

# Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system

Buelow, Elena; Bayjanov, Jumamurat R; Majoor, Eline; Willems, Rob J L; Bonten, Marc J M; Schmitt, Heike; van Schaik, Willem

DOI:  
[10.1093/femsec/fiy087](https://doi.org/10.1093/femsec/fiy087)

License:  
Other (please specify with Rights Statement)

Document Version  
Peer reviewed version

Citation for published version (Harvard):  
Buelow, E, Bayjanov, JR, Majoor, E, Willems, RJL, Bonten, MJM, Schmitt, H & van Schaik, W 2018, 'Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system', *FEMS Microbiology Ecology*, vol. 94, no. 7, fiy087. <https://doi.org/10.1093/femsec/fiy087>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**  
Checked for eligibility 13/06/2018

This is a pre-copyedited, author-produced version of an article accepted for publication in *FEMS Microbiology Ecology* following peer review. The version of record Elena Buelow, Jumamurat R Bayjanov, Eline Majoor, Rob JL Willems, Marc JM Bonten, Heike Schmitt, Willem van Schaik, Limited influence of hospital wastewater on the microbiome and resistome of wastewater in a community sewerage system, *FEMS Microbiology Ecology*, Volume 94, Issue 7, July 2018, fiy087, is available online at: [xxhttps://doi.org/10.1093/femsec/fiy087](https://doi.org/10.1093/femsec/fiy087).

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

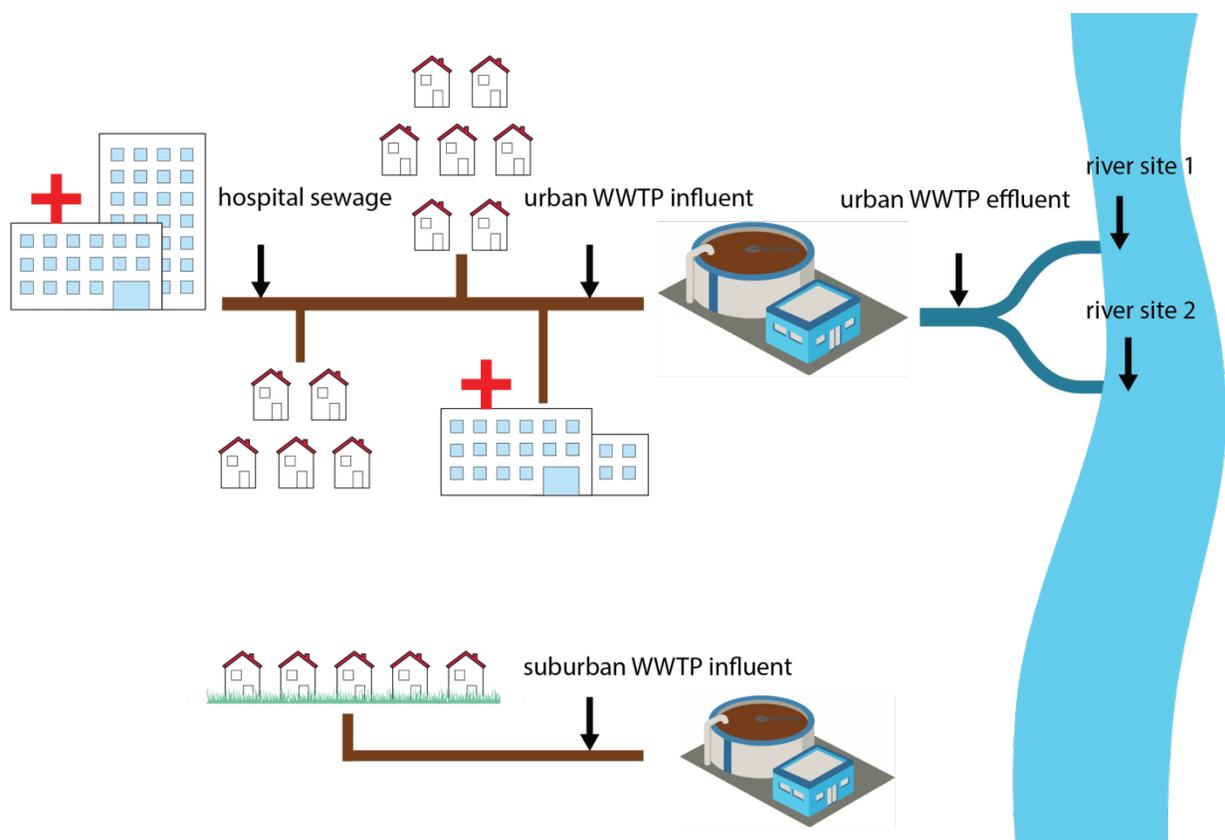
## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

1 **Supplementary Figures and Tables**

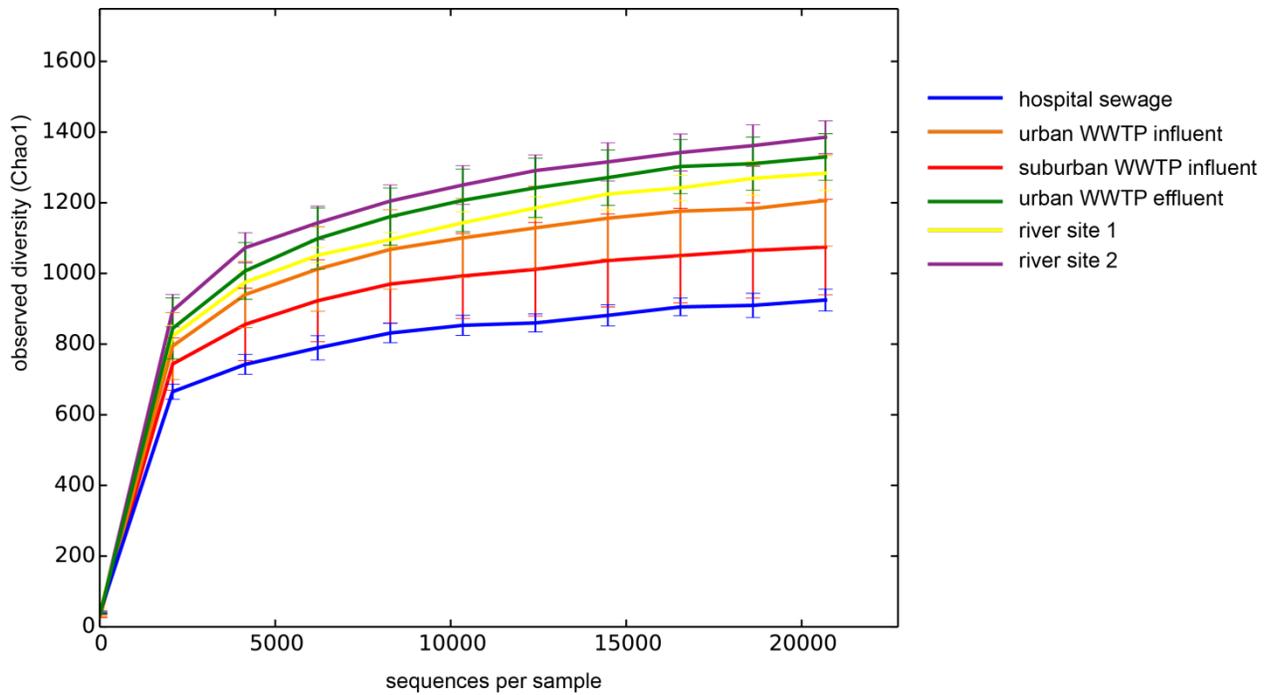
2



3

4 **Supplementary Figure 1: Schematic representation of sampling locations.** Sampling was carried out  
5 on four different days within the course of 2.5 weeks (Monday 31 March 2014; Wednesday 2 April 2014;  
6 Monday 7 April 2014; Monday 14 April 2014). The different sampling locations are indicated by black  
7 arrows. The vector graph depicting the sewage treatment plant was provided by Tracey Saxby, Integration  
8 and Application Network, University of Maryland Center for Environmental Science.

9



10

11

12

13 **Supplementary Figure 2: Observed diversity for the different sampling sites.** Rarefaction curves  
 14 depict the Chao1 index which indicates the species diversity per sampling site as a function of the sub-  
 15 sampling depth with the maximum threshold of 20681 sequences per sample.

16

17

18

19

20

21 **Supplementary Table 1. Primers used in this study.** Primers were developed to target the indicated  
 22 ARGs. Primer sequences in bold indicate ARGs which were detected in  $\geq 1$  sample. Grey background  
 23 indicates modifications to primer sequences compared to those published in Buelow *et al.*, 2017.

Antimicrobial resistance gene	Accession number	Forward primer	Reverse primer
<b>catB3</b>	<b>ABD58915.1</b>	<b>AAGGCAAGCTGCTTTCTGAG</b>	<b>TCGAATGAGTGCCCATGATA</b>
<i>acrF</i>	CAR04877.2	ACTGACACCGTTGATGTGA	GAGCAATAATCGAGGCGTTC
<i>tolC</i>	BAG78840.1	CTGAAAGAAGCCGAAAAACG	CGTCGGTAAGTGACCATCCT
<i>acrA</i>	ACI36997.1	GAAGGTAGCGACATCGAAGC	CTTTCGCCAGATCACCTTTC
<i>aph(3')-III</i>	ACB90577.1	CCGGTATAAAGGGACCACCT	CTTTGGAACAGGCAGCTTTC
<i>aph(2'')-Ib</i>	AF207840.1	ATCAAATCCCTGCGGTAGTG	CAAGGGCATCCTTTTCCTTT
<i>aadE</i> -like gene	AAW34138.1	GCATGATTTCTGGCTGATT	CCACAATTCCTCTGGGACAT
<i>aac(6')-aph(2'')</i>	ABY79711.1	<b>TCCAAGAGCAATAAGGGCATA</b>	<b>TGCCCTCGTGAATTCATGT</b>
<i>aac(6')-li</i>	WP_002293989.1	AGACAGCTCGGCAGAAGAAG	ACCGTATTGAGGGATTGCAC
<i>aac(3')-II(acde)</i>	HQ246166.1	TGACGTATGAGATGCCGATG	GAGAATGCCGTTTGAATCGT
<i>aac(6')-Ib</i>	KM387722.1	TTGCAATGCTGAATGGAGAG	TGGTCTATTCCGCGTACTCC
<b>aadA</b>	<b>ADW23165.1</b>	<b>CAGCGGAGGAATTCTTTGAC</b>	<b>GCTGCGAGTTCATAGCTTC</b>
<b>aac(6')-IIa</b>	<b>ACR24243.1</b>	<b>GGAACACTACCTGCCAGAG</b>	<b>GCGACGTACGACTGAGCATA</b>
<i>aph(2'')-I(de)</i>	AAC14693.1	CGGAGGTGGTTTTACAGGA	TTGCTTCGGCAGATTATTGA
<i>aph(3')-Ia, -Ic</i>	CAQ58482.1	<b>ATTCTCACCGATTCACTCG</b>	<b>GATTCCGACTCGTCCAACAT</b>
<i>strB</i>	CAJ77026.1	GGCGATTATAGCCGATCAAA	CGCGACTGGAGAACATGATA
<i>bacA_2</i>	ABR38862.1	GAGGCATTGATCCTTGGTGT	AAACAATGCCGAACCGATAG
<i>bacA_1</i>	CAH05846.1	GGCTGCGTTACTGTCTGTTTT	GGCCAATGATAAATGCATCC
<i>bacA</i>	ACL18936.1	AACTTCCCGTTCTGGTGCTA	CATAACGGGGATAGCGAGAA
<b>bla<sub>GES</sub></b>	<b>ABG47465.1</b>	<b>GACCCACACCATTGAGAGGT</b>	<b>GCGCAGGTACCAGTTTTCTC</b>
<i>bla<sub>IMP</sub></i>	AJ640197.1	GCTACCGCAGCAGAGTCTTT	CCCACCCGTTAACTTCTTCA
<i>bla<sub>VIM</sub></i>	AM183120.1	TGTCCGTGATGGTATGAGT	TTTCAATCTCCGCGAGAAGT
<i>bla<sub>ACC</sub></i>	AJ870923.1	TTGTTACGCTACGTGCAAGC	CGATTTGAAATAGCCGGTGT
<i>bla<sub>DHA</sub></i>	AHN96243.1	AAAGTGCGCAAAGCCAGTAT	AAGATTCGCATCAAGCTGT
<i>bla<sub>IMI</sub></i>	U50278.1	AGTCGATCCCAGCAGCTTTA	CCAAGAACTGTGCATTCCA
<i>bla<sub>CMY-1/MOX</sub></i>	AF357598.1	GATCTGCTGCGTTTTGTGAA	CTACCGAGTAATGCCCTTGG
<i>bla<sub>AMPc</sub></i>	ABF06289.1	ACCGCTAAACAGTGAATGG	GCAAGTCGCTTGAGGATTTT
<i>cepA</i>	CR626927.1	ATGTCCTGCCCTGGTAGTTG	CTTGCCCGTCGATAATGACT
<i>cepA_2</i>	AE016945.1	TGCACCAAGACGAAAGTCTG	ACAGTGCTTCTTTGCGGAAT
<i>bla<sub>BIC-1</sub></i>	GQ260093.1	CCATCAGCGCACACATAGT	CCAGAACGTTTTCCAGAAGC
<i>cbIA</i>	AAA66962.1	TGCCTGCGACATCTTGATAG	CCGTCTTCTGTTCCGAGAG
<i>cfxA</i>	AY769933.1	GCGCAAATCCTCCTTTAACA	ACAATAACCGCCACACCAAT
<i>bla<sub>CMY-2</sub></i>	AAZ99133.1	CGATCCGGTACGAAATACT	CCTGCCGTATAGGTGGCTAA
<i>bla<sub>CTX-M</sub></i>	ABG46354.1	ACTATGGCACCAACGAT	GGTTGAGGCTGGGTGAAGTA
<i>bla<sub>TEM</sub></i>	NP_775035.1	AAGCCATACCAAACGACGAG	TTGCCGGGAAGCTAGAGTAA
<b>bla<sub>SHV</sub></b>	<b>AAV83796.1</b>	<b>CTTTCCCATGATGAGCACCT</b>	<b>CAGATCTGCTGCGGATAGT</b>
<b>bla<sub>NDM</sub></b>	<b>CAZ39946.1</b>	<b>TGGATCAAGCAGGAGATCAA</b>	<b>ATTGGCATAAGTCGCAATCC</b>
<i>bla<sub>OXA</sub></i>	AAP70012.1	GTGGCATCGATTATCGGAAT	AGAGCACAACCTACGCCCTGT
<i>bla<sub>KPC</sub></i>	AEL12451.1	TGGCTAAAGGGAACACGAC	TAGTCATTTGCCGTGCCATA
<i>cat</i>	ABO92401.1	CAATCCTCAATCGACACGAA	GATTGTGTAGCAAGGCAGCA
<i>mdtL</i>	CAR15381.2	CGGACAAACCACGAGAAAAT	GAAGGTGAGGATCACCGAAA
<i>mdtF</i>	KEL93478.1	GGACCCGCAAAAACCTCAATA	AGTTGACCACCGGAAATCTG

Antimicrobial resistance gene	Accession number	Forward primer	Reverse primer
<i>ermF</i>	BAD66041.1	AGCACCCGCTTTTTCTTAT	GATCAAGAGGGGCTTTAGGG
<i>ermB</i>	BAH18720.1	GGTTGCTCTGCACACTCAA	CTGTGGTATGGCGGGTAAGT
<i>ermG</i>	122586.NMB0066	TGCTGTCTTTTACAGGCCACT	GCATATGTTCCAGTCCCTTCA
<i>ermC</i>	BAE05991.1	TGAAATCGGCTCAGGAAAAG	GGTCTATTTCAATGGCAGTTACG
<i>mefA_10</i>	583346.CKR_2320	CCTGCAAATGGCGATTATTT	CCAAAGACCGCATAGGGTAA
<i>mefA_3</i>	286636.M6_SPY1166	TTACCCTATGCGGTCTTTGG	GAACCAGCTGCTGCGATAAT
<i>macB</i>	ACR63203.1	GGCTGGAAGACCGTACAGAG	GTTGGTTCATCGGCAAGAAT
<i>fosB</i>	NP_372857.1	AGCTTGCAGGCCTATGGAT	TGCCAATATTTAAATTCGCTGTC
<i>cfr</i>	YP_003896025.1	CAAACGAAGGGCAGGTAGAA	GACCACAAGCAGCGTCAATA
<i>mfsA</i>	WP_002584949.1	AATATGCTCTCCGGGCTTTT	TTTGCACACCGTAAAATGGA
<i>ermA</i>	AB047088.2	GAGGGGTTTACCGCTTCTTT	ATCGGATCAGGAAAAGGACA
<i>mecA</i>	YP_184944.1	TCCAGGAATGCAGAAAGACC	GGCCAATCCACATTGTTTC
<i>arnA</i>	CAR03684.2	GAAATTCACCGTCTGGTCGT	GTGGTGCAACAGAAATCACC
<i>mdtO</i>	ASB78387.1	TTGTTGGCCTCTATCCAACC	TTAAGCGCTTGATGCATTTG
<i>qacA</i>	YP_536864	GACCCTTCTGGTACCCAACA	TCCCCATTTATCAGCAAAGG
<i>qacC</i>	CAA86016.1	TGGGCGGGACTAGGTTTAG	ACGAAACTACGCCACTATGA
<i>cm1A1</i>	ABI50481.1	AATGGGATGCCTGATAGCTG	ACCCACTAGCCACATTGGAG
<i>qacE</i>	NC_001735.4	TCGGTGTGCTTATGCAGTC	ATCAAGCTTTTGCCCATGAA
<i>qnrA</i>	ACA43024.1	ATTTCTCAGCCAGGATTTG	ACTGCAATCCTCGAAACTGG
<i>qnrB</i>	AFD54601.1	CGATCTGACCAATTCGGAGT	ACGATGCCTGGTAGTTGTCC
<i>qnrC</i>	ACK75961.1	GCAGAATTCAGGGGTGTGAT	AACTGCTCCAAAAGCTGCTC
<i>qnrS</i>	AEG74318.1	TGAAACCTACCGTCACACA	AATCGCATCGGATAAAGGTG
<i>spc</i>	AAL05549.1	TGACGAACGCAATGTGATT	TCAGTGCCAGATCTTTTGA
<i>vatA</i>	AAF24087.1	AACAGCTTCTGCAGCAATGA	CCTTGAAAGGGGACATTGAA
<i>vatB</i>	AAA86871.1	TGGGAAAAAGCAACTCCATC	TTCTGACCAATCCACACATCA
<i>aadE</i>	CAZ55809.1	TGTGCCGCAAAGAGATACTG	AACCTTCCACGACATCATCC
<i>sul1</i>	ADB23338.1	AGGCTGGTGGTTATGCACTC	AAGAACCGCACAATCTCGTC
<i>tetQ</i>	Y08615.1	GCAAAGGAAGGCATACAAGC	AAACGCTCCAAATTCACACC
<i>tetX</i>	ABQ05845.1	CGGTACGCTGGATTTACACA	CATCGGAATTGCCTTTTTGT
<i>tetW</i>	ACD97480.1	GGTGCAATTGGAGTTGTTT	AAATGACGGAGGGTTCCTTT
<i>tetM</i>	ACO22036.1	TTGATGCGGGAAAACTACC	TACCTCTGTCCACGCTTCTT
<i>tetO</i>	EAQ71799.1	GCGTCAAAGGGGAATCACTA	CGGTATACTTCCGCCAAAAA
<i>tetB</i>	AAL09908.1	CAAAACTTGCCCCTAACCAA	GCTTTCAGGGATCACAGGAG
<i>dfrA27</i>	ACD45689.1	TTGTTGTTTCTGGTGGTGA	TTCCGGAAAAGAAAACATTGC
<i>dfrF</i>	AEBU01000146.1	GATTGTTGCGAGGTCAAAGAA	CGCCCCATAATAACCACATT
<i>vanUG</i>	ACR77286.1	ATTTGCGAAACTCGGAAAAA	ACACCTCATTTTCGGGTACG
<i>vanR</i>	CAJ68489.1	TGAAGCTGTATGGGAGAAAA	TTTCGGGTTTTTAGAAGTTCA
<i>vanA</i>	ACP19236.1	GTGCGGTATTGGGAAACAGT	TGCGTTTTTCAGAGCCTTTTT
<i>vanB</i>	WP_032489746.1	CCTGCCTGGTTTTACATCGT	GCTGTCAATCAGTGCAGGAA
<i>vanX</i>	NP_878017.1	CCGTTGACGGTTATGAAGT	CAGCCAGTCTTTTGCCTTC
<i>cfr_2</i>	AJ249217.1	GCCGGAGCTTTTCTCTACT	GGTGCCGAAAGTCAAACAT
<i>sulA</i>	AAB03082.1	GATATTCGCGGTTTTCCAGAACC	GTCTTGACCCGAATGCATAAC
16S rRNA	a	CAACGCGARGAACCTTACC	ACAACACGAGCTGACGAC

a The primers for the 16S rRNA gene were previously described by Gloor *et al.* (2010), PLoS ONE 5(10): e15406. doi:10.1371/journal.pone.0015406

24  
25  
26

27 **Supplementary Table 2.** Results 16S rRNA gene profiling (aggregated to order level). Numbers represent the abundance, as percentage of the  
 28 total microbiome, of each indicated taxa.

	hospital sewage t1	hospital sewage t2	hospital sewage t3	hospital sewage t4	urban WWTP influent t1	urban WWTP influent t2	urban WWTP influent t3	urban WWTP influent t4	suburban WWTP influent t1	suburban WWTP influent t2	suburban WWTP influent t3	suburban WWTP influent t4
Clostridiales	29.50	27.42	27.41	30.42	26.94	13.34	17.65	14.53	18.40	21.10	19.63	16.84
Lactobacillales	15.18	18.49	16.99	9.31	15.23	20.57	8.61	7.43	10.85	9.42	8.69	14.43
Pseudomonadales	8.03	7.01	4.99	7.95	9.21	3.92	5.25	2.05	4.58	4.55	3.82	3.39
Bacteroidales	6.88	7.57	4.35	5.92	4.33	5.78	4.19	2.24	5.36	5.19	7.34	3.65
Burkholderiales	6.87	5.32	9.08	7.47	5.59	3.26	5.35	6.16	4.73	4.94	5.10	4.38
Aeromonadales	4.96	6.70	3.78	4.15	2.16	19.29	11.11	9.91	13.06	7.54	11.43	17.26
Actinomycetales	3.86	2.29	4.04	5.14	5.02	3.42	3.59	3.35	4.12	3.03	3.97	4.67
Campylobacteriales	3.77	6.08	5.63	6.61	4.31	10.06	17.55	29.03	16.95	15.13	18.24	12.36
Bifidobacteriales	3.71	4.36	4.21	4.82	5.34	2.05	3.77	2.57	2.68	2.30	3.06	2.46
Coriobacteriales	2.67	2.50	2.81	2.89	2.85	1.43	2.41	2.15	1.72	1.77	1.98	2.00
Flavobacteriales	1.43	0.90	1.76	1.64	0.76	0.33	0.79	0.46	0.39	0.93	0.37	0.28
Rhodocyclales	1.31	0.89	1.57	1.42	1.36	2.49	2.57	3.54	3.48	3.05	3.91	3.21
Enterobacteriales	1.11	1.24	1.58	1.27	1.41	1.94	2.00	1.45	1.46	1.60	1.56	1.95
Rhodobacterales	0.83	0.37	1.04	0.78	1.04	0.21	0.66	0.66	0.34	0.72	0.28	0.37
Neisseriales	0.73	0.64	0.99	0.72	0.65	0.33	0.70	0.89	0.41	0.64	0.64	0.42
Rhizobiales	0.52	0.39	0.70	0.60	0.64	0.17	0.47	0.54	0.23	0.59	0.20	0.24
Xanthomonadales	0.50	0.22	0.60	0.50	0.33	0.09	0.26	0.37	0.12	0.30	0.13	0.12
Fusobacteriales	0.48	0.14	0.12	0.31	1.07	1.99	1.79	0.52	1.82	2.49	1.08	2.08
Erysipelotrichales	0.47	0.49	0.89	0.61	0.59	0.12	0.21	0.30	0.38	0.23	0.21	0.21
Desulfovibrionales	0.32	0.28	0.38	0.37	0.29	0.59	0.39	0.24	0.61	0.36	0.81	0.67
ASSO-13- (Rhodocyclales)	0.32	0.26	0.49	0.22	0.29	0.07	0.30	0.48	0.15	0.29	0.13	0.12
Synergistales	0.27	0.09	0.05	0.16	0.98	0.79	0.98	0.98	0.92	2.15	1.24	1.25
Unassigned	3.77	4.85	3.95	4.27	4.03	5.56	4.42	4.77	4.91	4.29	3.82	5.14
others	2.48	1.48	2.58	2.45	5.55	2.20	4.96	5.36	2.31	7.39	2.37	2.48

	urban effluent t1	urban effluent t2	urban effluent t3	urban effluent t4	river water site 1 t1	river water site 1 t2	river water site 1 t3	river water site 1 t4	river water site 2 t1	river water site 2 t2	river water site 2 t3	river water site 2 t4
Clostridiales	9.83	10.70	7.24	11.31	6.28	3.75	6.13	5.94	6.65	8.45	7.78	9.21
Lactobacillales	1.42	2.85	1.06	2.09	1.10	0.97	1.04	1.22	1.32	0.94	1.58	1.55
Pseudomonadales	0.89	2.72	0.60	0.79	0.37	0.57	0.32	0.46	0.66	0.90	0.83	1.04
Bacteroidales	1.95	1.73	1.12	2.11	0.66	0.83	1.11	1.40	1.25	1.25	1.67	2.35
Burkholderiales	12.56	13.08	10.98	8.45	14.40	11.96	8.17	8.32	9.41	12.89	11.97	7.66
Aeromonadales	1.17	1.98	0.65	1.23	0.43	0.28	0.58	1.07	0.77	0.67	1.48	1.69
Actinomycetales	12.08	23.05	18.25	19.38	13.50	11.93	17.20	17.91	17.09	16.85	14.27	17.41
Campylobacterales	2.71	2.63	1.28	3.46	1.63	0.97	0.93	1.41	0.92	1.91	3.15	3.27
Bifidobacteriales	0.61	0.47	0.46	0.83	0.58	0.37	0.43	0.58	0.55	0.48	0.54	0.77
Coriobacteriales	0.67	1.00	0.53	0.93	0.41	0.30	0.47	0.43	0.54	0.53	0.84	0.84
Flavobacteriales	0.44	10.96	0.46	0.40	0.38	0.56	0.53	0.58	0.42	0.41	0.50	0.49
Rhodocyclales	7.46	4.69	9.52	8.69	13.89	16.47	12.32	13.90	9.49	7.81	9.79	8.48
Enterobacteriales	0.46	0.59	0.30	0.89	0.18	0.13	0.18	0.29	0.38	0.31	0.40	0.68
Rhodobacterales	0.56	0.32	0.96	0.80	0.87	1.56	1.48	1.60	1.42	0.79	0.96	0.95
Neisseriales	1.97	0.18	1.32	0.78	1.04	0.82	0.49	0.41	0.75	1.89	1.33	0.55
Rhizobiales	1.05	0.44	1.40	1.28	1.31	2.04	1.96	2.26	1.76	1.14	1.39	1.47
Xanthomonadales	0.30	0.28	0.56	0.39	0.72	0.86	0.71	0.82	0.47	1.37	0.54	0.50
Fusobacteriales	0.22	0.16	0.12	0.06	0.02	0.05	0.13	0.07	0.08	0.11	0.13	0.08
Erysipelotrichales	0.08	0.08	0.03	0.11	0.03	0.04	0.05	0.05	0.06	0.05	0.10	0.12
Desulfovibrionales	0.10	0.20	0.11	0.18	0.06	0.03	0.06	0.08	0.09	0.09	0.15	0.13
ASSO-13 (Rhodocyclales)	0.02	0.01	0.01	0.03	0.05	0.04	0.03	0.06	0.03	0.03	0.04	0.07
Synergistales	0.22	0.22	0.34	0.36	0.15	0.05	0.33	0.18	0.22	0.20	0.10	0.32
Unassigned	3.97	3.59	3.59	3.65	1.88	3.32	3.03	2.94	3.68	3.72	3.51	2.90
others	39.26	18.05	39.10	31.80	40.05	42.08	42.30	38.01	41.99	37.20	36.93	37.46



30 **Supplementary Table 3:** Aggregated cumulative abundance of ARG classes relative to the 16S rRNA gene.

	aminoglycosides	bacitracin	$\beta$ -lactams	chloramphenicols	efflux	macrolides	polymyxins
hospital sewage t1	1429.19	11.30	513.11	20.76	47.37	361.11	2.11
hospital sewage t2	1423.39	12.57	568.49	20.60	47.32	375.27	2.15
hospital sewage t3	1660.78	12.89	605.79	16.78	51.58	403.89	2.15
hospital sewage t4	1633.67	12.06	590.51	20.00	46.05	358.19	1.75
urban WWTP influent t1	87.02	5.83	38.72	9.31	27.77	163.60	0.92
urban WWTP influent t2	247.49	4.23	27.49	14.39	15.23	235.73	0.86
urban WWTP influent t3	106.03	3.46	41.34	15.22	21.21	107.09	0.92
urban WWTP influent t4	159.04	4.49	45.60	20.35	25.42	134.69	0.96
suburban WWTP influent t1	124.07	3.30	30.57	10.86	18.79	64.30	0.70
suburban WWTP influent t2	119.86	7.26	43.60	13.32	23.07	135.36	0.86
suburban WWTP influent t3	118.46	5.28	37.64	9.23	18.83	164.84	0.77
suburban WWTP influent t4	193.30	3.02	32.04	15.53	21.74	232.83	1.07
urban WWTP effluent t1	34.35	2.74	14.75	1.95	4.60	20.79	0.24
urban WWTP effluent t2	36.57	1.48	10.30	1.88	3.72	24.04	0.27
urban WWTP effluent t3	25.47	1.63	10.03	1.48	2.69	11.21	ND
urban WWTP effluent t4	43.17	5.00	23.40	3.43	7.61	27.53	0.40
river water site 1 t1	29.28	2.37	10.27	1.47	3.90	22.08	0.23
river water site 1 t2	25.94	0.97	4.82	0.38	1.64	15.71	ND
river water site 1 t3	26.28	1.39	7.29	0.98	2.48	14.97	ND
river water site 1 t4	30.90	1.84	10.63	1.74	3.47	19.36	ND
river water site 2 t1	18.36	2.30	9.70	1.37	3.39	13.78	ND
river water site 2 t2	39.02	2.35	10.50	1.41	3.23	19.80	0.20
river water site 2 t3	39.99	2.57	14.78	2.04	4.62	19.37	ND
river water site 2 t4	58.06	4.57	26.27	4.12	7.35	31.70	0.42

31

32

	QAC resistance genes	quinolones	streptogramins	sulphonamides	tetracyclines	trimethoprim	vancomycin
hospital sewage t1	344.47	9.17	ND	216.50	384.68	12.17	386.38
hospital sewage t2	337.67	6.91	0.07	214.56	387.78	14.31	302.88
hospital sewage t3	388.42	7.15	ND	234.68	402.38	11.04	377.20
hospital sewage t4	371.23	6.61	ND	243.17	390.20	15.34	457.12
urban WWTP influent t1	45.50	5.58	1.38	29.45	238.76	10.80	2.03
urban WWTP influent t2	93.93	6.87	0.90	59.21	197.41	2.41	ND
urban WWTP influent t3	82.65	4.96	1.34	54.48	186.89	5.54	1.81
urban WWTP influent t4	88.49	7.14	1.43	59.19	180.66	6.47	3.63
suburban WWTP influent t1	10.83	7.56	1.11	8.26	86.37	4.59	0.00
suburban WWTP influent t2	68.33	6.05	1.62	45.47	215.08	4.66	3.30
suburban WWTP influent t3	77.72	4.90	1.10	47.07	209.39	3.82	0.29
suburban WWTP influent t4	93.17	5.59	1.30	58.94	189.38	3.93	0.18
urban WWTP effluent t1	60.35	0.91	0.00	38.49	45.90	1.43	0.00
urban WWTP effluent t2	25.52	0.81	0.00	17.44	47.16	0.77	1.31
urban WWTP effluent t3	70.48	0.45	0.00	43.60	30.13	0.79	0.00
urban WWTP effluent t4	76.09	2.39	0.00	54.03	70.46	1.79	0.00
river water site 1 t1	53.19	0.80	ND	35.46	39.29	1.17	ND
river water site 1 t2	61.59	0.32	ND	43.29	23.08	0.68	0.25
river water site 1 t3	60.13	0.33	ND	49.54	30.12	0.92	ND
river water site 1 t4	67.05	0.81	ND	49.71	35.37	0.96	ND
river water site 2 t1	56.33	0.57	ND	42.26	33.41	0.79	ND
river water site 2 t2	71.04	0.76	ND	45.97	42.81	0.87	0.76
river water site 2 t3	68.26	0.74	ND	52.54	45.71	1.25	ND
river water site 2 t4	90.82	1.82	ND	59.42	63.83	2.15	1.49

33

34 Abundance for each individual ARG was calculated relative to the CT-value of 16S rRNA in each sample, as described in the Materials and  
35 Methods. Abundances of each ARG detected per ARG class were then aggregated to determine the relative abundance of ARG classes. ND:  
36 not detectable.

