

Supporting information for

High throughput analysis of integron gene cassettes in wastewater environments

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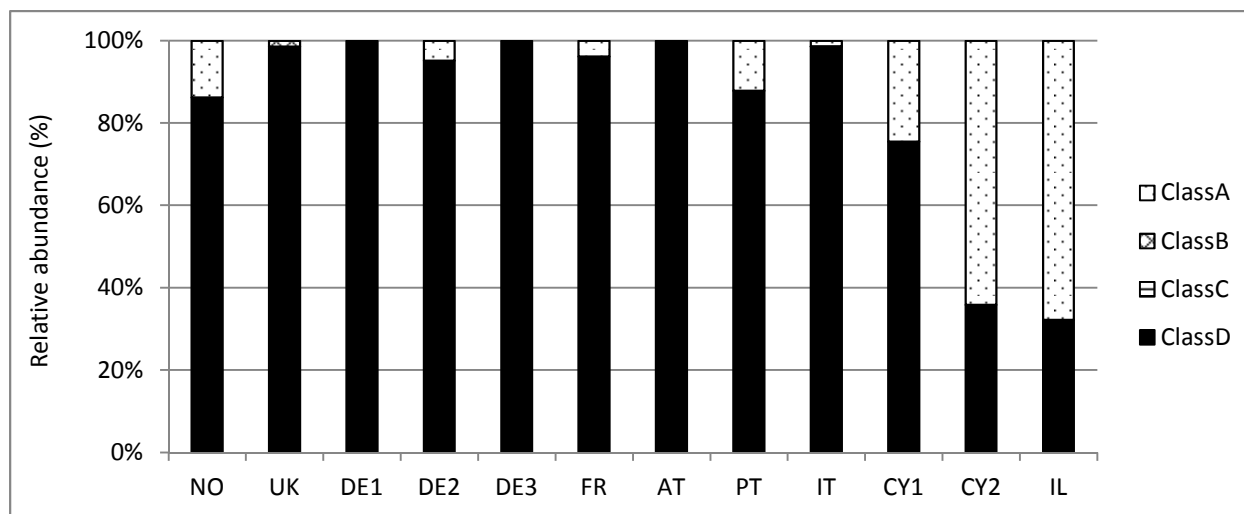
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The SI contains 19 pages of additional information, including 5 figures and 18 tables.

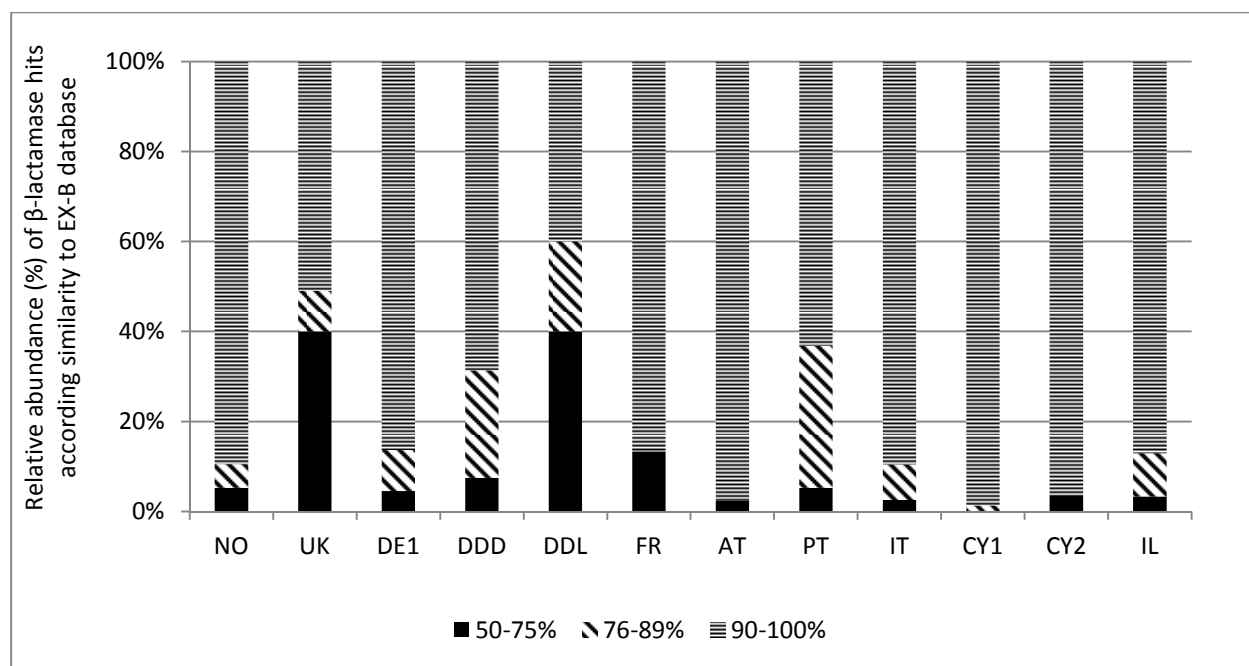
Supplementary figures



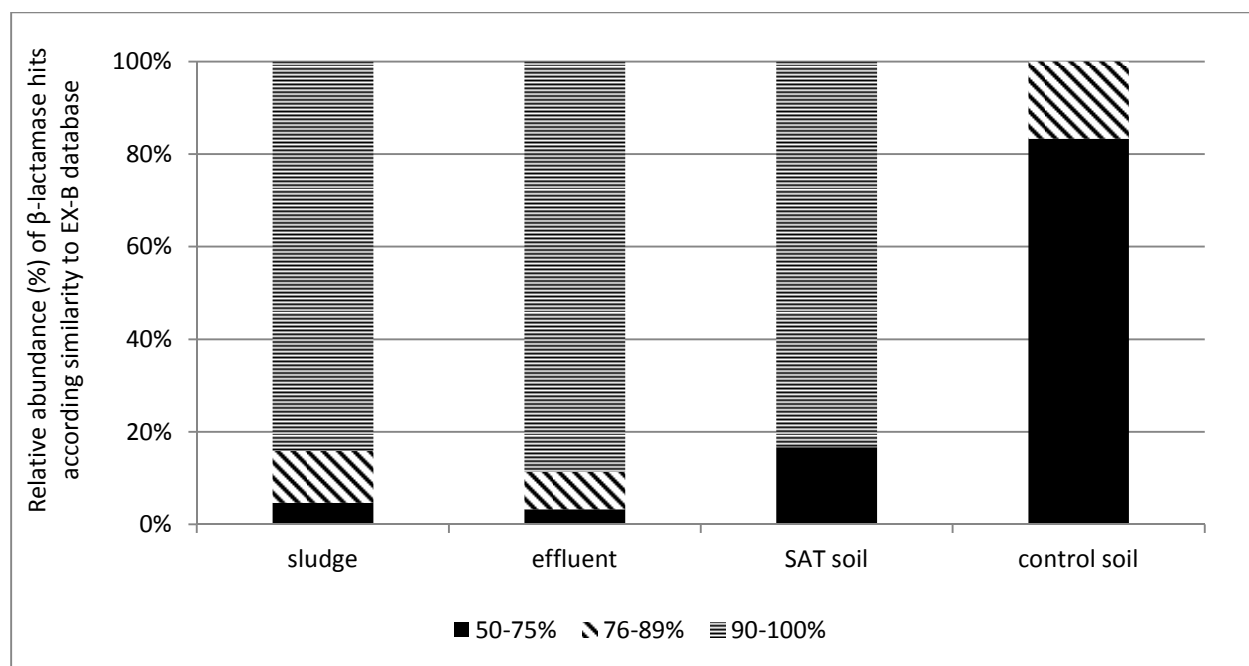
Supplementary Figure S1. Relative abundance of molecular classes of β -lactamases in European WWTP integron gene cassettes.

Gene target		Gel picture
PCR	<i>bla</i> _{OXA-group 2}	
	<i>bla</i> _{OXA-group 10}	
	<i>bla</i> _{GES}	
qPCR	<i>bla</i> _{OXA-group 2}	
	<i>bla</i> _{OXA-group 10}	
	<i>bla</i> _{GES}	

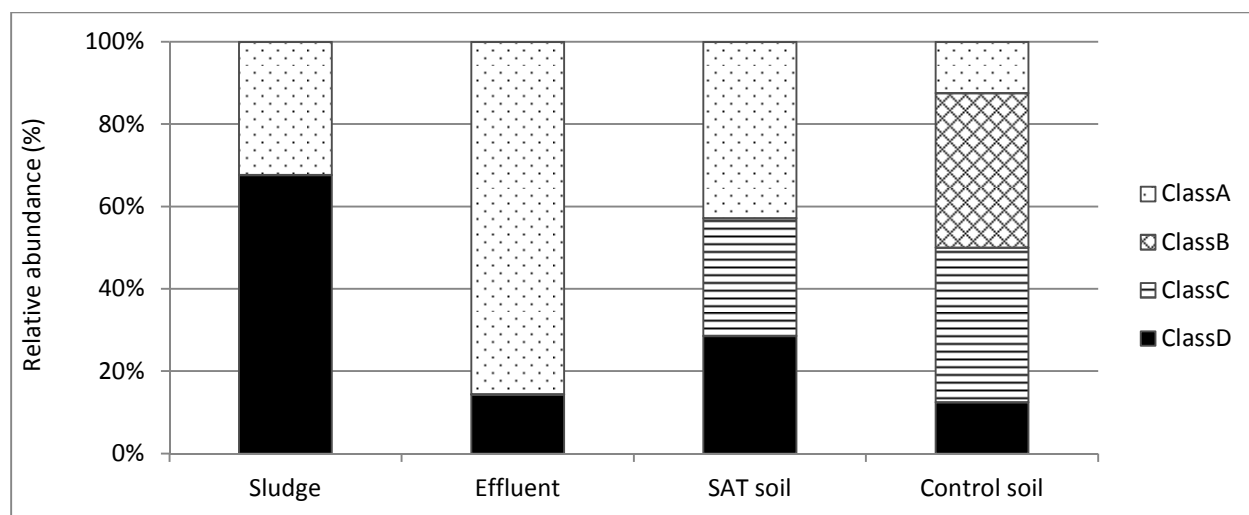
Supplementary Figure S2. Validation experiments showing agarose gels of PCR and qPCR products for *bla*_{OXA} and *bla*_{GES} genes. PCR amplification of Shafdan samples: S. sludge, E. Effluent, I., SAT soil, C. control soil and NC. negative control. qPCR amplification of European WWTP samples: IL. Israel, CY1 and CY2. Cyprus, NO. Norway, DE1, DE2, DE3. Germany, IT. Italy, AT. Austria, UK. England, FR. France, PT. Portugal, NC. negative control and ST: Positive control. M: 1 kb marker in PCR gels and 100 bp marker in qPCR gels.



Supplementary Figure S3. Distribution of β -lactamase hits in the European effluent samples based on % similarity to EX-B database entries.



Supplementary Figure S4. Distribution of β -lactamase hits in the Shafdan samples based on % similarity to EX-B database entries.



Supplementary Figure S5. Relative abundance of molecular classes of β -lactamases in Shafdan integron gene cassettes.

Supplementary Tables

Supplementary table S1. Contigs generated from assembly of integron GC reads performed using $k\text{-mer} = 91$.

Sample	Number of contigs	contigs >500bp	contigs >1000bp	larg. contig	total length (bp)	N50 >500bp	N75 >500bp	GC (%)	Selected Contigs (≥150bp)
NO	23814	211	1	1037	177147	583	537	53.23	9496
UK	7515	226	4	1340	139589	592	537	54.15	3069
DD	5792	141	1	1683	86945	594	549	51.94	2605
DE2	2035	108	4	1484	68187	610	546	51.71	1028
DE3	1878	147	5	1118	92938	613	552	48.20	1163
FR	7307	207	2	1259	126918	598	535	53.63	3063
AT	4892	90	1	1356	55988	592	544	54.38	1822
PT	4407	37	0	930	21943	571	535	52.31	1199
IT	6644	200	4	1233	107403	605	557	49.89	2290
CY1	6870	131	2	1125	79424	594	529	54.91	2441
CY2	687	108	25	2231	87636	820	624	51.98	483
IL	11623	159	6	1170	102674	615	555	52.31	2871
Sludge	7759	102	7	1419	69592	659	590	50.25	1812
Effluent	5802	117	14	2193	83908	691	595	50.00	1654
SAT soil	1878	126	8	1841	86411	666	575	54.19	2338
Control soil	1308	163	29	1756	122434	728	588	52.58	818

Supplementary table S2. Validation of integron gene cassette sequences based on cloning and Sanger sequencing.

GC amplicon name	Closest INTEGRALL BLAST hits (#)	Reference	Sequence length and identity of integron GC hits (bp)
GC1	No detected identities		
GC2	No detected identities		
GC3	Uncultured bacterium ORF1 for putative integron gene cassette protein	Koenig et. al., 2008. A new pool of integron gene cassettes encoding homologs of xenobiotic degrading proteins. unpublished	115/123 (93%)
GC5	<i>Pseudomonas aeruginosa</i> blaOXA-20	Mazzariol et. al., 2009. Class 1 integron bearing VIM-14 metallo-beta-lactamase. unpublished	794/801 (99%)

Supplementary Table S3. Primers and PCR conditions applied in this study.

Primer	Sequence (5' to 3')	Target gene (including gene variants)	Product size (bp)	Reaction (50µl)	Conditions	Reference
HS286	TCSGCTKGARCGAMTTGTTAGVC	Class 1 integron 59-base element family of recombination sites	variable	10 ng of template DNA, 100 pmol of each primer, 200 nM deoxynucleoside triphosphate (dNTP) mix, 2 mM MgCl ₂ , and 1 U of Dream Taq DNA polymerase (Thermo Scientific) in the reaction buffer supplied with the enzyme	94°C for 3', 30 cycles of 94°C for 30'', 55°C for 30'', 72°C for 2', and 72°C for 5'	Stokes et al., 2001
HS287	GCSGCTKANCTCVRRCGTTAGSC					
OXA-2F	AAGAAACGCTACTCGCCTGC	b-lactamases: <i>blaOXA2</i> , <i>blaOXA3</i> , <i>blaOXA210</i> , <i>blaOXA32</i> , <i>blaOXA34</i> , <i>blaOXA53</i> , <i>blaOXA121</i> , <i>blaOXA141</i> , <i>blaOXA161</i> , <i>blaOXA210</i> , <i>blaOXA226</i> , <i>blaOXA415</i>	478	Each 25µL reaction mixture contained 12.5 µL Fast SYBR green Master mix (Thermo Fisher Scientific), 1 µL of each primer (10µM), 2 µL of DNA template and 8.5 µL ultra pure PCR grade water.	Regular PCR: 96°C for 5', 30 cycles of 96°C for 30'', 55°C for 30'', 72°C for 1', and 72°C for 5' qPCR: 95°C for 10', 40 cycles of 95°C for 10'', 60°C for 30''	Bert et al., 2002
OXA-2R	CCACTCAACCCATCCTACCC					
OXA-10F	TCAACAAATCGCCAGAGAAG	β-lactamases: <i>blaOXA7</i> , <i>blaOXA10</i> , <i>blaOXA13</i> , <i>blaOXA14</i> , <i>blaOXA16</i> , <i>blaOXA17</i> , <i>blaOXA19</i> , <i>blaOXA28</i> ,	276	Each 25µL reaction mixture contained 12.5 µL Fast SYBR green Master mix (Thermo Fisher Scientific), 1 µL of each primer (10µM), 2 µL of DNA template and 8.5 µL ultra pure PCR grade water.	96°C for 5', 30 cycles of 96°C for 30'', 50°C for 30'', 72°C for 1', and 72°C for 5'.	Bert et al., 2002

OXA-10R	TCCCACACCAGAAAAACCAG	<i>blaOXA35,</i> <i>blaOXA56,</i> <i>blaOXA74,</i> <i>blaOXA101,</i> <i>blaOXA128,</i> <i>blaOXA129,</i> <i>blaOXA145,</i> <i>blaOXA233,</i> <i>blaOXA240,</i> <i>blaOXA246</i>			qPCR: 95°C for 10', 40 cycles of 95°C for 10'', 60°C for 30''	
GES-F	GCGGGTTTTCTAAAGATTGG	β-lactamases: <i>blaGES1,</i> <i>blaGES2,</i> <i>blaGES3,</i> <i>blaGES4,</i> <i>blaGES5,</i> <i>blaGES6,</i> <i>blaGES7,</i> <i>blaGES11,</i> <i>blaGES13,</i> <i>blaGES14,</i> <i>blaGES16,</i> <i>blaGES17,</i> <i>blaGES18,</i> <i>blaGES22,</i> <i>blaGES23,</i> <i>blaGES24,</i> <i>blaGES25,</i> <i>blaGES26,</i> <i>blaGES27,</i> <i>blaGES28,</i> <i>blaGES29</i>	196	Each 25μL reaction mixture contained 12.5 μL Fast SYBR green Master mix (Thermo Fisher Scientific), 1 μL of each primer (10μM), 2 μL of DNA template and 8.5 μL ultra pure PCR grade water.	95°C for 5', 30 cycles of 94°C for 30'', 52°C for 30'', 72°C for 1', and 72°C for 10' qPCR: 95°C for 10', 40 cycles of 95°C for 10'', 52°C for 30''	Designed according <i>blaGES</i> sequences of EX-B database
GES-R	AATAACTTGACCGACAGAGGC					

Supplementary Table S4. Number of high quality reads and β -lactamase hits obtained from European WWTP integron GCs.

	WWTPs											
	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
N of reads (after quality control)	172995	81456	93596	41662	51612	104358	89287	81271	90456	104520	38100	93227
β-lactamases hits	19	55	22	67	5	15	41	19	941	77	28	302
% of β-lactamases hits in filtered data	0.010	0.067	0.023	0.160	0.009	0.014	0.045	0.023	1.040	0.073	0.073	0.323

Supplementary Table S5. Number of high quality reads and β -lactamase hits obtained from Shafdan WWTP integron GCs.

	Shafdan Sites			
	sludge	effluent	SAT soil	Control soil
N of reads (after quality control)	130024	113048	124241	99204
β-lactamases hits	1200	4342	6	6
% of β-lactamases hits in filtered data	0.922	3.840	0.004	0.006

Supplementary Table S6. Prominent integron-associated genes in European WWTP effluents based on comparison to the nr database.

		NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
Unknown Function	Integron gene cassette protein	60055	23276	24112	6708	7784	11010	42556	10827	2665	4362	994	2390
	Hypothetical protein	8505	7240	10681	6630	7403	13089	5381	6653	11205	11407	5367	6751
	Cupin	5	9	7	7	18	78	31	0	7	757	0	44
	Aldehyde-activating protein	19	10	49	27	27	17	61	10	14	47	0	34
Membrane Protein	Membrane protein	210	68	39	277	165	180	62	96	148	190	214	339
antibiotic resistance and detoxification	Antibiotic biosynthesis monooxygenase	27	7	241	69	55	100	304	242	48	164	0	61
	Aminoglycoside acetyltransferase	344	81	125	171	509	199	142	50	298	118	71	124
	Beta-lactamase	14	32	19	50	2	11	37	13	673	60	26	178
	Glyoxalase	150	134	179	58	114	145	67	15	100	40	20	334
Toxin and Antitoxin	Antitoxin	0	95	146	87	100	43	84	14	80	110	0	39
	Killer protein	0	53	51	4	5	18	11	40	28	55	0	6
Motility and chemotaxis	Twitching motility protein PilT	8	162	91	53	8	9	72	15	35	140	0	17
Regulation	Diguanylate cyclase	0	13	0	8	8	0	20	13	43	2	61	0
	Histidine kinase	0	9	0	4	12	0	3	6	29	5	109	6
	Transcriptional regulator	18	138	56	75	42	71	81	65	163	667	386	142
Transport	ABC transporter	4	36	0	37	6	5	15	57	113	7	255	55
Mobile element	Transposase	12	30	12	12	12	6	19	20	69	13	47	42
DNA replication, transport and repair	DNA-binding protein	3	6	5	47	31	94	1	3	41	32	0	83
	Pyrimidine dimer DNA glycosylase	0	5	8	0	0	6	39	0	6	36	39	19
	DNA lyase	0	6	9	0	0	0	43	4	10	56	0	50

Supplementary Table S7. Prominent integron-associated genes in European WWTP effluents based on comparison to the SEED database.

		NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
Antibiotic Resistance and Biosynthesis	Beta lactamase	21	53	23	61	10	18	41	20	944	80	42	286
	aminoglycoside acetyltransferase	187	313	294	212	452	272	232	105	335	197	46	86
	Streptomycin3''-O-adenylyltransferase	2	0	3	5	1	4	4	9	1211	28	43	43
	Glyoxalase	832	197	244	25	88	72	78	22	106	86	14	447
	Lactoylglutathionelyase	22	188	33	14	45	58	40	9	32	3	5	32
	Dihydrofolate reductase	3	1	3	4	88	1	4	0	23	48	9	10
Membrane Associated	Membrane proteins	30	30	199	31	115	104	33	32	94	31	91	63
Toxin and Antitoxin	StbD replicon	2	25	86	4	6	18	95	14	43	35	86	5
	Toxin Protein	13	218	328	56	119	75	52	92	91	341	23	69
Unknown Functions	LeukotrieneC4	181	0	0	0	2	5	1	0	1	0	0	1
	Cupin	3	0	0	4	0	1	2	1	2	16	1	35
	GII1166 proteins	0	1	216	104	4	124	6	4	23	0	1	1
	Gfa-like protein	10	4	31	36	25	72	75	17	13	45	7	24
	Hypothetical protein	3552	5783	6344	3927	3498	4662	4374	2759	3463	4735	2668	3903
DNA repair	Pyrimidine Dimer	4	14	18	7	1	18	87	14	18	96	23	86
Phages and Mobile elements	Mobile element	8	30	14	18	14	8	20	18	58	0	60	25
	Phage Protein	107	63	29	56	84	193	45	100	255	39	311	69
	CRISPR-associated RAMP	0	3	0	0	0	10	1	0	2	0	163	1
	Transposes	8	84	0	48	42	23	18	20	148	0	49	40
Motility and Chemotaxis	PilT protein	0	168	99	53	8	12	85	15	34	150	1	17
Transcriptional regulations	Translational Elongation	3	8	3	3	2	0	5	10	0	75	43	4
	Transcriptional anti-terminator	27	7	126	100	69	123	136	204	27	160	0	16

Supplementary Table S8. Prominent integron-associated antibiotic resistance genes in European WWTP effluents based on comparison to the ARDB database

	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
aminoglycoside 6''-N-acetyltransferase	258	0	0	4	3	4	0	0	24	131	0	0
aminoglycoside adenyltransferase	1	0	2	2	1	3	3	3	1092	21	34	15
hypothetical protein	0	0	1	1	0	0	1	0	3	2	6	2
multidrug efflux	1	0	0	0	0	0	1	1	4	1	6	3
beta-lactamase	15	43	21	51	3	15	36	16	681	72	20	230
Dihydrofolate Reductase	1	0	2	3	80	1	2	0	12	44	0	8
Tetracycline	0	0	0	1	0	0	0	0	0	1	1	0
VanHB	0	0	0	0	0	0	0	0	0	1	2	0
ABC transporter	0	0	0	0	0	0	0	0	0	0	2	1
aminoglycoside resistance	0	0	0	0	0	0	0	0	0	0	4	0
macrolide transporter	2	2	0	2	0	0	0	2	5	0	8	1
penicillin-binding protein	0	0	0	0	1	0	0	0	1	0	5	0
catB2	0	0	0	1	0	0	0	0	0	0	6	0
Fused aminoglycoside	0	0	0	0	0	0	0	7	11	0	0	27
ATP-binding protein	0	0	0	0	0	0	0	0	0	0	0	2
aminoglycoside resistance	29	0	0	0	0	0	0	0	22	0	0	0

Supplementary Table S9. Prominent integron-associated β -lactamases in European WWTP effluents based on comparison to the EX-B database.

	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
β-lactamase	0	0	0	0	0	0	0	0	1	0	0	0
classD	9	25	1	21	2	5	27	5	834	44	6	42
blaBEL	0	0	0	1	0	0	0	0	12	1	0	0
blaGES	2	0	0	1	0	0	0	2	2	19	19	196
blaIMP	0	0	0	0	0	0	0	0	1	0	0	0
blaOKPA	0	0	0	0	0	0	0	0	1	0	0	0
blaOXA	8	30	22	44	3	10	14	12	90	13	3	64

Supplementary table S10. Classification of β -lactamase genes according to molecular classes.

Molecular class	Genes
Class A	blaTEM, blaSHV, blaCTX-M, blaKPC, blaGES, blaSME, blaIMI, blaPER, blaVEB, blaCphA, blaBEL, blaCARB, blaLEN, blaOKP, blaPSE, blaTOHO, BlaZ, blaCME, blaCFXA, blaKLUY, blaFONA, blaKLUA, blaOXY
Class B	Metallo-b-lactamases, blaIMP, blaVIM, blaNDM, blaIND, blaCcrA, blaGOB, blaFEZ, blaCAU, CphA, blaSIM, blab, blaEBR, blaJHON, blaSPM, blaCcrA, blaKHM, blaTUS, blaLRA, blaCAU, blaTHIN-B
Class C	AmpC, blaCMY, blaACT, blaACC, blaFOX, blaMIR, cephalosporinases, blaLAT, blaDHA, blaMOX, blaSRT, blaCFE
Class D	blaOXA, blaLCR

References:

1. K Bush and G Jacoby. Updated functional classification of β -lactamases. Antimicrobial agents and chemotherapy. 54:969-976 (2010).
2. A Srivastava, N Singhal, M Goel, J Viridi, M Kumar. CBMAR: a comprehensive β -lactamase molecular annotation resource. Database 1-8 (2014).

Supplementary Table S11. Closest BLAST identities of gene cassette amplicon-associated *bla*OXA gene variants in European effluents and Shafdan samples.

group	Variant	Shafdan WWTP				European WWTPs											
		Sludge	Effluent	SAT soil	Control soil	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
<i>bla</i> OXA-10 group	<i>bla</i> OXA10	2	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	<i>bla</i> OXA129	141	114	0	0	3	4	2	7	2	5	2	0	18	1	2	13
	<i>bla</i> OXA145	26	20	0	0	1	0	1	0	1	0	0	0	6	0	0	2
	<i>bla</i> OXA17	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0
	<i>bla</i> OXA28	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA7	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA74	39	88	0	0	2	11	0	0	0	0	0	1	3	4	0	18
	<i>bla</i> OXA240	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>bla</i> OXA-2 group	<i>bla</i> OXA2	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
	<i>bla</i> OXA21	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA3	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Other groups	<i>bla</i> OXA18	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA19	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA251	13	29	0	0	0	1	0	0	0	0	0	0	3	4	0	1
	<i>bla</i> OXA256	1	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	<i>bla</i> OXA34	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA36	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	<i>bla</i> OXA37	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0
	<i>bla</i> OXA5	322	337	0	0	1	9	18	37	0	5	10	8	52	1	0	28
	<i>bla</i> OXA50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
	<i>bla</i> OXA53	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	<i>bla</i> OXA56	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	<i>bla</i> OXA85	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Supplementary Table S12. Closest BLAST identities of gene cassette amplicon-associated *bla*GES gene variants in European effluents and Shafdan samples.

Variant	Shafdan WWTP				European WWTPs											
	Sludge	Effluent	SAT soil	Control soil	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY2	CY1	IL
<i>bla</i> GES2	78	682	0	0	0	0	0	0	0	0	0	0	0	0	1	26
<i>bla</i> GES3	35	223	0	0	0	0	0	0	0	0	0	2	0	1	2	21
<i>bla</i> GES4	0	16	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>bla</i> GES6	2	56	0	0	0	0	0	0	0	0	0	0	0	5	1	4
<i>bla</i> GES7	13	105	0	0	0	0	0	0	0	0	0	0	0	0	0	8
<i>bla</i> GES8	255	2554	3	0	2	0	0	1	0	0	0	0	2	13	14	135
<i>bla</i> GES10	2	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>bla</i> GES12	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>bla</i> GES15	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>bla</i> GES18	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>bla</i> GES19	1	18	0	1	0	0	0	0	0	0	0	0	0	0	0	1
<i>bla</i> GES21	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table S13. Prominent integron GC genes in the Shafdan samples based on comparison to the nr database.

		Sludge	Effluent	SAT soil	Control soil
Unknown Function	Hypothetical protein	6182	10565	11385	9543
	Integron gene cassette protein	2509	1574	2753	148
	Aldehyde-activating protein	29	312	16	0
	HIT family protein	24	20	0	0
	Lipoprotein	24	3	3	21
	Cupin	21	24	1075	287
Membrane	Membrane protein	1105	733	78	249
Antibiotic resistance and detoxification	Lactoylglutathione lyase family protein	17	20	0	0
	Glyoxalase	785	935	35	73
	Beta-lactamase	654	2586	3	23
	Aminoglycoside acetyltransferase	177	141	62	209
	Antibiotic biosynthesis monooxygenase	30	24	1080	26
Motility and chemotaxis	Twitching motility protein PilT	145	41	32	0
	Pilus assembly protein HicB	285	30	0	11
DNA replication, transport and repair	DNA lyase	110	37	6	0
	Pyrimidine dimer DNA glycosylase	74	44	9	0
Stress response	Molecular chaperone DnaJ	126	92	4	51
Regulation	Transcriptional regulator	140	93	310	515
Transport	ABC transporter ATP-binding protein	25	24	116	680
Mobile element	Transposase	22	19	26	117
Toxin and Antitoxin	Killer protein	17	173	0	22
	Toxin	3	0	74	85

Supplementary Table S14. Prominent integron GC genes in the Shafdan samples based on comparison to the SEED database.

		Sludge	Effluent	SAT soil	Control soil
Antibiotic Resitance and Biosynthesis	Beta lactamase	542	2081	0	9
	Aminoglycoside acetyltransferase	105	20	21	11
	Streptomycin3''-O-adenylyltransferase	28	27	0	0
	Glyoxalase	592	616	48	22
	Dihydrofolate reductase	4	4	36	107
Membrane Associated	Membrane proteins	47	9	0	15
Phage and Mobile Elements	Mobile element	29	15	10	54
	Transposes	30	15	23	29
	Phage Protein	0	24	17	12
Toxin and Antitoxin	Toxin Protein	22	17	442	0
Unknown Functions	Cupin	13	28	0	41
	Gfa-like protein	14	8	0	0
	Hypothetical protein	3419	4836	6846	3147
DNA repair	Pyrimidine Dimer	133	36	0	0
Motility and Chemotaxis	PilT protein	79	18	0	0
Transcriptional regulations	Translational Elongation	5	4	17	20
	Transcriptional regulators	5	10	60	197

Supplementary Table S15. Prominent integron GC antibiotic resistance genes in Shafdan samples based on comparison to the ARDB database.

	Sludge	Effluent	SAT soil	Control soil
Beta-lactamase	1549	5696	3	24
Aminoglycoside adenytransferase	69	80	0	2
Quinolone resistance	14	6	1	8
Hypothetical protein	11	7	16	59
Macrolide resistance	2	4	17	105
Multidrug resistance	2	3	4	33
Vancomycin resistance	2	4	8	51
Aminoglycoside-3N-acetyltransferase	0	6	13	10
Dihydrofolate reductase	0	10	0	0
Penicilline binding protein	0	0	0	18

Supplementary Table S16. Prominent integron GC β -lactamases in Shafdan samples based on comparison to the EX-B database.

	Sludge	Effluent	SAT soil	Control soil
β-lactamase	1	0	1	1
blaGES	388	3717	3	1
blaOXA	557	598	0	1
class D	254	27	2	0
blaB	0	0	0	1
blaGOB	0	0	0	1
metallo-β-lactamase	0	0	0	1

Supplementary Table S17. Distribution of β -lactamase hits based on % similarity to EX-B database entries in the European WWTP effluent samples.

	NO	UK	DE1	DE2	DE3	FR	AT	PT	IT	CY1	CY2	IL
Number of hits between 90-100% of similarity	17	28	19	46	2	13	40	12	843	76	27	263
Number of hits between 76-89% of similarity	1	5	2	16	1	0	0	6	74	1	0	29
Number of hits between 50-75% of similarity	1	22	1	5	2	2	1	1	24	0	1	10
Percent of hits between 90-100% similarity	89.47	50.90	86.36	68.65	40	86.6	97.56	63.15	89.58	98.70	96.42	87.08
Percent of hits between 76-89% similarity	5.26	9.09	9.09	23.88	20	0	0	31.5	7.86	1.29	0	9.60
Percent of hits between 50-75% similarity	5.26	40	4.54	7.46	40	13.3	2.43	5.26	2.55	0	3.57	3.31

Supplementary Table S18. Distribution of β -lactamase hits based on % similarity to EX-B database entries in the Shafdan samples.

	sludge	effluent	SAT soil	control soil
Number of hits between 90-100% of similarity	1010	3849	5	0
Number of hits between 76-89% of similarity	134	350	0	1
Number of hits between 50-75% of similarity	56	143	1	5
Percent of hits between 90-100% similarity	84.16	88.64	83.3	0
Percent of hits between 76-89% similarity	11.16	8.06	0	16.6
Percent of hits between 50-75% similarity	4.66	3.29	16.6	83.3