Supporting Information

Developing surrogate markers for predicting antibiotic resistance 'hot spots' in rivers where limited data are available

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Table S20. Spearman correlations between river water concentrations of total antibiotics, amoxicillin and ciprofloxacin (both antibiotics detected in the catchment above their PNECs), total antibiotic resistant genes (ARGs) and ARGs reported by antibiotic class for the river catchment (n=30). Correlation values only shown for $P < 0.05$ with P values corrected for multiple testing with the Benjamini Hochberg approach.

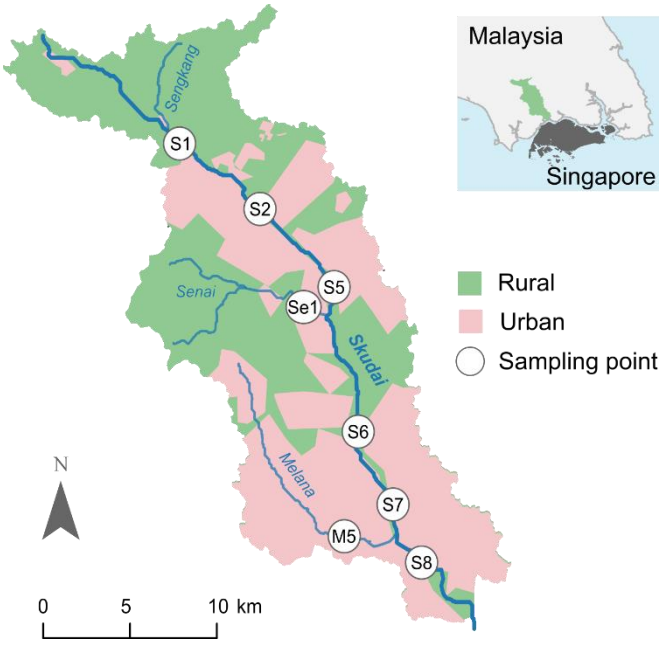
Methods S1: Additional details on antibiotics analysis

Duplicate 500 mL river water samples were filtered with glass microfiber and 0.45 µm cellulose acetate filter paper (both VWR). Samples were adjusted to pH 3.0 with hydrochloric acid, 2.5 mL of Na_4EDTA (100 g/L) was added, samples were spiked with 50 µL of isotopically labelled internal standards (ILISs at 100 ng), and stored for a maximum of 48 h at 4ºC in the dark prior to SPE. ILISs used in this study included ceftazidime-d_5, meropenem-d_6, ciprofloxacin-d_8, lincomycin-d_3, clindamycin-d_3, azithromycin-d_3, clarithromycin-d_3, erythromycin-d_6, sulfamethazine-d_4, sulfamethoxazole-d_4, trimethoprim-d_3, tetracycline-d_6, and chloramphenicol-d_5.[9–11]

The SPE cartridges (chromabond HR-X cartridges 6 mL, 500 mg, Macherey-Nagel) were preconditioned with 5 mL of methanol, followed by 5 mL of acidified Milli Q water (pH 3) at a flow rate of 3 mL/min. Subsequently, 500 mL of spiked and acidified surface water samples were loaded onto the cartridges at a flow rate of 5 mL/min. After all water samples were passed through SPE cartridges, the cartridges were rinsed with 5 mL of acidified Milli-Q water (pH 3.0) in order to remove weakly bound impurities and Na_4EDTA. Then the SPE cartridges were dried for 30 min under vacuum. Elution of the target analytes from the SPE cartridges were performed using 5 mL of methanol at a flow rate of 1 mL/min. The resulting extracts containing the target analytes were dried under a gentle stream of nitrogen at 35 °C. The dried extracts were finally dissolved again with 1 mL of a mixture of methanol and Milli-Q water (50:50, v/v). The final aliquots were transferred into 2 mL amber vials and stored at -20 °C until UHPLC-MS/MS analyses.

Quantification of target antibiotics in water samples was performed using 13 ILISs, which corrects for losses during sample preparation and matrix effects during HPLC-MS/MS. The relative SPE recoveries for target antibiotics in the water samples varied from 85.6 to 117%

(SI Table S5). Method quantification limits (MQL) of target antibiotics ranged from 0.1 to 50 ng/L, depending on the compound (SI Table S5).



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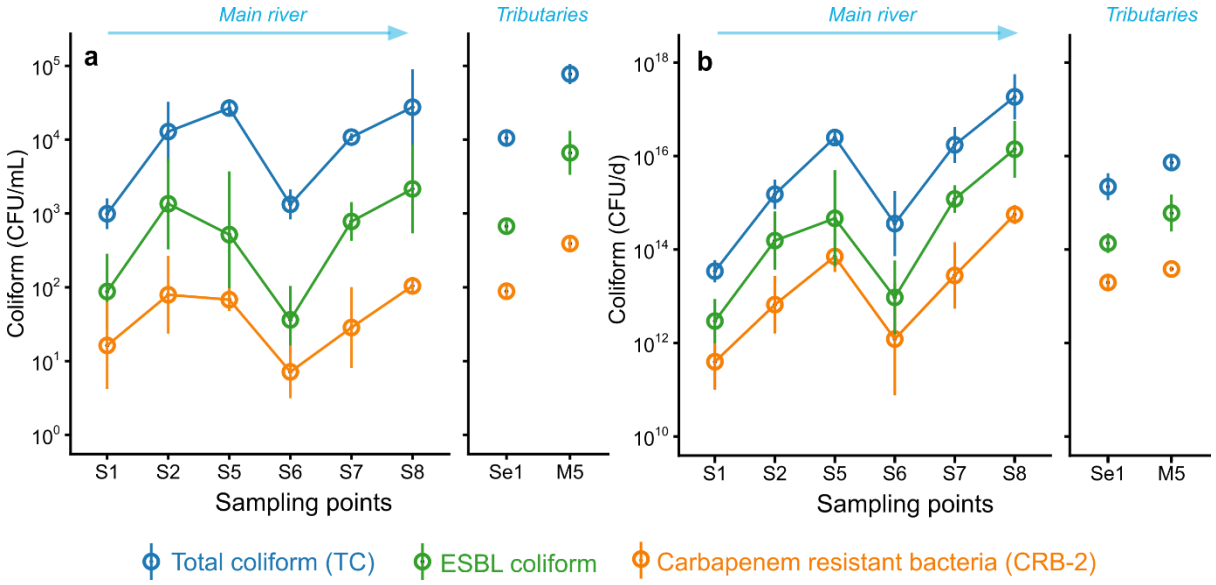

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169 **Figure S2.** Total coliform (TC), ESBL coliform and carbapenem resistant bacteria screened for with 2 µg/mL
170 meropenem (CRB-2) concentrations (a) and mass loadings (b) in the river catchment. Data represented is based
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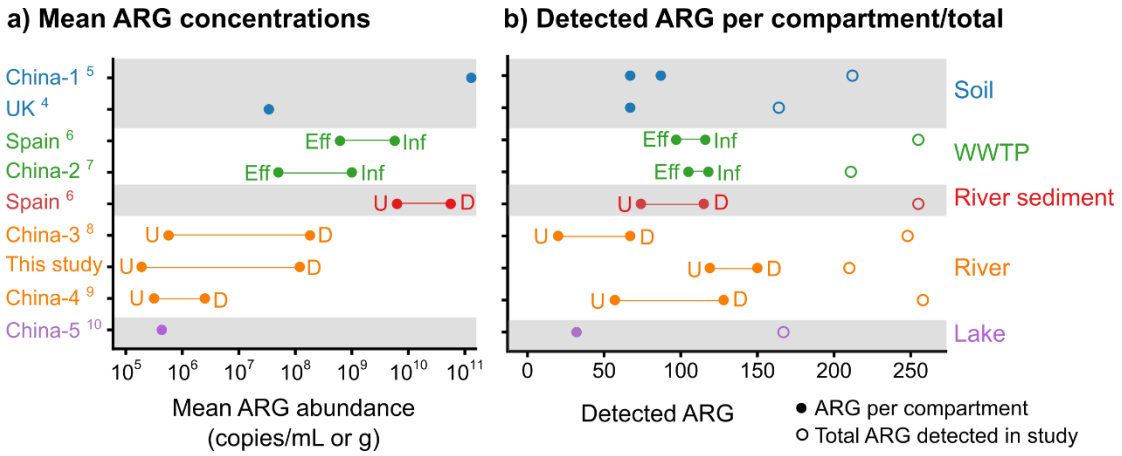
172 tributaries (Se1, M5).



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174 **Figure S3.** Comparison of high-throughput (HT) qPCR antibiotic resistance gene (ARG) concentrations (a) and
175 detections (b; out of 283 ARGs) across different environmental compartments (e.g., soil, sewage, river, lake).
176 Selected studies monitored ARG levels in soil (UK [1]; China [2]), wastewater treatment plant (WWTP) influent (I)
177 and effluent (E) (Spain [3]; China [4]), river sediment upstream (U) and downstream (D) (Spain [3]); river water
178 upstream (U) and downstream (D) (China [5,6]; Malaysia – this study) and in lake water phases (China [7]). To the
179 best of our knowledge, all studies applied the same HT-qPCR assay, screening for 283 ARGs with the same
180 primer sets. For all studies, 3/3 technical qPCR replicates had to amplify for a positive result, except for the
181 Spanish study, where 2/3 technical qPCR replicates were sufficient for a positive result. For ARG abundance
182 data (a), mean concentrations per environmental compartment are reported. For detected ARG (b), mean
183 numbers of ARG per environmental compartment and total ARG detected per study are reported.

Table S1. Sampling point coordinates.

Sampling point	Latitude	Longitude
S1	1°41'05.9"N	103°34'22.0"E
S2	1°38'58.8"N	103°36'58.1"E
S5	1°36'22.6"N	103°39'04.5"E
S6	1°32'54.6"N	103°39'41.8"E
S7	1°31'11.3"N	103°40'41.4"E
S8	1°29'57.2"N	103°40'59.0"E
Se1	1°36'09.3"N	103°38'45.7"E
M5	1°30'13.5"N	103°38'59.1"E

Table S2. Modified water quality classes for Malaysia based on the Malaysian Department of Environment (DOE) Water Quality Index (WQI, classifications clean - slightly polluted - polluted) and National Water Quality Standards for Malaysia (NWQS, classes I-V, see below). To unify both approaches, we translated the thresholds from the five NWQS classes to match the three WQI groups.[8]

DOE NWQS	Class I	Class II	Class III	Class IV	Class V
Assigned classes (based on DOE WQI)	Clean	Slightly polluted	Polluted		
NH3-N	0.1	0.3	0.9	2.7	>2.7
BOD	1	3	6	12	>12
COD	10	25	50	100	>100
DO	7	5	3	1	<1
pH	7	6	5	<5	>5
TSS	25	50	150	300	>300
WQI	92.7	76.5	51.9	31	>31

Table S3. Department of Environment (DOE) data for the Skudai catchment (2018). DOE sampling point 3SI09 = S1 and DOE sampling point 3SI05 = S8.

Sampling point	Month	DO	NH_3-N	COD
3SI09	January	7.49	0.11	19
3SI05	January	1.11	2.21	17
3SI09	March	9.36	0.05	14
3SI05	March	0.31	3.21	36
3SI09	May	6.902	0.11	14
3SI05	May	1.461	2.66	26
3SI09	July	7.719	0.07	15
3SI05	July	3.424	5.51	44
3SI09	September	6.771	0.14	14
3SI05	September	1.596	7.42	67
3SI09	November	7.329	0.1	21
3SI05	November	4.534	1.02	29

COD: chemical oxygen demand. DO: dissolved oxygen. NH_3-N: ammonia.

Table S4. Monitored antibiotics (ng/L) in aqueous phase of the Skudai river catchment, Malaysia during four sampling campaigns (two March 2018, two July 2018) with n=30.

Antibiotic class	Antibiotic name	Abbr.	Detected[1]	DL	Min - Max	Mean [1]	PNEC [2]
β-Lactam	Meropenem	MER	0/30*	1	n.a.	n.a.	64
	Cefixime	CEFX	0/30 *	1	n.a.	n.a.	64
	Ceftazidime	CFZ	0/30 *	15	n.a.	n.a.	500
	Amoxicillin	AMX	24/30	15	<15 - 3336	510 ± 906	250
	Ampicillin	AMP	17/30 *	1	<1 - 17	n.a.	250
Lincosamides	Clindamycin	CLI	30/30	0.02	1 - 4	2 ± 1	1000
	Lincomycin	LIN	30/30	0.02	3 - 21	10 ± 5	2000
Macrolide	Azithromycin	AZT	30/30	0.02	0.1 - 28	8 ± 8	250
	Clarithromycin	CLAR	30/30	0.03	0.4 -27	7 ± 7	250
	Dehydrated Erythromycin	ERY-H2O	30/30	0.05	3 - 167	54 ± 48	n.a.
	Erythromycin	ERY	0/30 *	0.05	n.a.	n.a.	1000
Quinolones/ Fluoroquinolones	Ciprofloxacin	CIPX	22/30	0.5	<0.5 - 705	131 ± 162	64
	Enrofloxacin	ENFLX	18/30	0.05	<0.5 - 13	2 ± 3	64
	Ofloxacin	OFLX	25/30	1	<1 - 13	2 ± 3	500
Sulfonamides	Sulfamethazine	SMZ	24/30	0.03	<0.03 - 39	5 ± 9	n.a.
	Sulfamethoxazole	SMX	27/30	0.05	<0.05 - 1933	181 ± 383	16000
Tetracyclines	Chlortetracycline	CTC	0/30 *	1	n.a.	n.a.	n.a.
	Minocycline	MIN	0/30 *	10	n.a.	n.a.	1000
	Oxytetracycline	OXY	0/30 *	7.5	n.a.	n.a.	500
	Tetracycline	TET	0/30 *	4.5	n.a.	n.a.	1000
Others	Chloramphenicol	CAP	1/30 *	0.3	<0.3 - 8	n.a.	8000
	Trimethoprim	TMP	30/30	0.06	2 - 26	10 ± 6	500

DL: detection limit. n.a.: not available. PNEC: predicted no effect concentration. * no mean was calculated as over 40% of values were under detection limit. [1] only calculated for when over 60% of values were detected. [2] PNEC from [12] where available.

Table S5. Relative SPE recovery, method detection limit (MDL) and method quantification limit (MQL) for antibiotics in surface water. For antibiotic abbreviations, see Table S4.

Target antibiotics	Method validation data	
	SPE recovery, Mean ± RSD (%)	MQL (ng/L)
AMP	117.0 ± 9.1	1.5
AMX	106.2 ± 13.4	40
AZT	103.2 ± 0.8	0.1
CAP	101.1 ± 4.4	1.0
CEFX	111.5 ± 9.1	0.3
CFZ	104.7 ± 8.5	50
CIPX	97.6 ± 9.7	1.5
CLAR	99.6 ± 13.8	0.1
CLI	100.8 ± 4.3	0.1
CTC	107.7 ± 10.9	3.0
ENFLX	102.5 ± 14.9	0.5
ERY	98.3 ± 9.3	0.2
ERY-H_2O	99.7 ± 8.4	0.2
LIN	101.5 ± 13.1	0.1
MER	102.7 ± 2.3	4.0
MIN	85.6 ± 11.4	30
OFLX	107.1 ± 7.7	0.6
OXY	109.2 ± 10.6	25
SMX	100.7 ± 5.7	0.15
SMZ	103.3 ± 7.6	0.1
TET	106.3 ± 3.6	15
TMP	100.2 ± 8.0	0.2

Table S6. Antibiotic resistant gene (ARG) and mobile genetic element (MGE) primer list for high-throughput qPCR. Colouring represents the ARG and MGE classifications from Figure 3 and 4 in the manuscript.

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
16S rRNA	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG	16S	Not applicable
catA1	GGGTGAGTTTCACCAGTTTTGATT	CACCTTGTCGCCTTGCGTATA	FCA	deactivate
catB3	GCACTCGATGCCTTCCAAAA	AGAGCCGATCCAAACGTCAT		deactivate
catB8	CACTCGACGCCTTCCAAAG	CCGAGCCTATCCAGACATCATT		deactivate
Cfr	GCAAAATTCAGAGCAAGTTACGAA	AAAATGACTCCCAACCTGCTTTAT		deactivate
cmlA1-01	TAGGAAGCATCGGAACGTTGAT	CAGACCGAGCACGACTGTTG		efflux
cmlA1-02	AGGAAGCATCGGAACGTTGA	ACAGACCGAGCACGACTGTTG		efflux
cmx(A)	GCGATCGCCATCCTCTGT	TCGACACGGAGCCTTGGT		efflux
floR	ATTGTCTTCACGGTGTCCGTTA	CCGCGATGTCGTCGAACT		efflux
qnrA	AGGATTTCTCACGCCAGGATT	CCGCTTTCAATGAAACTGCAA		unknown
Aac	CCCTGCGTTGTGGCTATGT	TTGGCCACGCCAATCC	Aminoglycoside	deactivate
aac(6')I1	GACCGGATTAAGGCCGATG	CTTGCCTTGATATTCAGTTTTTATAACCA		deactivate
aac(6')-Ib(aka aacA4)-02	CGTCGCCGAGCAACTTG	CGGTACCTTGCCTCTCAAACC		deactivate
aac(6')-Ib(aka aacA4)-01	GTTTGAGAGGCAAGGTACCGTAA	GAATGCCTGGCGTGTTTGA		deactivate
aac(6')-Ib(aka aacA4)-03	AGAAGCACGCCCGACACTT	GCTCTCCATTCAGCATTGCA		deactivate
aac(6')-II	CGACCCGACTCCGAACAA	GCACGAATCCTGCCTTCTCA		deactivate
aac(6')-Iy	GCTTTGCGGATGCCTCAAT	GGAGAACAAAAATACCTTCAAGGAAA		deactivate
aacA/aphD	AGAGCCTTGGGAAGATGAAGTTT	TTGATCCATACCATAGACTATCTCATCA		deactivate
aacC	CGTCACTTATTCGATGCCCTTAC	GTCGGGCGCGGCATA		deactivate
aacC1	GGTCGTGAGTTCGGAGACGTA	GCAAGTTCCCGAGGTAATCG		deactivate
aacC2	ACGGCATTCTCGATTGCTTT	CCGAGCTTCACGTAAGCATTT		deactivate
aacC4	CGGCGTGGGACACGAT	AGGGAACCTTTGCCATCAACT		deactivate
aadA-01	GTTGTGCACGACGACATCATT	GGCTCGAAGATACCTGCAAGAA		deactivate
aadA-02	CGAGATTCTCCGCGCTGTA	GCTGCCATTCTCCAAATTGC		deactivate
aadA1	AGCTAAGCGCGAACTGCAAT	TGGCTCGAAGATACCTGCAA		deactivate
aadA-1-01	AAAAGCCCGAAGAGGAACTTG	CATCTTTCACAAAGATGTTGCTGTCT		deactivate
aadA-1-02	CGGAATTGAAAAAACTGATCGAA	ATACCGGCTGTCCGTCATTT		deactivate
aadA2-01	ACGGCTCCGCAGTGGAT	GGCCACAGTAACCAACAAATCA		deactivate
aadA2-02	CTTGTCGTGCATGACGACATC	TCGAAGATACCCGCAAGAATG		deactivate
aadA2-03	CAATGACATTCTTGCGGGTATC	GACCTACCAAGGCAACGCTATG		deactivate
aadA5-01	ATCACGATCTTGCGATTTTGCT	CTGCGGATGGGCCTAGAAG		deactivate
aadA5-02	GTTCTTGCTCTTGCTCGCATT	GATGCTCGGCAGGCAAAC		deactivate
aadA9-01	CGCGGCAAGCCTATCTTG	CAAATCAGCGACCGCAGACT		deactivate
aadA9-02	GGATGCACGCTTGGATGAA	CCTCTAGCGGCCGGAGTATT		deactivate
aadD	CCGACAACATTTCTACCATCCTT	ACCGAAGCGCTCGTCGTATA		deactivate
aadE	TACCTTATTGCCCTTGGAAGAGTTA	GGAACTATGTCCCTTTTAATTCTACAATCT		deactivate
aph	TTTCAGCAAGTGGATCATGTTAAAAT	CCAAGCTGTTTCCACTGTTTTTC		deactivate
aph(2')-Id-02	TAAGGATATACCGACAGTTTTGGAAA	TTTAATCCCTCTTCATACCAATCCATA		deactivate
aph(2')-Id-01	TGAGCAGTATCATAAGTTGAGTGAAAAG	GACAGAACAATCAATCTCTATGGAATG		deactivate
aph6ia	CCCATCCCATGTGTAAGGAAA	GCCACCGCTTCTGCTGTAC		deactivate
aphA1(aka kanR)	TGAACAAGTCTGGAAAGAAATGCA	CCTATTAATTTCCCCTCGTCAAAAA		deactivate
spcN-01	AAAAGTTCGATGAAACACGCCTAT	TCCAGTGGTAGTCCCCGAATC		deactivate
spcN-02	CAGAATCTTCCTGAAAAGTTTGATGAA	CGCAGACACGCCGAATC		deactivate
str	AATGAGTTTTGGAGTGTCTCAACGTA	AATCAAAACCCCTATTAAAGCCAAT		deactivate
strA	CCGGTGGCATTTGAGAAAAA	GTGGCTCAACCTGCGAAAAG		deactivate
strB	GCTCGGTCGTGAGAACAATCT	CAATTTCGGTCGCCTGGTAGT		deactivate
ampC/blaDHA	TGGCCGCAGCAGAAAGA	CCGTTTTATGCACCCAGGAA	β-Lactam	deactivate
ampC-01	TGGCGTATCGGGTCAATGT	CTCCACGGGCCAGTTGAG		deactivate
ampC-02	GCAGCACGCCCCGTAA	TGTACCCATGATGCGCGTACT		deactivate
ampC-04	TCCGGTGACGCGACAGA	CAGCACGCCGGTGAAAGT		deactivate
ampC-05	CTGTTCGAGCTGGGTTCTATAAGTAAA	CAGTATCTGGTCACCGGATCGT		deactivate
ampC-06	CCGCTCAAGCTGGACCATAC	CCATATCCTGCACGTTGGTTT		deactivate

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
ampC-07	CCGCCCAGAGCAAGGACTA	GCTCGACTTCACGCCGTAAG		deactivate
ampC-09	CAGCCGCTGATGAAAAAATATG	CAGCGAGCCCACTTCGA		deactivate
bla1	GCAAGTTGAAGCGAAAGAAAAGA	TACCAGTATCAATCGCATATACACCTAA		deactivate
bla-ACC-1	CACACAGCTGATGGCTTATCTAAAA	AATAAACGCGATGGGTTCCA		deactivate
blaCMY	CCGCGGCGAAATTAAGC	GCCACTGTTTGCCTGTCAGTT		deactivate
blaCMY2-01	AAAGCCTCATGGGTGCATAAA	ATAGCTTTTGTTTGCCAGCATCA		deactivate
blaCMY2-02	GCGAGCAGCCTGAAGCA	CGGATGGGCTTGTCCTCTT		deactivate
blaCTX-M-04	CTTGGCGTTGCGCTGAT	CGTTCATCGGCACGGTAGA		deactivate
blaCTX-M-01	GGAGGCGTGACGGCTTTT	TTCAGTGCGATCCAGACGAA		deactivate
blaCTX-M-02	GCCGCGGTGCTGAAGA	ATCGGATTATAGTTAACCAGGTCAGATTT		deactivate
blaCTX-M-03	CGATACCACCACGCCGTTA	GCATTGCCCAACGTCAGATT		deactivate
blaCTX-M-05	GCGATAACGTGGCGATGAAT	GTCGAGACGGAACGTTTCGT		deactivate
blaCTX-M-06	CACAGTTGGTGACGTGGCTTAA	CTCCGCTGCCGGTTTTATC		deactivate
blaGES	GCAATGTGCTCAACGTTCAAG	GTGCCTGAGTCAATTCTTTCAAAG		deactivate
bla-L1	CACCGGGTTACCAGCTGAAG	GCGAAGCTGCGCTTGTAGTC		deactivate
blaMOX/blaCMY	CTATGTCAATGTGCCGAAGCA	GGCTTGTCCTCTTTCGAATAGC		deactivate
blaIMP-02	AAGGCAGCATTTCCTCTCATTTT	GGATAGATCGAGAATTAAGCCACTCT		deactivate
blaIMP-01	AACACGGTTTGGTGGTTCTTGTA	GCGCTCCACAAACCAATTG		deactivate
blaOCH	GGCGACTTGCGCCGTAT	TTTTCTGCTCGGCCATGAG		deactivate
blaOKP	GCCGCCATCACCATGAG	GGTGACGTTGTCACCGATCTG		deactivate
blaOXA1/blaOXA30	CGGATGGTTTGAAGGGTTTATTAT	TCTTGGCTTTTATGCTTGATGTTAA		deactivate
blaOXA10-01	CGCAATTATCGGCCTAGAAACT	TTGGCTTTCCGTCCCATTT		deactivate
blaOXA10-02	CGCAATTATCGGCCTAGAAACT	TTGGCTTTCCGTCCCATTT		deactivate
blaOXY	CGTTCAGGCGGCAGGTT	GCCGCGATATAAGATTTGAGAATT		deactivate
blaPAO	CGCCGTACAACCGGTGAT	GAAGTAATGCGGTTCTCCTTTCA		deactivate
blaPER	TGCTGGTTGCTGTTTTTGTGA	CCTGCGCAATGATAGCTTCAT		deactivate
blaPSE	TTGTGACCTATTCCCCTGTAATAGAA	TGCGAAGCACGCATCATC		deactivate
blaROB	GCAAAGGCATGACGATTGC	CGCGCTGTTGTCGCTAAA		deactivate
blaSFO	CCGCCGCCATCCAGTA	GGGCCGCCAAGATGCT		deactivate
blaSHV-01	TCCCATGATGAGCACCTTTAAA	TTCGTCACCGGCATCCA		deactivate
blaSHV-02	CTTTCCCATGATGAGCACCTTT	TCCTGCTGGCGATAGTGGAT		deactivate
blaTEM	AGCATCTTACGGATGGCATGA	TCCTCCGATCGTTGTCAGAAGT		deactivate
blaTLA	ACACTTTGCCATTGCTGTTTATGT	TGCAAATTTCGGCAATAATCTTT		deactivate
blaVEB	CCCGATGCAAAGCGTTATG	GAAAGATTCCCTTTATCTATCTCAGACAA		deactivate
blaVIM	GCACTTCTCGCGGAGATTG	CGACGGTGATGCGTACGTT		deactivate
blaZ	GGAGATAAAGTAACAAATCCAGTTAGATATGA	TGCTTAATTTTCCATTTGCGATAAG		deactivate
cepA	AGTTGCGCAGAACAGTCCTCTT	TCGTATCTTGCCCGTCGATAAT		deactivate
cfiA	GCAGCGTTGCTGGACACA	GTTCGGGATAAACGTGGTGACT		deactivate
cfxA	TCATTCCTCGTTCAAGTTTTCAGA	TGCAGCACCAAGAGGAGATGT		deactivate
cphA-01	GCGAGCTGCACAAGCTGAT	CGGCCCAGTCGCTCTTC		deactivate
cphA-02	GTGCTGATGGCGAGTTTCTG	GGTGTGGTAGTTGGTGTTGATCAC		deactivate
fox5	GGTTTGCCGCTGCAGTTC	GCGGCCAGGTGACCAA		deactivate
mecA	GGTTACGGACAAGGTGAAATACTGAT	TGTCTTTTAATAAGTGAGGTGCGTTAATA		protection
NDM1	ATTAGCCGCTGCATTGAT	CATGTCGAGATAGGAAGTG		deactivate
pbp	CCGGTGCCATTGGTTTAGA	AAAATAGCCGCCCCAAGATT		protection
pbp2x	TTTCATAAGTATCTGGACATGGAAGAA	CCAAAGGAAACTTGCTTGAGATTAG		protection
Pbp5	GGCGAACTTCTAATTAATCCTATCCA	CGCCGATGACATTCTTCTTATCTT		protection
penA	AGACGGTAACGTATAACTTTTTGAAAGA	GCGTGTAGCCGGCAATG		protection
intl-1(clinic) = clintl1	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA	MGEs/ Integron	integrase
intl-1LC (int1)	GGCATCCAAGCAGCAAG	AAGCAGACTTGACCTGA		integrase
int2	TGCTTTTCCCACCCTTACC	GACGGCTACCCTCTGTTATCTC		integrase
int3	GCCACCACTTGTTTGAGGA	GGATGTCTGTGCCTGCTTG		integrase
IS613	AGGTTCGGACTCAATGCAACA	TTCAGCACATACCGCCTTGAT	MGEs/ Transposase	transposase
tnpA-01	CATCATCGGACGGACAGAATT	GTCGGAGATGTGGGTGTAGAAAGT		transposase
tnpA-02	GGGCGGGTCGATTGAAA	GTGGGCGGGATCTGCTT		transposase
tnpA-03	AATTGATGCGGACGGCTTAA	TCACCAAACTGTTTATGGAGTCGTT		transposase

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
tnpA-04	CCGATCACGGAAAGCTCAAG	GGCTCGCATGACTTCGAATC		transposase
tnpA-05	GCCGCACTGTCGATTTTTATC	GCGGGATCTGCCACTTCTT		transposase
tnpA-07	GAAACCGATGCTACAATATCCAATTT	CAGCACCGTTTGCAGTGTAAG		transposase
Tp614	GGAAATCAACGGCATCCAGTT	CATCCATGCGCTTTTGTCTCT		transposase
carB	GGAGTGAGGCTGACCGTAGAAG	ATCGGCGAAACGCACAAA	MLSB	efflux
ereA	CCTGTGGTACGGAGAATTCATGT	ACCGCATTCGCTTTGCTT		deactivate
ereB	GCTTTATTTCAGGAGGCGGAAT	TTTTAAATGCCACAGCACAGAATC		deactivate
erm(34)	GCGCGTTGACGACGATTT	TGGTCATACTCGACGGCTAGAAC		protection
erm(35)	TTGAAAACGATGTTGCATTAAGTCA	TCTATAATCACAACTAACCACTTGAACGT		protection
erm(36)	GGCGGACCGACTTGCAT	TCTGCGTTGACGACGGTTAC		protection
ermA	TTGAGAAGGGATTTGCGAAAAG	ATATCCATCTCCACCATTAATAGTAAACC		protection
ermA/ermTR	ACATTTTACCAAGGAACTTGTGGAA	GTGGCATGACATAAACCTTCATCA		protection
ermB	TAAAGGGCATTTAACGACGAAACT	TTTATACCTCTGTTTGTTAGGGAATTGAA		protection
ermC	TTTGAAATCGGCTCAGGAAAA	ATGGTCTATTTCAATGGCAGTTACG		protection
ermF	CAGCTTTGGTTGAACATTTACGAA	AAATTCCTAAAATCACAACCGACAA		protection
ermJ/ermD	GGACTCGGCAATGGTCAGAA	CCCCGAAACGCAATATAATGTT		protection
ermK-01	GTTTGATATTGGCATTGTCAGAGAAA	ACCATTGCCGAGTCCACTTT		protection
ermK-02	GAGCCGCAAGCCCCTTT	GTGTTTCATTTGACGCGGAGTAA		protection
ermT-01	GTTCACTAGCACTATTTTTAATGACAGAAGT	GAAGGGTGTCTTTTTAATACAATTAACGA		protection
ermT-02	GTAAAATCCCTAGAGAATACTTTCATCCA	TGAGTGATATTTTTGAAGGGTGTCTT		protection
ermX	GCTCAGTGGTCCCCATGGT	ATCCCCCCGTCAACGTTT		protection
ermY	TTGTCTTTGAAAGTGAAGCAACAGT	TAACGCTAGAGAACGATTTGTATTGAG		protection
lmrA-01	TCGACGTGACCGTAGTGAACA	CGTGACTACCCAGGTGAGTTGA		efflux
lnuA-01	TGACGCTCAACACACTCAAAAA	TTCATGCTTAAGTTCCATACGTGAA		deactivate
lnuB-01	TGAACATAATCCCCTCGTTTAAAGAT	TAATTGCCCTGTTTCATCGTAAATAA		deactivate
lnuB-02	AAAGGAGAAGGTGACCAATACTCTGA	GGAGCTACGTCAAACAACCAGTT		deactivate
lnuC	TGGTCAATATAACAGATGTAAACCAGATTT	CACCCCAGCCACCATCAA		deactivate
matA/mel	TAGTAGGCAAGCTCGGTGTTGA	CCTGTGCTATTTTAAGCCTTGTTTCT		efflux
mdtA	CCTAACGGGCGTGACTTCA	TTCACCTGTTTCAAGGGTCAAA		efflux
mefA	CCGTAGCATTGGAACAGCTTTT	AAACGGAGTATAAGAGTGCTGCAA		efflux
mphA-01	CTGACGCGCTCCGTGTT	GGTGGTGCATGGCGATCT		deactivate
mphA-02	TGATGACCCTGCCATCGA	TTCGCGAGCCCCTCTTC		deactivate
mphB	CGCAGCGCTTGATCTTGTAG	TTACTGCATCCATACGCTGCTT		deactivate
mphC	CGTTTGAAGTACCGAATTGGAAA	GCTGCGGGTTTGCCTGTA		deactivate
msrA-01	CTGCTAACACAAGTACGATTCCAAAT	TCAAGTAAAGTTGTCTTACCTACACCATT		efflux
msrC-01	TCAGACCGGATCGGTTGTC	CCTATTTTTTGGAGTCTTCTCTCTAATGTT		efflux
oleC	CCCGGAGTCGATGTTCGA	GCCGAAGACGTACACGAACAG		efflux
pikR1	TCGACATGCGTGACGAGATT	CCGCGAATTAGGCCAGAA		protection
pikR2	TCGTGGGCCAGGTGAAGA	TTCCCCTTGCCGGTGAA		protection
vatB-01	GGAAAAAGCAACTCCATCTCTTGA	TCCTGGCATAACAGTAACATTCTGA		deactivate
vatB-02	TTGGGAAAAAGCAACTCCATCT	CAATCCACACATCATTTCCAACA		deactivate
vatC-01	CGGAAATTGGGAACGATGTT	GCAATAATAGCCCCGTTTCCTA		deactivate
vatC-02	CGATGTTTGGATTGGACGAGAT	GCTGCAATAATAGCCCCGTTT		deactivate
vatE-01	GGTGCCATTATCGGAGCAAAT	TTGGATTGCCACCGACAAT		deactivate
vatE-02	GACCGTCCTACCAGGCGTAA	TTGGATTGCCACCGACAATT		deactivate
vgaA-01	CGAGTATTGTGGAAAGCAGCTAGTT	CCCGTACCGTTAGAGCCGATA		efflux
vgaA-02	GACGGGTATTGTGGAAAGCAA	TTTCCTGTACCATTAGATCCGATAATT		efflux
vgb-01	AGGGAGGGTATCCATGCAGAT	ACCAAATGCGCCCGTTT		deactivate
vgbB-01	CAGCCGGATTCTGGTCCTT	TACGATCTCCATTCAATTGGGTAAA		efflux
vgbB-02	ATACGAGCTGCCTAATAAAGGATCTT	TGTGAACCACAGGGCATTATCA		deactivate
acrA-01	CAACGATCGGACGGGTTTC	TGGCGATGCCACCGTACT	Non-specific	efflux
acrA-02	GGTCTATCACCCTACGCGCTATC	GCGCGCACGAACATACC		efflux
acrA-03	CAGACCCGCATCGCATATT	CGACAATTTCGCGCTCATG		efflux
acrA-04	TACTTTGCGCGCCATCTTC	CGTGCGCGAACGAACAT		efflux
acrB-01	AGTCGGTGTTCGCCGTTAAC	CAAGGAAACGAACGCAATACC		efflux
acrR-01	GCGCTGGAGACACGACAAC	GCCTTGCTGCGAGAACAAA		efflux

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
acrR-02	GATGATACCCCCTGCTGTGAGA	ACCAAACAAGAAGCGCAAGAA		efflux
adeA	CAGTTCGAGCGCCTATTTCTG	CGCCCTGACCGACCAAT		efflux
acrA-05	CGTGCGCGAACGAACA	ACTTTGCGCGCCATCTTC		efflux
acrF	GCGGCCAGGCACAAAA	TACGCTCTTCCCACGGTTTC		efflux
ceoA	ATCAACACGGACCAGGACAAG	GGAAAGTCCGCTCACGATGA		efflux
cmeA	GCAGCAAAGAAGAAGCACCAA	AGCAGGGTAAGTAAAACTAAGTGGTAAATCT		efflux
cmr	CGGCATCGTCAGTGGAATT	CGGTTCCGAAAAAGATGGAA		efflux
emrD	CTCAGCAGTATGGTGGTAAGCATT	ACCAGGCGCCGAAGAAC		efflux
marR-01	GCGGCGTACTGGTGAAGCTA	TGCCCTGGTCGTTGATGA		efflux
mdetl1	ATACAGCAGTGGATATTGGTTTAATTGT	TGCATAAGGTGAATGTTCCATGA		efflux
mdtE/yhiU	CGTCGGCGCACTCGTT	TCCAGACGTTGTACGGTAACCA		efflux
mepA	ATCGGTCGCTCTTCGTTCAC	ATAAATAGGATCGAGCTGCTGGAT		efflux
mexA	AGGACAACGCTATGCAACGAA	CCGGAAAGGGCCGAAAT		efflux
mexD	TTGCCACTGGCTTTCATGAG	CACTGCGGAGAACTGTCTGTAGA		efflux
mexE	GGTCAGCACCGACAAGGTCTAC	AGCTCGACGTACTTGAGGAACAC		efflux
mexF	CCGCGAGAAGGCCAAGA	TTGAGTTCGGCGGTGATGA		efflux
mtrC-01	GGACGGGAAGATGGTCCAA	CGTAGCGTTCCGGTTCGAT		efflux
mtrC-02	CGGAGTCCATCGACCATTTG	ATCGTCGGCAAGGAGAATCA		efflux
mtrD-02	GGTCGGCACGCTCTTGTC	TGAAGAATTTGCGCACCACTAC		efflux
mtrD-03	CCGCCAAGCCGATATAGACA	GGCCGGGTTGCCAAA		efflux
oprD	ATGAAGTGGAGCGCCATTG	GGCCACGGCGAACTGA		efflux
oprJ	ACGAGAGTGGCGTCGACAA	AAGGCGATCTCGTTGAGGAA		efflux
pmrA	TTTGCAGGTTTTGTTCCTAATGC	GCAGAGCCTGATTTCTCCTTTG		efflux
putative multidrug	AATTTTGCCGATTATTGCTGAAA	GATTGTCATCATTCGTTTATCACCAA		efflux
qac	CAATAATAACCGAAATAATAGGGACAAGTT	AATAAGTGTTCCTAGTGTTGGCCATAG		efflux
qacA	TGGCAATAGGAGCTATGGTGTTT	AAGGTAACACTATTTTCGGTCCAAATC		efflux
qacA/qacB	TTTAGGCAGCCTCGCTTCA	CCGAATCCAAATAAAACCCAATAA		efflux
qacEdelta1-01	TCGCAACATCCGCATTAAAA	ATGGATTTCAGAACCAGAGAAAGAAA		efflux
qacEdelta1-02	CCCCTTCCGCCGTTGT	CGACCAGACTGCATAAGCAACA		efflux
qacH-01	GTGGCAGCTATCGCTTGGAT	CCAACGAACGCCCACAA		efflux
qacH-02	CATCGTGCTTGTGGCAGCTA	TGAACGCCCAGAAGTCTAGTTTT		efflux
rarD-02	TGACGCATCGCGTGATCT	AAATTTTCTGTGGCGTCTGAATC		efflux
sdeB	CACTACCGCTTCCGCACTTAA	TGAAAAAACGGGAAAAGTCCAT		efflux
tolC-01	GGCCGAGAACCTGATGCA	AGACTTACGCAATTCCGGGTTA		efflux
tolC-02	CAGGCAGAGAACCTGATGCA	CGCAATTCCGGGTTGCT		efflux
tolC-03	GCCAGGCAGAGAACCTGATG	CGCAATTCCGGGTTGCT		efflux
ttgA	ACGCCAATGCCAAACGATT	GTCACGGCGCAGCTTGA		efflux
ttgB	TCGCCCTGGATGTACACCTT	ACCATTGCCGACATCAACAAC		efflux
yceE/mdtG-01	TGGCACAAAATATCTGGCAGTT	TTGTGTGGCGATAAGAGCATTAG		efflux
yceE/mdtG-02	TTATCTGTTTTCTGCTCACCTTCTTTT	GCGTGGTGACAAACAGGCTTA		efflux
yceL/mdtH-01	TCGGGATGGTGGGCAAT	CGATAACCGAGCCGATGTAGA		efflux
yceL/mdtH-02	CGCGTGAAACCTTAAGTGCTT	AGACGGCTAAACCCCATATAGCT		efflux
yceL/mdtH-03	CTGCCGTTAAATGGATGTATGC	ACTCCAGCGGGCGATAGG		efflux
yidY/mdtL-01	GCAGTTGCATATCGCCTTCTC	CTTCCCGGCAAACAGCAT		efflux
yidY/mdtL-02	TGCTGATCGGGATTCTGATTG	CAGGCGCGACGAACATAAT		efflux
fabK	TTTCAGCTCAGCACTTTGGTCAT	AAGGCATCTTTTTCAGCCAGTTC	Other	deactivate
imiR	CCGGACTAGAGCTTCATGTAAGC	CCCACGCGGTACTCTTGTAAA		unknown
nisB	GGGAGAGTTGCCGATGTTGTA	AGCCACTCGTTAAAGGGCAAT		unknown
speA	GCAAGAGGTATTTGCTCAACAAGA	CAGGGTCACCCTCATAAAGAAAA		unknown
bacA-01	CGGCTTCGTGACCTCGTT	ACAATGCGATACCAGGCAAAT		deactivate
bacA-02	TTCCACGACACGATTAAGTCATTG	CGGCTCTTTCGGCTTCAG		deactivate
fosB	TCACTGTAACTAATGAAGCATTAGACCAT	CCATCTGGATCTGTAAAGTAAAGAGATC		deactivate
fosX	GATTAAGCCATATCACTTTAATTGTGAAAG	TCTCCTTCCATAATGCAAATCCA		deactivate
nimE	TGCGCCAAGATAGGGCATA	GTCGTGAATTCGGCAGGTTTA		unknown
pncA	GCAATCGAGGCGGTGTTC	TTGCCGCAGCCAATTCA		unknown
sat4	GAATGGGCAAAGCATAAAAACTTG	CCGATTTTGAAACCACAATTATGATA		deactivate
dfrA1	GGAATGGCCCTGATATTCCA	AGTCTTGCGTCCAACCAACAG	Sulfonamide	deactivate
dfrA12	CCTCTACCGAACCGTCACACA	GCGACAGCGTTGAAACAACTAC		deactivate

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
folA	CGAGCAGTTCCTGCCAAAG	CCCAGTCATCCGGTTCATAATC		deactivate
sul1	CAGCGCTATGCGCTCAAG	ATCCCGCTGCGCTGAGT		protection
sul2	TCATCTGCCAAACTCGTCGTTA	GTCAAAGAACGCCGCAATGT		protection
sulA/folP-01	CAGGCTCGTAAATTGATAGCAGAAG	CTTTCCTTGCGAATCGCTTT		protection
sulA/folP-03	CACGGCTTCGGCTCATGT	TGCCATCCTGTGACTAGCTACGT		protection
tet(32)	CCATTACTTCGGACAACGGTAGA	CAATCTCTGTGAGGGCATTTAACA		protection
tet(34)	CTTAGCGCAAACAGCAATCAGT	CGGTGATACAGCGCGTAAACT		unknown
tet(35)	ACCCCATGACGTACCTGTAGAGA	CAACCCACACTGGCTACCAGTT		unknown
tet(36)	AGAATACTCAGCAGAGGTCAGTTCCT	TGGTAGGTCGATAACCCGAAAAT		protection
tet(36)	TGCAGGAAAGACCTCCATTACAG	CTTTGTCCACACTTCCACGTACTATG		protection
tet(37)	GAGAACGTTGAAAAGGTGGTGAA	AACCAAGCCTGGATCAGTCTCA		unknown
tetA-01	GCTGTTTGTTCTGCCGGAAA	GGTTAAGTTCCTTGAACGCAAACT		efflux
tetA-02	CTCACCAGCCTGACCTCGAT	CACGTTGTTATAGAAGCCGCATAG		efflux
tetB-01	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA		efflux
tetB-02	GCCCAGTGCTGTTGTTGTCAT	TGAAAGCAAACGGCCTAAATACA		efflux
tetC-01	CATATCGCAATACATGCGAAAAA	AAAGCCGCGGTAAATAGCAA		efflux
tetC-02	ACTGGTAAGGTAAACGCCATTGTC	ATGCATAAACCAGCCATTGAGTAAG		efflux
tetD-01	TGCCGCGTTTGATTACACA	CACCAGTGATCCCGGAGATAA		efflux
tetD-02	TGTCATCGCGCTGGTGATT	CATCCGCTTCCGGGAGAT		efflux
tetE	TTGGCGCTGTATGCAATGAT	CGACGACCTATGCGATCTGA		efflux
tetG-01	TCAACCATTGCCGATTCGA	TGGCCCGGCAATCATG		efflux
tetG-02	CATCAGCGCCGGTCTTATG	CCCCATGTAGCCGAACCA		efflux
tetH	TTTGGGTCATCTTACCAGCATTAA	TTGCGCATTATCATCGACAGA		efflux
tetJ	GGGTGCCGCATTAGATTACCT	TCGTCCAATGTAGAGCATCCATA		efflux
tetK	CAGCAGTCATTGGAAAATTATCTGATTATA	CCTTGTACTAACCTACCAAAAATCAAAATA		efflux
tetL-01	AGCCCGATTTATTCAAGGAATTG	CAAATGCTTTCCCCCTGTTCT	Tetracycline	efflux
tetL-02	ATGGTTGTAGTTGCGCGCTATAT	ATCGCTGGACCGACTCCTT		efflux
tetM-01	CATCATAGACACGCCAGGACATAT	CGCCATCTTTTGCAGAAATCA		protection
tetM-02	TAATATTGGAGTTTTAGCTCATGTTGATG	CCTCTCTGACGTTCTAAAAGCGTATTAT		protection
tetO-01	ATGTGGATACTACAACGCATGAGATT	TGCCTCCACATGATATTTTTCCT		protection
tetW-01	ATGAACATTCCCACCGTTATCTTT	ATATCGGCGGAGAGCTTATCC		protection
tetPA	AGTTGCAGATGTGTATAGTCGTAAACTATCTATT	TGCTACAAGTACGAAAACAAAACTAGAA		efflux
tetPB-01	ACACCTGGACACGCTGATTTT	ACCGTCTAGAACGCGGAATG		protection
tetPB-02	TGATACACCTGGACACGCTGAT	CGTCCAAAACGCGGAATG		protection
tetPB-03	TGGGCGACAGTAGGCTTAGAA	TGACCCTACTGAAACATTAGAAATATACCT		protection
tetPB-05	CTGAAGTGGAGCGATCATTCC	CCCTCAACGGCAGAAATAACTAA		protection
tetQ	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT		protection
tetR-02	CGCGATAGACGCCTTCGA	TCCTGACAACGAGCCTCCTT		efflux
tetR-03	CGCGATGGAGCAAAAGTACAT	AGTGAAAAACCTTGTTGGCATAAAA		efflux
tetS	TTAAGGACAAACTTTCTGACGACATC	TGTCTCCCATTGTTCTGGTTCA		protection
tetT	CCATATAGAGGTTCCACCAAATCC	TGACCCTATTGGTAGTGGTTCTATTG		protection
tetU-01	GTGGCAAAGCAACGGATTG	TGCGGGCTTGCAAAACTATC		unknown
tetV	GCGGGAACGACGATGTATATC	CCGCTATCTCACGACCATGAT		efflux
tetX	AAATTTGTTACCGACACGGAAGTT	CATAGCTGAAAAAATCCAGGACAGTT		unknown
vanA	AAAAGGCTCTGAAAACGCAGTTAT	CGGCCGTTATCTTGTAAAAACAT		protection
vanB-01	TTGTCGGCGAAGTGGATCA	AGCCTTTTTCCGGCTCGTT		protection
vanC-01	ACAGGGATTGGCTATGAACCAT	TGACTGGCGATGATTTGACTATG		protection
vanC-02	CCTGCCACAATCGATCGTT	CGGCTTCATTCGGCTTGATA		protection
vanC-03	AAATCAATACTATGCCGGGCTTT	CCGACCGCTGCCATCA		protection
vanC1	AGGCGATAGCGGGTATTGAA	CAATCGTCAATTGCTCATTTCC		protection
vanC2/vanC3	TTTGACTGTCGGTGCTTGTGA	TCAATCGTTTCAGGCAATGG	Vancomycin*	protection
vanG	ATTTGAATTGGCAGGTATACAGGTTA	TGATTTGTCTTTGTCCATACATAATGC		protection
vanHB	GAGGTTTCCGAGGCGACAA	CTCTCGGCGGCAGTCGTAT		protection
vanHD	GTGGCCGATTATACCGTCATG	CGCAGGTCATTCAGGCAAT		protection
vanRA-01	CCCTTACTCCCACCGAGTTTT	TTCGTCGCCCCATATCTCAT		protection
vanRA-02	CCACTCCGGCCTTGTCATT	GCTAACCACATTCCCCTTGTTTT		protection
vanRB	GCCCTGTCGGATGACGAA	TTACATAGTCGTCTGCCTCTGCAT		protection

Assay ID	Forward Primer	Reverse Primer	Classification	Mechanism
vanRC	TGCGGGAAAAACTGAACGA	CCCCCCATACGGTTTTGATTA		protection
vanRC4	AGTGCTTTGGCTTATCTCGAAAA	TCCGGCAGCATCACATCTAA		protection
vanRD	TTATAATGGCAAGGATGCACTAAAGT	CGTCTACATCCGGAAGCATGA		protection
vanSA	CGCGTCATGCTTTCAAAATTC	TCCGCAGAAAGCTCAATTTGTT		protection
vanSB	GCGCGGCAAATGACAAC	TTTGCCATTTTATTCGCACTGT		protection
vanSC-01	ATCAACTGCGGGAGAAAAGTCT	TCCGCTGTTCCGCTTCTT		protection
vanSC-02	GCCATCAGCGAGTCTGATGA	CAGCTGGGATCGTTTTTCCTT		protection
vanTC-01	CACACGCATTTTTTCCCATCTAG	CAGCCAACAGATCATCAAAACAA		protection
vanTC-02	ACAGTTGCCGCTGGTGAAG	CGTGGCTGGTCGATCAAAA		protection
vanTE	GTGGTGCCAAGGAAGTTGCT	CGTAGCCACCGCAAAAAAAT		protection
vanTG	CGTGTAGCCGTTCCGTTCTT	CGGCATTACAGGTATATCTGGAAA		protection
vanWB	CGGACAAAGATACCCCCTATAAAG	AAATAGTAAATTGCTCATCTGGCAC AT		protection
vanWG	ACATTTTCATTTTGGCAGCTTGTAC	CCGCCATAAGAGCCTACAATCT		protection
vanXA	CGCTAAATATGCCACTTGGGATA	TCAAAAGCGATTCAGCCAACT		protection
vanXB	AGGCACAAAATCGAAGATGCTT	GGGTATGGCTCATCAATCAACTT		protection
vanXD	TAAACCGTGTTATGGGAACGAA	GCGATAGCCGTCCCATAAGA		protection
vanYB	GGCTAAAGCGGAAGCAGAAA	GATATCCACAGCAAGACCAAGCT		protection
vanYD-01	AAGGCGATACCCTGACTGTCA	ATTGCCGGACGGAAGCA		protection
vanYD-02	CAAACGGAAGAGAGGTCACTTACA	CGGACGGTAATAGGGACTGTTC		protection

FCA: fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol ARGs). MLSB: macrolide-lincosamide-streptogramin B ARGs. *: For consistency, ARG categories are based on previous studies using the same Ht-qPCR assay.[1–4] However, note that vancomycin is a specific antibiotic in the class 'glycopeptide'. The van operon is not confined to resistance to vancomycin and confers resistance to most glycopeptide antibiotics.

Table S7. Box-cox transformations prior statistical analysis (significance testing and effect size calculations).

Parameter	Transformation
Physical parameters (river flow)	Log_{10}
Physical parameters (DO)	None
Chemical concentrations (DO, NH_3-N, TN, TP)	None
Chemical concentration (COD)	Log_{10}
Chemical mass loadings (NH_3-N, TN, TP, COD)	Log_{10}
Coliform concentrations and mass loadings (TC, ESBL coliform, CPB-0.5, CPB-2)	Log_{10}
Total antibiotic concentration and mass loading	Log_{10}
Detected ARGs and MGEs	None
ARG and MGE concentrations and mass loadings	Log_{10}
Normalized ARG and MGE cell concentrations	None

ARGs: antibiotic resistant genes. COD: chemical oxygen demand. CPB: carbapenem resistant bacteria. DO: dissolved oxygen. MGEs: mobile genetic elements. NH_3-N: ammonia. TC: total coliform. TN: total nitrogen. TP: total phosphate.

Table S8. Physical parameters per sampling point (mean of biological replicates with standard deviations). Data for S1-S8 based on four biological replicates except for flow wet and dry season. Data for Se1 and M5 based on three biological replicates except for flow wet and dry season. Fold-change calculated by dividing the mean S8 with the mean S1 values.

	Flow (m^3/s)			Temperature (ºC)	pH	Conductivity (µS/cm)	DO (mg/L)
	All (n= 3-4 per site)	Wet season (n= 1-2 per site)	Dry season (n=2 per site)				
S1	0.45 ± 0.29	0.59 ± 0.41	0.31 ± 0	27.18 ± 0.52	5.88 ± 0.44	75.53 ± 10.3	7.52 ± 0.5
S2	1.55 ± 0.96	1.88 ± 1.47	1.19 ± 0.16	27.88 ± 0.98	6.08 ± 0.59	297.78 ± 240.44	4.39 ± 0.62
S5	11.96 ± 6.47	7.83 ± 2.98	16.15 ± 6.92	27.45 ± 0.95	6.16 ± 0.39	357.25 ± 12.04	2.63 ± 1.05
S6	6.2 ± 4.16	8.16 ± 1.91	4.24 ± 5.73	28.1 ± 1.19	6.3 ± 0.4	299 ± 61.66	5.35 ± 0.22
S7	25.38 ± 24.94	40.39 ± 30.79	10.32 ± 2.84	28.12 ± 2.27	6.15 ± 0.35	1306.5 ± 1882.71	2.03 ± 0.59
S8	82.7 ± 30.66	78.07 ± 50.9	87.02 ± 9.46	28.67 ± 1.69	6.25 ± 0.36	3349.25 ± 4818.75	1.32 ± 0.26
Se1	2.64 ± 1.4	1.79	3.06 ± 1.68	27.03 ± 0.67	5.62 ± 0.26	97.07 ± 3.76	6.39 ± 0.55
M5	1.11 ± 0.26	0.81	1.23 ± 0.11	28.8 ± 2.33	6 ± 0.36	317.67 ± 23.29	1.61 ± 2.08
S8/S1	184	140	281	1	1	44	0.2 (S1/S8 = 6)

DO: dissolved oxygen.

Table S9. Chemical concentrations per sampling point (mean of biological replicates with standard deviations). Total antibiotics summarize all antibiotics and antibiotic derivates detected in at least 60% of the samples above the detection limit (amoxicillin, ciprofloxacin, sulfamethoxazole, dehydrated erythromycin, lincomycin, trimethoprim, azithromycin, clarithromycin, clindamycin, sulfamethazine, ofloxacin, enrofloxacin). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates. Fold-change calculated by dividing the mean S8 with the mean S1 values.

	COD (mg/L)	NH_3-N (mg/L)	TN (mg/L)	TP (mg/L)	Total antibiotics (mg/L)
S1	5.8 ± 4.77	0.05 ± 0.03	1.09 ± 0.51	0.43 ± 0.08	0.07 ± 0.05
S2	10.29 ± 6.46	2.85 ± 1.13	4.8 ± 2	0.95 ± 0.42	1.19 ± 1.67
S5	20.08 ± 6.92	4.7 ± 1.01	7.59 ± 0.63	2.25 ± 2.37	0.67 ± 0.25
S6	11.83 ± 5.67	3.08 ± 1.06	6.2 ± 1.27	6.94 ± 1.17	0.35 ± 0.16
S7	15.83 ± 7.17	3.65 ± 1.22	6.08 ± 1.71	5.25 ± 2.36	0.62 ± 0.16
S8	25.25 ± 16.04	4.94 ± 2	6.08 ± 2.36	3.95 ± 2.01	1.27 ± 0.98
Se1	9.61 ± 8.18	0.82 ± 0.09	1.92 ± 0.56	0.41 ± 0.18	0.88 ± 0.14
M5	28.67 ± 9.94	10.03 ± 1.06	12.11 ± 0.75	2.13 ± 0.46	2.79 ± 2.04
S8/S1	4	99	6	9	17

COD: chemical oxygen demand. NH_3-N: ammonia. TN: total nitrogen. TP: total phosphate.

Table S10. Chemical mass loadings per sampling point (mean of biological replicates with standard deviations). Total antibiotics summarize all antibiotics and antibiotic derivates detected in at least 60% of the samples above the detection limit (amoxicillin, ciprofloxacin, sulfamethoxazole, dehydrated erythromycin, lincomycin, trimethoprim, azithromycin, clarithromycin, clindamycin, sulfamethazine, ofloxacin, enrofloxacin). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates. Fold-change calculated by dividing the mean S8 with the mean S1 values.

	COD (kg/d)	NH_3-N (kg/d)	TN (kg/d)	TP (kg/d)	Total antibiotics (g/d)
S1	223 ± 180	2 ± 3	40 ± 22	17 ± 13	73 ± 46
S2	1,154 ± 633	329 ± 112	567 ± 232	113 ± 48	1,187 ± 1,669
S5	19,641 ± 8,009	4,914 ± 2666	7,924 ± 4,551	1,659 ± 878	668 ± 255
S6	5,026 ± 3,724	1,488 ± 1,142	3,234 ± 2,269	3,521 ± 2,273	348 ± 156
S7	42,955 ± 59,766	6,442 ± 4,378	11,045 ± 7,638	7,905 ± 2,650	622 ± 161
S8	205,902 ± 189,333	34,392 ± 17,377	42,610 ± 22,092	28,830 ± 17,632	1,266 ± 979
Se1	2,088 ± 1,516	183 ± 81	405 ± 124	90 ± 46	878 ± 141
M5	2,877 ± 1,584	973 ± 307	1,164 ± 306	210 ± 85	2,785 ± 2,039
S8/S1	923	14,270	1,060	1,650	17

COD: chemical oxygen demand. NH_3-N: ammonia. TN: total nitrogen. TP: total phosphate.

Table S11. Coliform concentrations per sampling point in colony forming units (CFU) per mL river water (mean of biological replicates with standard deviation or minimum and maximum values). ESBL E. coli, CRB-0.5 *E. coli* and CRB-2 *E. coli* measurements were under detection limit in more than 40% of the sample, so the R2D substitution method was not applied. For these parameters we report minimum and maximum values instead of means. CRB-2 and CRB-2 *E. coli* was only measured for trips II-IV, so the mean and standard deviation are based on three biological replicates. For all other parameters, data for S1-S8 is based on four biological replicates and data for Se1 and M5 is based on three biological replicates. Fold-change calculated by dividing the mean S8 with the mean S1 values.

	TC	*E. coli*	ESBL coliform	ESBL *E. coli*	CRB-0.5	CRB-0.5 *E. coli*	CRB-2	CRB-2 *E. coli*
S1	$(1.1 \pm 0.5) \times 10^3$	$(3.5 \pm 2) \times 10^1$	$(1.5 \pm 1.3) \times 10^2$	$(<0.5 - 2) \times 10^1$	$(9 \pm 3.8) \times 10^1$	$<0.5 \times 10^1$	$(3.1 \pm 4.1) \times 10^1$	$<0.5 \times 10^1$
S2	$(1.7 \pm 1.3) \times 10^4$	$(3.4 \pm 5.5) \times 10^3$	$(3.5 \pm 5.6) \times 10^3$	$(<0.1 - 3.5) \times 10^2$	$(0.9 \pm 1.3) \times 10^3$	$<0.5 / 1 \times 10^1$	$(1.1 \pm 0.9) \times 10^2$	$<0.5 \times 10^1$
S5	$(2.7 \pm 0.6) \times 10^4$	$(1.1 \pm 0.2) \times 10^3$	$(1.1 \pm 0.8) \times 10^3$	$(<1 - 8) \times 10^1$	$(4.8 \pm 3.8) \times 10^2$	$<1 \times 10^1$	$(7.1 \pm 2.7) \times 10^1$	$<0.5 \times 10^1$
S6	$(1.4 \pm 0.7) \times 10^3$	$(3.3 \pm 1.2) \times 10^1$	$(5.4 \pm 4.3) \times 10^1$	$(<0.5 - 1) \times 10^1$	$(4.3 \pm 2) \times 10^1$	$<0.5 \times 10^1$	$(9 \pm 8) \times 10^0$	$<0.5 \times 10^1$
S7	$(1.1 \pm 0.1) \times 10^4$	$(4.9 \pm 1.3) \times 10^2$	$(9.4 \pm 5.4) \times 10^2$	$(<0.05 - 1) \times 10^2$	$(2.5 \pm 1.9) \times 10^2$	$<0.5 / 1 \times 10^1$	$(4.2 \pm 3.1) \times 10^1$	$<0.5 \times 10^1$
S8	$(4.1 \pm 3.3) \times 10^4$	$(2.8 \pm 2.1) \times 10^3$	$(4 \pm 4.1) \times 10^3$	$(<0.1 - 5) \times 10^2$	$(3.8 \pm 3.4) \times 10^2$	$<0.5 / 1 \times 10^1$	$(1.1 \pm 0.2) \times 10^2$	$<0.5 \times 10^1$
Se1	$(1.1 \pm 0.2) \times 10^4$	$(6.4 \pm 0.2) \times 10^2$	$(7.2 \pm 1.2) \times 10^2$	$(<1 - 4) \times 10^1$	$(4 \pm 1.7) \times 10^2$	$<1 \times 10^1$	$(8.9 \pm 1.5) \times 10^1$	$<0.5 \times 10^1$
M5	$(8 \pm 2.7) \times 10^4$	$(1.3 \pm 0.3) \times 10^4$	$(8.3 \pm 5.9) \times 10^3$	$(<0.1 - 6) \times 10^2$	$(1.9 \pm 1.5) \times 10^3$	$(<1 - 1) \times 10^1$	$(4 \pm 1) \times 10^2$	$(<0.5 - 1) \times 10^1$
S8/S1	37	80	27	NA	4	NA	3	NA

CRB: carbapenem resistant bacteria. CRB-0.5: CRB screened with 0.5 μg/mL meropenem. CRB-2: CRB screened with 2 μg/mL meropenem. ESBL: extended-spectrum β-lactamase. NA: not applicable. TC: total coliform (TC).

Table S12. Coliform mass loading per sampling point in colony forming units (CFU) per river water per day (mean of biological replicates with standard deviation). ESBL *E. coli,* CRB-0.5 *E. coli* and CRB-2 *E. coli* mass loadings were not calculated as more than 40% of measurements were under detection limit. CRB-2 mass loadings were only measured for trips II-IV, so the mean and standard deviation data is based on three biological replicates. For all other parameters, data for S1-S8 is based on four biological replicates and data for Se1 and M5 is based on three biological replicates. Fold-change calculated by dividing the mean S8 with the mean S1 values. NA = not applicable.

	TC	*E. coli*	ESBL coliform	CRB-0.5	CRB-2
S1	$(4 \pm 2) \times 10^{13}$	$(1 \pm 0.5) \times 10^{12}$	$(4 \pm 3) \times 10^{12}$	$(4 \pm 4) \times 10^{12}$	$(0.8 \pm 1) \times 10^{12}$
S2	$(2 \pm 1) \times 10^{15}$	$(4 \pm 6) \times 10^{14}$	$(4 \pm 6) \times 10^{14}$	$(1 \pm 1) \times 10^{14}$	$(1 \pm 1) \times 10^{13}$
S5	$(3 \pm 0.9) \times 10^{15}$	$(1 \pm 0.7) \times 10^{15}$	$(1 \pm 0.9) \times 10^{15}$	$(5 \pm 4) \times 10^{14}$	$(1 \pm 0.8) \times 10^{14}$
S6	$(6 \pm 5) \times 10^{14}$	$(2 \pm 1) \times 10^{13}$	$(3 \pm 3) \times 10^{13}$	$(3 \pm 2) \times 10^{13}$	$(5 \pm 7) \times 10^{12}$
S7	$(2 \pm 2) \times 10^{16}$	$(9 \pm 7) \times 10^{14}$	$(2 \pm 0.9) \times 10^{15}$	$(4 \pm 2) \times 10^{14}$	$(6 \pm 5) \times 10^{13}$
S8	$(3 \pm 2) \times 10^{17}$	$(2 \pm 2) \times 10^{16}$	$(3 \pm 3) \times 10^{16}$	$(3 \pm 3) \times 10^{15}$	$(7 \pm 3) \times 10^{14}$
Se1	$(3 \pm 2) \times 10^{15}$	$(1 \pm 0.8) \times 10^{14}$	$(2 \pm 0.8) \times 10^{14}$	$(8 \pm 3) \times 10^{13}$	$(2 \pm 0.9) \times 10^{13}$
M5	$(8 \pm 3) \times 10^{15}$	$(1 \pm 0.5) \times 10^{15}$	$(9 \pm 8) \times 10^{14}$	$(2 \pm 2) \times 10^{14}$	$(4 \pm 0.6) \times 10^{13}$
S8/S1	7,500	20,000	7,500	750	875

CRB: carbapenem resistant bacteria. CRB-0.5: CRB screened with 0.5 μg/mL meropenem. CRB-2: CRB screened with 2 μg/mL meropenem. ESBL: extended-spectrum β-lactamase. NA: not applicable. TC: total coliform (TC).

Table S13. Summarized antibiotic resistance gene (ARG) and mobile genetic element (MGE) levels in river water (mean of biological replicates with standard deviation). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates. Fold-change calculated by dividing the mean S8 with the mean S1 values.

	Detected (number)		River water concentration (copies/mL)		River water mass loading (copies/d)		Normalised cell concentration (copies/cell)	
	ARG	MGE	ARG	MGE	ARG	MGE	ARG	MGE
S1	119 ± 14	10 ± 1	$(1.9 \pm 1.7) \times 10^{5}$	$(1.3 \pm 1.1) \times 10^{5}$	$(6.3 \pm 4.1) \times 10^{15}$	$(4.3 \pm 2.6) \times 10^{15}$	0.1 ± 0.1	0.1 ± 0
S2	161 ± 15	12 ± 1	$(3.9 \pm 3.3) \times 10^{7}$	$(3.1 \pm 3.6) \times 10^{7}$	$(4.5 \pm 3.5) \times 10^{18}$	$(3.6 \pm 4) \times 10^{18}$	1.3 ± 0.5	0.9 ± 0.7
S5	154 ± 7	11 ± 1	$(6.5 \pm 1.4) \times 10^{7}$	$(3.7 \pm 0.8) \times 10^{7}$	$(7.2 \pm 5.1) \times 10^{19}$	$(4.1 \pm 3.1) \times 10^{19}$	1.1 ± 0.2	0.7 ± 0.2
S6	120 ± 5	11 ± 0	$(7.5 \pm 4.6) \times 10^{6}$	$(6.9 \pm 2.7) \times 10^{6}$	$(4 \pm 3.7) \times 10^{18}$	$(3.7 \pm 3) \times 10^{18}$	0.7 ± 0.1	0.8 ± 0.2
S7	145 ± 3	11 ± 1	$(2.2 \pm 0.6) \times 10^{7}$	$(1.9 \pm 0.9) \times 10^{7}$	$(5.5 \pm 6.9) \times 10^{19}$	$(5.1 \pm 7.2) \times 10^{19}$	1.2 ± 0.2	1 ± 0.3
S8	150 ± 8	11 ± 1	$(1.2 \pm 0.9) \times 10^{8}$	$(1.1 \pm 0.9) \times 10^{8}$	$(8.6 \pm 7.2) \times 10^{20}$	$(8.1 \pm 7.3) \times 10^{20}$	1.7 ± 0.6	1.6 ± 0.6
Se1	165 ± 3	12 ± 0	$(2.2 \pm 1.5) \times 10^{7}$	$(1.4 \pm 0.8) \times 10^{7}$	$(4.4 \pm 7.1) \times 10^{18}$	$(2.9 \pm 1.3) \times 10^{18}$	2 ± 0.6	1.3 ± 0.3
M5	156 ± 9	12 ± 1	$(3.1 \pm 2.6) \times 10^{8}$	$(2.3 \pm 1.9) \times 10^{8}$	$(3.1 \pm 2.6) \times 10^{19}$	$(2.3 \pm 2) \times 10^{19}$	2.6 ± 1.2	1.9 ± 1
S8/S1	1.3	1.1	632	846	136,508	188,372	17	16

Table S5. Detected antibiotic resistance genes (ARGs) per class in river water (mean of biological replicates with standard deviation). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates. Numbers in brackets indicate total number of ARGs per class included in the high-throughput qPCR assay.

	Amino-glycoside (36)	β-Lactams (52)	FCA (9)	MLSB (46)	Non-specific (51)	Sulfon-amide (7)	Tetra-cycline (39)	Vanco-mycin (32)	Others (11)
S1	17 ± 3	25 ± 4	6 ± 1	14 ± 3	32 ± 1	3 ± 1	16 ± 3	4 ± 0	3 ± 1
S2	27 ± 2	34 ± 4	7 ± 1	21 ± 3	35 ± 1	4 ± 1	22 ± 4	7 ± 1	4 ± 1
S5	25 ± 1	34 ± 2	7 ± 1	20 ± 2	34 ± 2	4 ± 1	23 ± 2	4 ± 1	4 ± 0
S6	20 ± 1	24 ± 3	6 ± 1	15 ± 1	27 ± 2	3 ± 1	18 ± 2	5 ± 1	2 ± 1
S7	25 ± 1	31 ± 1	6 ± 1	18 ± 1	34 ± 1	4 ± 1	21 ± 1	4 ± 1	3 ± 1
S8	25 ± 1	33 ± 3	7 ± 1	18 ± 2	35 ± 1	4 ± 1	21 ± 2	4 ± 1	3 ± 0
Se1	25 ± 1	38 ± 4	7 ± 1	21 ± 2	35 ± 1	4 ± 1	26 ± 3	5 ± 1	4 ± 1
M5	25 ± 2	34 ± 2	7 ± 1	19 ± 3	34 ± 2	4 ± 0	24 ± 1	5 ± 3	4 ± 0

FCA: fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol ARGs. MLSB: macrolide-lincosamide-streptogramin B ARGs.

Table S6. Antibiotic resistance gene (ARG) concentrations in river water (copies/mL) per class (mean of biological replicates with standard deviation). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates.

	Amino-glycoside	β-Lactams	FCA	MLSB	Non-specific	Sulfon-amide	Tetra-cycline	Vanco-mycin	Others
S1	(3.6 ± 3.7) x 10^4	(3 ± 3.3) x 10^4	(5.7 ± 4.7) x 10^3	(7.5 ± 7.4) x 10^3	(8 ± 5.7) x 10^4	(1.1 ± 1.1) x 10^4	(2.1 ± 1.4) x 10^4	(3.4 ± 2.6) x 10^3	(1 ± 0.9) x 10^3
S2	(1.1 ± 1.2) x 10^7	(4.3 ± 4.7) x 10^6	(1.5 ± 1.8) x 10^6	(1.2 ± 1.4) x 10^6	(9 ± 7.6) x 10^6	(9.2 ± 3.4) x 10^6	(2.7 ± 3.5) x 10^6	(6.8 ± 4) x 10^4	(9.8 ± 15) x 10^4
S5	(1.6 ± 0.3) x 10^7	(6.3 ± 1.8) x 10^6	(2 ± 0.6) x 10^6	(2.1 ± 0.5) x 10^6	(1.8 ± 0.32) x 10^7	(1.6 ± 0.6) x 10^7	(3.2 ± 0.8) x 10^6	(1.6 ± 0.6) x 10^5	(6 ± 1.2) x 10^4
S6	(1.4 ± 0.7) x 10^6	(3.7 ± 2.2) x 10^5	(8.7 ± 4.4) x 10^4	(2.8 ± 1.5) x 10^5	(2.9 ± 1.7) x 10^6	(2.1 ± 1.7) x 10^6	(2.3 ± 0.9) x 10^5	(2.9 ± 1.8) x 10^4	(8.7 ± 7) x 10^3
S7	(6.1 ± 1) x 10^6	(2.2 ± 0.4) x 10^6	(7.5 ± 1.6) x 10^5	(9.2 ± 2.5) x 10^5	(6.9 ± 2.1) x 10^6	(3.7 ± 2.3) x 10^6	(1.1 ± 0.4) x 10^6	(6.7 ± 2.7) x 10^4	(1.8 ± 0.7) x 10^4
S8	(3.8 ± 2.7) x 10^7	(1.6 ± 1.2) x 10^7	(5.9 ± 4.3) x 10^6	(4.8 ± 3.5) x 10^6	(3.7 ± 2.5) x 10^7	(1.7 ± 1.2) x 10^7	(5.9 ± 3.4) x 10^6	(2.4 ± 1.8) x 10^5	(9 ± 5.4) x 10^4
Se1	(8.8 ± 6.4) x 10^6	(3.3 ± 2.5) x 10^6	(1.6 ± 1.6) x 10^6	(5.4 ± 2.4) x 10^5	(5.6 ± 3.2) x 10^6	(5.1 ± 2.9) x 10^5	(1.5 ± 0.6) x 10^6	(1.7 ± 0.9) x 10^4	(1.8 ± 0.2) x 10^4
M5	(9 ± 8.5) x 10^7	(3.6 ± 2.8) x 10^7	(9 ± 6.8) x 10^6	(1.2 ± 1.2) x 10^7	(9.1 ± 7.3) x 10^7	(5.8 ± 4.1) x 10^7	(1.4 ± 1.2) x 10^7	(2.9 ± 1.4) x 10^5	(3.1 ± 2.2) x 10^5

FCA: fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol ARGs. MLSB: macrolide-lincosamide-streptogramin B ARGs.

Table S7. Normalized antibiotic resistance gene (ARG) cell concentrations in river water (copies/cell) per class (mean of biological replicates with standard deviation). Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates.

	Amino-glycoside	β-Lactams	FCA	MLSB	Non-specific	Sulfon-amide	Tetra-cycline	Vanco-mycin	Others
S1	0.02 ± 0.02	0.01 ± 0.01	0 ± 0	0 ± 0	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0 ± 0	0 ± 0
S2	0.32 ± 0.22	0.13 ± 0.08	0.04 ± 0.04	0.04 ± 0.03	0.3 ± 0.11	0.37 ± 0.1	0.08 ± 0.07	0 ± 0	0 ± 0
S5	0.29 ± 0.07	0.11 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.33 ± 0.03	0.29 ± 0.07	0.06 ± 0.01	0 ± 0	0 ± 0
S6	0.14 ± 0.05	0.04 ± 0.01	0.01 ± 0	0.03 ± 0.01	0.28 ± 0.07	0.19 ± 0.07	0.03 ± 0.01	0 ± 0	0 ± 0
S7	0.33 ± 0.05	0.12 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.37 ± 0.05	0.19 ± 0.09	0.06 ± 0.01	0 ± 0	0 ± 0
S8	0.51 ± 0.24	0.22 ± 0.1	0.08 ± 0.04	0.07 ± 0.03	0.51 ± 0.15	0.24 ± 0.05	0.09 ± 0.02	0 ± 0	0 ± 0
Se1	0.78 ± 0.31	0.29 ± 0.12	0.13 ± 0.09	0.05 ± 0.01	0.51 ± 0.11	0.05 ± 0.01	0.15 ± 0.02	0 ± 0	0 ± 0
M5	0.71 ± 0.45	0.29 ± 0.13	0.07 ± 0.03	0.09 ± 0.07	0.79 ± 0.36	0.47 ± 0.18	0.12 ± 0.06	0 ± 0	0 ± 0

FCA: fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol ARGs. MLSB: macrolide-lincosamide-streptogramin B ARGs.

Table S17. Mobile genetic element (MGE) levels in river water (mean of biological replicates with standard deviation). All measurements based on technical triplicates. Data for S1-S8 based on four biological replicates. Data for Se1 and M5 based on three biological replicates.

	Detected (number)		River water concentration (copies/mL)		Normalised cell concentration (copies/cell)	
	Int	Tran	Int	Tran	Int	Tran
S1	2 ± 1	8 ± 0	(6 ± 4.1) x 104	(7.4 ± 6.8) x 104	0.03 ± 0.02	0.04 ± 0.03
S2	4 ± 1	8 ± 0	(7.6 ± 5.8) x 106	(2.4 ± 3.1) x 107	0.26 ± 0.09	0.66 ± 0.61
S5	4 ± 1	8 ± 1	(1.4 ± 0.4) x 106	(2.3 ± 0.5) x 107	0.26 ± 0.07	0.41 ± 0.09
S6	3 ± 0	8 ± 0	(1.7 ± 1) x 106	(5.3 ± 1.8) x 106	0.16 ± 0.04	0.61 ± 0.22
S7	3 ± 1	8 ± 0	(5.1 ± 2.5) x 106	(1.4 ± 0.6) x 107	0.27 ± 0.09	0.73 ± 0.24
S8	3 ± 1	8 ± 0	(3.5 ± 2.9) x 107	(7.9 ± 5.8) x 107	0.47 ± 0.23	1.09 ± 0.38
Se1	4 ± 0	8 ± 0	(5.7 ± 3.5) x 106	(8.5 ± 4.7) x 106	0.51 ± 0.13	0.78 ± 0.2
M5	4 ± 1	8 ± 0	(8.9 ± 7.6) x 107	(1.4 ± 1.2) x 108	0.73 ± 0.37	1.19 ± 0.58

Int: integrons. Tran: transposases

Table S8. Seasonal effects. Paired t-tests and Cohen's D effect sizes comparing mass loadings (ML), concentrations (C) and detected numbers (D) for water quality and antibiotic resistant parameters between the dry and wet season. For applied transformations, see Table S.

				Paired t-test			Cohen's D effect size		
Type	Parameter	Type	Unit	P value	Degrees of freedom	t value	Value	95% confidence interval range	
Water quality	DO	C	mg/L	0.4200	7	-0.85662	-0.18	-0.64	0.27
	NH_3-N	C	mg/L	0.0328	7	2.65340	0.34	0.06	0.62
		ML	kg/d	0.6597	7	0.45969	0.03	-0.11	0.17
	COD	C	mg/L	0.8636	7	0.17825	0.07	-0.73	0.87
		ML	kg/d	0.5765	7	-0.58559	-0.08	-0.36	0.21
	TN	C	kg/d	0.0213	7	2.95380	0.41	0.1	0.72
		ML	mg/L	0.4207	7	0.85535	0.08	-0.12	0.27
	TP	C	kg/d	0.3615	7	0.97614	0.25	-0.3	0.79
		ML	mg/L	0.7489	7	-0.33296	-0.03	-0.22	0.16
Plating	TC	C	CFU/mL	0.0299	7	2.71750	0.36	0.07	0.66
		ML	CFU/d	0.2749	7	1.18430	0.15	-0.13	0.43
	E. coli	C	CFU/mL	0.0770	7	2.07240	0.3	-0.02	0.61
		ML	CFU/d	0.1381	7	1.67380	0.21	-0.06	0.47
	ESBL coliform	C	CFU/mL	0.0059	7	3.89890	0.67	0.26	1.07
		ML	CFU/d	0.0197	7	3.01030	0.4	0.1	0.7
	CRB-0.5	C	CFU/mL	0.1287	7	1.72210	0.39	-0.11	0.89
		ML	CFU/d	0.2891	7	1.14700	0.23	-0.21	0.67
Antibiotic	Total antibiotics	C	ng/L	0.0224	7	2.91970	0.9	0.12	1.69
		ML	g/d	0.0746	7	2.09300	0.35	-0.02	0.72
S16 rRNA	S16 rRNA	C	copies/mL	0.0200	7	2.99840	0.32	0.08	0.55
		ML	copies/d	0.3778	7	0.94143	0.12	-0.16	0.4
Detected MGEs/ARGs	Detected ARGs	D	NA	0.0124	7	3.34140	0.49	0.16	0.82
	Detected MGEs	D	NA	0.0796	7	2.04940	0.67	-0.1	1.44
Abundance MGEs/ARGs	Abundance ARGs	C	copies/mL	0.0105	7	3.46360	0.34	0.12	0.56
		ML	copies/d	0.1449	7	1.64040	0.19	-0.06	0.43
	Abundance MGEs	C	copies/mL	0.0073	7	3.73210	0.4	0.16	0.64
		ML	copies/d	0.1013	7	1.88570	0.22	-0.03	0.46
Normalized ARGs/MGEs	Normalised ARGs	C	copies/cell	0.0239	7	2.87330	0.43	0.09	0.77
		C	copies/cell	0.0329	7	2.65190	0.57	0.07	1.08

ARGs: antibiotic resistant genes. CFU: colony forming units. COD: chemical oxygen demand. CRB-0.5: carbapenem resistant bacteria selected for with 0.5 µg/mL meropenem. ESBL: extended-spectrum β-lactamase. DO: dissolved oxygen. MGE: mobile genetic elements. NA: not applicable. NH_3-N: ammonia. TC: total coliform. TN: total nitrogen. TP: total phosphorus.

Table S9. Spatial effects. Welch's t-test and Cohen's D effect size effect-sizes comparing mass loadings (ML), concentrations (C) and detected numbers (D) for water quality and antibiotic resistant parameters between the up- (S1) and downstream (S8). For applied transformations, see Table S.

				Welch's t-test			Cohen's D effect-size		
Type	Parameter	Type	Unit	P value	Degrees of freedom	t value	Value	95% confidence interval range	
Water quality	DO	C	mg/L	0.0000	4.52	22.02	15.57	5.89	25.25
	NH3-N	C	mg/L	0.0163	3.00	-4.89	-3.46	-6.19	-0.73
		ML	kg/d	0.0000	4.84	-17.49	-12.37	-20.13	-4.61
	COD	C	mg/L	0.0895	3.53	-2.33	-1.64	-3.65	0.36
		ML	kg/d	0.0001	5.99	-9.77	-6.91	-11.47	-2.34
	TN	C	kg/d	0.0218	3.28	-4.12	-2.91	-5.39	-0.43
		ML	mg/L	0.0000	5.99	-17.33	-12.25	-19.95	-4.56
	TP	C	kg/d	0.0393	3.01	-3.50	-2.47	-4.77	-0.18
		ML	mg/L	0.0000	5.80	-14.70	-10.39	-16.98	-3.80
Plating	Coliform	C	CFU/mL	0.0067	3.95	-5.21	-3.68	-6.52	-0.84
		ML	CFU/d	0.0001	4.38	-13.79	-9.75	-15.96	-3.54
	E. coli	C	CFU/mL	0.0104	3.68	-4.83	-3.42	-6.13	-0.70
		ML	CFU/d	0.0004	3.63	-12.36	-8.74	-14.36	-3.12
	ESBL coliform	C	CFU/mL	0.0131	5.85	-3.52	-2.49	-4.79	-0.18
		ML	CFU/d	0.0001	5.66	-9.55	-6.75	-11.23	-2.27
	CRB-0.5	C	CFU/mL	0.0888	4.21	-2.20	-1.56	-3.53	0.42
		ML	CFU/d	0.0001	5.99	-9.93	-7.02	-11.66	-2.39
Antibiotic	Antibiotics	C	ng/L	0.0032	5.75	-4.86	-3.43	-6.16	-0.71
		ML	g/d	0.0001	4.55	-12.61	-8.92	-14.64	-3.19
S16 rRNA	S16 rRNA	C	copies/mL	0.0025	3.57	-7.63	-5.40	-9.12	-1.67
		ML	copies/d	0.0000	4.89	-19.67	-13.91	-22.59	-5.23
Detected MGE/ARG	Detected ARG	D	NA	0.0141	4.55	-3.87	-2.74	-5.14	-0.33
		D	NA	0.0300	6.00	-2.83	-2.00	-4.12	0.12
Abundance MGE/ARG	Abundance ARG	C	copies/mL	0.0002	5.17	-9.20	-6.50	-10.84	-2.17
		ML	copies/d	0.0000	5.13	-18.04	-12.75	-20.74	-4.76
	Abundance MGE	C	copies/mL	0.0001	5.31	-9.68	-6.85	-11.38	-2.32
		ML	copies/d	0.0000	4.99	-18.07	-12.78	-20.79	-4.77
Normalized ARG/MGE	Normalised ARG	C	copies/cell	0.0120	3.07	-5.36	-3.79	-6.68	-0.90
		C	copies/cell	0.0120	3.07	-5.36	-3.47	-6.21	-0.73

ARGs: antibiotic resistant genes. CFU: colony forming units. COD: chemical oxygen demand. CRB-0.5: carbapenem resistant bacteria selected for with 0.5 µg/mL meropenem. ESBL: extended-spectrum β-lactamase. DO: dissolved oxygen. MGE: mobile genetic elements. NA: not applicable. NH_3-N: ammonia. TC: total coliform. TN: total nitrogen. TP: total phosphorus

Table S20. Spearman correlations between river water concentrations of total antibiotics, amoxicillin and ciprofloxacin (both antibiotics detected in the catchment above their PNECs), total antibiotic resistant genes (ARGs) and ARGs reported by antibiotic class for the river catchment (n=30). Correlation values only shown for $P < 0.05$ with P values corrected for multiple testing with the Benjamini Hochberg approach.

	Total antibiotics	Amoxicillin	Ciprofloxacin
ARG	0.70*	0.39	0.56*
Aminoglycoside	0.78*	0.49*	0.6*
β-Lactam	0.76*	0.46	0.62*
FCA	0.79*	0.48*	0.61*
MLSB	0.74*	0.40	0.58*
Non-specific	0.68*	-	0.56*
Sulfonamide	0.51*	-	0.5*
Tetracycline	0.77*	0.48*	0.57*
Vancomycin	0.58*	-	0.55*
Other	0.71*	0.41	0.58*

*: $P < 0.01$. FCA: fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol ARGs. MLSB: macrolide-lincosamide-streptogramin B ARGs.

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