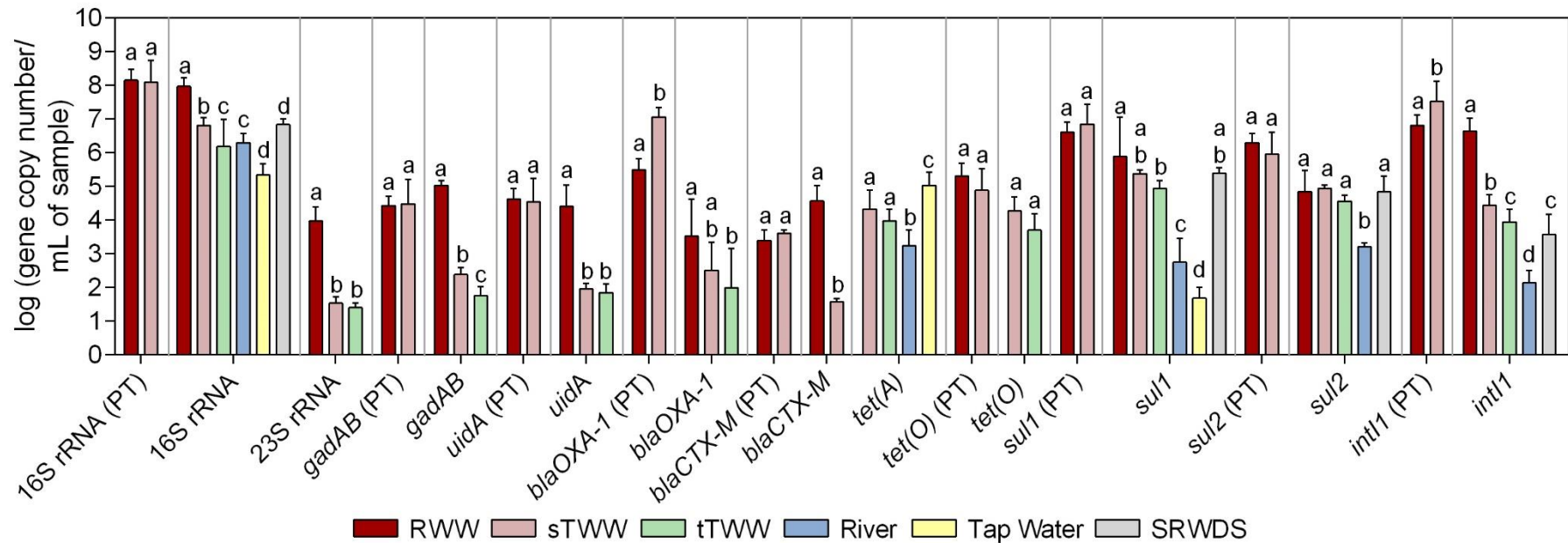


Figure S2 – Absolute abundance values (gene copy number/mL of sample) of the target genes in the six water types: RWW: wastewater treatment plant influent; sTWW: wastewater collected after secondary wastewater treatment; tTWW: wastewater collected after UV disinfection wastewater treatment; River; Tap water and SRWDS: *simulated* reclaimed water distribution system. PT refers to data obtained of samples collected in Portugal, the other data presented refer to data obtained of samples collected in United States. Error bars represent the standard deviation (n=3 and n=2 independent samples, for US and PT, respectively). In PT samples, the genes 23S rRNA and *tet(A)* were not analysed. In US, the absence of the genes *bla_{OXA-1}*, *tet(A)*, *tet(O)*, *sul2* and *int11* is due to their quantification below the limit of detection while the absence of the genes 23S rRNA, *gadAB*, *uidA* and *bla_{CTX-M}* was due to genes quantification below the limit of quantification. a, b, c, and d indicate significantly (p<0.01) different groups comparing the different types of water.