Supporting information

Table S1 PCR and qPCR primers for target *tet* genes, *sul* genes, integrons and the 16S rRNA gene

Target gene	Primer	Primer sequence	Annealing Temperature (℃)	Amplicon length (bp)	Ref.
*tet*A	*tet*A-F	GCTACATCCTGCTTGCCTTC	60	210	(Ng et al., 2001)
	*tet*A-R	CATAGATCGCCGTGAAGAGG			
*tet*B	*tet*B-F	GGCAGGAAGAATAGCCACTAA	63	151	(Ng et al., 2001)
	*tet*B-R	AGCGATCCCACCACCAG			
*tet*C	*tet*C-F	CTTGAGAGCCTTCAACCCAG	60	418	(Ng et al., 2001)
	*tet*C-R	ATGGTCGTCATCTACCTGCC			
*tet*G	*tet*G-F	GCACGCTGGTTTGGCTACA	56	176	(Mao et al., 2015)
	*tet*G-R	TGGCTGTGATTAGTCTCCTTGA			
*tet*M	*tet*M-F	ACAGAAAGCTTATTATATAAC	55	171	(Luo et al., 2010)
	*tet*M-R	TGGCGTGTCTATGATGTTCAC			
*tet*O	*tet*O-F	GATGGCATACAGGCACAGACC	57	172	(Luo et al., 2010)
	*tet*O-R	GCCCAACCTTTTGCTTCACTA			
*tet*Q	*tet*Q-F	AGAATCTGCTGTTTGCCAGTG	62	169	(Aminov et al., 2001)
	*tet*Q-R	CGGAGTGTCAATGATATTGCA			
*tet*S	*tet*S-F	GAAAGCTTACTATACAGTAGC	50	169	(Roberts, 2005)
	*tet*S-R	AGGAGTATCTACAATATTTAC			
sul1	*sul1*-F	CACCGGAAACATCGCTGCA	55	158	(Luo et al., 2010)
	sul1-R	AAGTTCCGCCGCAAGGCT			
sul2	*sul2*-F	CTCCGATGGAGGCCGGTAT	60	190	(Luo et al., 2010)
	sul2-R	GGGAATGCCATCTGCCTTGA			
intI 1	*intI* 1-F	GGCTTCGTGATGCCTGCTT	57	145	(Luo et al., 2010)
	intI 1-R	CATTCCTGGCCGTGGTTCT			
16S r RNA gene	1369F	CGGTGAATACGTTCYCGG	55	143	(Gaze et al., 2011)

Table S2 Standard curves of ARGs

Genes	Standard curve[a)]	R^2	Amplification efficiency (%)
*tet*A	$Y = -3.522 \lg X + 45.824$	0.997	92.3
*tet*B	$Y = -3.437 \lg X + 47.068$	0.998	95.4
*tet*C	$Y = -3.554 \lg X + 44.867$	0.998	91.1
*tet*G	$Y = -3.206 \lg X + 43.497$	0.998	105.0
*tet*M	$Y = -3.437 \lg X + 45.875$	0.997	95.4
*tet*O	$Y = -3.463 \lg X + 45.465$	0.998	94.4
*tet*Q	$Y = -3.371 \lg X + 44.800$	0.998	98.0
*tet*S	$Y = -3.465 \lg X + 45.604$	0.997	94.4
*sul*I	$Y = -3.387 \lg X + 47.095$	0.998	97.4
*sul*II	$Y = -3.377 \lg X + 43.838$	0.998	97.7
*intI*1	$Y = -3.323 \lg X + 44.530$	0.997	99.9
16s rRNA gene	$Y = -3.234 \lg X + 43.877$	0.997	103.8

Notes: a) Y represents cycle thershold (Ct), and X represents copies

Table S3 Log reductions of ARGs and *intI*1 during pretreatments and anaerobic digestion experiments [a)]

Treatments		tetracycline								sulfonamide		MGEs
		*tet*A	*tet*B	*tet*C	*tet*G	*tet*M	*tet*O	*tet*Q	*tet*S	*sul*I	*sul*II	*intI*1
Pre-treatment	AP	+ 0.40	+ 2.03	+ 0.65	+ 0.37	+ 0.81	+ 0.03	–0.08	+ 0.95	+ 0.49	+ 0.20	+ 0.43
		(0.06)	(0.05)	(0.06)	(0.06)	(0.10)	(0.04)	(0.06)	(0.08)	(0.05)	(0.07)	(0.09)
	THP	–3.30	–7.67	–3.34	–3.40	–8.87	–9.04	–9.08	–7.97	–3.77	–4.04	–3.68
		(0.18)	(0.04)	(0.15)	(0.02)	(0.09)	(0.06)	(0.01)	(0.10)	(0.06)	(0.06)	(0.01)
	UP	+ 0.02	+ 0.07	+ 0.07	+ 0.13	+ 0.04	–0.03	–0.17	–0.03	+ 0.13	+ 0.17	+ 0.03
		(0.04)	(0.06)	(0.02)	(0.05)	(0.08)	(0.06)	(0.00)	(0.08)	(0.04)	(0.13)	(0.01)
Anaerobic digestion	RS-AD	–0.85	+ 0.87	–0.79	–0.57	+ 0.93	+ 1.03	+ 0.98	–0.15	–0.73	–0.83	–1.28
		(0.02)	(0.06)	(0.19)	(0.03)	(0.03)	(0.02)	(0.04)	(0.07)	(0.02)	(0.04)	(0.02)
	AP-AD	–1.11	–0.77	–0.86	–0.44	+ 0.03	+ 1.12	+ 1.09	–1.26	–1.08	–0.84	–1.49
		(0.03)	(0.02)	(0.02)	(0.02)	(0.06)	(0.06)	(0.09)	(0.07)	(0.04)	(0.04)	(0.04)
	THP-AD	–0.16	+ 1.86	–0.06	–0.48	+ 0.24	+ 0.63	+ 1.58	–0.33	–0.26	–0.12	–0.57
		(0.03)	(0.04)	(0.05)	(0.03)	(0.07)	(0.05)	(0.08)	(0.04)	(0.06)	(0.07)	(0.06)
	UP-AD	–0.53	+ 0.89	–0.53	–0.70	+ 0.58	+ 0.47	+ 1.25	–1.39	–1.04	–0.93	–1.35
		(0.03)	(0.01)	(0.05)	(0.02)	(0.05)	(0.04)	(0.06)	(0.07)	(0.09)	(0.03)	(0.06)

Notes: a) Standard deviations are shown in the parenthesis; “+”: the gene abundance increased through the pretreatments and anaerobic digestion experiments; “–”: the gene abundance decreased through the pretreatments and anaerobic digestion experiments; the values were achieved by log10 of each gene minus the value on the initial

Table S4 Pearson correlation coefficients between the Target genes (*tet*M, *tet*Q and *intI*1) and predominant phyla and classes

Target genes	Parameters	Predominant phyla			Predominant classes		
		Proteobacteria	*Bacteroidetes*	*Firmicutes*	*Betaproteobacteria*	*Bacteroidia*	*Clostridia*
*tet*M	r [a)]	–0.859**	0.875**	0.822**	–0.863**	0.881**	0.827**
	p [b)]	0.001	0.000	0.002	0.001	0.000	0.002
*tet*Q	r	–0.861**	0.904**	0.914**	–0.809**	0.896**	0.914**
	p	0.001	0.000	0.000	0.003	0.000	0.000
*intI*1	r	0.779**	–0.742**	–0.702*	0.769**	–0.721**	–0.682*
	p	0.003	0.006	0.011	0.003	0.008	0.014

Notes: a) r: correlation coefficient; b) p: p-value, *$p < 0.05$; **$p < 0.01$

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