

Supplementary Information

Wastewater-Based Epidemiology for Monitoring Community derived Antimicrobials and Resistance genes: a one-year longitudinal study

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Water quality indicators (WQIs) methodology

Water quality indicators (WQIs) were analysed at Wessex Water. An Aquakem (Thermo Scientific) analyser was used for the quantitative measurement of water quality indicators, with the exception of COD. COD was analysed spectrophotometrically. Briefly, samples, either shaken or settled, were oxidised in tubes with chromic acid, a mixture of potassium dichromate, sulphuric acid in the presence of silver sulphate as a catalyst and mercuric ions to counteract interference from chloride. The sealed tubes are heated to 150 °C for three hours, cooled, and the degree of oxidation determined by spectrophotometry. The procedure is calibrated by processing a solution of potassium hydrogen phthalate as a standard material.

Al, Fe and Mn were analysed via inductively coupled plasma mass spectrometry (ICP/MS), firstly digestion of sample at $80 \pm 5^\circ\text{C}$ overnight in an acidic medium occurs to bring the metals into solution. Turbid samples are filtered by digestion before diluting and dosed with internal standard (Scandium, Gallium, Indium and Thallium). Al: Limits of quantification (LOQ), 0.01 mg/L, range: 0.01-2.00 mg/L. Fe: LOQ, 0.02 mg/L, range: 0.01-2.00 mg/L. Mn: LOQ, 0.001 mg/L, range: 0.001-0.500 mg/L.

Dissolved organic carbon (DOC) and total organic carbon (TOC). A SKALAR analyser was utilised for the analysis of dissolved organic carbon (DOC) and for total organic carbon (TOC). For TOC, samples are acidified to $\text{pH} < 3$ to allow for the removal of all inorganic carbon prior to a digestion step. Digestion is achieved by pumping the digested mixture into a quartz mixing coil around a ultraviolet (UV) light source. Following this, dialysis is performed where a dialyzer separates the sample stream with the colour reagent stream by a CO_2 semi-permeable membrane. For dissolved organic carbon, an aliquot of samples are first filtered through a 0.4 μm GF/F Glass Microfiber Filters Diameter 47 mm. Limit of detection (LOD) is 0.4 mg/L with LOQ of 0.45 mg/L, range: 0-12 mg/L. Suspended solids: a known volume of sample is filtered through a previously washed, dried and weighed filter paper. After drying, the filter paper is re-weighed and the suspended solids deposited on the paper can be calculated. LOD, 5 mg/L if 200 mL of sample is filtered and 2 mg/L if 1000 mL of sample is filtered.

Biological oxygen demand (BOD): samples are diluted as necessary before incubating at $20 \pm 0.5^\circ\text{C}$ in the dark for five days (117.5 ± 4.5 hrs). Following this, the sample is then seeded with bacteria of appropriate activity. The concentration of dissolved oxygen in the sample can then be determined both before and after the incubation period. The difference between these two readings can be used to calculate the BOD of the sample.

Phosphorus levels was determined via inductively couple plasma optical emission spectrometry (ICP-OES) using an Agilent 5110 system. Calibration range, 0-2 ppm.

Ammonia N: utilises ammonia reaction with sodium salicylate and hypochlorite ions, which are generated in situ by the alkaline hydrolysis of sodium dichloroisocyanurate. The absorbance of a blue product formed at $\text{pH} 12.6$ in the presence of sodium nitroprusside is measured spectrophotometrically at 660 nm and related to the ammonia concentration in the sample by means of a calibration curve (LOQ, 0.02 mg/L, range: 0.02-100 mg/L).

N total (TON): Nitrate is reduced to nitrite by hydrazine under alkaline conditions, using copper (II) ions as a catalyst. The total nitrite is then treated with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride under acidic conditions (in the presence of orthophosphoric acid). The absorbance of a characteristic pink azo – dye is measured spectrophotometrically at 540 nm and related to the total oxidised nitrogen concentration in the sample by means of a calibration curve (LOQ, 0.3 mg/L, range: 0.3-50 mg/L).

Nitrite: The diazotisation of sulphanilamide by nitrite in the presence of orthophosphoric acid, at $\text{pH} 1.9$, leads to the formation of an azo-dye with N-1-naphthylethylenediamine. Its absorbance is then

measured at 540 nm and is related to the nitrite concentration by means of a calibration curve (LOQ, 0.03 mg/L, range: 0.03-10 mg/L).

Nitrate: Nitrate is calculated using TON minus Nitrite. The calculation takes place after the samples have been analysed for both chemistries.

Orthophosphate: Orthophosphate ions react with a solution containing molybdic acid, ascorbic acid and antimony (II) ions in the presence of acid, to form a 12-molybdophosphoric acid. This is reduced in situ to a blue heteropoly compound (phosphomolybdenum blue) in which antimony is incorporated. The absorbance of the compound is measured spectrophotometrically at 880 nm and related to the orthophosphate concentration in the sample by means of a calibration curve. Soluble reactive phosphorus uses the same method as above, but the sample is filtered through a 0.45µm filter prior to analysing (LOQ, 0.06 mg/L, range 0.6-20 mg/L).

Chloride: Chloride ions were mixed with acid chloride colour reagent containing mercury (II) thiocyanate. The released thiocyanate ions then react in acid solution with iron (III) nitrate to give a reddish-brown coloured iron (III) thiocyanate complex. The resulting intensity of the stable colour produced is measured at a wavelength of 480 nm and is related to the chloride concentration by means of a calibration curve (LOQ 1 mg/L, range 1-1000 mg/L).

Table S1: Chemical information of AA targets, ordered by class groupings, table taken from Holton and Kasprzyk-Hordern, 2021, <https://rdcu.be/cxqhT>

Grouping	Chemical	Abbrev	Class A	Class B	CAS No.	Salt form θ	Molec. Formula	M.I. mass	Supplier	
Sulfonamide & Trimethoprim	Sulfadiazine	SDZ	Sulfonamide	Parent	68-35-9		C10H10N4O2S	250.05	Sigma-Aldrich	
	Sulfapyridine	SPY	Sulfonamide	Parent	144-83-2		C11H11N3O2S	249.06	Sigma-Aldrich	
	Sulfamethoxazole	SMX	Sulfonamide	Parent	723-46-6		C10H11N3O3S	253.05	Sigma-Aldrich	
	Sulfasalazine	SLZ	Sulfonamides	Parent	599-79-1		C18H14N4O5S	398.07	Sigma-Aldrich	
	Trimethoprim	TMP	Trimethoprim	Parent	738-70-5		C14H18N4O3	290.14	Sigma-Aldrich	
	N-acetyl sulfadiazine	aSDZ	Sulfonamide	Metabolite	127-74-2		C12H12N4O3S	292.06	TRC	
	N-acetyl sulfapyridine	aSPY	Sulfonamide	Metabolite	19077-98-6		C13H13N3O3S	291.07	TRC	
	N-acetyl sulfamethoxazole	aSMX	Sulfonamide	Metabolite	21312-10-7		C12H13N3O4S	295.06	TRC	
Macrolide & Lincomycin	4-hydroxy-trimethoprim	hTMP	Trimethoprim	Metabolite	112678-48-5		C14H18N4O4	306.13	TRC	
	Azithromycin	AZM	Macrolide	Parent	83905-01-5		C38H72N2O12	748.51	LCG	
	Erythromycin	ERY	Macrolide	Parent	114-07-8		C37H67NO13	733.46	Sigma-Aldrich	
	Clarithromycin	CLR	Macrolide	Parent	81103-11-9		C38H69NO13	747.48	Sigma-Aldrich	
	Clindamycin	CLI	Lincomycin	Parent	18323-44-9	Hydrochloride	C18H33ClN2O5S	424.18	Sigma-Aldrich	
	N-desmethyl azithromycin	dmAZM	Macrolide	Metabolite	172617-84-4		C37H70N2O12	734.49	TRC	
	N-desmethyl erythromycin A	dmERY	Macrolide	Metabolite	992-62-1		C36H65NO13	719.45	TRC	
β-LACTAMS	N-desmethyl clarithromycin	dmCLR	Macrolide	Metabolite	101666-68-6		C37H67NO13	733.46	TRC	
	N-desmethyl clindamycin	dmCLI	Lincomycin	Metabolite	22431-45-4		C17H31ClN2O5S	410.16	TRC	
	Penicillin	Amoxicillin	AMX	Penicillin	Parent	26787-78-0		C16H19N3O5S	365.10	Fluka
		Ampicillin	AMP	Penicillin	Parent	69-53-4	Trihydrate	C16H19N3O4S	349.11	Fluka
		Flucloxacillin	FLX	Penicillin	Parent	5250-39-5	Sodium	C19H17ClFN3O5S	453.06	Fluka
		Penicillin G	PenG	Penicillin	Parent	113-98-4	Sodium	C16H18N2O4S	334.10	Fluka
		Penicillin V	PenV	Penicillin	Parent	132-98-9	Potassium	C16H18N2O5S	350.09	Sigma-Aldrich
		Amoxicilloic acid	AMXa	Penicillin	Metabolite	42947-63-7 ¥	Trisodium salt	C16H21N3O6S	383.12	TRC
		Ampicilloic acid	AMPa	Penicillin	Metabolite	32746-94-4		C16H21N3O5S	367.12	TRC
		Penicilloic G acid	PenGa	Penicillin	Metabolite	11039-68-2		C9H14N2O5S	262.06	TRC
Cefalosporin		Cefalexin	LEX	Cefalosporin	Parent	23325-78-2	Monohydrate	C16H17N3O4S	347.09	Fluka

	Cefixime	CFM	Cefalosporin	Parent	79350-37-1	Trihydrate	C16H15N5O7S2	453.04	Fluka
	Ceftiofur	CTF	Cefalosporin	Parent	104010-37-9		C19H17N5O7S3	523.03	Fluka
	Ceftriaxone	CRO	Cefalosporin	Parent	104376-79-6	Disodium hemi(heptahydrate)	C18H18N8O7S3	554.05	Sigma-Aldrich
Monobactam	Aztreonam	ATM	Monobactam	Parent	78110-38-0		C13H17N5O8S2	435.05	TRC
Carbapenem	Imipenem	IPM	Carbapenem	Parent	64221-86-9		C12H17N3O4S	299.09	Sigma-Aldrich
	Meropenem	MEM	Carbapenem	Parent	119478-56-7	Trihydrate	C17H25N3O5S	383.15	Sigma-Aldrich
Quinolone	Besifloxacin	BSF	Quinolone	Parent	405165-61-9	Hydrochloride	C19H21ClFN3O3	393.13	MCE
	Ciprofloxacin	CIP	Quinolone	Parent/Metab.	85721-33-1		C17H18FN3O3	331.13	Fluka
	Danofloxacin	DFX	Quinolone	Parent	119478-55-6	Mesylate	C19H20FN3O3	357.15	LCG
	Enrofloxacin	ENR	Quinolone	Parent	93106-60-6		C19H22FN3O3	359.16	Sigma-Aldrich
	Flumequine	FLU	Quinolone	Parent	42835-25-6		C14H12FNO3	261.08	Fluka
	Gatifloxacin	GAT	Quinolone	Parent	112811-59-3		C19H22FN3O4	375.16	TRC
	Lomefloxacin	LOM	Quinolone	Parent	98079-52-8	Hydrochloride	C17H19F2N3O3	351.14	Sigma-Aldrich
	Moxifloxacin	MXF	Quinolone	Parent	268545-13-7	Hydrochloride	C21H24FN3O4	401.18	MCE
	Nadifloxacin	NAD	Quinolone	Parent	124858-35-1		C19H21FN2O4	360.15	MCE
	Nalidixic acid	NAL	Quinolone	Parent	389-08-2	Sodium	C12H12N2O3	232.08	Sigma-Aldrich
	Norfloxacin	NOR	Quinolone	Parent	70458-96-7		C16H18FN3O3	319.13	Sigma-Aldrich
	Ofloxacin (Levofloxacin) *	OFX	Quinolone	Parent	82419-36-1		C18H20FN3O4	361.14	Sigma-Aldrich
	Prulifloxacin	PFLX	Quinolone	Parent (prodrug)	123447-62-1		C21H20FN3O6S	461.11	Sigma-Aldrich
Grouping	Chemical	Abbrev	Class A	Class B	CAS No.	Salt form 0	Molec. Formula	M.I. mass	Supplier
	Sarafloxacin	SRF	Quinolone	Parent	91296-87-6	Hydrochloride	C20H17F2N3O3	385.12	Sigma-Aldrich
	Desethylene ciprofloxacin	deCIP	Quinolone	Metabolite	528851-31-2	Hydrochloride	C15H16FN3O3	305.12	TRC
	Hydroxy-norfloxacin	hNOR	Quinolone	Metabolite	109142-49-6		C16H18FN3O4	335.13	TRC
	Ofloxacin N-oxide	OFXo	Quinolone	Metabolite	104721-52-0	Acetic acid salt	C18H20FN3O5	377.14	TRC
	Desmethyl-ofloxacin	dmOF X	Quinolone	Metabolite	82419-52-1		C17H18FN3O4	347.13	TRC
	Ulifloxacin	UFX	Quinolone	Metabolite	112984-60-8		C16H16FN3O3S	349.09	TRC
TB (1st line)	Isoniazid	INH	Isoniazid	Parent	54-85-3		C6H7N3O	137.06	Sigma-Aldrich
	Pyrazinamide	PZA	Pyrazinamide	Parent	98-96-4		C5H5N3O	123.04	Sigma-Aldrich
	Ethambutol	EMB	Ethambutol	Parent	74-55-5	Dihydrochloride	C10H24N2O2	204.18	Sigma-Aldrich
	Rifampicin	RMP	Rifamycin	Parent	13292-46-1		C43H58N4O12	822.41	Sigma-Aldrich

	Rifabutin	RFB	Rifamycin	Parent	72559-06-9		C46H62N4O11	846.44	Sigma-Aldrich
	Isonicotinic acid	INa	Isoniazid	Metabolite	55-22-1		C6H5NO2	123.03	Sigma-Aldrich
	Acetyl-isoniazid	aINH	Isoniazid	Metabolite	1078-38-2		C8H9N3O2	179.07	Sigma-Aldrich
	5-Hydroxy-pyrazinoic acid	hPZA	Pyrazinamide	Metabolite	34604-60-9		C5H4N2O3	140.02	Sigma-Aldrich
	25-desacetyl rifampicin	daRMP	Rifamycin	Metabolite	16783-99-6		C41H56N4O11	780.39	Sigma-Aldrich
	25-O-desacetyl rifabutin	daRFB	Rifamycin	Metabolite	100324-63-8		C44H60N4O10	804.43	TRC
TB (MDR)	Capreomycin IA ≠	CAP1a	Aminoglycoside	Parent	1405-37-4	Sulfate	C25H44N14O8	668.35	TRC
	Capreomycin IB ≠	CAP1b	Aminoglycoside	Parent	1405-37-4	Sulfate	C25H44N14O7	652.35	TRC
	Gentamycin C1 ≠	GEN1	Aminoglycoside	Parent	1405-41-0	Sulfate salt hydrate	C21H43N5O7	477.32	Fluka
	Gentamycin C1a ≠	GEN1a	Aminoglycoside	Parent	1405-41-0	Sulfate salt hydrate	C19H39N5O7	449.28	Fluka
	Gentamycin C2 C2a C2b ≠	GEN2	Aminoglycoside	Parent	1405-41-0	Sulfate salt hydrate	C20H43N5O7	465.32	Fluka
	Kanamycin A ≠	KAN	Aminoglycoside	Parent	25389-94-0	Sulfate	C18H36N4O11	484.24	Sigma-Aldrich
	Streptomycin A ≠	STR	Aminoglycoside	Parent	3810-74-0	Sulfate	C21H39N7O12	581.27	Sigma-Aldrich
	D-cycloserine	DCS	Isoxazole	Parent/Metab.	68-41-7		C3H6N2O2	102.04	TRC
TB (other)	Delamanid	DMD	Nitroimidazole	Parent	681492-22-8		C25H25F3N4O6	534.17	Sigma-Aldrich
	Bedaquiline	BDQ	Diarylquinoline	Parent	843663-66-1		C32H31BrN2O2	554.16	Sigma-Aldrich
	Linezolid	LZD	Oxazolidinone	Parent	165800-03-3		C16H20FN3O4	337.14	Sigma-Aldrich
	Thalidomide	THAL	Thalidomide	Parent	50-35-1		C13H10N2O4	258.06	Sigma-Aldrich
OTHER									
Amphenicol	Chloramphenicol	CHL	Amphenicol	Parent	56-75-7		C11H12Cl2N2O5	322.01	Sigma-Aldrich
	Florfenicol	FLO	Amphenicol	Parent	73231-34-2		C12H14Cl2FNO4S	357.00	MCE
	2-Amino-1-(4-nitrophenyl)-1,3-propanediol	ANP	Amphenicol	Metabolite	2964-48-9		C9H12N2O4	212.08	Sigma-Aldrich
Cycline	Doxycycline	DOX	Cycline	Parent	24390-14-5	Hyclate	C22H24N2O8	444.15	Sigma-Aldrich
	Oxytetracycline	OTC	Cycline	Parent	2058-46-0	Hydrochloride	C22H24N2O9	460.15	TRC
	Tetracycline	TET	Cycline	Parent	64-75-5	Hydrochloride	C22H24N2O8	444.15	Sigma-Aldrich
Nitrofurantoin	Nitrofurantoin	NIT	Nitrofurantoin	Parent	67-20-9		C8H6N4O5	238.03	Sigma-Aldrich
	1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione	NPAHD	Nitrofurantoin	Metabolite	623145-57-3		C10H8N4O4	248.05	TRC
Azole	Metronidazole	MTZ	Azole	Parent	443-48-1		C6H9N3O3	171.06	Sigma-Aldrich

	Ketoconazole	KTC	Azole	Parent	65277-42-1	C ₂₆ H ₂₈ Cl ₂ N ₄ O ₄	530.15	Sigma-Aldrich
	Hydroxy-metronidazole	hMTZ	Azole	Metabolite	1215071-08-1	C ₆ H ₉ N ₃ O ₄	187.06	TRC
	Deacetyl-ketoconazole	daKTC	Azole	Metabolite	67914-61-8	C ₂₄ H ₂₆ Cl ₂ N ₄ O ₃	488.14	TRC
Antiretroviral	Emtricitabine	FTC	ARV	Parent	143491-57-0	C ₈ H ₁₀ FN ₃ O ₃ S	247.04	TRC
	Lamivudine	3TC	ARV	Parent	134678-17-4	C ₈ H ₁₁ N ₃ O ₃ S	229.05	TRC

Monoisotopic mass (M.I. mass), multi-drug resistant (MDR), tuberculosis (TB), nucleoside reverse transcriptase inhibitor (NRTI)

LC-MS method is not chiral (*); one standard used for all forms within the drug complex (≠); CAS for chiral free acid (¥); salt corrections considered in all calculations, i.e., analysis of the free base (θ)

Table S2. Internal standards used along with instrument and method detection limits (IDLs and MDLs) and instrument and method quantification limits (IQLs and MQLs) table adapted from Holton and Kasprzyk-Hordern, 2021, <https://rdcu.be/cxqhT>

Class	Abbrev	Internal Standard	Mobile phase (ng L ⁻¹)		Influent wastewater (ng L ⁻¹)		
			IDL	IQL	MDL	MQL	
Sulfonamide & Trimethoprim	SDZ	Sulfamethoxazole D4	0.015	0.050	0.230	0.76	
	SPY	Sulfamethoxazole D4	0.003	0.010	0.026	0.088	
	SMX	Sulfamethoxazole D4	0.002	0.005	0.014	0.046	
	SLZ	Sulfamethoxazole D4	1.500	5.000	5.130	17.09	
	TMP	Trimethoprim D9	0.150	0.500	0.970	3.22	
	aSDZ	Sulfamethoxazole D4	0.021	0.070	0.230	0.77	
	aSPY	Sulfamethoxazole D4	0.167	0.556	1.350	4.51	
	aSMX	Sulfamethoxazole D4	0.019	0.063	0.200	0.66	
	hTMP	Trimethoprim D9	0.004	0.013	0.030	0.1	
	Macrolide & Lincomycin	AZM	Clarithromycin D3	0.015	0.050	-	-
ERY		Clarithromycin D3	0.002	0.005	0.016	0.053	
CLR		Clarithromycin D3	0.002	0.005	0.014	0.046	
CLI		Flumequine 13C3	0.150	0.500	-	-	
dmAZM		Clarithromycin D3	0.375	1.250	-	-	
dmERY		Clarithromycin D3	0.002	0.007	0.020	0.07	
dmCLR		Clarithromycin D3	0.005	0.017	0.070	0.25	
dmCLI		Flumequine 13C3	0.002	0.005	-	-	
β-LACTAMS Penicillin	AMX	Sulfamethoxazole D4	1.500	5.000	-	-	
	AMP	Ampicillin D5	1.500	5.000	55.600	185	
	FLX	Flumequine 13C3	0.150	0.500	1.630	5.45	
	PenG	Penicillin G D7	0.150	0.500	5.470	18.2	
	PenV	Penicillin G D7	3.000	10.000	81.300	271	
	AMXa	Sulfamethoxazole D4	0.300	1.000	146.000	488	
	AMPa	Ampicillin D5	0.150	0.500	31.800	106	
	PenGa	Penicillin G D7	0.150	0.500	15.400	51.5	
	Cefalosporin	LEX	Trimethoprim D9	0.375	1.250	5.910	19.7
		CFM	Trimethoprim D9	1.500	5.000	13.200	44
CTF		Flumequine 13C3	0.150	0.500	-	-	
CRO		Trimethoprim D9	7.500	25.000	48.400	161	
Monobactam	ATM	Trimethoprim D9	0.300	1.000	-	-	
Carbapenem	IPM	Metronidazole D4	1.500	5.000	-	-	
	MEM	Trimethoprim D9	1.500	5.000	-	-	
Quinolone	BSF	Flumequine 13C3	0.375	1.250	3.150	10.5	
	CIP	Desmethyl-ofloxacin D8	0.150	0.500	1.130	3.77	
	DFX	Desmethyl-ofloxacin D8	1.500	5.000	13.900	46.3	
	ENR	Desmethyl-ofloxacin D8	0.030	0.100	0.250	0.82	
	FLU	Flumequine 13C3	0.003	0.010	0.030	0.1	
	GAT	Desmethyl-ofloxacin D8	0.003	0.010	0.030	0.09	
	LOM	Desmethyl-ofloxacin D8	0.030	0.100	0.280	0.95	
	MXF	Desmethyl-ofloxacin D8	0.375	1.250	3.040	10.1	
	NAD	Flumequine 13C3	0.300	1.000	2.680	8.92	

	NAL	Flumequine 13C3	0.003	0.010	0.030	0.11
	NOR	Desmethyl-ofloxacin D8	0.003	0.010	0.021	0.069
	OFX	Desmethyl-ofloxacin D8	0.030	0.100	0.410	1.37
	PFLX	Desmethyl-ofloxacin D8	0.300	1.000	1.780	5.95
	SRF	Desmethyl-ofloxacin D8	0.150	0.500	1.500	5.01
	deCIP	Desmethyl-ofloxacin D8	0.150	0.500	1.440	4.82
	hNOR	Desmethyl-ofloxacin D8	3.600	12.000	38.600	129
	OFXo	Desmethyl-ofloxacin D8	3.600	12.000	36.500	122
	dmOFX	Desmethyl-ofloxacin D8	0.150	0.500	1.340	4.45
	UFX	Desmethyl-ofloxacin D8	1.500	5.000	11.400	38.1
TB DRUGS	INH	Isoniazid D4	0.150	0.500	1.520	5.06
TB (1st line)	PZA	Metronidazole D4	1.500	5.000	-	-
	EMB	Metronidazole D4	0.003	0.010	0.130	0.42
	RMP	Rifabutin D7	0.375	1.250	-	-
	RFB	Rifabutin D7	0.150	0.500	1.680	5.62
	INa	Isoniazid D4	0.150	0.500	7.200	24
	aINH	Isoniazid D4	0.150	0.500	3.580	11.9
	hPZA	Metronidazole D4	0.030	0.100	-	-
	daRMP	Rifabutin D7	1.500	5.000	15.000	50
	daRFB	Rifabutin D7	0.030	0.100	0.330	1.11
TB (MDR)	CAPIa	Metronidazole D4	6.621	22.071	4528.000	15094
	CAPIb	Metronidazole D4	6.621	22.071	-	-
	GEN1	Metronidazole D4	2.820	9.400	-	-
	GEN1a	Metronidazole D4	2.070	6.900	-	-
	GEN2	Metronidazole D4	5.250	17.500	-	-
	KAN	Metronidazole D4	3.563	11.875	-	-
	STR	Metronidazole D4	12.000	40.000	2650.000	8835
	DCS	Metronidazole D4	0.150	0.500	-	-
TB (other)	DMD	Rifabutin D7	0.150	0.500	-	-
	BDQ	Rifabutin D7	1.500	5.000	-	-
	LZD	Chloramphenicol D5	0.030	0.100	0.270	0.89
	THAL	Trimethoprim D9	0.300	1.000	3.480	11.6
OTHER	CHL	Chloramphenicol D5	0.150	0.500	1.310	4.35
Amphenicol	FLO	Chloramphenicol D5	3.000	10.000	21.900	73.1
	ANP	Metronidazole D4	1.500	5.000	42.300	141
Cycline	DOX	Rifabutin D7	0.375	1.250	12.700	42.2
	OTC	Desmethyl-ofloxacin D8	0.300	1.000	6.350	21.2
	TET	Desmethyl-ofloxacin D8	0.150	0.500	1.700	5.66
Nitrofurantoin	NIT	Nitrofurantoin 13C3	0.300	1.000	3.460	11.54
	NPAHD	Chloramphenicol D5	0.030	0.100	0.350	1.18
Azole	MTZ	Metronidazole D4	0.030	0.100	0.270	0.91
	KTC	Flumequine 13C3	0.003	0.010	0.030	0.1
	hMTZ	Metronidazole D4	0.030	0.100	1.010	3.38
	daKTC	Flumequine 13C3	0.375	1.250	-	-
Antiviral	FTC	Metronidazole D4	0.150	0.500	1.570	5.24
	3TC	Metronidazole D4	0.300	1.000	9.510	31.7

Figure S1. Influent wastewater flow data (m3/day) and daily rainfall data (mm) for Bath and Keynsham over the days that were sampled only during the sampling period of 2018-2019.

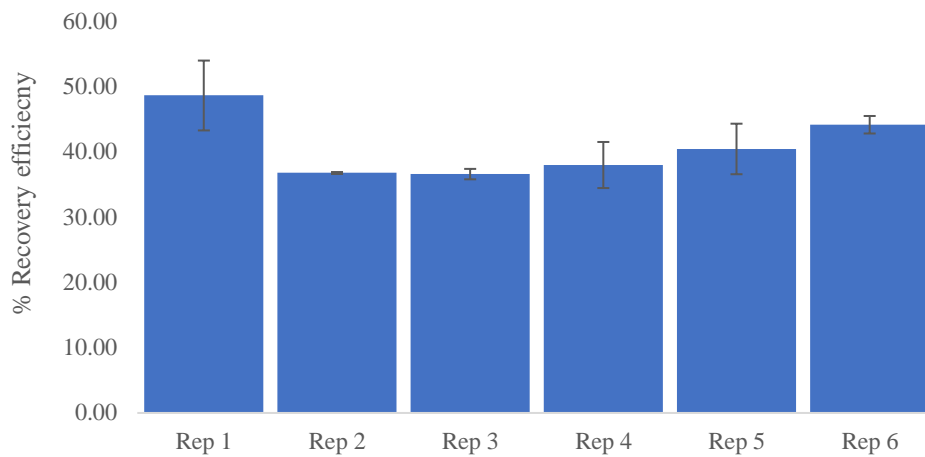
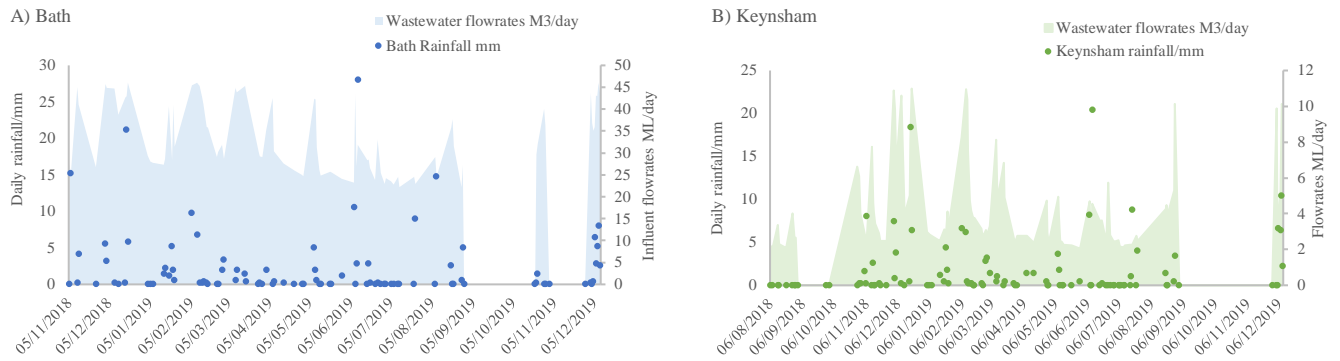


Figure S2: Recovery efficiency (%) of TaqMan™ Universal DNA Spike in Control from one wastewater sample extracted out six times (rep = replicates)

Table S3. p values for seasonal T-test results for AAs in Bath and Keynsham

	Saltford			Keynsham		
	Winter '18 vs Summer '19	Winter '18 vs Spring '19	Spring '19 vs Summer '19	Winter '18 vs Summer '19	Winter '18 vs Spring '19	Spring '19 vs Summer '19
CLR	0.0000	0.0004	0.0017	0.0000	0.0328	0.9634
dmCLR	0.0000	0.0293	0.0017	0.0185	0.2547	0.9684
ERY	0.0007	0.0000	0.0000	0.0001	0.1987	0.0002
dmERY	0.0000	0.0789	0.0061	-	-	-
INa	0.0002	0.0000	0.0117	0.0000	0.0000	0.8644
HPZA	0.9101	0.8481	0.9217	0.1570	0.3625	0.0874
MTZ	0.7254	0.0012	0.3003	0.0019	0.1314	0.4331
hMTZ	0.0111	0.0000	0.0889	0.0053	0.0126	0.9513
KTC	0.0008	0.0365	0.6252	0.0047	0.0039	0.5656
Lam	0.6512	0.0028	0.0339	0.0236	0.1215	0.7903
EMT	0.3880	0.0001	0.0130	0.9540	0.0570	0.1380
SPY	0.4669	0.1470	0.4605	0.7779	0.1261	0.0862
aSPY	0.0000	0.0072	0.0916	0.1446	0.0789	0.0330
SMX	0.5769	0.7892	0.4421	0.0003	0.1416	0.3101
aSMX	0.0010	0.1419	0.3336	0.0999	0.9581	0.1659
SLZ	0.0966	0.2900	0.0080	0.6975	0.0854	0.2104
FLX	0.1050	0.0446	0.6838	0.3324	0.0112	0.0195
Tet	0.0000	0.3600	0.0004	0.0040	0.0003	0.7901
OTC	0.0000	0.2852	0.0011	0.0277	0.0014	0.6613
CIP	0.0000	0.0016	0.5434	0.1224	0.0001	0.0035
OFX	0.3925	0.0644	0.8748	0.0002	0.2191	0.0005
TMP	0.0003	0.0273	0.1310	0.0046	0.1290	0.6980
Cli	0.0005	0.0728	0.3654	0.4820	0.0039	0.0841
dmCLI	0.9452	0.8982	0.8562	0.1524	0.0002	0.0442

*Statistically significant results are shown in **bold** ($p \leq 0.05$)

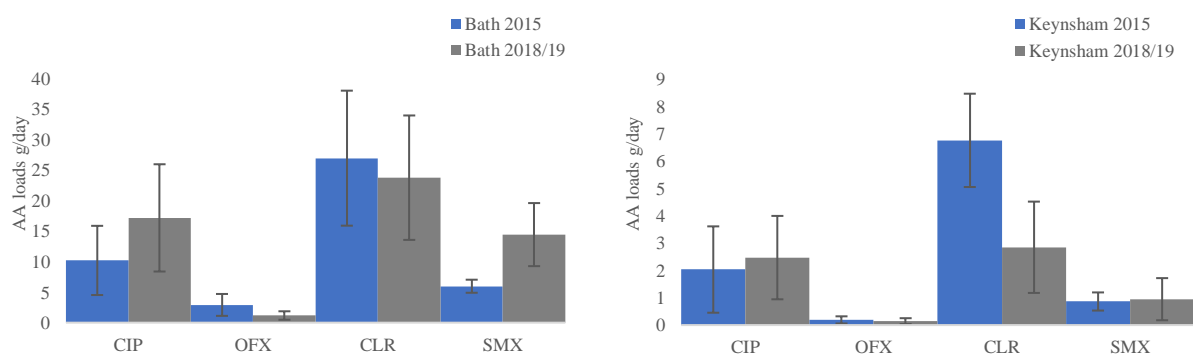


Figure S3. Comparison of averaged daily loads of AAs from 2015 (one week average) versus 2018/19 (12/13 months average for Bath and Keynsham respectively).

Table S4. ARG profiling from metagenomic sequencing data of influent wastewater sampled from Bath in November 2018 and March 2019. The % mapped donates the % coverage of each gene by the reads of each sample

Antibiotic Class	Gene Family*	Resistance Mechanism*	Observed Pathogen**	Gene	% mapped, only >80% shown	
					29/03/2019	13/11/2018
Aminoglycoside antibiotic	ANT(2")	antibiotic inactivation	Pseudomonas aeruginosa	ANT(2")-Ia	100	100
	APH(2")	antibiotic inactivation	Enterococcus gallinarum	APH(2")-IIIa	100	
	APH(3")	antibiotic inactivation	Pseudomonas aeruginosa	APH(3")-Ib	100	100
	APH(3')	antibiotic inactivation	Campylobacter coli	APH(3')-IIIa	100	
	APH(6)	antibiotic inactivation	Pseudomonas aeruginosa	APH(6)-Id	100	100
	ANT(3")	antibiotic inactivation	Pseudomonas aeruginosa	aadA11		100
	ANT(3")	antibiotic inactivation	Acinetobacter lwoffii	aadA27	100	100
	ANT(3")	antibiotic inactivation	Pseudomonas aeruginosa	aadA6		100
	ANT(3")	antibiotic inactivation	Pseudomonas aeruginosa	aadA6/aadA10	81	95
ANT(3")	antibiotic inactivation	Escherichia coli	aadA		82	
Rifamycin antibiotic	rifamycin-resistant beta-subunit of RNA polymerase (rpoB)	antibiotic target alteration; antibiotic target replacement	Bifidobacterium adolescentis	Bifidobacterium adolescentis rpoB conferring resistance to rifampicin	97	88
Penam	CARB beta-lactamase	antibiotic inactivation	Acinetobacter baumannii	CARB-10	96	
	RCP beta-lactamase	antibiotic inactivation	Rhodobacter capsulatus	RCP-1	100	
Mupirocin	antibiotic resistant isoleucyl-tRNA synthetase (ileS)	antibiotic target alteration	Bifidobacterium bifidum	Bifidobacteria intrinsic ileS conferring resistance to mupirocin Campylobacter coli chloramphenicol acetyltransferase	82	
Phenicol antibiotic	chloramphenicol acetyltransferase (CAT)	antibiotic inactivation	Campylobacter coli	chloramphenicol acetyltransferase	82	.
Macrolide antibiotic	macrolide esterase	antibiotic inactivation	Riemerella anatipestifer	EreD	100	
	major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic efflux	Photobacterium damsela	mefC	100	98
	macrolide phosphotransferase (MPH)	antibiotic inactivation	uncultured bacterium	mphE	100	100
	macrolide phosphotransferase (MPH) intrinsic colistin resistant	antibiotic inactivation	Photobacterium damsela	mphG	100	100
Peptide antibiotic	phosphoethanolamine transferase	antibiotic target alteration	Moraxella osloensis	ICR-Mo	100	97
	undecaprenyl pyrophosphate related proteins	antibiotic target alteration	Escherichia coli	bacA	89	
Fluoroquinolone antibiotic	quinolone resistance protein (qnr)	antibiotic target protection	Salmonella enterica	QnrS2	88	

	small multidrug resistance (SMR) antibiotic efflux pump	antibiotic efflux	Vibrio cholerae	qacH	100	100
Lincosamide antibiotic	lincosamide nucleotidyltransferase (LNU)	antibiotic inactivation	Enterococcus faecium	lnuB	90	
	lincosamide nucleotidyltransferase (LNU)	antibiotic inactivation	Enterococcus faecalis	lnuG	88	
Sulfonamide antibiotic	sulfonamide resistant sul major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic target replacement	Vibrio fluvialis	suI		98
Tetracycline antibiotic	major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic efflux	Acinetobacter sp.	tet(39)	100	100
	major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic efflux	uncultured bacterium	tet(40)	97	
	major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic efflux	Aeromonas salmonicida	tet(C)	100	80
	tetracycline-resistant ribosomal protection protein	antibiotic target protection	uncultured bacterium	tet(W/N/W)	86	
	tetracycline-resistant ribosomal protection protein	antibiotic target protection	Staphylococcus aureus	tetM	98	100
	tetracycline-resistant ribosomal protection protein	antibiotic target protection	Campylobacter jejuni	tetO	100	89
	tetracycline-resistant ribosomal protection protein	antibiotic target protection	Bacteroides fragilis	tetQ	98	
	tetracycline-resistant ribosomal protection protein	antibiotic target protection	Butyrivibrio fibrisolvens	tetW	100	100
Nucleoside antibiotic; acridine dye	major facilitator superfamily (MFS) antibiotic efflux pump	antibiotic efflux	Escherichia coli	mdtP		81
Multidrug resistance						
macrolide antibiotic; fluoroquinolone antibiotic; penam	resistance-nodulation-cell division (RND) antibiotic efflux pump	antibiotic efflux	Escherichia coli	CRP		91
macrolide antibiotic; lincosamide antibiotic; streptogramin antibiotic	Erm 23S ribosomal RNA methyltransferase	antibiotic target alteration	Enterococcus faecium	ErmB	100	100
macrolide antibiotic; lincosamide antibiotic; streptogramin antibiotic; tetracycline antibiotic; oxazolidinone antibiotic; phenicol antibiotic; pleuromutilin antibiotic	ABC-F ATP-binding cassette ribosomal protection protein	antibiotic target protection	Enterococcus faecalis	lsaE	100	100
macrolide antibiotic; lincosamide antibiotic; streptogramin antibiotic; tetracycline antibiotic; oxazolidinone antibiotic; phenicol antibiotic; pleuromutilin antibiotic	ABC-F ATP-binding cassette ribosomal protection protein	antibiotic target protection	Streptococcus pyogenes	mel	100	100
macrolide antibiotic; lincosamide antibiotic; streptogramin antibiotic; tetracycline antibiotic; oxazolidinone antibiotic; phenicol antibiotic; pleuromutilin antibiotic	ABC-F ATP-binding cassette ribosomal protection protein	antibiotic target protection	Acinetobacter baumannii	mrsE	100	100
fluoroquinolone antibiotic; cephalosporin; glycylicline; penam; tetracycline antibiotic; rifamycin antibiotic; phenicol antibiotic; triclosan	resistance-nodulation-cell division (RND) antibiotic efflux pump	antibiotic efflux	Escherichia coli	Escherichia coli acrA	85	
fluoroquinolone antibiotic; cephalosporin; glycylicline; penam; tetracycline antibiotic; rifamycin antibiotic; phenicol antibiotic; triclosan	resistance-nodulation-cell division (RND) antibiotic efflux pump	antibiotic efflux	Escherichia coli	Escherichia coli acrA	85	
cephalosporin; penam	NPS beta-lactamase	antibiotic inactivation	Pseudomonas aeruginosa	NPS-1		86
cephalosporin; penam	OXA beta-lactamase	antibiotic inactivation	Acinetobacter johnsonii	OXA-211	92	84

cephalosporin; penam	OXA beta-lactamase	antibiotic inactivation	Acinetobacter johnsonii	OXA-333	98	
cephalosporin; penam	OXA beta-lactamase	antibiotic inactivation	Citrobacter freundii	OXA-101		83
cephalosporin; penam	OXA beta-lactamase	antibiotic inactivation	Pseudomonas aeruginosa	OXA-205	83	

*Drug class, gene family and resistance mechanisms are fields in CARD database and describe the corresponding AMR genes. **Observed pathogen is a known pathogen of public health importance that harbours that gene. This does not mean that this gene was found in this pathogen or couldn't be found elsewhere

Table S5. Absolute (not *16S rRNA* normalised) concentrations and loads of ARGs in wastewater in Bath

Date	<i>16S rRNA</i>				<i>ermB</i>				<i>sulI</i>				<i>IntI1</i>				<i>qnrS</i>			
	Copies/L	STD	Daily loads average copies/day	STD	Copies/L	STD	Daily loads average copies/day	STD	Copies/L	STD	Daily loads average copies/day	STD	Copies/L	STD	Daily loads average copies/day	STD	Copies/L	STD	Daily loads average copies/day	STD
05/11/18	3.49E+09	8.53E+08	9.61E+13	2.34E+13	4.66E+09	7.11E+08	1.28E+14	1.95E+13	7.30E+07	3.38E+07	2.01E+12	9.30E+11	6.27E+07	8.52E+06	1.72E+12	2.34E+11	1.69E+06	2.98E+05	4.64E+10	8.20E+09
06/11/18	4.01E+09	1.79E+08	1.02E+14	4.56E+12	3.38E+09	1.11E+09	8.62E+13	2.83E+13	4.83E+07	1.10E+06	1.23E+12	2.81E+10	7.32E+07	1.47E+07	1.87E+12	3.74E+11	6.82E+05	2.57E+05	1.74E+10	6.55E+09
11/11/18	6.19E+09	4.24E+07	2.79E+14	1.91E+12	6.16E+09	2.55E+08	2.78E+14	1.15E+13	6.86E+07	1.12E+07	3.09E+12	5.06E+11	6.49E+07	8.51E+06	2.92E+12	3.83E+11	9.27E+05	1.23E+05	4.17E+10	5.55E+09
25/11/18	6.65E+09	8.84E+08	1.78E+14	2.37E+13	6.20E+09	3.31E+08	1.66E+14	8.89E+12	7.22E+07	2.82E+07	1.94E+12	7.55E+11	7.46E+07	7.67E+06	2.00E+12	2.06E+11	8.76E+05	2.16E+05	2.35E+10	5.80E+09
02/12/18	5.77E+09	1.08E+09	2.64E+14	4.95E+13	4.96E+09	2.92E+09	2.26E+14	1.33E+14	4.00E+07	2.78E+07	1.83E+12	1.27E+12	8.26E+06	7.04E+06	3.77E+11	3.22E+11	5.20E+05	4.04E+05	2.38E+10	1.85E+10
03/12/18	3.57E+09	1.58E+09	1.60E+14	7.10E+13	5.29E+09	8.42E+08	2.38E+14	3.78E+13	4.67E+07	1.62E+06	2.10E+12	7.27E+10	7.06E+07	0.00E+00	3.17E+12	0.00E+00	1.40E+06	5.10E+04	6.29E+10	2.29E+09
12/12/18	4.78E+09	1.30E+09	1.85E+14	5.03E+13	2.14E+09	2.36E+09	8.28E+13	9.13E+13	6.21E+07	2.04E+07	2.40E+12	7.90E+11	6.98E+07	9.20E+06	2.70E+12	3.56E+11	6.23E+05	1.17E+05	2.41E+10	4.54E+09
19/12/18	5.89E+09	1.15E+09	2.53E+14	4.92E+13	3.31E+09	1.79E+09	1.43E+14	7.68E+13	6.46E+07	2.46E+07	2.78E+12	1.06E+12	4.88E+07	3.07E+06	2.10E+12	1.32E+11	5.23E+05	8.87E+04	2.25E+10	3.81E+09
07/01/19	5.13E+09	5.63E+08	1.43E+14	1.57E+13	3.58E+09	4.26E+08	9.95E+13	1.19E+13	6.70E+07	6.40E+06	1.86E+12	1.78E+11	5.68E+07	9.05E+06	1.58E+12	2.52E+11	2.34E+06	4.80E+05	6.50E+10	1.34E+10
15/01/19	4.56E+09	1.16E+09	1.24E+14	3.15E+13	4.80E+09	2.70E+08	1.31E+14	7.37E+12	4.78E+07	3.44E+07	1.30E+12	9.39E+11	1.95E+07	7.13E+06	5.32E+11	1.94E+11	7.73E+05	2.03E+05	2.11E+10	5.54E+09
21/01/19	5.49E+09	1.80E+09	1.55E+14	5.09E+13	6.39E+09	1.42E+09	1.81E+14	4.02E+13	6.50E+07	5.77E+06	1.84E+12	1.63E+11	6.27E+07	7.59E+06	1.77E+12	2.15E+11	3.32E+06	2.61E+05	9.40E+10	7.38E+09
23/01/19	5.84E+09	5.83E+08	1.83E+14	1.83E+13	4.15E+09	1.71E+08	1.30E+14	5.34E+12	5.73E+07	3.02E+07	1.79E+12	9.47E+11	4.32E+07	3.19E+07	1.35E+12	1.00E+12	1.57E+06	2.89E+03	4.92E+10	9.06E+07
10/02/19	4.18E+09	1.20E+09	1.92E+14	5.53E+13	6.53E+09	6.60E+08	3.00E+14	3.04E+13	5.45E+07	2.52E+07	2.51E+12	1.16E+12	5.47E+07	1.37E+07	2.52E+12	6.30E+11	1.34E+06	6.35E+05	6.15E+10	2.92E+10
11/02/19	4.58E+09	1.80E+08	1.99E+14	7.83E+12	2.29E+09	7.99E+08	9.97E+13	3.48E+13	9.26E+07	4.89E+07	4.03E+12	2.13E+12	7.06E+07	4.05E+07	3.07E+12	1.76E+12	1.06E+06	2.83E+04	4.60E+10	1.23E+09
17/02/19	6.59E+09	1.31E+09	2.35E+14	4.67E+13	4.47E+09	1.06E+09	1.60E+14	3.79E+13	3.78E+07	1.18E+07	1.35E+12	4.23E+11	4.07E+07	1.70E+07	1.45E+12	6.06E+11	7.53E+05	1.02E+05	2.69E+10	3.63E+09
25/02/19	6.84E+09	2.24E+09	2.07E+14	6.77E+13	5.61E+09	1.27E+09	1.70E+14	3.86E+13	5.84E+07	1.89E+07	1.77E+12	5.74E+11	2.07E+07	2.84E+07	6.28E+11	8.61E+11	8.68E+05	4.01E+04	2.63E+10	1.21E+09
01/03/19	3.71E+09	3.11E+08	1.06E+14	8.88E+12	6.07E+09	1.61E+08	1.74E+14	4.60E+12	3.75E+07	4.41E+06	1.07E+12	1.26E+11	4.30E+07	5.54E+06	1.23E+12	1.58E+11	1.31E+06	4.55E+05	3.76E+10	1.30E+10
10/03/19	5.40E+09	6.66E+08	2.42E+14	2.98E+13	6.03E+09	1.44E+09	2.70E+14	6.47E+13	3.52E+07	3.02E+07	1.58E+12	1.35E+12	1.90E+07	1.27E+07	8.50E+11	5.70E+11	6.70E+05	2.46E+04	3.00E+10	1.10E+09
17/03/19	4.32E+09	9.26E+08	1.96E+14	4.19E+13	2.80E+09	7.99E+07	1.27E+14	3.62E+12	3.65E+07	5.64E+06	1.66E+12	2.55E+11	3.81E+07	4.45E+06	1.72E+12	2.02E+11	3.53E+05	3.24E+04	1.60E+10	1.47E+09
29/03/19	5.22E+09	1.89E+08	1.52E+14	5.49E+12	5.57E+09	1.72E+09	1.62E+14	4.98E+13	5.57E+07	1.47E+06	1.62E+12	4.27E+10	4.62E+07	6.94E+06	1.34E+12	2.01E+11	1.10E+06	2.21E+04	3.20E+10	6.40E+08
07/04/19	7.50E+09	2.20E+09	3.19E+14	9.37E+13	3.04E+09	8.69E+08	1.29E+14	3.69E+13	4.32E+06	4.23E+06	1.83E+11	1.80E+11	2.84E+07	3.03E+07	1.20E+12	1.29E+12	5.25E+05	6.36E+05	2.23E+10	2.70E+10
08/04/19	3.71E+09	2.78E+08	1.13E+14	8.43E+12	2.27E+09	5.90E+08	6.90E+13	1.79E+13	5.06E+07	3.28E+06	1.53E+12	9.94E+10	4.78E+07	7.73E+06	1.45E+12	2.34E+11	1.02E+06	2.25E+05	3.10E+10	6.81E+09
15/04/19	6.50E+09	1.29E+09	1.80E+14	3.57E+13	5.74E+09	5.08E+08	1.59E+14	1.40E+13	5.80E+07	1.25E+07	1.60E+12	3.45E+11	5.55E+07	1.67E+07	1.54E+12	4.61E+11	2.05E+06	3.89E+04	5.67E+10	1.08E+09
29/04/19	5.52E+09	3.25E+07	1.44E+14	8.45E+11	1.65E+09	1.65E+08	4.28E+13	4.30E+12	6.05E+07	1.83E+07	1.57E+12	4.77E+11	5.36E+07	9.16E+06	1.39E+12	2.38E+11	3.49E+05	1.76E+05	9.07E+09	4.59E+09
09/05/19	8.77E+09	7.01E+08	3.71E+14	2.96E+13	1.19E+09	1.30E+08	5.01E+13	5.48E+12	3.25E+07	7.18E+05	1.37E+12	3.03E+10	2.88E+07	1.07E+06	1.22E+12	4.54E+10	2.69E+05	1.84E+04	1.14E+10	7.79E+08

10/05/19	5.30E+09	1.42E+09	1.65E+14	4.43E+13	9.06E+08	1.39E+08	2.83E+13	4.33E+12	4.13E+07	1.55E+07	1.29E+12	4.84E+11	4.16E+07	1.11E+07	1.30E+12	3.46E+11	7.89E+05	2.96E+04	2.46E+10	9.24E+08
20/05/19	4.91E+09	1.19E+09	1.26E+14	3.05E+13	2.36E+09	7.94E+07	6.05E+13	2.04E+12	4.21E+07	1.04E+07	1.08E+12	2.66E+11	4.43E+07	5.11E+06	1.14E+12	1.31E+11	5.65E+05	7.51E+04	1.45E+10	1.93E+09
21/05/19	3.20E+09	1.02E+08	8.21E+13	2.61E+12	4.75E+09	1.20E+09	1.22E+14	3.07E+13	6.57E+07	1.86E+07	1.68E+12	4.78E+11	6.16E+07	1.35E+07	1.58E+12	3.46E+11	1.73E+06	3.08E+05	4.44E+10	7.90E+09
17/06/19	5.56E+09	1.51E+08	1.58E+14	4.27E+12	5.25E+09	4.30E+08	1.49E+14	1.22E+13	7.59E+07	1.14E+07	2.15E+12	3.22E+11	6.55E+07	8.91E+05	1.86E+12	2.53E+10	3.51E+06	4.75E+05	9.96E+10	1.35E+10
18/06/19	6.87E+09	4.08E+08	1.96E+14	1.16E+13	6.02E+09	1.15E+09	1.72E+14	3.28E+13	6.25E+07	1.02E+07	1.78E+12	2.90E+11	5.51E+07	1.67E+06	1.57E+12	4.76E+10	4.48E+06	9.11E+04	1.28E+11	2.60E+09
19/06/19	7.77E+09	1.06E+09	2.07E+14	2.83E+13	3.56E+09	2.03E+08	9.45E+13	5.41E+12	7.76E+07	1.76E+07	2.06E+12	4.69E+11	8.13E+07	2.35E+07	2.16E+12	6.24E+11	1.68E+06	2.33E+05	4.46E+10	6.18E+09
25/06/19	3.90E+09	5.78E+07	1.29E+14	1.91E+12	5.38E+09	6.72E+08	1.78E+14	2.22E+13	5.13E+07	2.76E+05	1.70E+12	9.15E+09	5.85E+07	1.36E+07	1.94E+12	4.50E+11	2.78E+06	4.99E+05	9.21E+10	1.65E+10
30/06/19	2.58E+09	4.59E+08	5.94E+13	1.05E+13	3.78E+09	5.08E+08	8.70E+13	1.17E+13	7.32E+07	1.93E+07	1.68E+12	4.44E+11	7.11E+07	1.15E+07	1.63E+12	2.64E+11	2.56E+06	1.94E+05	5.89E+10	4.46E+09
01/07/19	2.69E+09	3.69E+08	6.54E+13	8.97E+12	5.26E+09	1.58E+09	1.28E+14	3.84E+13	5.14E+07	2.19E+06	1.25E+12	5.32E+10	6.63E+07	1.61E+07	1.61E+12	3.92E+11	5.92E+06	2.37E+06	1.44E+11	5.77E+10
05/07/19	4.56E+09	5.70E+08	1.07E+14	1.34E+13	4.71E+09	1.31E+09	1.11E+14	3.09E+13	7.70E+07	1.33E+07	1.81E+12	3.14E+11	6.82E+07	8.47E+06	1.61E+12	2.00E+11	3.33E+06	1.18E+05	7.86E+10	2.77E+09
10/07/19	6.01E+09	7.53E+07	1.47E+14	1.84E+12	7.24E+09	8.89E+08	1.77E+14	2.18E+13	7.01E+07	3.42E+06	1.72E+12	8.37E+10	6.37E+07	2.13E+06	1.56E+12	5.21E+10	6.45E+06	4.37E+05	1.58E+11	1.07E+10
22/07/19	3.56E+09	1.18E+09	8.73E+13	2.90E+13	5.62E+09	9.06E+08	1.38E+14	2.22E+13	6.71E+07	7.81E+06	1.65E+12	1.92E+11	5.78E+07	3.84E+06	1.42E+12	9.42E+10	4.70E+05	1.99E+04	1.15E+10	4.89E+08
07/08/19	4.28E+09	7.07E+08	9.77E+13	1.61E+13	5.29E+09	6.46E+08	1.21E+14	1.47E+13	8.29E+07	2.02E+06	1.89E+12	4.60E+10	7.37E+07	4.09E+06	1.68E+12	9.33E+10	5.65E+06	1.55E+05	1.29E+11	3.54E+09
08/08/19	5.64E+09	1.61E+08	1.33E+14	3.78E+12	4.27E+09	1.70E+08	1.01E+14	3.99E+12	3.87E+07	3.51E+06	9.10E+11	8.25E+10	5.17E+07	6.29E+06	1.22E+12	1.48E+11	5.77E+06	1.92E+05	1.36E+11	4.53E+09
19/08/19	7.18E+09	4.52E+08	1.97E+14	1.24E+13	6.17E+09	2.62E+09	1.69E+14	7.18E+13	3.91E+07	8.77E+06	1.07E+12	2.41E+11	4.63E+07	9.21E+06	1.27E+12	2.53E+11	1.76E+06	2.40E+05	4.82E+10	6.58E+09
20/08/19	7.93E+09	6.16E+08	3.27E+14	2.54E+13	4.91E+09	8.73E+08	2.03E+14	3.60E+13	7.58E+07	3.86E+07	3.13E+12	1.59E+12	5.74E+07	1.24E+07	2.37E+12	5.11E+11	3.26E+06	1.05E+05	1.35E+11	4.35E+09
22/10/19	7.09E+09	1.98E+08	2.10E+14	5.85E+12	4.13E+09	7.55E+08	1.22E+14	2.23E+13	6.29E+07	1.58E+07	1.86E+12	4.68E+11	6.56E+07	1.13E+07	1.94E+12	3.34E+11	1.28E+06	1.59E+05	3.78E+10	4.72E+09
23/10/19	9.35E+09	1.18E+09	2.99E+14	3.79E+13	2.78E+09	1.87E+07	8.91E+13	5.97E+11	5.76E+07	2.83E+06	1.84E+12	9.05E+10	5.56E+07	1.46E+05	1.78E+12	4.66E+09	2.79E+06	2.49E+03	8.92E+10	7.96E+07
28/10/19	4.98E+09	8.36E+08	1.99E+14	3.35E+13	3.00E+09	1.77E+09	1.20E+14	7.09E+13	8.10E+07	5.60E+06	3.24E+12	2.24E+11	7.01E+07	5.88E+06	2.80E+12	2.35E+11	5.08E+06	4.56E+05	2.03E+11	1.83E+10
29/10/19	5.13E+09	2.22E+09	1.86E+14	8.04E+13	2.95E+09	1.02E+09	1.07E+14	3.70E+13	6.01E+07	1.59E+07	2.18E+12	5.78E+11	5.89E+07	1.85E+07	2.13E+12	6.68E+11	4.28E+06	3.22E+05	1.55E+11	1.17E+10
02/12/19	8.24E+09	1.97E+07	3.60E+14	8.62E+11	3.55E+09	1.77E+09	1.55E+14	7.74E+13	5.93E+07	6.69E+06	2.59E+12	2.92E+11	6.56E+07	9.57E+06	2.87E+12	4.18E+11	3.56E+06	3.81E+04	1.55E+11	1.66E+09
05/12/19	6.15E+09	5.92E+08	2.21E+14	2.13E+13	3.45E+09	3.83E+08	1.24E+14	1.38E+13	4.94E+07	1.31E+07	1.77E+12	4.70E+11	4.74E+07	4.53E+06	1.70E+12	1.63E+11	1.67E+06	7.32E+04	6.00E+10	2.63E+09
07/12/19	5.14E+09	8.51E+08	2.21E+14	3.65E+13	2.50E+09	2.97E+08	1.07E+14	1.28E+13	6.08E+07	4.75E+06	2.61E+12	2.04E+11	6.01E+07	1.17E+07	2.58E+12	5.02E+11	8.98E+05	1.55E+04	3.86E+10	6.66E+08
08/12/19	6.50E+09	1.38E+09	3.00E+14	6.35E+13	4.76E+09	6.81E+08	2.20E+14	3.15E+13	5.58E+07	1.60E+07	2.58E+12	7.39E+11	5.85E+07	1.39E+07	2.70E+12	6.43E+11	1.11E+06	2.06E+04	5.11E+10	9.51E+08
09/12/19	5.52E+09	7.30E+08	2.41E+14	3.19E+13	5.57E+09	6.64E+08	2.43E+14	2.90E+13	6.93E+07	2.57E+07	3.03E+12	1.12E+12	7.55E+07	9.04E+06	3.30E+12	3.95E+11	1.12E+06	4.86E+05	4.87E+10	2.12E+10
Average:	5.49E+09	7.94E+08	1.86E+14	2.76E+13	4.33E+09	8.56E+08	1.43E+14	2.94E+13	5.75E+07	1.40E+07	1.89E+12	4.88E+11	5.45E+07	1.07E+07	1.79E+12	3.65E+11	1.99E+06	2.41E+05	6.17E+10	7.60E+09

Table S6. Relative loads of ARGs (*I6S rRNA* normalised) and daily loads per capita of ARGs (absolute gene loads normalised to Bath city population size) in wastewater

Date	<i>ermB</i>			<i>sulI</i>			<i>Int11</i>			<i>qnrS</i>						
	Relative daily loads copies/day	STD	Daily loads per capita copies/day	STD	Relative daily loads copies/day	STD	Daily loads per capita copies/day	STD	Relative daily loads copies/day	STD	Daily loads per capita copies/day	STD	Relative daily loads copies/day	STD	Daily loads per capita copies/day	STD
05/11/2018	1.35E+00	1.25E-01	1.07E+12	1.63E+11	2.03E-02	4.72E-03	1.67E+10	7.74E+09	1.88E-02	7.02E-03	1.43E+10	1.95E+09	4.86E-04	3.33E-05	3.86E+08	6.83E+07
06/11/2018	8.23E-01	2.35E-01	7.18E+11	2.36E+11	1.18E-02	8.02E-04	1.02E+10	2.34E+08	1.79E-02	2.79E-03	1.55E+10	3.11E+09	1.69E-04	7.05E-05	1.45E+08	5.45E+07
11/11/2018	9.97E-01	4.81E-02	2.31E+12	9.57E+10	1.11E-02	1.89E-03	2.57E+10	4.21E+09	1.05E-02	1.45E-03	2.43E+10	3.19E+09	1.50E-04	2.09E-05	3.48E+08	4.62E+07
25/11/2018	9.37E-01	7.47E-02	1.38E+12	7.40E+10	1.07E-02	2.82E-03	1.61E+10	6.29E+09	1.12E-02	3.40E-04	1.67E+10	1.71E+09	1.31E-04	1.51E-05	1.96E+08	4.83E+07
02/12/2018	8.26E-01	3.50E-01	1.89E+12	1.11E+12	6.60E-03	3.58E-03	1.52E+10	1.06E+10	1.34E-03	9.69E-04	3.14E+09	2.68E+09	9.85E-05	8.85E-05	1.98E+08	1.54E+08
03/12/2018	1.70E+00	9.88E-01	1.98E+12	3.15E+11	1.44E-02	5.91E-03	1.75E+10	6.05E+08	2.19E-02	9.69E-03	2.64E+10	0.00E+00	4.31E-04	1.76E-04	5.24E+08	1.91E+07
12/12/2018	3.95E-01	3.86E-01	6.89E+11	7.60E+11	1.41E-02	8.10E-03	2.00E+10	6.57E+09	1.54E-02	6.13E-03	2.25E+10	2.96E+09	1.39E-04	6.23E-05	2.01E+08	3.78E+07
19/12/2018	5.43E-01	1.97E-01	1.19E+12	6.39E+11	1.08E-02	2.08E-03	2.31E+10	8.80E+09	8.49E-03	2.17E-03	1.75E+10	1.10E+09	9.19E-05	3.29E-05	1.87E+08	3.17E+07
07//01/2019	7.33E-01	1.06E-02	8.29E+11	9.87E+10	1.38E-02	1.29E-04	1.55E+10	1.48E+09	1.18E-02	3.09E-03	1.32E+10	2.10E+09	4.88E-04	1.50E-04	5.41E+08	1.11E+08
15/01/2019	1.08E+00	2.15E-01	1.09E+12	6.13E+10	1.18E-02	1.05E-02	1.08E+10	7.82E+09	4.62E-03	2.74E-03	4.43E+09	1.62E+09	1.69E-04	1.56E-06	1.76E+08	4.61E+07
21/01/2019	1.18E+00	1.29E-01	1.50E+12	3.34E+11	1.27E-02	5.21E-03	1.53E+10	1.36E+09	1.23E-02	5.41E-03	1.48E+10	1.79E+09	6.47E-04	2.60E-04	7.82E+08	6.14E+07
23/01/2019	7.12E-01	4.20E-02	1.08E+12	4.45E+10	1.01E-02	6.19E-03	1.49E+10	7.88E+09	7.71E-03	6.24E-03	1.13E+10	8.33E+09	2.70E-04	2.75E-05	4.10E+08	7.54E+05
10/02/2019	1.65E+00	6.32E-01	2.50E+12	2.53E+11	1.27E-02	2.36E-03	2.09E+10	9.63E+09	1.31E-02	5.05E-04	2.10E+10	5.25E+09	3.11E-04	6.25E-05	5.12E+08	2.43E+08
11/02/2019	5.05E-01	1.94E-01	8.30E+11	2.89E+11	2.00E-02	9.90E-03	3.35E+10	1.77E+10	1.53E-02	8.25E-03	2.56E+10	1.47E+10	2.31E-04	1.53E-05	3.83E+08	1.02E+07
17/02/2019	6.76E-01	2.68E-02	1.33E+12	3.15E+11	6.04E-03	3.00E-03	1.12E+10	3.52E+09	6.57E-03	3.88E-03	1.21E+10	5.05E+09	1.15E-04	7.41E-06	2.24E+08	3.02E+07
25/02/2019	8.98E-01	4.80E-01	1.42E+12	3.21E+11	9.49E-03	5.87E-03	1.47E+10	4.78E+09	2.49E-03	3.34E-03	5.23E+09	7.17E+09	1.33E-04	3.76E-05	2.19E+08	1.01E+07
01/03/2019	1.65E+00	1.81E-01	1.45E+12	3.83E+10	1.01E-02	3.43E-04	8.93E+09	1.05E+09	1.17E-02	2.48E-03	1.02E+10	1.32E+09	3.50E-04	9.34E-05	3.13E+08	1.08E+08
10/03/2019	1.14E+00	4.08E-01	2.25E+12	5.39E+11	6.21E-03	4.81E-03	1.31E+10	1.12E+10	3.39E-03	1.94E-03	7.08E+09	4.75E+09	1.25E-04	2.00E-05	2.50E+08	9.19E+06
17/03/2019	6.65E-01	1.61E-01	1.06E+12	3.01E+10	8.51E-03	5.17E-04	1.38E+10	2.13E+09	8.90E-03	8.75E-04	1.44E+10	1.68E+09	8.44E-05	2.55E-05	1.33E+08	1.22E+07
29/03/2019	1.07E+00	3.68E-01	1.35E+12	4.15E+11	1.07E-02	1.04E-04	1.34E+10	3.56E+08	8.87E-03	1.65E-03	1.12E+10	1.67E+09	2.11E-04	3.44E-06	2.67E+08	5.33E+06
07/04/2019	5.79E-01	3.61E-01	1.08E+12	3.08E+11	6.38E-04	4.76E-04	1.53E+09	1.50E+09	6.14E-03	7.38E-03	1.00E+10	1.07E+10	7.35E-05	7.98E-05	1.86E+08	2.25E+08
08/04/2019	6.21E-01	2.06E-01	5.74E+11	1.49E+11	1.37E-02	1.91E-03	1.28E+10	8.28E+08	1.30E-02	3.05E-03	1.21E+10	1.95E+09	2.74E-04	4.00E-05	2.58E+08	5.67E+07
15/04/2019	9.08E-01	2.59E-01	1.32E+12	1.17E+11	9.30E-03	3.77E-03	1.34E+10	2.88E+09	8.98E-03	4.35E-03	1.28E+10	3.84E+09	3.21E-04	5.79E-05	4.72E+08	8.96E+06
29/04/2019	2.98E-01	2.82E-02	3.56E+11	3.58E+10	1.09E-02	3.26E-03	1.31E+10	3.97E+09	9.69E-03	1.60E-03	1.16E+10	1.98E+09	6.30E-05	3.16E-05	7.55E+07	3.82E+07
09/05/2019	1.36E-01	2.57E-02	4.17E+11	4.56E+10	3.71E-03	2.15E-04	1.14E+10	2.53E+08	3.29E-03	1.40E-04	1.01E+10	3.78E+08	3.09E-05	4.57E-06	9.47E+07	6.49E+06

10/05/2019	1.81E-01	7.46E-02	2.35E+11	3.60E+10	8.48E-03	5.20E-03	1.07E+10	4.03E+09	8.44E-03	4.36E-03	1.08E+10	2.88E+09	1.55E-04	4.71E-05	2.05E+08	7.69E+06
20/05/2019	4.97E-01	1.37E-01	5.04E+11	1.69E+10	8.59E-03	3.21E-05	9.00E+09	2.21E+09	9.18E-03	1.18E-03	9.46E+09	1.09E+09	1.17E-04	1.30E-05	1.21E+08	1.60E+07
21/05/2019	1.49E+00	4.21E-01	1.01E+12	2.55E+11	2.04E-02	5.17E-03	1.40E+10	3.98E+09	1.92E-02	3.60E-03	1.31E+10	2.88E+09	5.40E-04	7.90E-05	3.70E+08	6.57E+07
17/06/2019	9.46E-01	1.03E-01	1.24E+12	1.02E+11	1.36E-02	1.67E-03	1.79E+10	2.68E+09	1.18E-02	4.80E-04	1.55E+10	2.11E+08	6.33E-04	1.03E-04	8.29E+08	1.12E+08
18/06/2019	9.21E-01	2.32E-01	1.43E+12	2.73E+11	9.55E-03	2.14E-03	1.48E+10	2.42E+09	8.39E-03	7.73E-04	1.31E+10	3.96E+08	6.82E-04	2.84E-05	1.06E+09	2.16E+07
19/06/2019	4.64E-01	8.96E-02	7.87E+11	4.50E+10	1.02E-02	3.67E-03	1.72E+10	3.90E+09	1.08E-02	4.49E-03	1.80E+10	5.19E+09	2.16E-04	4.02E-07	3.71E+08	5.15E+07
25/06/2019	1.38E+00	1.52E-01	1.48E+12	1.85E+11	1.32E-02	2.66E-04	1.41E+10	7.62E+07	1.50E-02	3.27E-03	1.61E+10	3.74E+09	7.14E-04	1.18E-04	7.67E+08	1.37E+08
30/06/2019	1.51E+00	4.64E-01	7.24E+11	9.72E+10	2.95E-02	1.27E-02	1.40E+10	3.69E+09	2.83E-02	9.47E-03	1.36E+10	2.19E+09	1.01E-03	2.55E-04	4.90E+08	3.71E+07
01/07/2019	2.01E+00	8.63E-01	1.07E+12	3.20E+11	1.93E-02	3.46E-03	1.04E+10	4.43E+08	2.44E-02	2.63E-03	1.34E+10	3.26E+09	2.16E-03	5.85E-04	1.20E+09	4.80E+08
05/07/2019	1.06E+00	4.20E-01	9.23E+11	2.58E+11	1.72E-02	5.08E-03	1.51E+10	2.62E+09	1.52E-02	3.76E-03	1.34E+10	1.66E+09	7.39E-04	1.18E-04	6.54E+08	2.31E+07
10/07/2019	1.21E+00	1.63E-01	1.48E+12	1.81E+11	1.17E-02	7.16E-04	1.43E+10	6.97E+08	1.06E-02	4.87E-04	1.30E+10	4.34E+08	1.07E-03	5.93E-05	1.31E+09	8.91E+07
22/07/2019	1.63E+00	2.86E-01	1.15E+12	1.85E+11	1.96E-02	4.31E-03	1.37E+10	1.59E+09	1.70E-02	4.58E-03	1.18E+10	7.84E+08	1.41E-04	5.24E-05	9.60E+07	4.07E+06
07/08/2019	1.27E+00	3.60E-01	1.01E+12	1.23E+11	1.96E-02	2.76E-03	1.58E+10	3.83E+08	1.74E-02	1.91E-03	1.40E+10	7.77E+08	1.34E-03	2.57E-04	1.07E+09	2.95E+07
08/08/2019	7.58E-01	8.52E-03	8.37E+11	3.33E+10	6.87E-03	8.18E-04	7.58E+09	6.87E+08	9.19E-03	1.38E-03	1.01E+10	1.23E+09	1.02E-03	4.97E-06	1.13E+09	3.77E+07
19/08/2019	8.72E-01	4.19E-01	1.41E+12	5.98E+11	5.48E-03	1.56E-03	8.93E+09	2.00E+09	6.50E-03	1.69E-03	1.06E+10	2.10E+09	2.46E-04	4.88E-05	4.01E+08	5.48E+07
20/08/2019	6.25E-01	1.59E-01	1.69E+12	3.00E+11	9.78E-03	5.63E-03	2.61E+10	1.33E+10	7.32E-03	2.13E-03	1.97E+10	4.25E+09	4.14E-04	4.54E-05	1.12E+09	3.63E+07
22/10/2019	5.81E-01	9.03E-02	1.02E+12	1.86E+11	8.91E-03	2.48E-03	1.55E+10	3.89E+09	9.28E-03	1.85E-03	1.62E+10	2.78E+09	1.80E-04	2.75E-05	3.15E+08	3.93E+07
23/10/2019	3.00E-01	4.00E-02	7.42E+11	4.97E+09	6.22E-03	1.09E-03	1.53E+10	7.53E+08	5.99E-03	7.74E-04	1.48E+10	3.88E+07	3.00E-04	3.83E-05	7.42E+08	6.63E+05
28/10/2019	5.81E-01	2.58E-01	1.00E+12	5.90E+11	1.66E-02	3.91E-03	2.70E+10	1.87E+09	1.44E-02	3.60E-03	2.33E+10	1.96E+09	1.03E-03	8.10E-05	1.69E+09	1.52E+08
29/10/2019	6.82E-01	4.95E-01	8.90E+11	3.08E+11	1.22E-02	2.16E-03	1.81E+10	4.81E+09	1.18E-02	1.52E-03	1.78E+10	5.57E+09	9.36E-04	4.68E-04	1.29E+09	9.70E+07
02/12/2019	4.31E-01	2.16E-01	1.29E+12	6.44E+11	7.19E-03	7.94E-04	2.16E+10	2.43E+09	7.96E-03	1.14E-03	2.39E+10	3.48E+09	4.32E-04	3.59E-06	1.29E+09	1.39E+07
05/12/2019	5.66E-01	1.17E-01	1.03E+12	1.15E+11	7.97E-03	1.36E-03	1.48E+10	3.91E+09	7.71E-03	6.08E-06	1.42E+10	1.35E+09	2.81E-04	1.51E-05	5.15E+08	2.19E+07
07/12/2019	4.87E-01	2.28E-02	8.92E+11	1.06E+11	1.19E-02	1.05E-03	2.17E+10	1.70E+09	1.17E-02	3.46E-04	2.15E+10	4.18E+09	1.77E-04	2.63E-05	3.21E+08	5.54E+06
08/12/2019	7.60E-01	2.66E-01	1.83E+12	2.62E+11	8.52E-03	6.56E-04	2.15E+10	6.15E+09	8.98E-03	2.40E-04	2.25E+10	5.35E+09	1.75E-04	4.01E-05	4.26E+08	7.92E+06
09/12/2019	1.03E+00	2.56E-01	2.03E+12	2.41E+11	1.24E-02	3.02E-03	2.52E+10	9.34E+09	1.39E-02	3.48E-03	2.75E+10	3.29E+09	1.98E-04	6.19E-05	4.06E+08	1.77E+08
Average:	8.86E-01	2.45E-01	1.19E+12	2.45E+11	1.17E-02	3.20E-03	1.57E+10	4.06E+09	1.13E-02	2.93E-03	1.49E+10	3.04E+09	4.10E-04	7.99E-05	5.14E+08	6.32E+07

Table S7. Seasonal T-tests for ARGs studied in influent wastewater in Bath, comparing winter 2018/19 and summer 2019

Winter 18/19: Summer 19	p values Relative (16S rRNA normalised) loads	Absolute loads
<i>ermB</i>	0.108	0.149
<i>sul1</i>	0.136	0.084
<i>qnrS</i>	0.002	0.001
<i>int11</i>	0.067	0.385

*Statistically significant results ($p \leq 0.05$) shown in bold

Table S8: Pearson correlations coefficients of ARGs and AAs quantified in influent wastewater from Bath (every sample)

	16S rRNA	<i>ermB</i>	<i>int11</i>	<i>sul1</i>	<i>qnrS</i>	<i>ermB</i> (16S normalised)	<i>int11</i> (16S normalised)	<i>sul1</i> (16S normalised)	<i>qnrS</i> (16S normalised)
16S rRNA				0.306		-0.873	-0.318	-0.281	-0.315
<i>ermB</i>				0.297		-0.280			
<i>int11</i>				0.772			0.746	0.623	
<i>sul1</i>	0.306	0.297	0.772			-0.314	0.512	0.714	
<i>qnrS</i>									0.867
<i>ermB</i> (16S normalised)	-0.873	-0.280		-0.314			0.364	0.325	0.444
<i>int11</i> (16S normalised)	-0.318		0.746	0.512		0.364		0.861	0.386
<i>sul1</i> (16S normalised)	-0.281		0.623	0.714		0.325	0.861		0.311
<i>qnrS</i> (16S normalised)	-0.315				0.867	0.444	0.386	0.311	
ERY					-0.308				-0.282
CLR	0.363	0.309				-0.394			
dmERY	0.705	0.458		0.338		-0.608			
dmCLR		0.282							
INa						-0.301			
hPZA		0.356			0.867				-0.292
MTZ		0.356							
KTC		0.349							
hMTZ	0.327				0.310	-0.338			
3TC									
FTC									
SPY					0.374				
SMX									
SLZ					0.311				0.287
aSPY		0.335							
aSMX									
FLX									
AMXa	0.380	0.535	0.422	0.365		-0.489			
TET			-0.319		-0.414				-0.344
OTC			-0.378		-0.322				
CIP		0.378				-0.279			
OFX									
TMP									
NPAHD						-0.349			
CLI	0.288	0.312							
dmCLI									

*Statistically significant results ($p \leq 0.05$) only shown

Table S9: Pearson correlations coefficients of ARGs and AAs quantified in influent wastewater from Bath (using monthly averages)

	16S rRNA	ermB	intl1	sul1	qnrS	ermB (16S normalised)	intl1 (16S normalised)	sul1 (16S normalised)	qnrS (16S normalised)
16S rRNA			0.637	0.595		-0.946			-0.387
ermB			0.404	0.543			0.504	0.574	
intl1	0.637	0.404		0.898		-0.518	0.766	0.650	
sul1	0.595	0.543	0.898			-0.568	0.756	0.820	
qnrS									0.929
ermB (16S normalised)	-0.946		-0.518	-0.568					0.488
intl1 (16S normalised)		0.504	0.766	0.756				0.900	
sul1 (16S normalised)		0.574	0.650	0.820			0.900		
qnrS (16S normalised)	-0.387				0.929	0.488			
ERY					-0.783	-0.387			-0.696
CLR		0.448			-0.447	-0.438			-0.478
dmERY	0.818	0.580	0.514	0.648	-0.524	-0.901	0.349	0.424	-0.650
dmCLR		0.479			-0.616				-0.548
INa	0.534	0.437	0.562	0.651		-0.521	0.307	0.383	
hPZA	-0.639		-0.715	-0.532		0.420	-0.406		
MTZ		0.555		0.330		-0.308		0.430	
KTC	-0.302	0.586	-0.376						
hMTZ	0.393	0.340	0.412	0.598	0.491	-0.505		0.328	0.307
3TC	-0.612	0.348				0.548			0.376
FTC	-0.506	0.367			0.387	0.351		0.290	0.497
SPY					0.482		-0.389		0.320
SMX					0.334		-0.322		0.283
SLZ	-0.537				0.323	0.520			0.523
aSPY		0.515				-0.375		0.295	
aSMX		0.425							
FLX		0.541						0.309	
AMXa	0.341	0.572	0.388	0.514		-0.469		0.295	
TET		0.414	-0.319		-0.728				-0.623
OTC	-0.351	0.319	-0.509		-0.534				-0.400
CIP		0.800	0.291	0.537		-0.408	0.339	0.568	
OFX		0.302		0.308	0.353			0.368	0.426
TMP		0.402				-0.289			
NPAHD		0.482		0.347	-0.411	-0.321		0.365	-0.385
CLI		0.429		0.342		-0.359			
dmCLI	-0.491		-0.604	-0.404		0.282	-0.586	-0.328	

*Statistically significant results ($p \leq 0.05$) shown in **bold**

	daKTC	-	-	-	-	-	-	-	-	-	-	-	-
Antiviral	FTC	-	-	0.31	0.13	-	-	-	-	0.56	0.09	0.43	0.18
	3TC	-	-	-	-	0.10	-	-	-	0.12	-	0.11	0.01

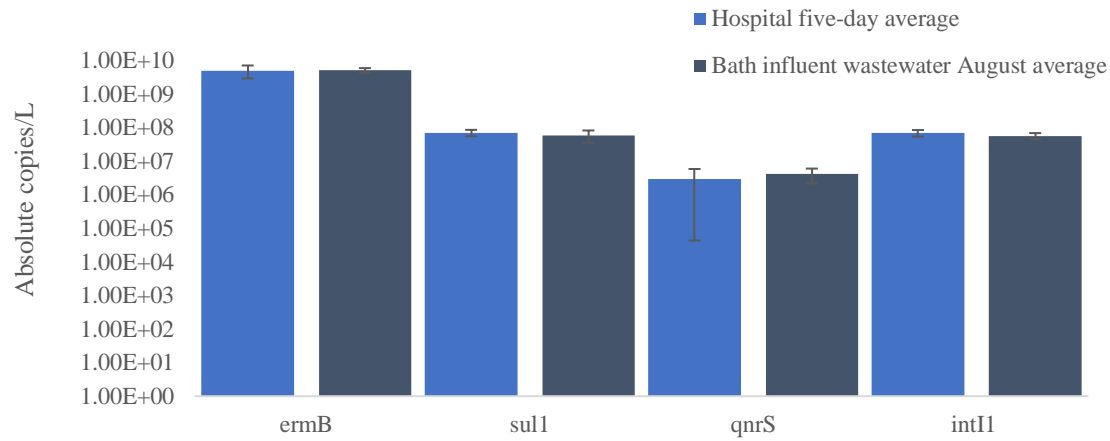


Figure S4. Comparison of absolute concentrations of ARGs in hospital effluent (sampled August 2019) and the averaged ARG concentration from Bath influent wastewater collected during August 2019.

Table S11: Absolute concentration of *16S rRNA* and ARGs in hospital effluent collected in August 2019

Gene	Associated Resistance	05/08/2019		06/08/2019		07/08/2019		08/08/2019		09/08/2019		Weekly Average	
		Copies/L	STD	Copies/L	STD	Copies/L	STD	Copies/L	STD	Copies/L	STD	copies/L	STD
<i>16S rRNA</i>	N/A	3979612000	587807261	5944449000	335602778	4451028750	682760741	3781230250	1533251100	5539616250	68685171	4739187250	957994515
<i>ermB</i>	Macrolide	3820612250	355052811	6456516500	2187016220	7306832750	2566327743	5540123750	302372648	2065687400	2343965783	5037954530	2105266876
<i>sulI</i>	Sulfonamide	87993801	10172295	74501406	273879	75533661	30616792	70360206	13240951	46025175	589960	70882850	15376002
<i>qnrS</i>	Quinolone	505327	80660	7514092	271095	4247888	59374	1250486	191179	1299921	150133	2963543	2919849
<i>intI1</i>	Anthropogenic pollution	90262935	23609612	52540257	7957145	78545166	15475049	57812370	53398	69860014	2396180	69804148	15303718
<i>tetM</i>	Tetracycline	3641760	463725	9941167	216685	3913100	598508	10350742	359487	15729617	861394	8715277	5054206
<i>blaTEM</i>	β -lactamase	340282	935	3343961	339632	5573404	128675	9724635	1857762	12893507	3600023	6375158	4998964

Table S12. Relative concentrations of ARGs in hospital effluent (normalised to *16S rRNA*) collected in August 2019

Gene	Associated Resistance	05/08/2019		06/08/2019		07/08/2019		08/08/2019		09/08/2019		Weekly Average	
		Copies/L	STD	Copies/L	STD	Copies/L	STD	Copies/L	STD	Copies/L	STD	Copies/L	STD
<i>ermB</i>	Macrolide	9.77E-01	2.34E-01	1.08E+00	3.07E-01	1.62E+00	3.29E-01	1.61E+00	7.34E-01	3.76E-01	4.28E-01	1.13E+00	5.16E-01
<i>sulI</i>	Sulfonamide	2.25E-02	5.89E-03	1.26E-02	6.63E-04	1.77E-02	9.59E-03	1.95E-02	4.41E-03	8.31E-03	2.10E-04	1.61E-02	5.68E-03
<i>qnrS</i>	Quinolone	1.27E-04	1.53E-06	1.27E-03	1.17E-04	9.65E-04	1.35E-04	3.72E-04	2.01E-04	2.35E-04	2.42E-05	5.93E-04	4.97E-04
<i>intI1</i>	Anthropogenic pollution	2.25E-02	2.61E-03	8.89E-03	1.84E-03	1.76E-02	7.79E-04	1.67E-02	6.77E-03	1.26E-02	3.65E-04	1.57E-02	5.15E-03
<i>TetM</i>	Tetracycline	9.16E-04	1.88E-05	1.68E-03	1.31E-04	8.79E-04	3.95E-07	2.96E-03	1.11E-03	2.84E-03	1.91E-04	1.85E-03	1.01E-03
<i>blaTEM</i>	β -lactamase	8.64E-05	1.25E-05	5.65E-04	8.90E-05	1.27E-03	2.24E-04	2.69E-03	6.01E-04	2.33E-03	6.79E-04	1.39E-03	1.12E-03

Table S13: Pearson correlations of ARGs concentrations in hospital effluent across five sampling days

	<i>16Sr RNA</i>	<i>ermB</i>	<i>intI1</i>	<i>sul1</i>	<i>qnrS</i>	<i>tetM</i>	<i>bla-tem</i>
<i>16Sr RNA</i>							
<i>ermB</i>	-0.11						
<i>intI1</i>	-0.47	-0.28					
<i>sul1</i>	-0.50	0.53	0.35				
<i>qnrS</i>	0.65	0.67	-0.54	0.13			
<i>tetM</i>	0.56	-0.54	-0.60	-0.90	-0.04		
<i>bla-tem</i>	0.17	-0.39	-0.39	-0.92	-0.28	0.81	

*Statistically significant results are shown in **bold** ($p \leq 0.05$)

Table S14: Pearson correlations of concentrations of ARGs normalised to 16S rRNA in hospital effluent across five sampling days

	<i>ermB</i>	<i>intI1</i>	<i>sul1</i>	<i>qnrS</i>	<i>tetM</i>	<i>bla-tem</i>
<i>ermB</i>						
<i>intI1</i>	0.28					
<i>sul1</i>	0.64	0.84				
<i>qnrS</i>	0.37	-0.60	-0.24			
<i>tetM</i>	-0.25	-0.43	-0.48	-0.32		
<i>bla-tem</i>	0.05	-0.22	-0.34	-0.28	0.86	

*Statistically significant results are shown in **bold** ($p \leq 0.05$)