

Comprehensive analysis of antimicrobial, heavy metal and pesticide residues in commercial organic fertilizers and their correlation with tigeccycline-resistant *tet(X)*-variant genes

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Running Head: heavy metals promoting spread of *tet(X)*-variant genes

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Figure S5. Redundancy analysis (RDA) of the relationships between *tet(X)*-variant

genes and concentrations of five heavy metals, five classes of antimicrobials, and all the pesticides. SAs: sulfanilamides, FQs: fluoroquinolones, MAs: macrolides, TCs: tetracyclines, AMs: amphenicols.

Figure S6. Heavy metals induced significant changes in the bacterial outer-membrane permeability. A. Changes in the outer membrane permeability of *tet*(X3)-positive *A. baumannii* ATCC17978 using the 1-N-Phenylnaphthylamine (NPN) probe on exposure to different heavy metals. B. Changes in the outer membrane permeability of *tet*(X4)-positive *E. coli* BL21 using the 1-N-Phenylnaphthylamine (NPN) probe on exposure to different heavy metals. Significant differences between outer membrane permeability with one concentration of the heavy metal and the corresponding control were tested using the unpaired *t*-test, * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Table S1. Sampling information in this study.

Provinces	Origin of raw materials for organic fertilizers				Number
	Chicken feces	Pig feces	Dairy feces	Plant waste ^a	Total
Zhejiang	3	5	0	8	16
Jiangsu	7	5	7	7	26
Hebei	4	3	11	0	18
Shandong	2	2	/	/	4
Henan	3	2	/	/	5
Liaoning	4	/	/	/	4
Heilongjiang	2	1	/	/	3
Hubei	4	4	/	/	8
Sichuan	5	5	/	/	10
Total	34	27	18	15	94

^a Plant waste origin means the organic fertilizers were made from plant materials such as straw from wheat, corn and rice.

Table S2. Previously reported *tet(X)*-variant-carrying plasmids.

Plasmid designation	<i>tet(X)</i> variants	Bacterial host	Genbank accession no.	Incompatibility group or <i>rep</i> gene	Conjugative	T4SS type
p34AB	<i>tet(X3)</i>	<i>Acinetobacter baumannii</i>	MK134375	<i>rep</i> -p34AB	Yes	Untypable
pB18-2	<i>tet(X3)</i>	<i>Acinetobacter indicus</i>	CP044457	<i>rep</i> -pB18-2	No	No
pCMG3-2-1	<i>tet(X3)</i>	<i>A. indicus</i>	CP044446	<i>rep</i> -pB18-2	No	No
pMMS9-2-1	<i>tet(X3)</i>	<i>A. indicus</i>	CP044451	<i>rep</i> -pB18-2	No	No
pHZE30-1-1	<i>tet(X3)</i>	<i>Acinetobacter schindleri</i>	CP044484	<i>rep</i> -pB18-2	No	No
pHZE33-1-1	<i>tet(X3)</i>	<i>A. schindleri</i>	CP044484	<i>rep</i> -pB18-2	No	No
pC15-1	<i>tet(X3)</i>	<i>A. indicus</i>	CP048655	<i>rep</i> -pB18-2	No	No
p18TQ-X3	<i>tet(X3)</i>	<i>A. indicus</i>	CP045132	<i>rep</i> -pB18-2	No	No
pAB17H194-1	<i>tet(X3)</i>	<i>Acinetobacter pittii</i>	CP040912	<i>rep</i> -pB18-2	No	No
pBDT2091-4	<i>tet(X3)</i>	<i>A. schindleri</i>	CP094838	<i>rep</i> -pB18-2	No	No
pYY76-1-2	<i>tet(X4)</i>	<i>Escherichia coli</i>	CP040929	IncX1	Yes	VirB/VirD (type P)
p54-tetX	<i>tet(X4)</i>	<i>Escherichia coli</i>	CP041286	IncX1	No	No
pYPE12-101k-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	CP041443	IncX1	No	No
pYPE10	<i>tet(X4)</i>	<i>E. coli</i>	CP041449	IncFIA(HI1)	Yes	Tra_F (type F)
pYPE3-92k-tetX4	<i>tet(X4)</i>	<i>E. coli</i>	CP041453	IncX1	No	No
pNT1F31-tetX4	<i>tet(X4)</i>	<i>E. coli</i>	CP045188	IncX1	No	No
p1916D18-1	<i>tet(X4)</i>	<i>E. coli</i>	CP045998	IncX1	Yes	VirB/VirD (type P)
p1916D6-2	<i>tet(X4)</i>	<i>E. coli</i>	CP046002	IncX1	Yes	VirB/VirD (type P)
p1919D3-1	<i>tet(X4)</i>	<i>E. coli</i>	CP046004	IncFIA(HI1)	Yes	Tra_F (type F)
p1919D62-1	<i>tet(X4)</i>	<i>E. coli</i>	CP046007	IncFIA(HI1)	Yes	Tra_F (type F)
pT16R-1	<i>tet(X4)</i>	<i>E. coli</i>	CP046717	IncFIA(HI1)	Yes	Tra_F (type F)
pZF31-tetX-119kb	<i>tet(X4)</i>	<i>E. coli</i>	CP047460	IncFIB(K)	No	No
pZF34-tetX-114kb	<i>tet(X4)</i>	<i>E. coli</i>	CP047466	IncFIB(K)	No	No
p2EC1-1	<i>tet(X4)</i>	<i>E. coli</i>	CP047572	IncI1	Yes	Tra_I (type I)
p94EC-2	<i>tet(X4)</i>	<i>E. coli</i>	CP047578	IncI1	Yes	Tra_I (type I)
pEC931_tetX	<i>tet(X4)</i>	<i>E. coli</i>	CP049121	IncX1	Yes	VirB/VirD (type P)
pT28R-1	<i>tet(X4)</i>	<i>E. coli</i>	CP049354	IncFIA(HI1)	Yes	Tra_F (type F)
pCD58-3-1	<i>tet(X4)</i>	<i>E. coli</i>	CP050037	IncX1	No	No
pCD63-2-1	<i>tet(X4)</i>	<i>E. coli</i>	CP050041	IncX1	No	No
pCD74-2-2	<i>tet(X4)</i>	<i>E. coli</i>	CP050046	IncX1	No	No
pSTB20-1T	<i>tet(X4)</i>	<i>E. coli</i>	CP050174	IncX1	No	No
pHNCF11W-tetX4	<i>tet(X4)</i>	<i>Escherichia fergusonii</i>	CP053047	IncX1	Yes	VirB/VirD (type P)
pSY3626C1_229k	<i>tet(X4)</i>	<i>E. coli</i>	CP058949	IncFIA(HI1)	Yes	Tra_F (type F)
pSY3626C1_315k	<i>tet(X4)</i>	<i>E. coli</i>	CP059044	IncFIA(HI1)	Yes	Tra_F (type F)
pSY3626_190k_tetX	<i>tet(X4)</i>	<i>E. coli</i>	CP059284	IncFIA(HI1)	Yes	Tra_F (type F)
pSal21GXH-tetX4	<i>tet(X4)</i>	<i>Salmonella enterica</i>	CP060586	IncFIA(HI1)	Yes	Tra_F (type F)
pb1-2L-1	<i>tet(X4)</i>	<i>Klebsiella pneumoniae</i>	CP072457	IncFIA(HI1)	Yes	Tra_F (type F)
pK-1L-1	<i>tet(X4)</i>	<i>K. pneumoniae</i>	CP072461	IncFIA(HI1)	Yes	Tra_F (type F)
p3Z-5L-2-X4	<i>tet(X4)</i>	<i>Klebsiella</i>	CP072517	IncFIA(HI1)	Yes	Tra_F

		<i>quasipneumoniae</i>				(type F)
pPK5074-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP072807	IncFII	Yes	Tra_F (type F)
pPK8217-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080124	IncFII	Yes	Tra_F (type F)
pPK8568-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080129	IncFII	Yes	Tra_F (type F)
pPK8277-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080134	IncFII	Yes	Tra_F (type F)
pPK8241-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080140	IncFII	Yes	Tra_F (type F)
pPK8276-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080147	IncQ1	No	No
pPK8261-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080156	IncFII	Yes	Tra_F (type F)
pPK8275-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080164	IncFII	Yes	Tra_F (type F)
pPK8566-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080175	IncFII	Yes	Tra_F (type F)
pPK5086-tetX	<i>tet</i> (X4)	<i>E. coli</i>	CP080371	IncFII	Yes	Tra_F (type F)
pLHC5-1-tetX	<i>tet</i> (X4)	<i>Citrobacter werkmanii</i>	CP084295	IncFIA(HI1)	Yes	Tra_F (type F)
pEC05-X4	<i>tet</i> (X4)	<i>E. coli</i>	MN436006	IncX1	No	No
pEC12-X4	<i>tet</i> (X4)	<i>E. coli</i>	MN436007	IncX1	No	No
pRB3-1_31K_tetX	<i>tet</i> (X4)	<i>E. coli</i>	MT197111	IncX1	No	No
pRF14-1	<i>tet</i> (X4)	<i>E. coli</i>	MT219822	IncX1	Yes	VirB/VirD (type P)
pRT18-1_294k_tetX	<i>tet</i> (X4)	<i>E. coli</i>	MT219824	IncFIA(HI1)	Yes	Tra_F (type F)
pXMPTW1-3	<i>tet</i> (X4)	<i>Citrobacter freundii</i>	MZ375739	IncX1	Yes	VirB/VirD (type P)
pSY3626_190k_tetX	<i>tet</i> (X4)	<i>E. coli</i>	NZ_JABXOE 010000002	IncFIA(HI1)	Yes	Tra_F (type F)
p47EC	<i>tet</i> (X4)	<i>E. coli</i>	NZ_MK1343 76	IncFIB(K)	Yes	Tra_F (type F)
pAB17H194-1	<i>tet</i> (X5)	<i>Acinetobacter pittii</i>	CP040912	rep-pAB17H194	No	No
pZN2-tetX-171kb	<i>tet</i> (X6)	<i>Proteus cibarius</i>	CP047350	IncA/C2	No	Tra_F (type F)
pZN3-tetX-171kb	<i>tet</i> (X6)	<i>Proteus vulgaris</i>	CP047345	IncA/C2	No	Tra_F (type F)
pZA25-tetX-168kb	<i>tet</i> (X6)	<i>Proteus mirabilis</i>	CP047353	IncA/C2	No	Tra_F (type F)

Table S3. qPCR primers used in this study.

Target genes	Genbank accession no. and location	Primers	Primer sequences (5'- 3')	Length (bp)	References
16S rRNA	—	16S rRNA-F	CCTACGGGAGGCAGCAG	194	(1)
		16S rRNA-R	ATTACCGCGGCTGCTGG		
<i>tet(X)/(X2)^a</i>	<i>tet(X)</i> (GU014535.1), <i>tet(X2)</i> (AJ311171)	<i>tet(X)/(X2)</i> -F	TGCGGCTAATGGCATCTCAC	227	(2)
		<i>tet(X)/(X2)</i> -R	GCTGCTACACATGACAACGTCGT		
<i>tet(X3)</i>	MK134375: 6174-7340	<i>tet(X3)</i> -F	GTGGATGCTTTGCTATTGTCTGA	125	(2)
		<i>tet(X3)</i> -R	TCTGTTGATTTCGTCTGCGTAT		
<i>tet(X4)</i>	MK134376: c1482-325	<i>tet(X4)</i> -F	TCGCTACAAAGAACTGATTCGTG	93	(2)
		<i>tet(X4)</i> -R	GGTCGCTTACTTCTCCAAGACTTAC		
<i>tet(X5)</i>	CP040912	<i>tet(X5)</i> -F	TGCCGTTGACCTACACAAAGG	161	(2)
		<i>tet(X5)</i> -R	TGTCAAACGATTTTCGGGTC		
<i>tet(X6)</i>	PRJNA593823	<i>tet(X6)</i> -F	CCGGGCCTGTTGGATTAACA	117	(2)
		<i>tet(X6)</i> -R	GCAGATCAAGTGTCCCACCA		
IncX1 plasmid <i>rep (tet(X4))</i>	MT219822: c38652-37816	IncX1 <i>rep</i> -F	CATCGTTGCCTTTGTCTGCC	115	This study
		IncX1 <i>rep</i> -R	AATGCATATTCCCGAGCCGT		
IncFIA (HI1) plasmid <i>rep (tet(X4))</i>	CP041449: 45613-46368	<i>repE</i> -F	CGACTGGATCATGGAACGCT	111	This study
		<i>repE</i> -R	ATTGGTGTCCGGCTGTTGAT		
IncFII plasmid <i>rep (tet(X4))</i>	CP072807: 90246-91103	<i>repA</i> -F	ACATCACGTTTCACTGGCT	256	This study
		<i>repA</i> -R	TCCTGACGTTCTCTGTTCGC		
IncII plasmid <i>rep (tet(X4))</i>	CP047572: 46-1077	<i>repII</i> -F	ACTTCACGAACAGCAGCAGA	84	This study
		<i>repII</i> -R	TCATCCTCACGCAGAACACC		
IncFIB(K) plasmid <i>rep (tet(X4))</i>	MK134376: c16538-15528	<i>repA2</i> -F	CGGTCTCTTCGTCCCAACTC	187	This study
		<i>RepA2</i> -R	GCGAATTATCCCCAGCCAGA		
pB18-2 plasmid <i>rep (tet(X3))</i>	CP044457: c42212-41040	<i>rep</i> -pB18-2-F	TGAGGAGCCTGCTGATAATGG	160	This study
		<i>rep</i> -pB18-2-R	ATTACCCCCAGCCAGTTGAG		
p34AB plasmid <i>rep (tet(X3))</i>	MK134375: 134004-134528	<i>rep</i> -34AB-F	ACTCCCTCAATCAGCTCCCA	119	This study
		<i>rep</i> -34AB-R	GCTCAACGTGTTCTGGGACA		
pAB17H194-1 plasmid <i>rep (tet(X5))</i>	CP040912: c73828-72656	<i>rep</i> -pAB-F	CCGTTAAGAAAGGCCGTCCA	253	This study
		<i>rep</i> -pAB-R	TGAGAGTCAGTGAGGCATCC		
pZn2 plasmid <i>rep (tet(X6))</i>	CP047350: 37-1101	<i>rep</i> -pZn2-F	GGGACAGCGAGAGGAAATCC	152	This study
		<i>rep</i> -pZn2-R	CGTTCAGGTTGTACCCGTGA		
<i>czcA</i>	CP045132	<i>czcA</i> -F	ATTTATTGGGCCCCGTCAGCA	211	This study
		<i>czcA</i> -R	GACGCACAATCCAGTCCTGA		
<i>merT</i>	MT219816	<i>merT</i> -F	GCTTGGATCGGCAACTTGAC	220	This study
		<i>merT</i> -R	TGACGTAGGGAAATCCGAGC		
<i>dotB</i>	MK134375: c256266-254971	<i>dotB</i> -F	TTTCTGGCATTCCCCCTCAC	108	This study
		<i>dotB</i> -R	CAGAACCAGTGATTCCGCCA		
<i>dotC</i>	MK134375: c257103-256294	<i>dotC</i> -F	ATGGGTTTAGTTGAGGGCCG	263	This study
		<i>dotC</i> -R	GGTGCAATTTGTTCAAGTTGGA		
<i>dotI</i>	MK134375: 260194-260877	<i>dotI</i> -F	TGGCTCAGAAAACAGGTGGA	101	This study
		<i>dotI</i> -R	GCAAGAACAGCACTCACGAT		
<i>dotG</i>	MK134375: 261860-263371	<i>dotG</i> -F	TGGTACGGAAATGGCGAACA	113	This study
		<i>dotG</i> -R	TCGGTGTTGTTACGTCAGCA		
<i>traA_F</i> (InFIB(K))	MK134376: 63626-63988	<i>traA</i> -F	ATATGCTTCGCCTGGTACGC	191	This study
		<i>traA</i> -R	CATGACAGCACCGACCAGAA		
<i>traK_F</i> (InFIB(K))	MK134376: 64903-65607	<i>traK</i> -F	TGCCACCGTCAACAAAAAGC	188	This study
		<i>traK</i> -R	TGGTCACAAGCAGGGATTCTG		
<i>traB_F</i> (InFIB(K))	MK134376: 65607-67034	<i>traB</i> -F	TAACGGCAAAATCCTCGGCT	133	This study
		<i>traB</i> -R	TTTCAGGACATCACCAGCCC		
<i>traC_F</i>	MK134376:	<i>traC</i> -F	AATGAGCTTCCCGCAGTTCA	130	This study

(InFIB(K))	68946-71573	<i>traC</i> -R	GGACTCATTCGCCCCATTGA		
<i>traG_F</i> (InFIB(K))	MK134376: 79640-82462	<i>traG</i> -F	GGGATGTATGGCGATGCTCA	101	This study
		<i>traG</i> -R	TCAATACTTGCCCGACCACC		
<i>virB2</i> (IncX1)	MT219822: 46274-46564	<i>virB2</i> -F	ACCCTGTCTGTCTTCATGGC	147	This study
		<i>virB2</i> -R	CGCAATGATAGTGATAGCTGGC		
<i>virB4</i> (IncX1)	MT219822: 46588-49347	<i>virB3</i> -F	AGCTGCCTACTACCTTCCCA	133	This study
		<i>virB3</i> -R	TTTGACTCCACGGTTAGCCC		
<i>virB8</i> (IncX1)	MT219822: 1087-1809	<i>virB8</i> -F	CGGAGGAATGTCAGGGATGG	129	This study
		<i>virB8</i> -R	GTCAACCTTTCAGCAACACCA		
<i>virB9</i> (IncX1)	MT219822: 1815-2744	<i>virB9</i> -F	ACCCCCAAAATACGACCGTT	112	This study
		<i>virB9</i> -R	GACCAACCTTGCGGAAAACC		
<i>virB10</i> (IncX1)	MT219822: 2741-3955	<i>virB10</i> -F	TTCGTGCAATGTCCAGCTCT	123	This study
		<i>virB10</i> -R	TGTCATCCCTCTGGCTCTGA		
<i>virD4</i> (IncX1)	MT219822: 5015-6850	<i>virD4</i> -F	GGAACGGAAGGGGCAAAAAC	168	This study
		<i>virD4</i> -R	AGAGTTGCTTCGGGATGTGG		
<i>traA_F</i> (IncFIA(HI1))	CP041449: 176552-176905	<i>traA</i> -F	TCTCGGTGGCTTATTCGGC	126	This study
		<i>traA</i> -R	GAGGAATACCAGCATCCAGGA		
<i>traK_F</i> (IncFIA(HI1))	CP041449: 178074-179306	<i>traK</i> -F	CTGGCACTACGACTGAGACC	119	This study
		<i>traK</i> -R	GTTATTTGACAACGCCCCGG		
<i>traB_F</i> (IncFIA(HI1))	CP041449: 179738-181096	<i>traB</i> -F	CCGACCCGAAACAGTCCAAT	135	This study
		<i>traB</i> -R	TTCACTTCCCGCAACAACCT		
<i>traC_F</i> (IncFIA(HI1))	CP041449: 183444-186125	<i>traC</i> -F	GCAATGGTGTGAAGGAGGGA	125	This study
		<i>traC</i> -R	CAGTCTGAAGGTGTGGGGTC		
<i>traG_F</i> (IncFIA(HI1))	CP041449: c108660-104671	<i>traG</i> -F	GTCACCACGAACCTTAGCCA	156	This study
		<i>traG</i> -R	GCTGTCACCACCCCATTGTA		

^a *tet*(X) and *tet*(X2) could not be distinguished by qPCR as they shared >99% sequence identity with each other

Table S4. The minimal inhibitory concentrations (MICs) of heavy metals against bacterial hosts.

MIC of heavy metals (µg/mL)						
	<i>A. baumannii</i> ATCC17978	<i>A. baumannii</i> 5AB	<i>E. coli</i> BL21-p47EC (<i>tet</i> (X4) ⁺)	<i>E. coli</i> BL21-pRF14-1 (<i>tet</i> (X4) ⁺)	<i>E. coli</i> BL21-pYPE10 (<i>tet</i> (X4) ⁺)	<i>E. coli</i> J53
	-p34AB (<i>tet</i> (X3) ⁺)					
As ⁵⁺	250	125	250	250	250	125
Pb ²⁺	15	15	15	15	15	15
Cd ²⁺	15	7.5	60	60	60	125
Cr ⁶⁺	15	3	30	15	30	15
Hg ²⁺	1.5	3	7.5	7.5	7.5	1.5

Table S5. Detected concentrations (mg/kg) of heavy metals among 96 organic fertilizer products.

	Hg	As	Cd	Cr	Pb	Total
Chinese National standard limits	≤2 mg/kg	≤15 mg/kg	≤3 mg/kg	≤150 mg/kg	≤50 mg/kg	
YJF-2020001	0.04	4.95	0.71	199.46^a	14.43	219.59
YJF-2020002	0.10	0.41	0.32	2.55	0.92	4.30
YJF-2020003	ND ^b	4.03	1.13	23.14	18.07	46.37
YJF-2020004	0.19	5.26	1.15	50.57	6.44	63.62
YJF-2020005	0.30	3.86	0.64	15.17	2.82	22.80
YJF-2020006	ND	4.42	0.98	95.73	66.00	167.13
YJF-2020007	0.07	5.00	0.98	33.73	38.56	78.33
YJF-2020008	0.07	4.64	1.31	20.11	39.93	66.06
YJF-2020009	0.39	7.94	1.46	31.21	92.14	133.13
YJF-2020010	0.61	3.79	0.40	9.15	2.03	15.97
YJF-2020011	0.12	6.47	0.38	10.51	1.17	18.64
YJF-2020012	ND	8.96	0.33	6.63	0.46	16.37
YJF-2020013	0.09	1.60	0.63	37.93	13.17	53.42
YJF-2020014	0.02	121.56	0.09	10.52	3.48	135.67
YJF-2020015	0.06	5.37	4.93	15.42	12.48	38.26
YJF-2020016	0.13	19.94	1.22	59.16	505.93	586.39
YJF-2020017	0.03	6.21	0.37	29.73	23.85	60.18
YJF-2020018	0.19	4.20	0.22	38.71	9.29	52.60
YJF-2020019	0.02	2.02	0.68	12.48	16.17	31.36
YJF-2020020	0.05	10.83	0.09	10.87	3.48	25.32
YJF-2020021	0.04	5.12	1.14	21.26	45.02	72.58
YJF-2020022	0.01	1.40	0.05	4.50	7.33	13.28
YJF-2020023	0.15	5.77	0.45	75.80	24.59	106.75
YJF-2020024	ND	1.17	0.01	5.63	0.93	7.74
YJF-2020025	0.02	12.25	0.89	27.57	24.89	65.62
YJF-2020026	0.03	5.47	1.02	20.99	6.48	33.98
YJF-2020027	0.04	3.23	0.14	25.85	7.57	36.83
YJF-2020028	0.02	8.44	4.92	42.91	16.33	72.61
YJF-2020029	ND	4.73	0.05	7.75	0.71	13.24
YJF-2020030	0.42	17.76	0.63	40.37	9.01	68.19
YJF-2020031	ND	9.53	0.06	6.11	0.33	16.03
YJF-2020032	0.21	6.15	1.12	77.40	18.19	103.08
YJF-2020033	0.24	5.23	0.21	42.42	11.69	59.79
YJF-2020034	ND	2.36	0.01	4.61	1.15	8.13
YJF-2020035	0.02	6.34	0.59	25.46	10.54	42.95
YJF-2020036	0.13	27.72	11.42	52.04	251.19	342.50
YJF-2020037	ND	5.62	0.10	8.43	2.87	17.01

YJF-2020038	0.26	9.67	0.20	26.65	4.05	40.82
YJF-2020039	0.09	7.16	2.71	48.99	42.43	101.38
YJF-2020040	0.09	4.52	0.27	22.24	15.72	42.83
YJF-2020041	0.49	5.59	0.04	3.62	1.00	10.74
YJF-2020042	0.04	8.68	1.43	28.88	45.55	84.58
YJF-2020043	0.04	6.03	1.13	25.94	46.73	79.88
YJF-2020044	0.02	6.03	0.65	40.46	26.74	73.89
YJF-2020045	ND	3.80	0.12	16.37	7.06	27.34
YJF-2020046	0.08	6.89	1.13	23.16	62.11	93.38
YJF-2020047	ND	2.99	0.04	7.30	15.75	26.08
YJF-2020048	ND	3.77	0.30	22.28	9.60	35.95
YJF-2020049	ND	4.18	0.12	13.37	7.27	24.93
YJF-2020050	0.32	2.93	0.34	21.51	28.12	53.22
YJF-2020051	0.29	6.46	1.05	60.06	7.89	75.77
YJF-2020052	0.05	11.23	0.59	28.00	23.50	63.36
YJF-2020053	0.06	5.95	0.63	23.44	42.89	72.97
YJF-2020054	0.17	4.53	0.61	21.81	26.20	53.33
YJF-2020055	0.01	4.06	0.31	31.76	11.43	47.58
YJF-2020056	ND	0.25	0.04	5.42	0.64	6.35
YJF-2020057	0.02	3.79	0.19	20.89	11.94	36.84
YJF-2020058	0.03	3.25	0.63	35.68	21.07	60.66
YJF-2020059	0.07	5.25	0.62	51.29	13.00	70.23
YJF-2020060	0.17	4.00	0.31	20.02	16.33	40.82
YJF-2020061	0.53	3.80	0.50	33.18	13.12	51.14
YJF-2020062	0.27	4.74	0.24	18.14	24.77	48.16
YJF-2020063	0.04	3.60	0.61	25.24	16.75	46.24
YJF-2020064	0.08	6.71	0.48	34.00	14.91	56.19
YJF-2020065	0.21	6.60	1.01	23.36	22.72	53.90
YJF-2020066	0.13	3.74	0.73	27.90	16.24	48.75
YJF-2020067	0.17	5.69	0.92	32.25	15.17	54.21
YJF-2020068	0.07	3.14	0.38	21.44	12.25	37.28
YJF-2020069	ND	3.90	0.62	40.61	13.67	58.80
YJF-2020070	ND	0.04	0.08	2.19	0.23	2.53
YJF-2020071	0.57	7.58	1.63	250.00	22.58	282.36
YJF-2020072	0.04	2.88	0.33	35.31	14.96	53.52
YJF-2020073	0.07	4.70	0.37	24.54	12.30	41.99
YJF-2020074	0.04	4.52	0.35	23.25	11.95	40.11
YJF-2020075	0.01	0.22	0.50	7.34	4.20	12.26
YJF-2020076	ND	0.96	0.01	2.25	1.42	4.64
YJF-2020077	ND	0.03	0.03	3.05	0.83	3.94
YJF-2020078	ND	3.74	0.25	15.00	8.82	27.80

YJF-2020079	1.47	6.35	0.85	63.66	28.94	101.27
YJF-2020080	0.11	11.41	2.80	27.39	49.35	91.06
YJF-2020081	ND	0.13	0.11	9.52	0.36	10.12
YJF-2020082	ND	0.87	0.20	13.25	5.31	19.62
YJF-2020083	0.01	4.55	0.35	20.58	7.60	33.09
YJF-2020084	0.14	5.72	1.31	27.24	26.80	61.21
YJF-2020085	ND	1.68	0.63	43.31	12.16	57.78
YJF-2020086	0.75	9.44	1.84	37.17	19.85	69.05
YJF-2020087	0.27	8.52	0.51	42.90	15.65	67.86
YJF-2020088	0.07	14.89	1.84	51.26	27.21	95.26
YJF-2020089	0.02	7.92	1.83	27.43	43.71	80.91
YJF-2020090	0.77	4.10	0.91	13.93	13.30	33.02
YJF-2020091	0.14	8.93	0.83	14.89	12.75	37.54
YJF-2020092	0.36	10.00	2.40	27.53	28.09	68.38
YJF-2020093	2.07	16.16	1.49	37.44	21.45	78.60
YJF-2020094	0.29	8.39	2.74	30.41	83.48	125.31

^a The values in bold were that which exceeded the limits requested in the national standards.

^b ND means not detected using the methods in this study.

Table S6. Detected concentrations of antimicrobials among 96 organic fertilizer products.

[illegible]

YJF-2020043								212.48		8.73								
YJF-2020044	5.03																	
YJF-2020045																		
YJF-2020046																		
YJF-2020047																		
YJF-2020048																		
YJF-2020049											7.4							
YJF-2020050																		
YJF-2020051																		
YJF-2020052										8.51								
YJF-2020053																		
YJF-2020054																		
YJF-2020055										8.36								
YJF-2020056																		
YJF-2020057																		
YJF-2020058																		
YJF-2020059																		
YJF-2020060																		
YJF-2020061										8.49								
YJF-2020062																		
YJF-2020063							11.47											
YJF-2020064							11.93							44.2				

YJF-2020087																		
YJF-2020088																64.16		
YJF-2020089																		
YJF-2020090																	44.46	
YJF-2020091																		
YJF-2020092					6.33													
YJF-2020093														23.1	56.31			
YJF-2020094								114.36										

^a SD, sulfadiazine; SMZ, sulfamethoxazole; SM2, sulfamethazine; SMM, sulfamonomethoxine; SQ, sulfaquinoxaline; ENR, enrofloxacin; CIP, ciprofloxacin; DFL, daifloxacin; SAR, sarafloxacin; AZM, azithromycin; TIL, tilmicosin; TYL, tylosin; TET, tetracycline; OTC, oxytetracycline; CTET, chlortetracycline; DOX, doxycycline; THI, thiamphenicol; FFC, florfenicol.

^b Blank means not detected using the detection methods in this study.

^c The values in bold were the concentrations that were more than 100 µg/kg.

Table S7. Comparison of residual concentrations of pesticides in fertilizer samples of different origins.

	Chicken feces origin (n=34)			Pig feces origin (n=27)			Dairy feces origin (n=18)			Plant waste origin (n=15)			Total (n=94)		
	Detection	Concentration	Mean ^a	Detection	Concentration	Mean	Detection	Concentration	Mean	Detection	Concentration	Mean	Detection	Concentration	Mean
	rate (%)	ranges (µg/kg)	(µg/kg)	rate (%)	ranges (µg/kg)	(µg/kg)	rate (%)	ranges (µg/kg)	(µg/kg)	rate (%)	ranges (µg/kg)	(µg/kg)	rate (%)	ranges (µg/kg)	(µg/kg)
avermectin	0	–	–	3.70	3.81	3.81	5.56	13.69	13.69	0	–	–	2.13	3.81-13.69	8.75
chlorpyrifos	2.94	8.41	8.41	0	–	–	0	–	–	6.67	20.66	20.66	2.13	8.41-20.66	14.54
fenprothrin	2.94	5.66	5.66	0	–	–	0	–	–	6.67	12.63	12.63	1.06	2.84-2.84	2.84
imidacloprid	2.94	2.16	2.16	0	–	–	0	–	–	13.33	2.21-19.64	10.93	3.19	2.16-19.64	8
tetramethrin	0	–	–	0	–	–	0	–	–	6.67	2.48	2.48	1.06	2.48	2.48
triazophos	0	–	–	0	–	–	0	–	–	40	2.32-13.88	6.26	6.38	2.32-13.88	6.26
tebuconazole	0	–	–	3.70	5.02	5.02	5.56	2.62	2.62	20	2.22-86.68	30.70	5.32	2.22-86.68	19.95
3-OH-Carbofuran	0	–	–	0	–	–	0	–	–	6.67	5.31	5.31	1.06	5.31	5.31
bifenthrin	0	–	–	0	–	–	0	–	–	6.67	2.84	2.84	1.06	2.84	2.84
carbendazim	0	–	–	0	–	–	0	–	–	20	2.28-2.93	2.67	3.19	2.28-2.93	2.67
dichlorvos	2.94	13.29	13.29	7.41	8.51	8.53	11.11	3.67	3.70	6.67	4.99	4.99	6.38	3.67-13.29	7.12
diethofencarb	0	–	–	3.70	2.55	2.55	0	–	–	26.67	2.44-13.74	6.34	5.32	2.44-13.74	5.58
isocarbophos	2.94	2.40	2.40	0	–	–	0	–	–	26.67	2.24-8.10	3.79	5.32	2.24-8.10	3.51
prochloraz	0	–	–	0	–	–	0	–	–	20	2.65-4.36	3.35	3.19	2.65-4.36	3.35
triazophos	0	–	–	0	–	–	0	–	–	6.67	3.29	3.29	1.06	3.29	3.29
cyromazine	32.35	4.62– 828.76	91.13	33.33	3.27-653.69	105.01	38.89	3.60-148.31	39.17	0	–	–	28.72	3.27-828.76	82.29
methyl parathion	0	–	–	0	–	–	0	–	–	13.33	10.78-10.99	10.89	2.13	10.78-10.99	10.89
carbaryl	0	–	–	0	–	–	0	–	–	6.67	2.71	2.71	1.06	2.71	2.71

^a The mean indicated the average value of the detected concentrations of pesticides.

Table S8. Relative abundances (copies/16S rRNA) of detected *tet(X)*-variants, two heavy metal resistance genes and plasmid *rep* genes among 96 organic fertilizer samples.

		<i>tet(X)</i> -variant genes					Heavy metal resistance genes		<i>rep</i> genes from <i>tet(X)</i> -variant-carrying plasmids								
		<i>tet(X)</i> /(X2)	<i>tet(X3)</i>	<i>tet(X4)</i>	<i>tet(X5)</i>	<i>tet(X6)</i>	<i>czcA</i>	<i>merT</i>	<i>tet(X4)</i>					<i>tet(X3)</i>		<i>tet(X5)</i>	<i>tet(X6)</i>
	Origins								IncX1	IncFIA(HI1)	IncFII	IncI1	IncFIB(K)	pB18-2	p34AB	pAB17H194-1	pZN2
YJF-2020001	Chicken feces		5.10×10 ⁻⁴				4.28×10 ⁻⁴							6.56×10 ⁻⁴			
YJF-2020002	plant waste	8.64×10 ⁻⁵															
YJF-2020003	plant waste																
YJF-2020004	Pig feces				5.21×10 ⁻⁴									4.58×10 ⁻⁴			
YJF-2020005	plant waste							4.36×10 ⁻⁴									
YJF-2020006	Pig feces						6.27×10 ⁻³		2.86×10 ⁻⁴	4.73×10 ⁻⁴							
YJF-2020007	Pig feces	2.81×10 ⁻⁴															
YJF-2020008	plant waste																
YJF-2020009	Chicken feces	1.44×10 ⁻⁴	9.40×10 ⁻⁵					7.55×10 ⁻⁴						2.13×10 ⁻³			
YJF-2020010	plant waste							6.75×10 ⁻⁴									
YJF-2020011	plant waste																
YJF-2020012	plant waste	7.36×10 ⁻⁵															
YJF-2020013	plant waste																
YJF-2020014 ^a pYPE10-like-1	Pig feces		5.42×10 ⁻⁴	2.78×10 ⁻⁴			6.59×10 ⁻⁴			7.56×10 ⁻⁴			6.32×10 ⁻⁴				
YJF-2020015	Chicken feces		2.30×10 ⁻⁴												3.67×10 ⁻⁴		
YJF-2020016 pYPE10-like-2	Pig feces			4.74×10 ⁻⁴		3.10×10 ⁻⁴			8.56×10 ⁻⁴	8.27×10 ⁻⁴			7.41×10 ⁻⁴				

YJF-2020017	Dairy feces						8.17×10^{-4}										
YJF-2020018	Dairy feces								3.77×10^{-4}								
YJF-2020019	plant waste																
YJF-2020020	Pig feces	1.73×10^{-4}					4.73×10^{-3}										
YJF-2020021	Dairy feces	3.88×10^{-4}															
YJF-2020022	plant waste																
YJF-2020023	Dairy feces								2.93×10^{-4}	6.89×10^{-4}							
YJF-2020024	plant waste	3.57×10^{-4}															
YJF-2020025	Pig feces						7.50×10^{-3}		4.24×10^{-3}								
YJF-2020026	plant waste																
YJF-2020027	plant waste																
YJF-2020028	Chicken feces		3.76×10^{-4}				2.33×10^{-2}							6.42×10^{-4}			
YJF-2020029	Dairy feces																
YJF-2020030	Pig feces			3.29×10^{-4}					6.93×10^{-4}	6.18×10^{-4}				5.33×10^{-4}			
YJF-2020031	Pig feces						7.52×10^{-4}		6.62×10^{-4}								
YJF-2020032	Chicken feces	1.43×10^{-4}						2.17×10^{-4}									
YJF-2020033	Chicken feces																
YJF-2020034	plant waste	2.62×10^{-4}															
YJF-2020035	Dairy feces						4.94×10^{-3}										
YJF-2020036	Chicken feces			5.30×10^{-4}					8.25×10^{-4}								
YJF-2020037	Dairy feces																
YJF-2020038	Pig feces														6.39×10^{-4}		2.47×10^{-4}
YJF-2020039	Chicken feces	2.90×10^{-4}															
YJF-2020040	Chicken feces						3.28×10^{-3}		6.13×10^{-4}								

YJF-2020041	plant waste																
YJF-2020042	Chicken feces		3.73×10^{-4}				2.47×10^{-3}							4.66×10^{-4}			
YJF-2020043	Dairy feces		2.12×10^{-4}														
YJF-2020044	Dairy feces												8.27×10^{-4}				
YJF-2020045	Dairy feces									8.54×10^{-4}							
YJF-2020046	Pig feces				8.94×10^{-5}											2.56×10^{-4}	
YJF-2020047	Dairy feces								7.31×10^{-4}					5.44×10^{-4}			
YJF-2020048	Dairy feces													8.27×10^{-4}			
YJF-2020049	Dairy feces										6.44×10^{-4}						
YJF-2020050	Chicken feces	3.47×10^{-4}															
YJF-2020051	Chicken feces								8.35×10^{-4}								
YJF-2020052	Chicken feces																
YJF-2020053	Pig feces		2.95×10^{-4}				1.93×10^{-3}										
YJF-2020054	Pig feces												8.94×10^{-4}				
YJF-2020055	Chicken feces								3.29×10^{-4}								
YJF-2020056	Dairy feces	3.78×10^{-4}															
YJF-2020057	Dairy feces																
YJF-2020058	Dairy feces													3.16×10^{-4}			
YJF-2020059	Dairy feces													5.73×10^{-4}			
YJF-2020060	Dairy feces																
YJF-2020061	Chicken feces						5.22×10^{-3}										
YJF-2020062	Chicken feces								7.52×10^{-4}								
YJF-2020063	Pig feces									6.23×10^{-4}							
YJF-2020064	Pig feces									5.79×10^{-4}							

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YJF-2020088	Pig feces																
YJF-2020089	Pig feces		3.55×10^{-4}				4.27×10^{-3}										
YJF-2020090	Pig feces							5.89×10^{-4}									
YJF-2020091	Chicken feces																
YJF-2020092	Chicken feces													1.48×10^{-3}			
YJF-2020093	Pig feces		7.90×10^{-5}	6.84×10^{-4}				4.63×10^{-3}	9.28×10^{-4}								
YJF-2020094	Pig feces		4.63×10^{-4}				9.80×10^{-3}										

^a The designations in bold mean that the *tet*(X4)-carrying plasmid was captured by *E. coli* from these samples.

Table S9. Correlation by Spearman correlation analysis.

	$\sum tet(X)$ -variants ^a	$tet(X)/(X2)$	$tet(X3)$	$tet(X4)$	$tet(X5)$	$tet(X6)$
<i>czcA</i>	0.178	-0.179	0.474***	-0.0506	-0.0759	-0.0534
<i>merT</i>	0.107	0.147	0.0828	0.266**	-0.0449	-0.0316
IncX1	0.100	-0.063	-0.091	0.318**	-0.034	0.152
IncFIA(HI1)	0.182	-0.130	0.0599	0.293**	-0.0361	0.427
IncFIB(K)	0.127	-0.121	0.0316	0.231*	-0.0335	0.357
pB18-2	0.113	-0.0513	0.264*	-0.0800	0.107	-0.0333
p34AB	0.0644	-0.0766	0.221*	-0.0442	-0.0213	-0.0184
Hg	0.0738	-0.0584	0.045	0.241*	0.0907	0.0635
As	0.232*	-0.148	0.285**	0.383***	0.0701	0.17
Cd	0.283**	-0.0786	0.325**	0.166	0.152	0.117
Cr	0.191	-0.0859	0.152	0.234*	0.0854	0.151
Pb	0.197	-0.0541	0.260*	0.158	0.0415	0.178
Total HMs ^b	0.369*** ^c	-0.0597	0.389***	0.346***	0.135	0.178
DFL	0.201	-0.00119	0.321**	-0.0618	-0.0349	-0.0246
OTC	0.0801	0.0775	0.102	0.275**	-0.0418	-0.0294
DOX	0.0848	-0.0712	-0.0588	0.257*	-0.0217	0.696****

^a $\sum tet(X)$ -variants means the total amount of $tet(X)/(X2)$, $tet(X3)$, $tet(X4)$, $tet(X5)$ and $tet(X6)$.

^b HMs, heavy metals; DFL, dafloxacin; OTC, oxytetracycline; DOX, doxycycline;

^c Values indicate the Spearman correlation coefficient (R). * means the significant level at the 0.05 level ($p < 0.05$, two-tailed); ** means the significant level at the 0.01 level ($p < 0.01$, 2-tailed); *** means the significant level at the 0.001 level ($p < 0.001$, 2-tailed), **** means the significant level at the 0.0001 level ($p < 0.0001$, 2-tailed). The chemical contaminants which has no value with significant difference are not shown.

Table S10. Coexistence of *tet*(X3) or *tet*(X4) with the heavy metal resistance genes in the same plasmid obtained from NCBI.

	<i>tet</i> (X)-variant gene	Bacterial species	Animal origin	Heavy metal resistance genes	Heavy metal resistance phenotype	Genbank no.
pCMG3-2-1	<i>tet</i> (X3)	<i>A. indicus</i>	goose	<i>czcC, B, A, D</i>	Cd/Zn/Co	NZ_CP044446
pHZE33-1-1	<i>tet</i> (X3)	<i>A. schindleri</i>	goose	<i>czcC, B, A, D</i>	Cd/Zn/Co	CP044475
pHZE23-1-1	<i>tet</i> (X3)	<i>A. schindleri</i>	goose	<i>czcC, B, A, D</i>	Cd/Zn/Co	NZ_CP044464
pFS42-2-1	<i>tet</i> (X3)	<i>A. indicus</i>	duck	<i>cusA/czcA</i>	Cd/Zn/Co	CP046596
p10FS3-1-3	<i>tet</i> (X3)	<i>Acinetobacter</i> spp.	pig	<i>czcC, B, A, D</i>	Cd/Zn/Co	CP039146
pYH12207-2	<i>tet</i> (X3)	<i>A. piscicola</i>	pig	<i>cusA/czcA</i>	Cd/Zn/Co	CP048661
p10FS3-1-3	<i>tet</i> (X3)	<i>Acinetobacter</i> spp.	pig	<i>copA/copB, cusA/czcA</i>	Cu, Cd/Zn/Co	CP039146
pMMS9-2-1	<i>tet</i> (X3)	<i>A. indicus</i>	soil	<i>cusA/czcA</i>	Cd/Zn/Co	CP044451
pAI01	<i>tet</i> (X3)	<i>A. indicus</i>	manure	<i>copA/copB, czcC, B, A, D</i>	Cu, Cd/Zn/Co	CP044019
pAT205	<i>tet</i> (X3)	<i>A. townneri</i>	pig	<i>copA/copB, czcC, B, A, D</i>	Cu, Cd/Zn/Co	CP048015
p18TQ-X3	<i>tet</i> (X3)	<i>A. indicus</i>	cow	<i>czcC, B, A, D, merR</i>	Cd/Zn/Co, mercury	CP045132
pB18-2	<i>tet</i> (X3)	<i>A. indicus</i>	pigeon	<i>copA/copB, czcC, B, A, D</i>	Cu, Cd/Zn/Co	CP044457
pYUSHP10-1	<i>tet</i> (X3)	<i>Acinetobacter</i> spp.	pig	<i>copA/copB, czcC, B, A, D</i>	Cu, Cd/Zn/Co	MT107270
pNT1F10-tetX4	<i>tet</i> (X4)	<i>Escherichia coli</i>	Pig	<i>merD/ merC/ merP/ merT</i>	Hg	NZ_CP075463
pNT1N34-tetX4	<i>tet</i> (X4)	<i>Escherichia coli</i>	Pig	<i>merD/ merC/ merP/ merT</i>	Hg	NZ_CP075467

pNT1W22-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merT/merD</i>	Hg	NZ_CP075470
pNT1F25-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP075471
pNT1N28-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merD/ merC/ merP/ merT</i>	Hg	NZ_CP075473
pNT1W25-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP075479
pNT1N31-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP075481
pNT1N25-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP075485
pNT1F34-tetX4	<i>tet(X4)</i>	<i>Escherichia coli</i>	Pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP075486
pT16R-2	<i>tet(X4)</i>	<i>Escherichia coli</i>	dog	<i>merE/merD/merC/merP/merT/mntB</i>	Hg/Mn	NZ_CP046718
pSTB20-1T	<i>tet(X4)</i>	<i>Escherichia coli</i>	pig	<i>merD/merC/merP/merT</i>	Hg	NZ_CP050174
pCD63-2-1	<i>tet(X4)</i>	<i>Escherichia coli</i>	broiler chicken	<i>merT/merP/merC/merD/merE</i>	Hg	NZ_CP050041

Figure S1.

A



B

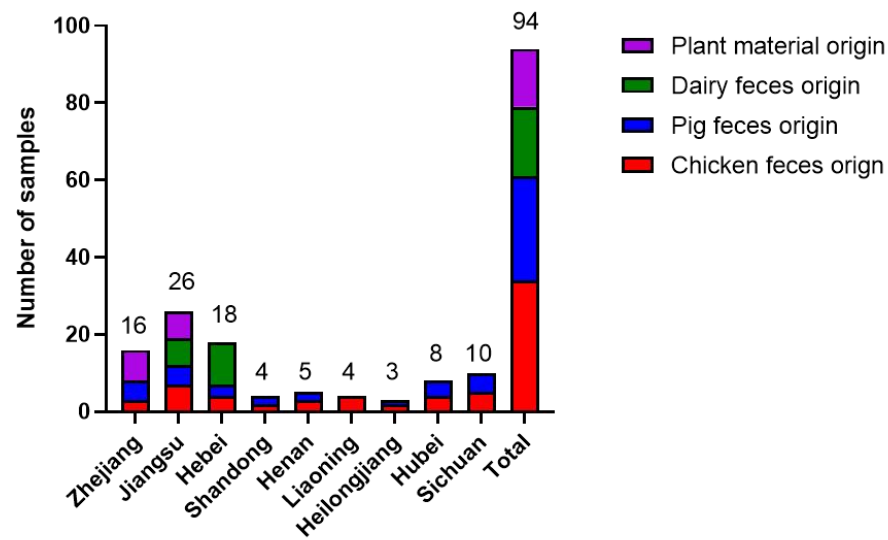


Figure S2.

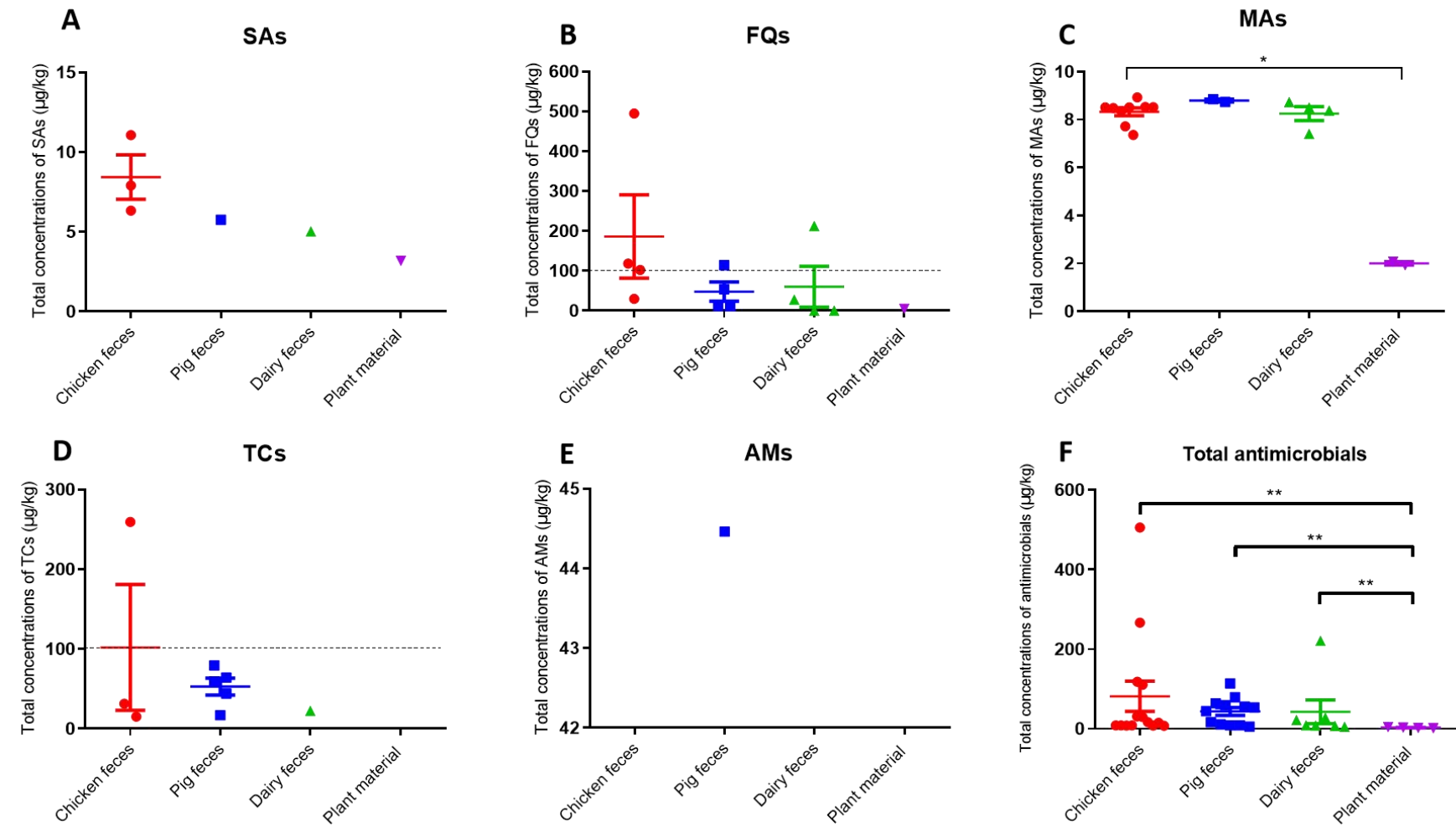


Figure S3.

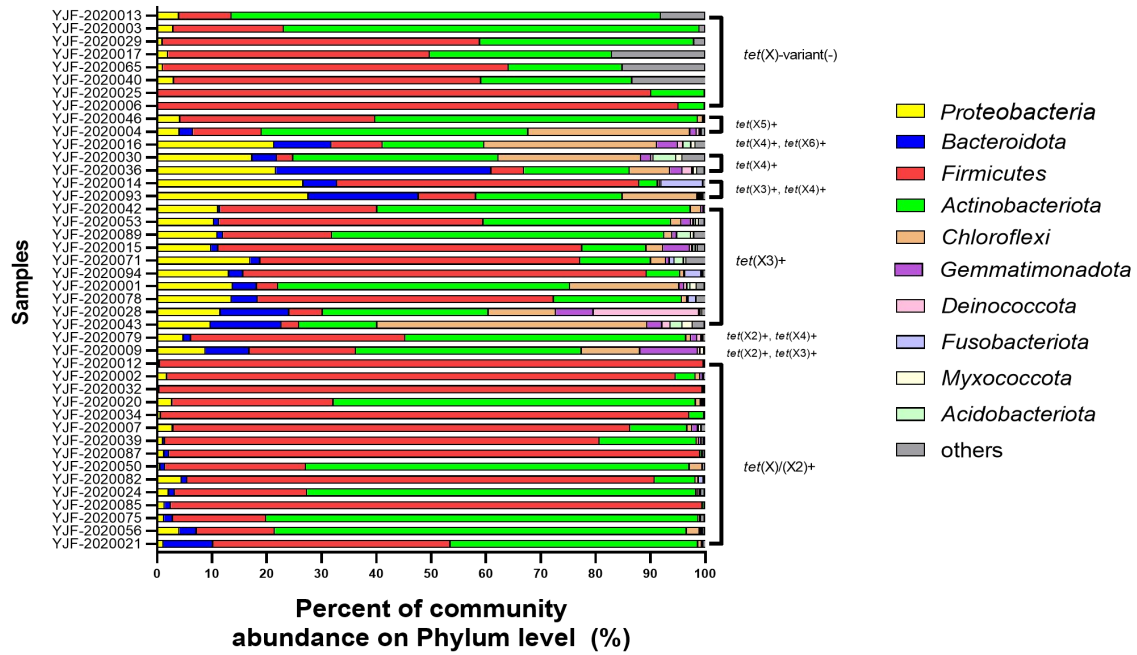
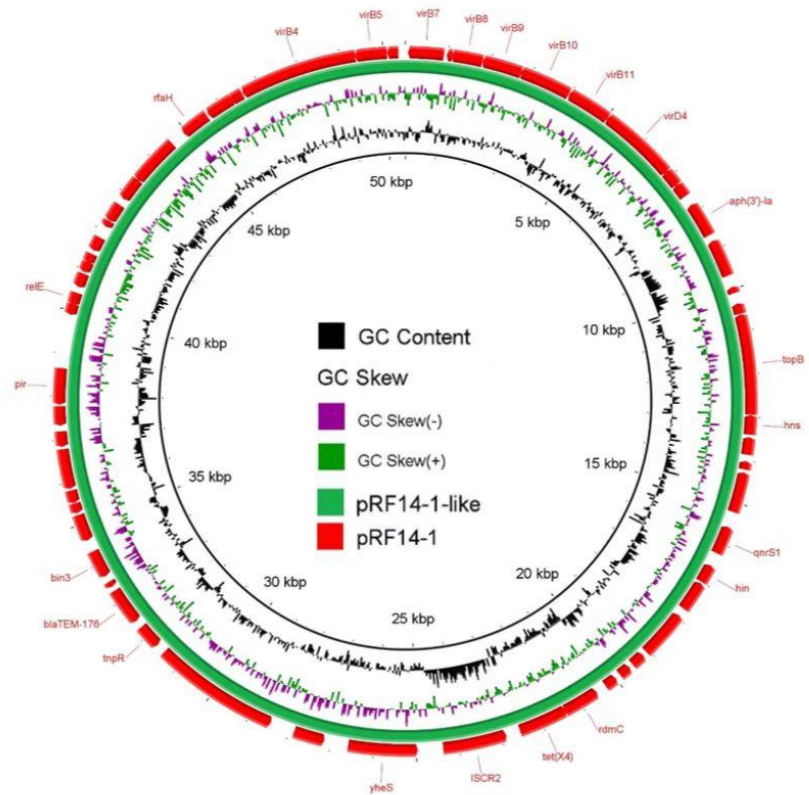


Figure S4.

A



B

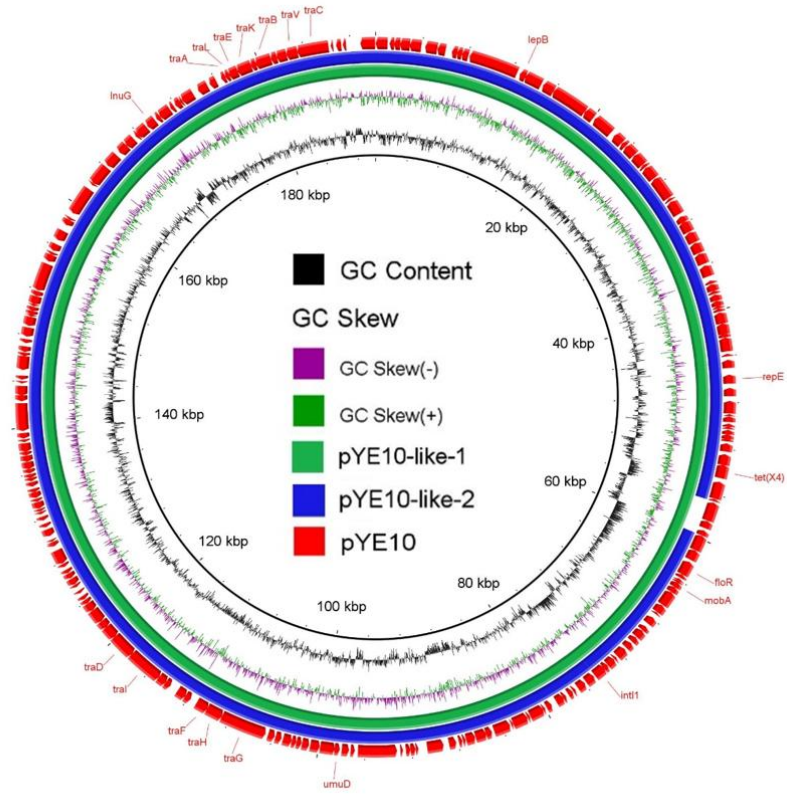


Figure S5.

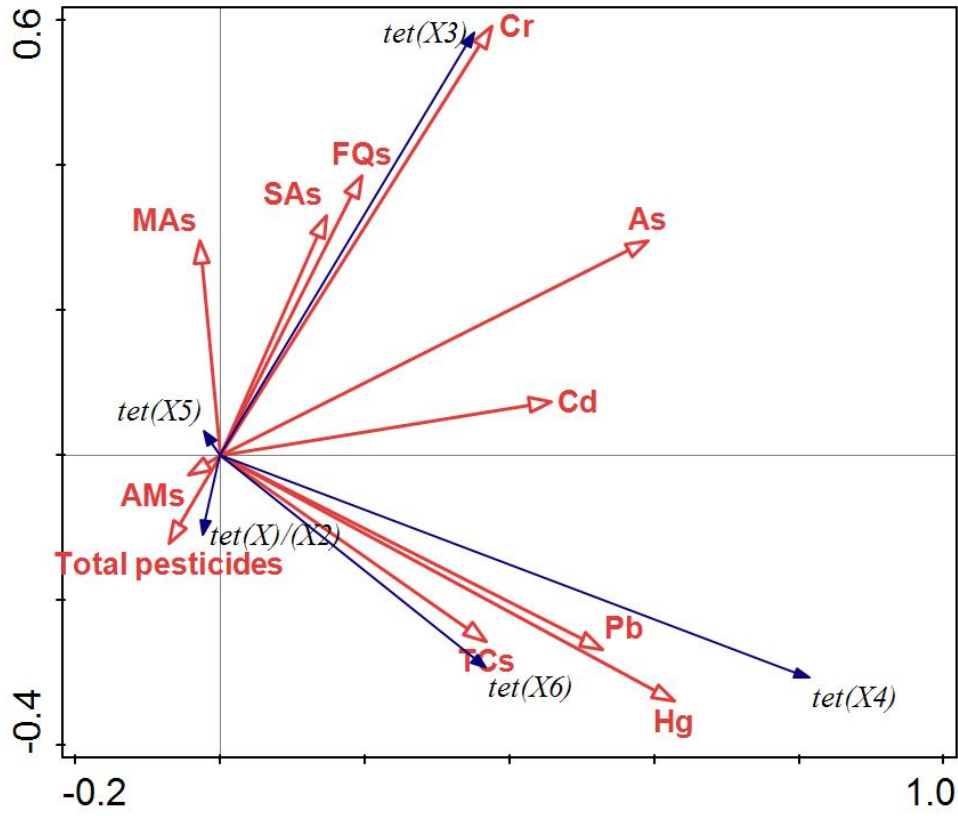
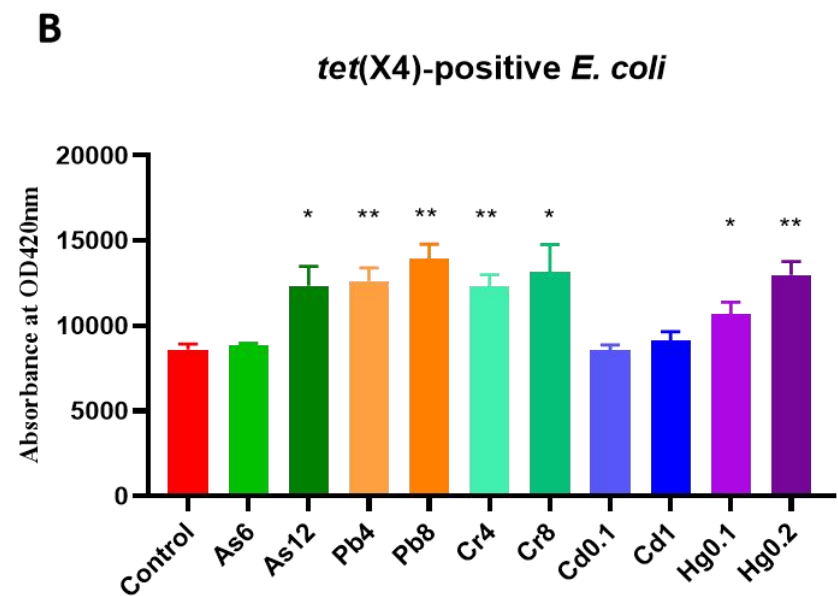
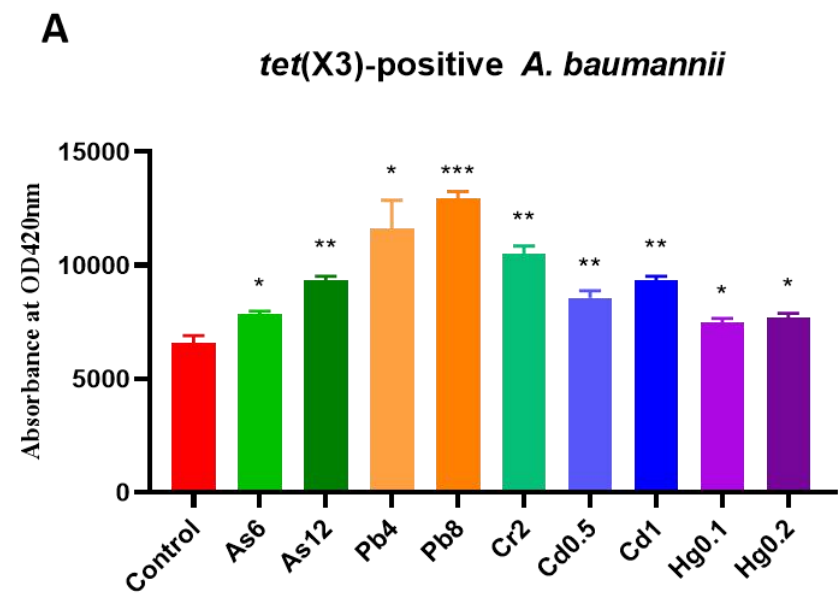


Figure S6.



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