Supplementary Information:

Abundance and Diversity of Tetracycline Resistance Genes in Soils Adjacent to Representative Swine Feedlots in China

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Fig S1. Three locations in China from which soil samples were originally collected.

Table S1 Primers and PCR conditions used in this study

Target genes	Primer	Sequences (5′—3′)	Amplicon size（bp）	Annealing temp (°C)	Reference
*tet*A	*tet*A-FW	GCTACATCCTGCTTGCCTTC	210	55	
	*tet*A-RV	CATAGATCGCCGTGAAGAGG			
*tet*C	*tet*C-FW	CTTGAGAGCCTTCAACCCAG	418	55	
	*tet*C-RV	ATGGTCGTCATCTACCTGCC			
*tet*E	*tet*E-FW	GTTATTACGGGAGTTTGTTGG	278	55	
	*tet*E-RV	AATACAACACCCACACTACGC			
*tet*G	*tet*G-FW	GCTCGGTGGTATCTCTGCTC	468	55	
	*tet*G-RV	AGCAACAGAATCGGGAACAC			
*tet*K	*tet*K-FW	CGAAAACAGACTCGCCAATC	169	55	(Ng, L.K., *et al.*)
	*tet*K-RV	TCCATAATGAGGTGGGGC			
*tet*L	*tet*L-FW	TCGTTAGCGTGCTGTCATTC	267	55	
	*tet*L-RV	GTATCCCACCAATGTAGCCG			
*tet*A/P	*tet*A/P-FW	CTTGGATTGCGGAAGAAGAG	676	55	
	*tet*A/P-RV	ATATGCCCATTTAACCACGC			
*tet*S	*tet*S-FW	CATAGACAAGCCGTTGACC	667	55	
	*tet*S-RV	ATGTTTTTGGAACGCCAGAG			
*tet*X	*tet*X-FW	CAATAATTGGTGGTGGACCC	468	58	
	*tet*X-RV	TTCTTACCTTGGACATCCCG			
*tet*M	*tet*M-FW	ACAGAAAGCTTATTATATAAC	171	45	
	*tet*M-RV	TGGCGTGTCTATGATGTTCAC			
*tet*O	*tet*O-FW	ACGGARAGTTTATTGTATACC	171	45	
	*tet*O-RV	TGGCGTATCTATAATGTTGAC			
*tet*Q	*tet*Q-FW	AGAATCTGCTGTTTGCCAGTG	169	55	
	*tet*Q-RV	CGGAGTGTCAATGATATTGCA			(Aminov, R.I., *et al.*)
*tet*T	*tet*T-FW	AAGGTTTATTATATAAAAGTG	169	40	
	*tet*T-RV	AGGTGTATCTATGATATTTAC			
*tet*W	*tet*W-FW	GAGAGCCTGCTATATGCCAGC	168	60	
	*tet*W-RV	GGGCGTATCCACAATGTTAAC			
*tet*B/P	*tet*B/P-FW	AAAACTTATTATATTATAGTG	169	40	
	*tet*B/P-RV	TGGAGTATCAATAATATTCAC			

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(2) Aminov, R. I.; Garrigues-Jeanjean, N.; Mackie, R. I., Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Applied and Environmental Microbiology* **2001,** *67*, (1), 22-32.

Table S2 Detection of *tet* genes in soils around nine swine feedlots

tet genes	Mechanism[a]	Sampling sites									Frequency index [b]
		BJ1	BJ2	BJ3	TJ1	TJ2	TJ3	JX1	JX2	JX3	
*tet*A	E	–	+	+	+	+	+	+	+	+	8/9
*tet*C	E	+	+	+	+	+	+	–	+	+	8/9
*tet*E	E	–	–	–	+	–	–	–	–	–	1/9
*tet*G	E	–	–	+	+	+	–	–	–	–	3/9
*tet*K	E	–	–	–	+	+	–	–	–	–	2/9
*tet*L	E	+	–	+	+	+	+	–	–	–	5/9
*tet*A/P	E	+	–	–	+	–	+	+	–	–	4/9
*tet*M	R	+	+	+	+	+	+	+	+	+	9/9
*tet*O	R	+	+	+	+	+	+	+	+	+	9/9
*tet*Q	R	+	+	+	+	+	+	+	+	+	9/9
*tet*S	R	–	–	–	+	–	–	–	–	–	1/9
*tet*T	R	+	+	–	–	+	+	+	–	–	5/9
*tet*W	R	+	+	+	+	+	+	+	+	+	9/9
*tet*B/P	R	+	+	+	–	+	+	+	+	–	7/9
*tet*X	M	+	+	+	–	+	+	+	+	+	8/9

+: present; –: absent.

[a] E = efflux pump; R = ribosomal protection proteins; M = enzymatic modification

[b] Frequency was calculated as the number of positive detections in total nine soil samples

Table S3 Copies of *tet* genes in soil at different sites, normalized to sample volume (copies/g dry soil). (Standard errors of the mean are presented in brackets)

Sample	**Gene copies/g dry soil**					
	***tet*M**	***tet*O**	***tet*Q**	***tet*W**	***tet*T**	**Sum of *tet* genes (*tet*M, *tet*O, *tet*Q, *tet*W)**
BJ1	4.90×10^7	2.60×10^7	1.99×10^7	1.44×10^8	5.43×10^6	2.39×10^8
	(6.72×10^6)	(1.07×10^6)	(3.13×10^5)	(8.75×10^6)	(2.84×10^5)	(1.56×10^7)
BJ2	1.43×10^9	4.09×10^8	4.30×10^8	3.48×10^8	1.41×10^7	2.62×10^9
	(5.91×10^7)	(9.83×10^6)	(5.24×10^7)	(2.60×10^7)	(1.82×10^6)	(1.03×10^8)
BJ3	4.29×10^6	4.80×10^7	4.85×10^6	2.77×10^7	ND	8.49×10^7
	(3.59×10^5)	(2.43×10^6)	(6.47×10^5)	(4.24×10^6)		(6.13×10^6)
TJ1	1.50×10^8	3.48×10^7	1.72×10^8	1.61×10^7	ND	3.73×10^8
	(6.94×10^5)	(5.14×10^6)	(8.13×10^6)	(1.38×10^6)		(5.09×10^6)
TJ2	5.47×10^7	1.83×10^7	8.40×10^6	2.11×10^7	3.25×10^5	1.03×10^8
	(2.42×10^6)	(2.00×10^6)	(4.66×10^5)	(1.69×10^6)	(1.22×10^4)	(4.03×10^6)
TJ3	7.66×10^5	1.02×10^7	1.36×10^6	1.82×10^6	7.86×10^4	1.41×10^7
	(4.81×10^4)	(4.90×10^5)	(3.85×10^4)	(7.05×10^4)	(1.29×10^3)	(5.50×10^5)
JX1	7.84×10^7	2.70×10^7	1.61×10^8	1.87×10^7	9.71×10^6	2.85×10^8
	(7.86×10^6)	(3.35×10^6)	(1.67×10^7)	(9.06×10^5)	(1.26×10^6)	(2.34×10^7)
JX2	4.75×10^6	3.83×10^6	1.80×10^6	2.51×10^6	ND	1.29×10^7
	(2.73×10^5)	(2.89×10^5)	(6.05×10^5)	(2.16×10^4)		(8.85×10^5)
JX3	2.96×10^5	3.68×10^5	6.63×10^5	1.38×10^5	ND	1.46×10^6
	(3.19×10^4)	(7.82×10^4)	(6.29×10^4)	(1.36×10^4)		(1.71×10^5)

Table S4 Copies of *tet* genes in soil at different sites, normalized to ambient 16S rRNA gene copies (Standard errors of the mean are presented in brackets)

Sample	Gene copies/16S rRNA gene copies					
	***tet*M**	***tet*O**	***tet*Q**	***tet*W**	***tet*T**	**Sum of *tet* genes (*tet*M, *tet*O, *tet*Q, *tet*W)**
BJ1	2.64×10^{-3} (3.62×10^{-4})	1.40×10^{-3} (5.76×10^{-5})	1.07×10^{-3} (1.68×10^{-5})	7.74×10^{-3} (4.71×10^{-4})	2.92×10^{-4} (1.53×10^{-5})	1.28×10^{-2} (9.07×10^{-4})
BJ2	2.41×10^{-2} (9.96×10^{-4})	6.89×10^{-3} (1.66×10^{-4})	7.25×10^{-3} (8.84×10^{-4})	5.86×10^{-3} (4.39×10^{-4})	2.38×10^{-4} (3.07×10^{-5})	4.41×10^{-2} (2.48×10^{-3})
BJ3	2.02×10^{-4} (1.69×10^{-5})	2.26×10^{-3} (1.14×10^{-4})	2.29×10^{-4} (3.05×10^{-5})	1.31×10^{-3} (2.00×10^{-4})	ND	4.00×10^{-3} (3.62×10^{-4})
TJ1	3.32×10^{-3} (1.54×10^{-5})	7.70×10^{-4} (1.14×10^{-4})	3.81×10^{-3} (1.80×10^{-4})	3.57×10^{-4} (3.04×10^{-5})	ND	8.25×10^{-3} (3.39×10^{-4})
TJ2	3.16×10^{-3} (1.40×10^{-4})	1.06×10^{-3} (1.15×10^{-4})	4.85×10^{-4} (2.69×10^{-5})	1.22×10^{-3} (9.75×10^{-5})	1.88×10^{-5} (7.04×10^{-7})	5.92×10^{-3} (3.79×10^{-4})
TJ3	3.97×10^{-5} (2.49×10^{-6})	5.29×10^{-4} (2.54×10^{-5})	7.05×10^{-5} (2.82×10^{-6})	9.43×10^{-5} (3.65×10^{-6})	4.07×10^{-6} (6.68×10^{-8})	7.33×10^{-4} (3.35×10^{-5})
JX1	6.09×10^{-3} (6.11×10^{-4})	2.10×10^{-3} (2.60×10^{-4})	1.25×10^{-2} (1.30×10^{-3})	1.45×10^{-3} (7.04×10^{-5})	7.55×10^{-4} (9.80×10^{-5})	2.22×10^{-2} (2.24×10^{-3})
JX2	3.14×10^{-4} (1.80×10^{-5})	2.53×10^{-4} (1.91×10^{-5})	1.19×10^{-4} (3.99×10^{-5})	1.66×10^{-4} (1.42×10^{-6})	ND	8.51×10^{-4} (7.85×10^{-5})
JX3	4.29×10^{-5} (4.63×10^{-6})	5.34×10^{-5} (1.13×10^{-5})	9.61×10^{-5} (9.12×10^{-6})	2.00×10^{-5} (1.97×10^{-6})	ND	2.12×10^{-4} (2.71×10^{-5})

Table S5 The mean chemical and biological conditions of each sample

	Samples								
	BJ1	BJ2	BJ3	TJ1	TJ2	TJ3	JX1	JX2	JX3
Organic Matter (%)	3.58 (0.23)	4.38 (0.04)	2.69 (0.35)	5.34 (0.48)	2.05 (0.29)	3.38 (0.38)	3.04 (0.18)	3.46 (0.11)	1.02 (0.36)
pH	7.98 (0.10)	7.07 (0.15)	7.32 (0.07)	7.66 (0.13)	8.12 (0.10)	7.86 (0.22)	6.32 (0.14)	5.94 (0.06)	8.01 (0.09)
16S rRNA gene copies/g dry soil	1.86×10^{10} (8.44×10^{8})	5.93×10^{10} (2.78×10^{9})	2.12×10^{10} (8.84×10^{8})	4.52×10^{10} (5.24×10^{9})	1.73×10^{10} (2.41×10^{9})	1.93×10^{10} (1.15×10^{9})	1.29×10^{10} (3.65×10^{8})	1.51×10^{10} (9.09×10^{8})	6.90×10^{9} (8.13×10^{8})

Fig. S2 (A) Relationship between log of the total *tet* gene copy numbers and total concentrations of tetracyclines. (B) Relationship between log of the total *tet* gene copy numbers and log of the 16S rRNA gene copy numbers. (C) Relationship between log of the total *tet* gene copy numbers and organic matter in soil

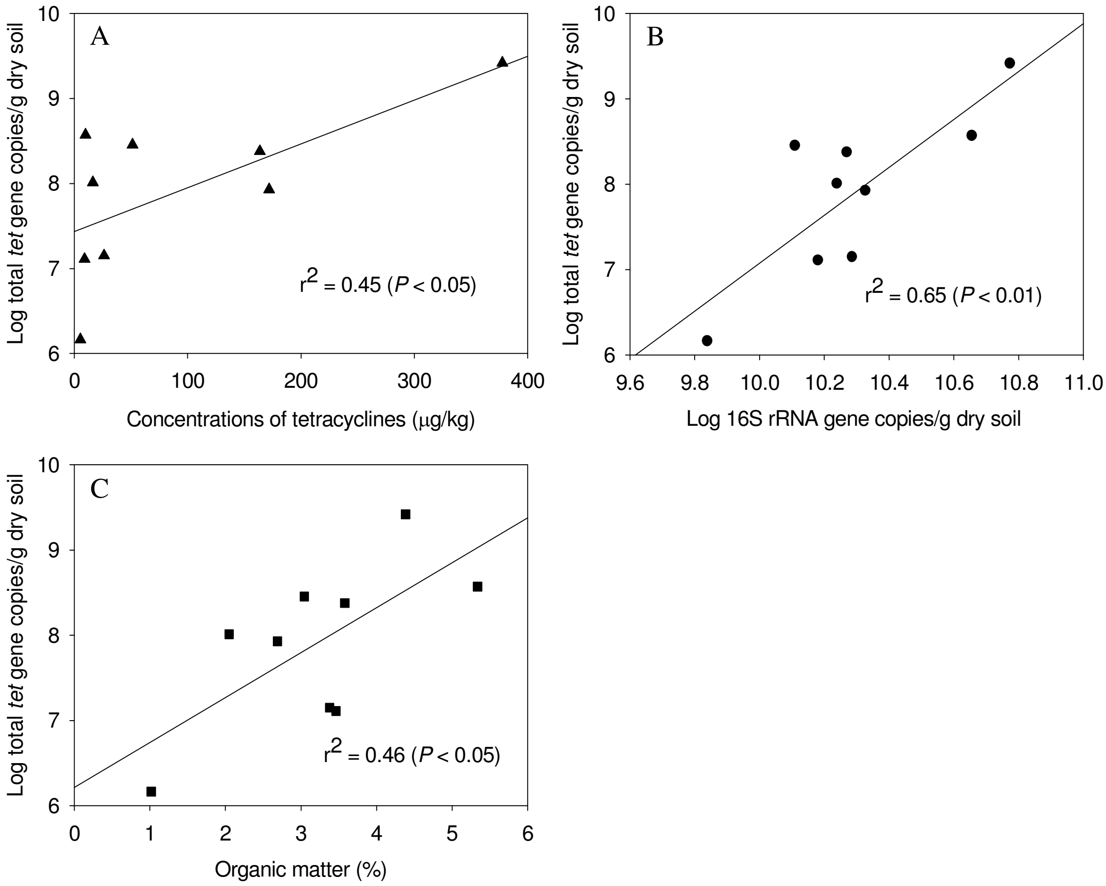

Fig. S3 Neighbor-joining phylogenetic tree of *tet*M gene sequences (171 bp fragment) detected in nine soil samples from Beijing, Tianjin, Jiaxing in China. The *tet*S gene sequence from *Streptococcus intermedius* was used as the outgroup to root the tree. Bootstrap values (>50) are indicated at branch points. The accession numbers of sequences used in this analysis are shown in parentheses