

Supplementary Information

“Evaluation of antibiotic resistance dissemination by wastewater treatment plant effluents with different catchment areas in Germany” with the authors Johannes Alexander, Norman Hembach, and Thomas Schwartz.

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Number of tables 5

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SI Table 1: Daily discharge of ARG and FPB cell equivalents by communal WWTPs. Displayed are the average discharge concentration for each investigated parameter per day and the median value and deviation per group (frequently, intermediately, rarely occurring, and bacteria). Each WWTP was samples 4 times.

WWTP discharge [m³/d]	841	1,730	1,647	860	660	1,216	720	314	1,481	1,825	495	median of summation	deviation	
WWTP acronym	C7	C2	C5	C8	C1	C3	C9	C10	C6	C4	C11		min.	max.
antibiotic resistance gene [cell equivalents/d]														
sulfonamid resistance (<i>su1</i>)	2.60E+14	2.63E+14	3.43E+14	2.43E+14	2.72E+13	6.29E+13	2.19E+13	1.91E+13	1.49E+14	1.60E+14	7.39E+12			
erythromycin resistance (<i>ermB</i>)	1.24E+14	3.21E+13	8.57E+13	3.00E+13	2.31E+13	2.84E+13	8.07E+12	6.86E+12	1.56E+13	9.17E+13	8.79E+11			
β-lactam resistance (<i>bla_{TEM}</i>)	7.25E+13	1.17E+13	1.79E+13	3.43E+14	3.90E+11	1.91E+14	5.66E+13	1.01E+14	3.93E+13	4.32E+13	1.34E+13			
tetracycline resistance (<i>tetM</i>)	2.26E+12	1.20E+12	7.93E+11	5.45E+11	8.20E+11	7.63E+11	6.37E+10	4.31E+10	3.16E+11	3.04E+12	1.63E+10			
summation frequently abundant ARG	4.59E+14	3.08E+14	4.47E+14	6.17E+14	5.15E+13	2.83E+14	8.66E+13	1.27E+14	2.04E+14	2.98E+14	2.17E+13	2.83E+14	2.17E+13	6.17E+14
cephalosporine resistance (<i>bla_{CTXM32}</i>)	1.31E+12	3.10E+10	1.01E+11	5.49E+10	2.24E+10	1.00E+11	2.52E+10	3.61E+04	1.51E+11	1.18E+11	1.78E+09			
carbapenem resistance (<i>bla_{OXA48}</i>)	4.95E+10	1.89E+11	1.01E+11	2.11E+09	7.16E+10	5.60E+10	1.92E+10	6.77E+04	7.38E+10	8.32E+11	1.00E+00			
cephalosporine resistance (<i>bla_{CTX-M15}</i>)	2.37E+10	3.83E+09	2.45E+09	3.76E+09	2.00E+09	6.87E+09	6.02E+08	2.27E+03	1.13E+10	4.36E+10	1.94E+08			
β-lactam resistance (<i>bla_{CMY-2}</i>)	3.49E+09	1.23E+09	5.16E+08	1.34E+09	1.36E+09	8.72E+08	5.76E+08	2.27E+02	6.75E+08	2.83E+09	1.00E+00			
summation intermediate abundant ARG	1.38E+12	2.26E+11	2.05E+11	6.21E+10	9.74E+10	1.64E+11	4.56E+10	1.06E+05	2.37E+11	9.97E+11	1.98E+09	1.64E+11	1.06E+05	1.38E+12
methicillin resistance (<i>mecA</i>)	9.00E+08	2.23E+08	6.62E+08	6.51E+07	1.31E+07	1.08E+08	7.49E+07	4.11E+07	2.13E+07	3.40E+09	9.58E+07			
carbapeneme resistance (<i>bla_{NDM-1}</i>)	4.26E+08	1.57E+08	3.80E+08	1.41E+09	3.38E+08	4.70E+08	1.00E+00	1.00E+00	1.90E+07	3.60E+08	8.21E+07			
colistin resistance (<i>mcr-1</i>)	5.82E+08	7.55E+08	8.29E+08	2.36E+08	2.66E+08	3.66E+08	2.68E+08	1.27E+08	1.34E+08	3.51E+08	0.00E+00			
vancomycin resistance (<i>vanA</i>)	2.39E+08	2.32E+07	1.00E+00	1.00E+00	1.17E+08	1.28E+07	1.22E+07	1.06E+07	9.55E+07	1.00E+00	4.04E+07			
summation rarely occuring ARG	2.15E+09	1.16E+09	1.87E+09	1.71E+09	7.34E+08	9.57E+08	3.55E+08	1.79E+08	2.70E+08	4.11E+09	2.18E+08	9.57E+08	1.79E+08	4.11E+09

facultative pathogenic bacteria [cell equivalents/d]														
<i>E. coli</i> (<i>yccT</i>)	4.74E+11	1.11E+11	6.20E+10	5.81E+10	8.87E+10	7.33E+10	2.74E+10	2.33E+10	3.81E+10	8.12E+11	2.09E+09			
enterococci (<i>23SrRNA</i>)	5.11E+10	9.90E+09	1.98E+10	1.62E+11	1.59E+11	2.09E+11	3.94E+09	4.87E+09	2.08E+10	3.43E+11	2.75E+09			
<i>K. pneumoniae</i> (<i>gltA</i>)	2.90E+10	1.20E+10	1.38E+10	2.70E+10	6.91E+10	3.94E+10	1.77E+09	1.03E+10	9.07E+09	1.69E+11	4.70E+08			
<i>A. baumannii</i> (<i>secE</i>)	4.39E+09	2.50E+09	8.04E+09	5.56E+09	5.41E+09	2.73E+09	3.19E+08	4.43E+08	3.82E+09	1.16E+10	1.14E+08			
<i>P. aeruginosa</i> (<i>ecfX</i>)	1.58E+09	6.01E+08	1.02E+09	3.86E+08	2.94E+08	3.70E+08	2.73E+08	2.90E+08	1.16E+09	3.71E+09	1.00E+00			
summation FPB	5.60E+11	1.36E+11	1.05E+11	2.53E+11	3.22E+11	3.24E+11	3.37E+10	3.92E+10	7.30E+10	1.34E+12	5.42E+09	1.36E+11	5.42E+09	1.34E+12

SI Table 2: Daily discharge of ARG and FPB cell equivalents by food production-impacted WWTPs. Displayed are the average discharge concentration for each parameter per day and the median value and deviation per group (frequently, intermediately, rarely occurring, and bacteria). Each WWTP was samples 4 times.

WWTP discharge [m ³ /d]	1,340	767	2,800	2,687	median of summation	deviation	
WWTP acronym	F1	F2	F3	F4		min.	max.
antibiotic resistance gene [cell equivalents/d]							
sulfonamid resistance (<i>su1</i>)	2.21E+14	5.82E+12	4.57E+14	4.09E+13			
erythromycin resistance (<i>ermB</i>)	4.57E+13	1.40E+12	6.86E+12	1.40E+12			
β-lactam resistance (<i>bla</i> TEM)	2.90E+13	1.26E+12	6.50E+12	4.88E+12			
tetracycline resistance (<i>tetM</i>)	2.97E+11	3.43E+10	2.56E+11	2.12E+11			
summation frequently abundant ARG	2.96E+14	8.51E+12	4.71E+14	4.74E+13	1.72E+14	8.51E+12	4.71E+14
cephalosporine resistance (<i>bla</i> CTXM32)	1.99E+11	4.87E+07	5.06E+09	1.20E+10			
carbapeneme resistance (<i>bla</i> OXA48)	6.08E+11	1.81E+06	3.03E+11	2.35E+11			
cephalosporine resistance (<i>bla</i> CTX-M15)	4.48E+09	1.48E+06	4.87E+08	4.96E+10			
β-lactam resistance (<i>bla</i> CMY-2)	4.78E+09	0.00E+00	8.87E+05	8.52E+05			
summation intermediate abundant ARG	8.15E+11	5.20E+07	3.08E+11	2.97E+11	3.02E+11	5.20E+07	8.15E+11
methicillin resistance (<i>mecA</i>)	8.50E+08	4.87E+07	1.29E+08	3.38E+08			
carbapeneme resistance (<i>bla</i> NDM-1)	0.00E+00	1.81E+06	4.72E+06	4.53E+06			
colistin resistance (<i>mcr-1</i>)	1.18E+08	1.48E+06	5.59E+07	3.70E+06			
vancomycin resistance (<i>vanA</i>)	3.33E+07	0.00E+00	0.00E+00	0.00E+00			
summation rarely occurring ARG	1.00E+09	5.20E+07	1.89E+08	3.46E+08	2.68E+08	5.20E+07	1.00E+09

facultative pathogenic bacteria [cell equivalents/d]							
<i>E. coli</i> (<i>yccT</i>)	1.84E+10	4.70E+09	2.53E+11	6.37E+10			
enterococci (<i>23S</i> rRNA)	1.69E+10	1.73E+08	2.03E+09	4.05E+09			
<i>K. pneumoniae</i> (<i>gltA</i>)	4.92E+10	1.43E+09	3.51E+11	2.30E+10			
<i>A. baumannii</i> (<i>secE</i>)	3.81E+09	8.51E+07	8.20E+08	2.59E+08			
<i>P. aeruginosa</i> (<i>ecfX</i>)	1.28E+08	6.44E+07	2.52E+09	2.61E+09			
summation FPB	8.85E+10	6.45E+09	6.10E+11	9.36E+10	9.10E+10	6.45E+09	6.10E+11

SI Table 3: Daily discharge of ARG and FPB cell equivalents by hospital-impacted WWTPs. Displayed are the average discharge concentration for each parameter per day and the median value and deviations per group (frequently, intermediately, rarely occurring, and bacteria). Each WWTP was samples 4 times.

WWTP discharge [m ³ /d]	2,895	2,583	1,581	662	2,136	3,240	7,421	34,306	median of summation	deviation	
WWTP acronym	H2	H1	H6	H7	H4	H5	H3	H8		min.	max.
antibiotic resistance gene [cell equivalents/d]											
sulfonamid resistance (<i>su1</i>)	1.91E+14	1.22E+14	1.08E+14	1.43E+14	6.86E+13	1.20E+14	2.35E+14	1.17E+13			
erythromycin resistance (<i>ermB</i>)	1.07E+14	6.70E+13	1.18E+13	8.30E+12	1.81E+13	3.12E+13	2.66E+13	1.10E+13			
β-lactam resistance (<i>bla</i> _{TEM})	2.64E+13	1.91E+15	5.55E+13	3.75E+12	4.26E+13	2.69E+14	6.60E+13	3.27E+14			
tetracycline resistance (<i>tetM</i>)	1.99E+12	1.26E+12	2.28E+11	5.06E+10	5.10E+10	4.55E+12	1.63E+11	2.57E+11			
summation frequently abundant ARG	3.27E+14	2.10E+15	1.75E+14	1.55E+14	1.29E+14	4.25E+14	3.28E+14	3.50E+14	3.27E+14	1.29E+14	2.10E+15
cephalosporine resistance (<i>bla</i> _{CTXM32})	2.34E+11	2.08E+11	4.79E+10	1.08E+10	4.72E+10	1.07E+11	4.01E+10	2.08E+10			
carbapeneme resistance (<i>bla</i> _{OXA48})	2.34E+11	6.01E+10	5.48E+10	1.31E+10	1.32E+10	1.40E+10	1.09E+11	6.84E+10			
cephalosporine resistance (<i>bla</i> _{CTX-M15})	1.96E+10	1.47E+10	1.24E+09	7.17E+08	4.32E+09	9.31E+09	4.45E+09	4.50E+09			
β-lactam resistance (<i>bla</i> _{CMY-2})	2.23E+09	1.39E+09	6.92E+08	1.00E+00	6.15E+08	3.79E+09	7.06E+08	8.46E+06			
summation intermediate abundant ARG	4.90E+11	2.84E+11	1.05E+11	2.46E+10	6.54E+10	1.34E+11	1.54E+11	9.38E+10	1.19E+11	2.46E+10	4.90E+11
methicillin resistance (<i>mecA</i>)	5.98E+08	2.56E+08	1.51E+08	5.85E+07	2.45E+08	3.65E+08	2.77E+07	2.98E+08			
carbapeneme resistance (<i>bla</i> _{NDM-1})	1.20E+10	5.74E+08	1.87E+08	1.81E+07	5.02E+08	7.60E+07	4.39E+07	4.50E+07			
colistin resistance (<i>mcr-1</i>)	1.60E+09	3.94E+08	2.50E+09	5.99E+07	5.34E+06	1.66E+08	1.41E+08	5.53E+08			
vancomycin resistance (<i>vanA</i>)	1.00E+00	9.79E+07	6.94E+07	1.00E+00	1.82E+07	2.66E+07	1.42E+07	0.00E+00			
summation rarely occurring ARG	1.42E+10	1.32E+09	2.90E+09	1.36E+08	7.71E+08	6.34E+08	2.27E+08	8.96E+08	8.33E+08	1.36E+08	1.42E+10
facultative pathogenic bacteria [cell equivalents/d]											
<i>E. coli</i> (<i>yccT</i>)	2.50E+11	7.47E+10	5.95E+10	7.28E+09	2.04E+10	5.39E+09	1.06E+12	2.76E+11			
enterococci (<i>23S</i> <i>rRNA</i>)	2.41E+11	1.72E+11	8.07E+09	1.40E+08	1.57E+09	4.04E+09	3.17E+08	1.20E+11			
<i>K. pneumoniae</i> (<i>gltA</i>)	7.93E+10	6.20E+10	3.80E+09	3.07E+09	2.02E+09	1.60E+10	1.65E+10	1.83E+11			
<i>A. baumannii</i> (<i>secE</i>)	1.69E+10	2.44E+09	1.89E+09	9.08E+08	1.26E+09	1.00E+00	1.00E+00	3.23E+09			
<i>P. aeruginosa</i> (<i>ecfX</i>)	2.18E+09	3.71E+08	1.73E+08	1.00E+00	3.15E+08	3.26E+07	1.55E+08	2.98E+09			
summation FPB	5.89E+11	3.12E+11	7.34E+10	1.14E+10	2.55E+10	2.55E+10	1.07E+12	5.85E+11	1.93E+11	1.14E+10	1.07E+12

SI Table 4: Full primer sequences of investigated ARGs and FPB with corresponding calibration curve, efficiency and LOD

target	primer sequence	calibration curve	amplicon	efficiency	R ²	LOD	reference strain	literature
facultative pathogenic bacteria								
enterococci	Fwd: AGAAATCCAAACGAACCTTG Rev: CAGTGCTCTACCTCCATCATT	$F(x) = -3.585x + 35.283$	93 bp	90.1 %	1.000	65	<i>E. faecium</i> DSM20477	(Frahm et al. 2003)
<i>P. aeruginosa</i>	Fwd: AGCGTTCGTCCTGCACAAGT Rev: TCCACCATGCTCAGGGAGAT	$F(x) = -3.282x + 35.276$	81 bp	101.7 %	0.999	3	<i>P. aeruginosa</i> DSM1117	(Clifford et al. 2012)
<i>K. pneumoniae</i>	Fwd: ACGGCCGAATATGACGAATTC Rev: AGAGTGATCTGCTCATGAA	$F(x) = -3.387x + 38.844$	68 bp	97.4 %	0.998	18	<i>K. pneumoniae</i> DSM30104	(Clifford et al. 2012)
<i>A. baumannii</i>	Fwd: GTTGTGGCTTTAGGTTTATTATACG Rev: AAGTTACTCGACGCAATTCG	$F(x) = -3.380x + 35.679$	94 bp	97.6 %	1.000	31	<i>A. baumannii</i> DSM30007	(Clifford et al. 2012)
<i>E. coli</i>	Fwd: GCATCGTGACCACCTTGA Rev: CAGCGTGGTGGCAAAA	$F(x) = -3.361x + 35.797$	59 bp	98.4 %	0.994	4	<i>E. coli</i> DSM1103	(Clifford et al. 2012)
antibiotic resistance genes								
<i>ermB</i>	Fwd: TGAATCGAGACTTGAGTGTGCAA Rev: GGATTCTACAAGCGTACCTT	$F(x) = -3.328x + 35.901$	71 bp	100 %	1.000	16	<i>S. hyointestinalis</i> DSM20770	(Alexander et al. 2015)
<i>Int1</i>	Fwd: GCCTTGATGTTACCCGAGAG Rev: GATCGGTGCAATGCGTGT	$F(x) = -3.472x + 34.720$	196 bp	94.1 %	1.000	126	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>tetM</i>	Fwd: GGTTCCTCTTGATACTTAAATCAATC Rev: CCAACCATAAATCCTTGTCRC	$F(x) = -3.424x + 38.747$	88 bp	95.9 %	0.998	4	<i>E. coli</i> DH5α	(Peak et al. 2007)
<i>bla</i> _{TEM}	Fwd: TTCCTGTTTTTGCTCACCCAG Rev: CTCAAGGATCTTACCGCTGTTG	$F(x) = -3.303x + 38.559$	112 bp	100.8 %	0.999	80	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>sul1</i>	Fwd: CGCACCGAAACATCGCTGCAC Rev: TGAAGTTCGCCGCAAGGCTCG	$F(x) = -3.387x + 39.802$	161 bp	97.6 %	0.999	80	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>bla</i> _{CTX-M15}	Fwd: CGCTTGCGATGTGCAG Rev: ACCGCGATATCGTTGGT	$F(x) = -3.504x + 34.255$	551 bp	92.9 %	1.000	93	<i>E. coli</i> pNORM	(Paterson et al. 2003, Rocha et al. 2018)
<i>bla</i> _{CTX-M-32}	Fwd: CGTCACGCTGTTGTTAGGAA Rev: CGCTCATCAGCACGATAAAG	$F(x) = -3.517x + 37.800$	155 bp	92.5 %	1.000	235	<i>E. coli</i> pNORM	(Rocha et al. 2018)
<i>bla</i> _{OXA-48}	Fwd: TGTTTTGGTGGCATCGAT Rev: GTAAMRATGCTTGGTTCGC	$F(x) = -3.540x + 36.913$	177 bp	91.6 %	0.998	92	<i>K. pneumoniae</i> TGH Isolate 2	(Monteiro et al. 2012)

target	primer sequence	calibration curve	amplicon	efficiency	R ²	LOD	reference strain	literature
<i>bla</i> _{CMY-2}	Fwd: CGTTAATCGCACCATCACC Rev: CGTCTTACTAACCGATCCTAGC	F(x)= -3.591x+34.026	172 bp	89.9 %	0.998	71	<i>K. pneumoniae</i> NRZ-01013	(Kurpiel and Hanson 2011)
<i>vanA</i>	Fwd: TCTGCAATAGAGATAGCCGC Rev: GGAGTAGCTATCCCAGCATT	F(x)= -3,541x+33,078	376 bp	91.6 %	1.000	43	<i>E. faecium</i> B7641	(Klein et al. 1998)
<i>mecA</i>	Fwd: CGCAACGTTCAATTTAATTTTGTTAA Rev: TGGTCTTTCTGCATTCTGGA	F(x)=-3,327x+34,887	91 bp	99.8 %	1.000	11	<i>S. aureus</i> A1	(Volkman et al. 2004)
<i>mcr-1</i>	Fwd: GGGCCTGCGTATTTTAAGCG Rev: CATAGGCATTGCTGTGCGTC	F(x)=-3,386x+35,349	183 bp	97.4 %	0.999	8	<i>E. coli</i> NRZ-14408	(Hembach et al. 2017)
<i>bla</i> _{NDM-1}	Fwd: TTGGCCTTGCTGTCCTTG Rev: ACACCAGTGACAATATCACCG	F(x)= -3,293x+35,877	82 bp	101.2 %	0.999	66	<i>K. pneumoniae</i> ATCC BAA-2146	(Monteiro et al. 2012)

SI Table 5: wastewater treatment setup of investigated WWTPs

WWTP acronym	WWTP feed	primary treatment		secondary treatment			
C1	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C2	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment and enhanced biological P-removal	P-elimination	secondary clarification	
C3	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C4	two-pipe drainage system	rake, sedimentation with skimmers	primary clarification	activated sludge treatment	P-elimination	secondary clarification	
C5	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C6	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C7	two-pipe drainage system	rake, sedimentation with skimmers	primary clarification	activated sludge treatment	P-elimination	secondary clarification	

C8	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C9	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C10	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
C11	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
F1	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
F2	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
F3	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
F4	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment and enhanced biological P-removal	P-elimination	secondary clarification	
H1	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
H2	mixed sewer system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
H3	mixed sewer system	rake, sedimentation with skimmers	primary clarification	activated sludge treatment	P-elimination	secondary clarification	
H4	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
H5	two-pipe drainage system	rake, sedimentation with skimmers	primary clarification	activated sludge treatment and enhanced biological P-removal	P-elimination	secondary clarification	
H6	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	

H7	two-pipe drainage system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	
H8	mixed sewer system	rake, sedimentation with skimmers		activated sludge treatment	P-elimination	secondary clarification	cloth filter