Impact of ZnO nanoparticles on the antibiotic resistance genes (ARGs) in estuarine water: ARG variations and their association with the microbial community

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Supporting Information

1. Sampling sites and estuarine water sample analysis along the Yangtze Estuary

Water samples were collected along the Yangtze Estuary at three sampling sites (Fig. S1), and the physicochemical properties of these three estuarine samples were shown in Table S1.



Figure S1. Sampling sites along the Yangtze Estuary.

Table S1. Physicochemical properties of three estuarine samples	

Sampling sites	ites Salinity Conductivity (‰) (µs/cm)		рН	DO (mg/L)	ORP (mV)	DOC (mg/L)
SDK	0.15	516	7.22	4.03	106.9	15.6
СҮ	1.44	4128	7.66	5.36	86.4	6.7
DH	3.38	6344.67	7.47	4.45	-142.4	7.4

Notes: DO, dissolved oxide; OPR, oxidation-reduction potential; DOC, dissolved organic carbon.

2. Characterization of ZnO NPs by TEM



Figure S2. Representative TEM images, EDX and SAED analysis of ZnO NPs particles.

3. Preliminary experiment

Preliminary experiment had been conducted in SDK water samples for long-term effect (up to 84 h) of ZnO NPs on the native cultivable bacteria. The results showed that the total bacteria viability had a clear time-dependent variation (Figure S3). At 12 h, ZnO NPs exhibited strong growth inhibition to bacteria and the viability of the bacteria at ZnO NPs concentration of 7.5mg/L, 25mg/L and 50mg/L were 42%, 19% and 18%, respectively. However, there was a growth recovery trend after 24h exposure time. The dramatic culturability variation of cultivable bacteria at different ZnO NP doses appears within 24h.



Figure S3. Cell viability assessment of native cultivable bacteria treated with 0, 7.5, 25 and 50 mg/L ZnO NPs at 12, 24, 48, 72 and 84 hours in SDK water sample.

4. Illumina MiSeq sequencing

The V4–V5 region of the 16S rRNA gene was amplified with the forward primer 515F (5'-GTGCCAGCMGCCGCGG-3') and reverse primer 907R (5'-CCGTCAATTCMTTTRAGTTT-3'), where the barcode is an eight-base sequence unique to each sample.¹ After DNA extraction, Polymerase chain reaction (PCR) was conducted in a 20 μ L reaction mixture containing 4 μ L of 5× FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of each primer (5 μ M), 0.4 μ L of FastPfu Polymerase, and 10 ng of template DNA following the cycling parameters: 2 min for 95 °C, 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and a final elongation at 72 °C for 10 min. After being purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using the QuantiFluorTM-ST system (Promega, USA), the amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform at Personal Biotechnology Co., Ltd., Shanghai, China, according to the standard protocols. The original paired Illumina MiSeq reads were deposited in the NCBI sequence read archive (accession number: SRP188045).

5. Quantitative PCR (qPCR)

5.1 PCR amplification and Plasmids construction

The PCR amplification was performed in 25 μ L reaction mixture containing 0.5 μ L of each primer (10 μ M), 1 μ L of template DNA, 12.5 μ L of Taq PCR Master Mix (2×, with blue dye), and sterile distilled water to a final volume of 25 μ L. And thermal cycling was conducted under the following conditions: an initial denaturing step at 94 °C for 10min, a touch-down thermal cycling of denaturation at 94 °C for 1 min, annealing temperature and time were listed in the Supporting Information (Table S2) and elongation at 72 °C for 0.5-1.0 min with 35 cycles. Finally, an extension step was carried out at 72 °C for 10 min. PCR products and their sizes were verified by agarose gel electrophoresis (1.0% weight/volume agarose) with ethidium bromide staining in 1× TAE buffer at 120 V for 20 min and visualized using an ultraviolet (UV) transilluminator. After that, the target gene fragments were purified using the Universal DNA purification Kit (Tiangen, China) and connected onto the pMD®19-T Vector, which conjugated into *Escherichia coli* DH5 α . Then, plasmids were extracted from *Escherichia coli* DH5 α using a SanPrep plasmid Mini Preparation Kit (Sangon, China) for standard curve construction. Plasmid DNA concentrations

were measured using a Nanodrop-2000 Spectrophotometer (Thermo, USA), and used as standards in a 10-fold dilution for making qPCR standard curve.

5.2 Copy number calculation

The calculation of the copy number was conducted as follows²: (1) The corresponding copy number of the six 10-fold serial dilution series of the target gene plasmid was calculated as follows: copy number μ l-1 = (A × 6.022 × 1023) (660 × B) ⁻¹, where A is the plasmid DNA concentration (g μ l-1), B is the plasmid length (bp) containing the cloned sequence, 6.022 × 1023 is Avogadro's number, and 660 is the average molecular weight of one base pair. (2) The six values (copy number of the 10-fold serial dilution series of the target gene plasmid) obtained from step (1) were subjected to log10 transformation. (3) The standard curve was generated through a linear regression of the six log10-transformed copy numbers (x-axis) and their corresponding CT values (y-axis). (4) The absolute number of the target genes in the experimental samples was derived after normalization to the corresponding standard curves.

5.3 qPCR thermocycling steps

qPCR was performed in a total volume of 25 μ L reaction volume containing 12.5 μ L of 2×SGExcel FastSYBR Mixture (with ROX) (Sangon, China), 1 μ L of standard plasmid or DNA extract, 0.5 μ L of each primer (10 μ M) and 10.5 μ L sterile distilled water. The thermocycling steps for qPCR amplification were as follows: 2min for 50°C,10 min for 95°C, followed by 40 cycles: 95°C for 30s, 53 -60°C for 30s, and 72°C for 0.5- 1min, the melt curve stage with temperature ramping from 66 to 95 °C (0.5 °C/5s). Primer specificity was confirmed by melting curves and gel electrophoresis. Primers, annealing temperature used in qPCR and the corresponding amplification efficiencies were summarized in Tables S2 and S3, respectively. Each gene was quantified in triplicate for each sample using a standard curve and a negative control. Negative controls containing no template DNA were subjected to detect and exclude any possible contamination. The target genes abundance was calculated based on the constructed standard curve (Table S3), and then converted into copies per milliliter of water samples.

5.4 ARGs analysis

Twenty-two ARGs, including sulfonamide resistance genes (*sul*1, *sul*2, *sul*3, *sul*A), fluoroquinolones resistant genes (*qnr*S, *qnr*B, *aac*(6')-Ib), tetracycline resistance genes (*tet*A, *tet*B, *tet*C, *tet*E, *tet*G, *tet*L, *tet*M, *tet*O, *tet*Q, *tet*S, *tet*T, *tet*W, *tet*X), macrolide resistance gene (*erm*B)

and chloramphenicol resistance gene (*Chl*), were detected, as they were corresponded to the five classes of antibiotics and had been reported in the various environments. Primers, annealing temperature used in PCR were summarized in Tables S2

Table S2. The primer sequences, expected amplicon size, and annealing temperature for each target gene used in this study.

Target genes	Primer Sequences (5'-3')	Amplicon size(bp)	Annealing temp(°C)	Reference
	CACCGGAAACATCGCTGCA	150	55	2
SUL I	AAGTTCCGCCGCAAGGCT	158	55	3
au 10	TCCGGTGGAGGCCGGTATCTGG	101	55	4
SU12	CGGGAATGCCATCTGCCTTGAG	191	33	4
totA	GCTACATCCTGCTTGCCTTC	210	60	5
lelA	CATAGATCGCCGTGAAGAGG	210	00	5
totW	GAGAGCCTGCTATATGCCAGC	169	60	6
leiw	GGGCGTATCCACAATGTTAAC	108	00	0
orm B	CGTTTACGAAATTGGAACAGG	361	58	7
ermD	GCGTGTTTCATTGCTTGATG	504	58	/
anrS	TCTAAACCGTCGAGTTCGGCG	128	56	8
<i>qn</i> 5	GCAAGTTCATTGAACAGGGT	420	50	0
$7nt\Delta$	TAACGCATTCGCTGTCACCA	134	60	
21111	TCCGCCAGAACATCACCATT	134	00	0
=ntB	GAAATCGACGCCTGTATAGCA	153	60)
21110	TTGACGCCAAATAACCCTGT	155	00	
int[1	CCTCCCGCACGATGATC	280	60	10
11111	TCCACGCATCGTCAGGC	200	00	10
Tn916/1545	GACAGTATTAAGCCATCAGAC	142	50	11
111/10/10/10/10	TCTTCCGAACACAATCATCT	1-12	50	11
16S rRNA	CCTACGGGAGGCAGCAG	193	60	10
100 1101/1	TTACCGCGGCTGCTGGCAC	175	00	10
sul3	CCCATACCCGGATCAAGAATAA	143	57/58	12
5415	CAGCGAATTGGTGCAGCTACTA	145	51150	12
sulA	GCACTCCAGCAGGCTCGTAA	198	56/57	12
Sull	CTCTGCCACCTGACTTTTCCA	170	50/57	12
tetB	CGAAGTAGGGGTTGAGACGC	192	56	12
ieiD	AGACCAAGACCCGCTAATGAA	1)2	50	12
tetC	CTTGAGAGCCTTCAACCCAG	418	55	5
ieic	GGCAGGTAGATGACGACCAT	410	55	5
t <i>et</i> F	AAACCACATCCTCCATACGC	278	55	5
	AAATAGGCCACAACCGTCAG	210	55	5
tetG	GCAGAGCAGGTCGCTGG	134	65	9

	CCYGCAAGAGAAGCCAGAAG			
tat	TCGTTAGCGTGCTGTCATTC	267	58/55	12
leiL	GTATCCCACCAATGTAGCCG	207	38/33	15
t otM	CCGTTGGGAAGTGGAATGC	106	57	5
lelivi	TCCGAAAATCTGCTGGGGTA	190	57	5
tat	GATGGCATACAGGCACAGACC	170	57	5
leiO	GCCCAACCTTTTGCTTCACTA	172	57	3
tat	AGAATCTGCTGTTTGCCAGTG	167	52	12
leiQ	CGGAGTGTCAATGATATTGCA	107	33	15
4.049	ATAGCGGTACAACGAAAACGG	100	56/55	5
tetS	GATTAGCAAAATAGCTCCATCCAA	188	30/33	3
t α/T	ATGCTACAACGACAACGGATTC	140	55	5
lel I	TCGCTCAACTTCGGCTATGA	149	33	3
t of V	AGCCTTACCAATGGGTGTAAA	270	60	10
leix	TTC TTA CCT TGG ACA TCC CG	278	00	10
CL1	GGAGTGAATACCACGACGAT	269	55	0
Chi	ACGGCATGATGAACCTGAAT	208	55	9
D	GATCGTGAAAGCCAGAAAGG	176	50	0
qnrв	ATGAGCAACGATGCCTGGTA	4/0	30	9
$\pi \pi \alpha (C')$ The	TTGCGATGCTCTATGAGTGGCTA	400	50	0
<i>aac(6')</i> -lb	CTCGAATGCCTGGCGTGTTT	482	38	9

Table S3. Standard curve and amplification efficiency of selected genes for qPCR.

Target genes	Standard curve	r^2	amplification efficiencies
sul1	y = -3.23 x + 34.57	0.999	104%
sul2	y = -3.70 x + 39.45	0.998	86.3%
tetA	y = -3.42 x + 34.17	0.999	96.1%
tetW	y = -3.33 x + 36.96	0.998	99.7%
ermB	y = -3.64 x + 40.65	0.994	88%
qnrS	y = -3.39 x + 42.88	0.996	97%
zntA	y = -3.61 x + 43.1	0.994	89.3%
zntB	y = -3.56 x + 33.98	0.999	91%
<i>int</i> I1	y = -3.41 x + 37.48	0.998	96.5%
Tn916/1545	y = -3.61 x + 43.16	0.992	89.1%
16s rRNA	y = -3.75 x + 41.94	0.993	84.7%

Notes: "y" means the threshold cycle number (C_T value) determined during the real-time PCR, "x" is the log gene copy number in the PCR mix.



Figure S4. Absolute gene copies of six ARGs and 16S rRNA in raw SDK, CY and DH samples.



Figure S5. Variations of absolute gene copies of 16S rRNA in SDK, CY and DH samples.



Figure S6. The normalized value (NV) of total ARGs during ZnO NPs exposure in SDK, CY and DH samples. The NV was calculated as relative abundance of total ARGs versus the corresponding blank values. The NV >1 indicated the abundances of ARGs were enhanced when exposed to ZnO NPs, at certain time compared to the corresponding blanks; the NV <1 indicated the abundances of ARGs were reduced when exposed to ZnO NPs, at certain time.

6. Hydrodynamic size and zeta-potential of ZnO NPs

Water samples were filtered through 1-µm glass fiber filters (PAUL, USA) and further ultrafiltered by cross-flow ultrafiltration with a 1-kDa regenerated cellulose Pellicon 2 PLAC ultrafiltration membrane to remove the indigenous colloids and microorganisms.¹⁴ Then, the filtrate (<1 kD) water was used as the aqueous medium for the aggregation test of ZnO NPs. In order to better mimic the real aquatic environmental conditions, ZnO NPs (10mg/L) were added in the filtrate (<1 kD) water samples, and the dynamic size and zeta-potential of ZnO NPs were analyzed using Zeta-Sizer (Malvern, Nano-ZS90, UK).



Figure S7. The particle size (A) and Zeta potential (B) of ZnO NPs in three estuarine water samples under the exposure of 10 mg/L ZnO NPs.



Figure S8. Dissolved Zn concentrations in SDK, CY and DH samples with exposure to ZnO Nos with different doses.

Committee altern	Turnet	T			A	RGs				MGEs	MR	Gs
Sampling sites	Treatment	Time	sul1	sul2	tetA	tetW	ermB	qnrS	intI1	Tn916/1545	zntA	zntB
		3h	46.020	3.534	6402.657	10.774	2581.240	54.562	11.724	641.950	826.801	70.456
	0.2 mg/I	6h	50.013	3.253	3410.704	10.401	3271.448	23.273	5.460	223.630	349.492	90.853
	0.2 mg/L	12h	40.806	3.488	2306.235	19.299	5538.642	22.651	5.409	615.453	428.557	92.571
		24h	29.120	2.700	4707.979	16.856	6462.501	22.301	13.768	439.547	1298.575	37.575
		3h	53.295	3.006	8104.901	18.006	5960.328	51.079	21.132	514.520	1020.844	75.117
CDV	1 m ~/I	6h	37.400	1.734	4576.129	25.785	6329.656	25.263	14.454	390.068	6545.967	145.863
SDK	1 mg/L	12h	32.152	1.270	4712.155	34.290	4038.256	151.261	12.620	247.576	1919.029	320.530
		24h	46.597	3.728	5053.302	34.331	5855.580	19.115	35.239	452.796	4039.972	19.681
		3h	2.531	8.587	120.809	59.199	260.269	6.390	4.700	657.113	810.730	49.647
	10	6h	4.708	7.210	54.051	56.432	248.728	5.831	2.259	560.557	283.961	54.927
	10 mg/L	12h	1.955	2.876	87.911	122.363	421.669	9.957	1.869	773.021	632.313	102.491
		24h	3.384	7.299	58.214	111.468	499.418	2.625	3.410	1198.697	2094.161	17.732
		3h	25.258	4.867	4018.273	3.873	10204.182	10.990	2.834	110.861	35.297	0.788
	0.2	6h	18.107	6.561	7698.584	19.853	1421.160	12.556	6.612	1408.672	1.907	1.627
	0.2 mg/L	12h	34.251	4.003	9786.557	13.466	229.989	4.101	21.200	370.208	0.242	0.778
		24h	24.651	2.566	4021.242	4.003	13159.805	67.493	12.196	705.588	0.603	0.974
		3h	47.296	8.699	6225.628	9.361	4065.207	12.771	8.940	171.873	56.594	1.218
CV	1 mg/L	6h	25.194	5.671	6207.767	4.964	837.798	8.515	4.252	935.287	1.347	1.160
CY		12h	42.880	7.908	8228.866	5.482	769.099	6.093	22.209	184.709	0.244	0.904
		24h	57.151	4.594	3446.036	4.818	3281.598	80.963	47.147	462.312	1.750	0.875
		3h	1.271	10.798	145.483	91.568	56.350	5.123	6.093	1622.249	275.909	6.104
	10	6h	0.485	9.584	86.029	67.409	196.600	1.788	1.018	13185.085	7.384	6.194
	10 mg/L	12h	0.609	9.585	135.129	94.122	140.169	0.546	2.266	2108.750	0.632	2.083
		24h	0.683	5.189	79.581	91.295	233.179	19.847	1.498	528.882	2.445	1.868
		3h	22.930	3.057	4206.153	5.189	38.293	57.287	2.738	671.288	55.280	1.024
	0.2 mg/I	6h	58.525	5.882	9517.490	16.835	54.262	34.860	12.639	3589.473	48.404	1.124
	0.2 mg/L	12h	103.471	5.157	6864.698	54.133	130.307	25.312	9.505	793.243	61.790	0.864
		24h	68.395	5.221	23920.918	38.878	734.408	40.231	15.576	2060.491	67.262	1.357
		3h	75.275	3.387	7965.747	1.494	135.050	74.652	7.318	1030.057	86.302	1.276
חח	1 mg/I	6h	193.393	3.020	10842.900	5.799	236.175	36.329	13.381	2575.133	53.570	1.275
Dn	I IIIg/L	12h	461.156	16.004	11012.770	20.789	1511.301	48.953	42.798	1876.082	137.521	2.174
		24h	327.609	22.394	22562.248	24.835	306.493	65.385	41.888	1845.516	95.780	1.750
		3h	2.056	8.133	224.048	127.040	121.579	27.683	0.396	49035.468	633.140	8.139
	10 mg/I	6h	1.285	7.920	147.753	58.689	68.432	8.541	0.897	21069.677	330.496	5.778
	10 mg/L	12h	4.721	14.845	92.444	114.333	13.788	2.790	3.879	10855.690	333.054	6.751
		24h	1.114	3.701	89.577	51.856	87.245	1.875	0.580	8357.609	307.855	4.193

Table S4. The normalized value (NV) of individual ARG, MGE and MRG during ZnO NPs exposure in SDK, CY and DH samples. The NV was calculated as relative abundance of individual ARG, MGE and MRG versus the corresponding blank values. The redder, the higher the NV, and the greener, the lower the NV.

Table S5. Correlation coefficients between MGEs (gene copies/16S rRNA copies) and Zn^{2+} (mg/L) in SDK, CY and DH respectively.

	SE	ЭК	(CY	DH				
	int[]	Tn916/1545	intIl	Tn916/1545	int[]	Tn916/1545			
Zn ²⁺	0.502*	0.529*	0.397	0.495*	0.201	0.826**			

* Significant at P < 0.05.

** Significant at P < 0.01.

Table S6. Correlation coefficients between MRGs and ARGs based on their relative abundance (gene copies/16S rRNA copies) in SDK, CY and DH respectively.

	SDK		CY		DH			
	zntA	zntB	zntA	zntB	zntA	zntB		
sul1	0.620**	0.534*	-0.140	-0.230	0.382	0.370		
sul2	0.358	0.301	0.189	0.297	0.841**	0.811**		
tetA	0.779**	0.789**	-0.025	0.056	0.397	0.282		
tetW	0.581*	0.493*	0.471	0.600^{*}	0.806**	0.696**		
ermB	0.632**	0.730**	-0.230	-0.186	0.591*	0.461		
qnrS	0.703**	0.819**	0.091	0.054	0.419	0.390		

* Significant at P < 0.05.

** Significant at P < 0.01.

Tractmont	Time				SDK				СҮ					DH					
Treatment	Time	reads	OTU	chao1	ACE	simpson	shannon	reads	OTU	chao1	ACE	simpson	shannon	reads	OTU	chao1	ACE	simpson	shannon
	0h	48409	2632	1511	1597	0.99	8.50	67453	2711	1172	1212	0.99	8.25	71766	3890	1698	1797	1.00	9.24
	3h	45012	2463	1532	1589	0.98	8.60	41117	2718	1393	1479	0.99	8.38	42733	2738	1764	1848	0.99	8.77
Blank	6h	42699	2455	1698	1704	0.99	9.00	40216	3065	1586	1690	0.99	8.40	65378	2865	1933	2079	0.99	8.82
	12h	44350	2377	1444	1518	0.99	8.60	47386	3053	1563	1662	0.99	8.71	68531	3071	1823	1966	0.99	8.88
	24h	42811	2454	1506	1531	0.99	8.70	44266	2175	1364	1400	0.91	7.30	35689	2708	1339	1434	0.99	8.02
	3h	35583	2491	1465	1523	0.99	8.80	40660	2552	1376	1482	0.99	8.27	48383	2647	1650	1783	0.99	8.58
0.2 mg/L	6h	33473	2472	1659	1762	0.99	8.70	41057	2545	1358	1463	0.99	8.15	52267	2720	1656	1797	0.97	8.10
ZnO NPs	12h	42356	2588	1686	1820	0.99	8.70	39332	2577	1767	1907	0.99	8.53	37789	2562	1757	1884	0.99	8.39
	24h	43970	2601	1752	1896	0.99	8.70	42814	2649	1609	1741	0.99	8.44	40256	2470	1641	1781	0.99	8.51
	3h	49230	2792	1738	1758	0.99	9.00	40649	1494	888	959	0.97	6.79	29835	2516	1293	1372	0.98	8.10
1 mg/L	6h	41816	2698	1656	1729	1.00	8.90	37436	2231	1026	1084	0.98	7.38	52162	2525	1453	1535	0.97	7.93
ZnO NPs	12h	39842	2309	1621	1699	0.99	8.40	43349	2286	1254	1294	0.99	7.91	37132	2735	1614	1692	0.97	8.21
	24h	34324	2423	1478	1550	0.99	8.90	52072	2009	1223	1303	0.99	7.88	34760	2579	1500	1575	0.98	7.87
	3h	49227	2181	1446	1510	0.99	8.70	68453	3250	1408	1514	0.98	7.88	73422	3505	1564	1684	0.99	8.18
10 mg/L	6h	46402	2083	1589	1620	0.99	8.90	74768	3346	1598	1731	0.99	8.56	70002	3611	1526	1656	0.99	8.05
ZnO NPs	12h	41249	2222	1633	1696	0.99	8.60	60787	3579	1621	1750	0.99	8.60	70473	3532	1689	1804	0.99	8.52
	24h	47495	2345	1659	1765	0.99	8.80	63898	2740	1010	1058	0.98	7.44	69683	3526	1466	1576	0.99	8.09

Table S7. The richness (Chao and Ace), diversity (Shannon and Simpson) and sequencing depth indexes for bacterial community of each sample.

Notes: The richness, diversity and sequencing depth indexes data for each treatment group are presented as the average values of the replicates.



Figure S9. PCA analysis of relative abundance of genera showed the evolution of the microbial community dosed with different concentrations of ZnO NPs at different exposure time.



Figure S10. Absolute and relative abundances of top four phyla in microbial community in SDK, CY and DH samples.



Figure S11. The NVs for the top 4 phyla in SDK, CY and DH samples. The values were calculated as the absolute abundance of each phylum at each treatment normalized by the corresponding blank values.



Figure S12. Heatmap of relative abundance of top 50 genera (values were log10- transformed) showed the evolution of the microbial community dosed with different concentration of ZnO NPs at different exposure time.



Figure S13. Venn map of the top 50 genera in this study.

			Treatment										
Sampling sites	Top 50 genera		0.2 m	g/L			1 n	ng/L			10 r	ng/L	
		3h	6h	12h	24h	3h	6h	12h	24h	3h	6h	12h	24h
	Streptomyces	0.2192	0.3989	0.5659	1.8347	0.1538	0.1453	0.5630	0.4756	0	0	0	0
	E1B-B3-114	0.5004	0.2485	0.2779	0.3608	0.1243	0.0782	0.0107	0.3553	0	0	0	0.0077
	Microbacterium	3.3680	0.9890	1.8943	5.0815	7.8888	3.6860	1.7077	21.4033	2.7299	2.2003	5.5552	49.9009
	Nocardioides	1.5619	0.7496	1.2718	2.8292	5.1972	3.1025	0.7665	8.1839	0.6847	0.5135	0.5353	3.7322
	Agromyces	2.6456	2.0220	1.7031	11.7868	0.4515	0.6408	1.6448	2.2879	0.0618	0.1609	0.2044	1.7647
	Rhodococcus	3.8544	0.8948	0.9015	4.1094	1.7246	2.1297	0.5927	4.0478	0	0.0073	0.0015	0
	Oerskovia	5.8294	1.6878	2.8293	11.6274	11.4413	5.8860	9.0265	40.5296	0	0	0.0157	0
	Yonghaparkia	3.2196	18.8755	2.3300	3.5306	8.7223	22.0537	1.2669	39.5163	0.1265	0.1632	0.2192	6.1766
	Pedobacter	1.4293	0.2404	1.4705	1.4459	0.2256	0.0029	0.0081	0.5345	0	0	0	0.0420
	Dyadobacter	0.3756	0.2449	0.3419	5.1782	2.0620	2.0236	6.6945	20.8883	0	0.2417	1.6965	2.0589
	Paludibacter	0.5779	0.1754	0.0291	0.2230	2.2596	0.7947	0.0809	1.3291	12.0016	5.4588	2.5319	17.5544
	Cloacibacterium	0.2584	0.0501	0.0099	0.0160	0.6565	0.1244	0.0037	0.0812	5.6520	1.8163	1.7932	17.6473
	Microbacter	1.1499	0.1225	0.2184	0.5946	5.2093	0.5077	0.2157	1.7721	31.4807	5.1852	5.8823	28.6072
	Chitinophaga	0.4817	0.3764	0.5859	0.1341	0.6561	0.0018	0.0051	0.6393	0	0.0058	0.0206	0.9605
	Flavobacterium	0.3947	0.0291	0.0604	0.0308	0.0757	0.0178	0.0038	0.0493	0.0851	0.0168	0.0266	0.8471
	Macellibacteroides	0.3756	0.0661	0.0376	0.0763	3.1400	0.3258	0.0243	0.4803	27.2752	4.7646	3.5287	16.2164
	Deinococcus	0.1931	0.1165	0.1011	0.1553	0.2289	0.0674	0.0492	0.2833	0.8672	0.4859	0.2412	2.2920
	Methanosaeta	1.6098	0.4330	0.3560	3.2481	8.7545	1.3624	0.5152	14.2165	38.3951	16.6899	6.0406	86.1189
	Methanosarcina	3.1525	0.8267	0.7678	3.3737	9.8126	2.8719	0.4999	16.3157	18.3638	6.8235	3.5672	32.9416
	Trichococcus	0.2739	0.0898	0.0432	0.0980	0.0800	0.0547	0.0029	0.0609	0.7184	0.4693	0.0891	0.6019
	Peptoclostridium	1.9931	1.2302	0.5825	3.5508	2.6046	1.5398	1.7984	3.9817	2.2289	1.5035	0.9630	6.3026
	Exiguobacterium	164.1997	12.9511	2.8986	18.0943	0	0.2484	0.0565	1.1691	0	0	0	0
	Acinetobacter	0.3263	0.3945	0.3956	0.8501	0.0037	0.0028	0.0010	0.0356	0.3365	0.2168	0.0653	0.4563
	Thermomonas	0.4930	0.3339	0.2249	0.5346	0.2246	0.0742	0.0428	0.6461	0.3608	0.2080	0.0926	1.4424
SDV	Pseudomonas	0.6081	0.2198	0.2091	0.4522	0.3237	0.1340	0.0173	0.6200	0.1681	0.0727	0.0464	0.2330
SDK	Pseudoxanthomonas	0.6218	0.1857	0.2473	0.2783	0.1518	0.0372	0.0211	0.1927	0.0021	0.0014	0.0000	0.0033

Table S8. The normalized values (NVs) for the top 50 genera in SDK, CY and DH samples. The values were calculated as the absolute abundance of each genus at each treatment normalized by the corresponding blank values. The redder, the higher the NV, and the greener, the lower the NV.

Lysobacter	0.3107	0.1359	0.2357	0.3988	0.1477	0.0284	0.0483	0.3754	0.1810	0.1025	0.0541	0.7084
Stenotrophomonas	2.0898	0.3830	0.3184	0.8096	2.5216	0.5651	0.0893	3.2655	0.0769	0.0419	0.0784	1.2475
Aeromonas	0.3465	0.2774	0.2257	0.9713	0.1679	0.1527	0.0204	0.5407	8.7040	4.2979	0.7831	4.9368
Bosea	0.5966	0.3044	0.4799	1.1024	0.1487	0.0870	0.1595	0.5249	0.2071	0.1834	0.1204	0.8464
Acidovorax	0.1227	0.1737	0.0975	0.2267	0.1433	0.0522	0.0429	0.2765	0.4513	0.2965	0.1287	1.2308
Brevundimonas	1.9094	0.4086	0.8703	0.7227	2.3995	0.1845	0.0403	3.1433	0	0.0007	0.0032	0.0208
Luteimonas	0.2943	0.0949	0.2230	0.3722	0.0980	0.0109	0.0170	0.1523	0.0735	0.0251	0.0178	0.4788
Aquabacterium	0.6820	0.3149	0.4381	1.2357	1.3887	0.3564	0.2610	5.9706	5.7126	3.1978	1.6450	15.2969
Arenimonas	0.1520	0.0700	0.0953	0.1629	0.0380	0.0167	0.0042	0.2075	0.1838	0.1155	0.1515	0.6827
Paracoccus	0.2130	0.0758	0.0953	0.2162	0.4910	0.2807	0.2005	0.7349	1.7778	0.9730	0.4405	4.7180
Achromobacter	0.1456	0.0700	0.1057	0.2763	0.2460	0.0340	0.0244	0.4717	0.4799	0.2813	0.1637	2.3172
Variovorax	0.1459	0.1041	0.0619	0.1698	0.2932	0.1236	0.0552	0.3315	0.7193	0.5802	0.4419	2.8816
Noviherbaspirillum	0.6778	0.8267	0.3084	0.4783	2.6922	1.6830	1.4715	2.8361	0.7193	0.4607	7.0020	5.1234
Dechloromonas	0.4188	0.2441	0.0324	0.3455	3.8911	0.7663	0.1677	1.6915	12.4717	9.9673	3.6171	19.3745
Novosphingobium	0.2576	0.1314	0.1315	0.4119	0.1958	0.1109	0.0475	1.0620	1.3438	0.7438	0.6859	5.5515
Brachymonas	0.2348	0.1218	0.0615	0.2648	0.4669	0.1756	0.2232	1.0522	1.8417	1.7000	0.4570	7.6839
Thiothrix	0.5151	0.0882	0.3236	1.0592	1.8030	0.4371	0.5032	2.4785	12.9600	6.5921	6.9418	30.8828
Defluviicoccus	0.9391	0.4822	0.3137	2.5356	1.8486	0.9338	1.2939	4.4214	4.7831	2.5944	2.5125	11.3905
Thauera	0.1480	0.0617	0.0906	0.1126	0.5239	0.0890	0.0958	0.4880	2.7784	1.7876	0.7161	5.2942
Rhodovulum	0.3389	0.0861	0.1456	0.3531	0.7997	0.1925	0.0359	0.6547	8.3615	2.1416	1.7537	9.8825
Rhodobacter	0.3302	0.0877	0.0539	0.1284	0.9350	0.2890	0.0439	0.7227	12.9652	4.3611	1.9892	11.4708
Limnobacter	0.0079	0.0056	0.0004	0.0062	0.0089	0.0140	0.0008	0.0207	0.0367	0.0857	0.0034	0.0781
Azoarcus	0.0826	0	0	0.0235	0.5649	0.0662	0.1120	1.8706	7.8405	3.6483	2.4198	15.0002
CandidatusCompetibacter	0.1736	0.0204	0	0.2463	0.5660	0.0809	0.4025	0.7178	3.1820	1.4021	1.9291	23.6802
Nocardioides	4.0858	1.1473	0.5868	4.2152	1.7796	1.3607	1.1447	3.1742	0.1057	0.0795	0.2650	0.4467
Microbacterium	2.9667	1.1123	0.6269	2.2078	0.8095	0.3796	0.4181	1.2601	0.0812	0.0665	0.2280	0.2931
Yonghaparkia	7.9035	1.6158	0.8478	1.0902	0.7909	0.3433	0.1887	0.4701	0.0645	0.0587	0.2628	0.2328
Agromyces	3.2462	1.3451	1.0639	1.7753	1.2113	0.6695	0.7435	1.2406	0.0205	0.0060	0.0276	0.0308
Ilumatobacter	2.1092	0.8120	0.7431	2.0854	1.8819	1.4509	1.1295	1.7640	0.3203	0.1413	0.2366	0.6142
Mycobacterium	2.0973	1.7331	1.2922	1.2258	2.3463	1.7104	1.1873	0.8058	1.8875	1.4206	1.7485	0.8198
E1B-B3-114	0.6353	0.1072	0.4328	0.6435	3.1447	0.6559	0.5702	7.1669	0.0093	0	0	22.6945
Streptomyces	0.5841	0.0684	0.0304	0.8720	0.1728	0.0667	0.0685	0.2128	0	0	0	0.0028

Cl	hryseobacterium	4.0022	0.1449	0.2286	1.2729	1.4103	0.5146	0.2163	1.9689	0.0388	0.0158	0.0806	0.2064
	Pontibacter	0.5233	0.1275	0.3417	0.6243	0.5954	0.4666	0.2966	0.1789	0.0189	0.0057	0.0093	0.0915
	Algoriphagus	1.6957	0.7054	0.4527	1.4420	2.6249	1.1520	0.7728	3.4378	6.6957	5.1131	3.6506	3.1158
	Nibribacter	1.2082	0.3742	0.6461	1.5445	1.9507	1.1666	0.7085	0.7631	0.0073	0.0129	0.0289	0.2087
F	Flavobacterium	0.5510	0.3657	0.9704	1.6940	1.2785	1.0664	0.6517	0.5298	1.1051	0.6280	0.3027	1.7418
1	Flavisolibacter	1.4784	0.6881	0.6485	1.5181	1.9905	1.9370	1.2152	1.1996	0.0334	0.0231	0.0764	1.0033
	Cytophaga	0.6825	0.3882	1.0181	1.8197	2.8745	1.9419	1.7652	2.4370	0.0800	0.0662	0.1110	0.1889
P	Parasegetibacter	2.7300	0.6825	0.5121	1.1400	1.9478	0.7659	0.7165	1.1376	5.8296	2.0300	3.7571	5.2767
	Dyadobacter	1.9800	0.7847	0.6066	3.2556	0.9256	1.2963	1.4937	2.2250	1.6879	1.0191	0.9205	1.8413
F	Flavihumibacter	4.2526	4.0268	1.5371	0	14.7026	10.7410	4.2174	0	0	0	0	0
	Hymenobacter	0	0	0	0.9653	0.3888	0	0	0.1244	33.1172	0	0	4.4998
j	Herpetosiphon	1.0369	0.3810	1.0647	1.4333	0.0982	0.0580	0.0835	0.9208	0	0.0001	0.0003	0
E	xiguobacterium	4.2490	0.4658	0.1021	3.8165	1.1537	0.6820	0.2956	2.0462	0.0112	0.0284	0.5577	0.0095
	Planococcus	4.3455	1.3215	0.7306	1.7555	0.0338	0.0551	0.0066	0.0897	0.0006	0.0018	0	0
Р	Planomicrobium	8.7198	0.6117	0.4666	3.3492	0.2734	0.1026	0.0135	0.1498	0	0	0	0.0084
	Pirellula	2.6995	0.9100	0.5076	1.8608	1.1090	0.6460	0.6158	1.3552	0.2521	0.0625	0.0923	0.0849
1	Rhodopirellula	1.7672	1.0080	0.4000	3.6506	1.0782	0.5030	0.4142	1.4298	0.2836	0.0693	0.0947	0.1423
	Planctomyces	2.1911	0.5784	0.7265	3.5523	0.8233	0.6425	0.5609	2.2828	0.7114	0.1550	0.2735	0.4904
	Thermomonas	1.4241	0.3609	0.3484	1.7131	1.3116	0.4688	0.5666	1.5000	0.0202	0.0044	0.0120	0.2222
	Acinetobacter	0.7522	0.0861	0.0793	0.0569	0.1437	0.1629	0.0349	0.0048	0.0244	0.0197	0.1183	0.4461
	Lysobacter	1.0715	0.5420	0.6082	1.5487	0.5761	0.5177	0.5921	2.0366	0.0037	0.0013	0.0029	0.0163
	Pseudomonas	1.0003	0.0893	0.5037	0.8170	1.1756	0.6593	0.4577	1.5987	0.0047	0.0050	0.0036	0.9268
	Paracoccus	0.8917	0.1596	0.2951	0.6526	0.0405	0.0379	0.0298	0.0533	0.0398	0.0111	0.0193	0.0331
	Luteimonas	1.3667	0.3443	0.4995	1.4898	0.1837	0.2919	0.4063	0.8999	0.0128	0.0051	0.0147	0.0375
	Massilia	5.5094	0.1770	0.2433	1.0643	9.5608	1.9090	0.7565	0.8652	3.1161	0.6753	0.6235	21.0029
	Rhodovulum	1.7200	0.3357	0.4368	1.3404	0.3364	0.1867	0.1978	0.6846	0.1043	0.0177	0.0336	0.1813
	Arenimonas	0.5756	0.3292	0.4730	0.9443	0.5115	0.2423	0.3631	0.8977	0.0393	0.0194	0.0465	0.0439
	Rhodobacter	1.1403	0.2592	0.4075	1.4237	0.3491	0.1797	0.1779	0.5895	0.2638	0.0473	0.0920	0.5640
	Simplicispira	1.0558	0.0389	0.0644	3.4383	0.5670	0.4835	0.1545	1.2656	0.0043	0.0007	0.0065	0.0112
	Devosia	1.0156	1.5680	0.3811	1.0419	0.5720	0.4779	0.5477	1.5326	0.0224	0.0128	0.0470	0.0373
	Aeromonas	0.4331	0	0	0.1902	0.3517	0.0947	0	0	0.0844	0.1419	1.7846	1.3647
E	Brevundimonas	1.3765	3.4527	1.2132	0.7275	1.1219	1.1666	0.5178	2.9862	0.6626	1.4429	1.2248	4.4913

	Noviherbaspirillum	2.0790	0.1346	0.3342	1.1326	2.1287	1.0344	0.7536	0.8759	0.6894	0.4573	0.2285	5.4780
	Novosphingobium	1.9751	0.2845	0.3039	0.3592	0.3525	0.1559	0.1781	0.2006	0.4337	0.1432	0.1586	0.4246
	Altererythrobacter	0.6417	0.2341	0.2692	0.2645	0.1728	0.2764	0.1340	0.4091	0.3501	0.1731	0.3974	0.7718
	Pseudoxanthomonas	1.0395	0.5641	0.1394	1.0297	2.3696	0.6407	0.4462	5.9724	0	0	0	4.1476
	Duganella	6.9301	0.2234	0.3680	1.3659	10.0667	1.9871	1.1806	0.8735	2.7800	0.5644	0.7737	13.8147
	Stenotrophomonas	1.0588	0.1911	0.1970	0.5148	1.9007	0.8381	0.1881	6.6692	1.4827	1.0894	0.7598	7.6692
	Sandaracinus	4.0557	0.9365	0.2402	2.2771	1.6493	0.2361	0.3476	0.9571	0	0	0	0
	Haliangium	0.2530	0.1966	0.2514	0.3547	0.3413	0.2595	0.3705	0.3997	0.0014	0	0.0007	0.0082
	Nannocystis	0.9574	0.9830	1.5332	4.3293	1.5278	9.6318	2.7597	8.3251	0	0	0	0
	Phaselicystis	1.6088	0.3984	0.2901	0.9608	1.3329	0.8912	0.6683	0.4486	0	0	0.0034	0
	Microbacterium	1.2896	0.5975	0.8004	0.8737	0.3632	0.5531	0.3489	0.5908	0.0730	0.2117	0.1969	1.4769
	Nocardioides	2.0148	0.7967	1.3411	1.3563	1.7928	2.3258	1.6017	2.7346	0.0298	0.0743	0.1670	0.1546
	Oerskovia	2.1174	2.5717	0.9814	1.4105	0.4306	0.6772	0.7805	1.6743	0.0053	0	0.1998	0
	Agromyces	1.5638	0.3646	0.6647	0.6126	0.0086	0.0361	0.0622	0.0682	0.0027	0.0027	0.0032	0.0034
	Lysinimicrobium	1.0093	0.2164	0.9067	0.9436	0.1260	0.1370	0.6389	0.2474	0.0197	0.0219	0.0539	0.0368
	Ilumatobacter	1.1232	1.0430	0.8145	1.4730	0.1051	0.1000	0.1044	0.0866	0.1832	0.3517	0.0869	0.1842
	Demequina	2.3856	1.2330	1.5493	2.0398	1.8142	1.6089	5.2232	3.2216	0.0371	0.0443	0.0538	0.0272
	Frondihabitans	3.0243	1.7147	1.0222	1.5412	16.7145	5.1235	5.9730	4.5725	0	0	0.0018	0.0600
	Streptomyces	0.0918	0.1460	0.2332	0.0773	0.0616	0.0502	0.0374	0.0031	0.0021	0.0010	0.0008	0.0002
	Candidatus_Aquiluna	0.3470	0.4076	0.3883	0.3268	0.5078	1.0877	0.7423	0.6856	2.8504	6.8498	1.4132	1.3350
	Yonghaparkia	0.7753	0.3925	0.5433	1.2843	0.4054	2.5319	2.2766	30.2720	0.0517	0.0562	0.5271	1.8515
	Paeniglutamicibacter	1.2899	1.1267	2.3125	1.1978	0.0000	0.0069	0.0373	0.0076	0	0.0012	0.0172	0
	Flavobacterium	0.4154	0.5847	0.2607	1.0837	0.2647	0.1461	0.1019	0.7505	0.0764	0.7701	0.3620	5.3491
	Algoriphagus	5.5664	3.0357	1.2265	5.2519	2.8347	2.2505	0.5927	3.3296	1.2141	0.3872	0.2567	3.1357
	Sediminicola	0.7961	0.6449	1.7125	3.0277	6.3231	0.2474	2.4578	1.6826	0.0002	0.0001	0.0006	0.0000
	Arenibacter	0.3702	0.5859	0.7125	0.4806	0.9062	1.0166	0.6454	0.4938	0.0086	0.0243	0.0157	0.0019
	Winogradskyella	0.6305	0.3549	0.6135	1.0725	1.1885	0.8747	0.9010	0.9696	0.1740	0.2277	0.2119	0.6387
	Formosa	0.1434	0.2184	0.2857	0	0.4904	0.4601	0.3159	0	0.0131	0.0099	0.0063	0
	Maribacter	1.0868	0	0	3.9152	5.5194	0	0	10.7274	0.0445	0	0	0.0747
	Subsaxibacter	0.1969	0.1243	0.2542	0.6113	0.5161	0.4220	0.1734	0.2332	0.0246	0.0326	0.0070	0.0322
	Gaetbulibacter	0	0.0923	0.2642	0.3585	0.1886	0.5669	0.1742	0.5470	0.1136	0.2073	0.0230	0.0711
	Pedobacter	4.9394	0.8554	1.0435	0.3600	0.4620	0.0705	7.1915	0.3686	0.0096	0.0046	0.0126	0

Planococcus	2.3525	1.3769	2.2139	3.6392	0.0000	0.0024	0.0047	0	0.0091	0.0025	0.0005	0.0040
Exiguobacterium	11.0284	6.4515	7.1796	4.8097	0.0687	0.0367	0.0229	0.0285	0.1202	0.0136	0.0040	0.0053
Planomicrobium	0.6728	1.5103	1.9853	1.7281	0	0	0	0	0.0072	0.0056	0.0049	0.0090
Paracoccus	0.1181	0.1015	0.1891	0.2086	0.0047	0.0099	0.0088	0.0116	0.0072	0.0126	0.0061	0.0117
Methylotenera	0.0394	0.0778	0.5963	0.9334	0.0907	0.0357	1.1468	0.3095	0.0095	0.0671	4.7610	0.2242
Altererythrobacter	0.4110	0.1399	0.3905	0.5008	0.0479	0.0430	0.0625	0.0554	0.0492	0.0450	0.0216	0.0265
Sandaracinus	1.0376	0.6421	0	0	0	0	0	0	0	0	0	0
Lysobacter	2.0880	0.9758	0.9938	0.4799	1.1324	0.4366	0.8155	0.0953	0	0	0	0
Erythrobacter	0.2794	0.0838	0.2972	0.4260	0.0588	0.0246	0.0762	0.1029	0.0626	0.0511	0.0310	0.0518
Devosia	3.2318	2.0438	1.2514	0.9284	0.1598	0.0505	0.1234	0.0442	0.0063	0.0122	0.0061	0.0020
Albirhodobacter	0.3501	0.9831	0.5943	0.3975	0.0065	0.0095	0	0.0064	0.0102	0.0138	0.0017	0.0060
Rheinheimera	2.2178	0.7184	1.0380	7.6962	0	0	0.0566	0	0.0061	0.0038	0.0748	0.6618
Arenimonas	0.5508	1.2067	0.3237	0.4040	0	0	0.0109	0	0.0005	0.0141	0.0029	0.0010
Loktanella	0.1786	0.2139	0.1188	0.3753	0.0696	0.0760	0.0224	0.0472	0.2940	0.5384	0.1103	0.4445
Rhodovulum	0.1978	0.2463	0.2611	0.2359	0.0317	0.0355	0.0075	0.0445	0.0250	0.0328	0.0089	0.0349
Brevundimonas	1.8792	2.6019	0.5111	1.6637	6.5188	4.3516	9.3588	7.5399	0.1043	0.0144	3.1787	0.0535
Ruegeria	0.3880	0.2838	0.2234	0.3451	0.0543	0.0410	0.0161	0.0511	0.0356	0.0533	0.0131	0.0538
Pseudomonas	3.9622	4.1372	0.6992	0.3903	1.0705	1.5631	0.6966	0.0369	0.0302	0.0597	0.0091	0.2918
Neorhizobium	3.1197	4.3001	1.0435	0.3310	0	0	0	0	0	0	0	0
Variovorax	0.7655	0.6832	0	0.7224	42.0104	1.9005	0	4.7198	0.0014	0.0036	0	0.0965
Acinetobacter	2.4792	2.3395	0.7084	0.5348	0.1943	0.1546	0.1610	0.6849	0.0111	0.2264	0.0463	80.6107
Hydrogenophaga	0.1150	0.3784	1.2385	1.2385	0.0651	0.0805	0.7351	0.9526	0.2546	0.6419	3.5329	5.8548
Psychrobacter	0.5169	0.9076	0.2043	0.6192	0	0	0	0	0.0564	0.0030	0.0006	0.0322
Roseicyclus	0.4880	0.4701	0.2534	0.8515	0.6962	0.5623	0.1625	0.3897	3.0997	5.5321	0.9337	3.3537
Lutimaribacter	0.8201	0.9499	0.4617	1.2901	1.5204	1.1486	0.2035	0.7361	6.2641	14.4081	0.9345	2.5092
Stenotrophomonas	2.2227	6.2684	1.3119	1.3159	11.3877	20.2319	19.0116	24.8925	0.0898	0.0994	3.9838	1.8698
Delftia	0	0	0	0	0	0	0	0	0	0	0	0
Marinobacterium	0	0	0	0	0	0	2.2936	0	0	0	54.6086	0

References

- 1 Q. Ye, Y. Wu, Z. Zhu, X. Wang, Z. Li and J. Zhang, Bacterial diversity in the surface sediments of the hypoxic zone near the Changjiang Estuary and in the East China Sea, *Microbiologyopen*, 2016, **5**, 323-339.
- 2 C. Lee, J. Kim, S. G. Shin and S. Hwang, Absolute and relative QPCR quantification of plasmid copy number in *Escherichia coli*, *J. Biotechnol.*, 2006, **123**, 273-280.
- 3 S. Koike, I. G. Krapac, H. D. Oliver, A. C. Yannarell, J. C. Chee-Sanford, R. I. Aminov and R. I. Mackie, Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period, *Appl. Environ. Microbiol.*, 2007, **73**, 4813-4823.
- 4 R. Pei, S. C. Kim, K. H. Carlson and A. Pruden, Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG), *Water Res.*, 2006, 40, 2427-2435.
- 5 L. K. Ng, I. Martin, M. Alfa and M. Mulvey, Multiplex PCR for the detection of tetracycline resistant genes, *Mol. Cell. Probes*, 2001, **15**, 209-215.
- 6 R. I. Aminov, N. Garrigues-Jeanjean and R. I. Mackie, Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins, *Appl. Environ. Microbiol.*, 2001, 67, 22-32.
- 7 T. R. Burch, M. J. Sadowsky and T. M. Lapara, Aerobic digestion reduces the quantity of antibiotic resistance genes in residual municipal wastewater solids, *Front. Microbiol.*, 2013, **4**, 17.
- 8 H. B. Kim, C. H. Park, C. J. Kim, E. C. Kim, G. A. Jacoby and D. C. Hooper, Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period, *Antimicrob. Agents Chemother.*, 2009, **53**, 639-645.
- 9 X. P. Guo, Y. Yang, D. P. Lu, Z. S. Niu, J. N. Feng, Y. R. Chen, F. Y. Tou, E. Garner, J. Xu, M. Liu and M. F. Hochella, Jr., Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze Estuary, *Water Res.*, 2018, 129, 277-286.
- 10 H. Schmitt, K. Stoob, G. Hamscher, E. Smit and W. Seinen, Tetracyclines and tetracycline resistance in agricultural soils: microcosm and field studies, *Microb. Ecol.*, 2006, **51**, 267-276.
- 11 H. Liao, X. Lu, C. Rensing, V. P. Friman, S. Geisen, Z. Chen, Z. Yu, Z. Wei, S. Zhou and Y. Zhu, Hyperthermophilic composting accelerates the removal of antibiotic resistance genes and mobile genetic elements in sewage sludge, *Environ. Sci. Technol.*, 2018, **52**, 266-276.
- 12 Y. Luo, D. Mao, M. Rysz, Q. Zhou, H. Zhang, L. Xu and J. J. A. P, Trends in antibiotic resistance genes occurrence in the Haihe River, China, *Environ. Sci. Technol.*, 2010, 44, 7220-7225.
- 13 R. I. Aminov, J. C. Chee-Sanford, N. Garrigues, B. Teferedegne, I. J. Krapac, B. A. White and R. I. Mackie, Development, validation, and application of PCR primers for detection of tetracycline efflux genes of gramnegative bacteria, *Appl. Environ. Microbiol.*, 2002, **68**, 1786-1793.
- 14 C. X. Yan, M. H. Nie, Y. Yang, J. L. Zhou, M. Liu, M. Baalousha and J. R. Lead, Effect of colloids on the occurrence, distribution and photolysis of emerging organic contaminants in wastewaters, *J. Hazard. Mater.*, 2015, 299, 241-248.