Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry

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This SI file includes:

17 pages, 8 tables, and 4 figures.

MATERIALS AND METHODS

Swine Manure Slurry

Manure slurry samples were collected from the sampling locations labeled in Figure S1. The wet weight and dry weight of the manure solids in the manure slurries from the finisher (BAC-manure), grower (CTC-manure), and sow and gilts (TYL-manure) were measured using gravimetric methods.

Field Site

This field study was conducted in May and June 2011 at the University of Nebraska Rogers Memorial Farm located 18 km east of Lincoln, Nebraska. The study site had been cropped using a long-term no-till management system with controlled wheel traffic. Soybeans (*Glycine max*) were planted during the 2010 season and herbicide (glyphosate) was applied as needed to control weed growth. The soil at the site developed in loess under prairie vegetation, and is the Aksarben silty clay loam (fine, smectitic, mesic Typic Argiudoll) containing 15% sand, 57% silt, and 28% clay [1].

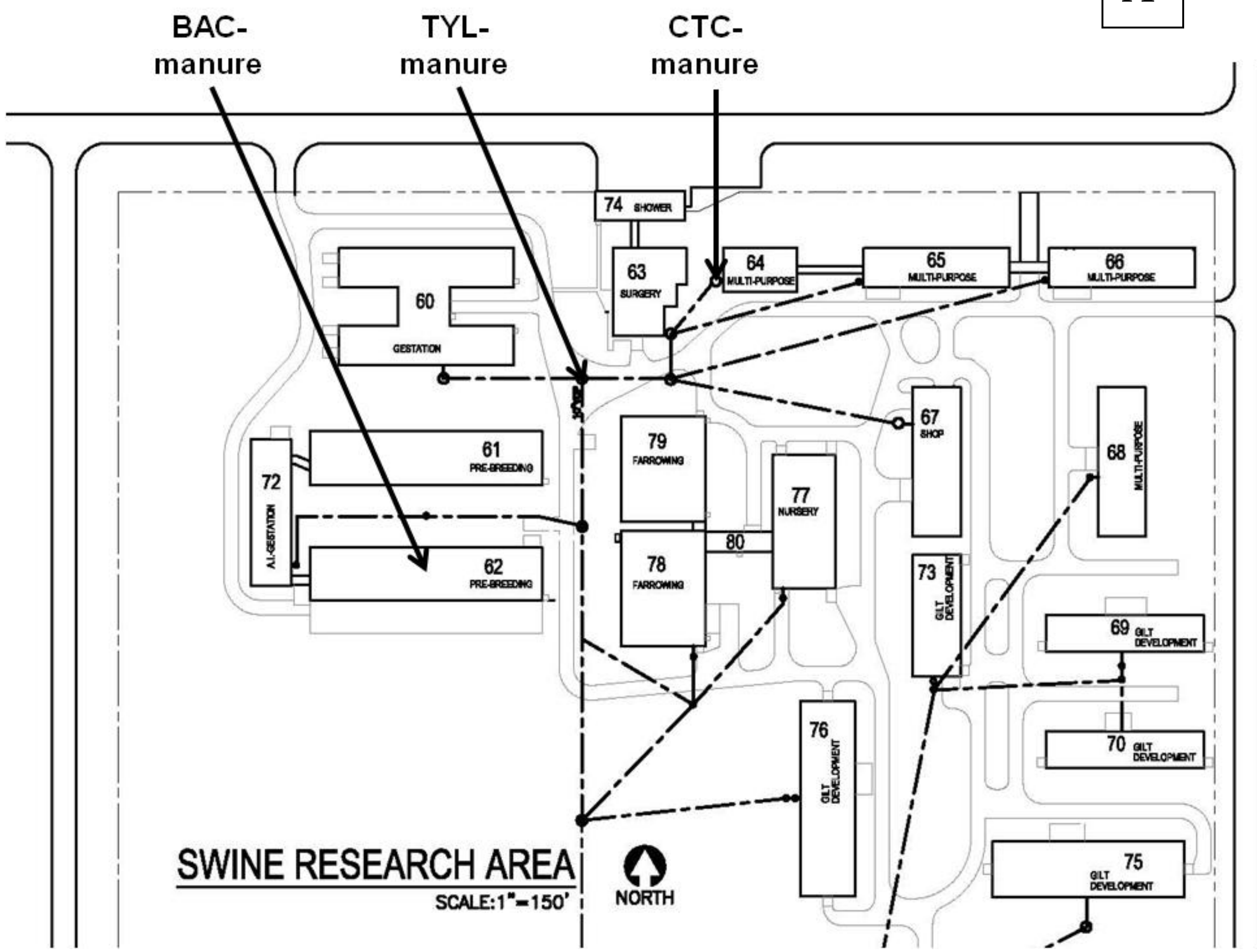
Soil samples for site characterization were obtained from the surface down to 2 cm just prior to manure application, and were air dried following collection. The study site had a mean slope gradient of 5.8%, an electrical conductivity (EC) of 0.38 dS m^{-1} and a pH of 6.8. The organic matter and total carbon content of the soil was 4.7% and 2.62%, respectively. Mean measured concentrations of Bray and Kurtz No. 1 P, water-soluble P, NO_3-N, and NH_4-N were 43, 5.2, 8, and 4 mg kg^{-1}, respectively. The initial soil moisture condition prior to swine slurry application was not measured.

Chemicals

Standards for roxithromycin, doxycycline, bactracin A, and fenbendazole were purchased from Sigma-Aldrich (Fluka Chemicals). Oleandomycin, tylosin A and chlortetracycline were obtained from ThermoFisher Scientific (ICN Biomedicals and MP Biomedicals). Roxithromycin and doxycycline were used as internal standards and oleandomycin was used as a surrogate. Analytes were chlortetracycline, bactracin A, tylosin, and fenbendazole. Because bacitracin A is rapidly hydrolyzed in water at near neutral pH, a standard for bacitracin F (one of its degradation products) was synthesized and used to quantify this compound in the manure, soil, and runoff samples [2].

FIGURES AND TABLES

A



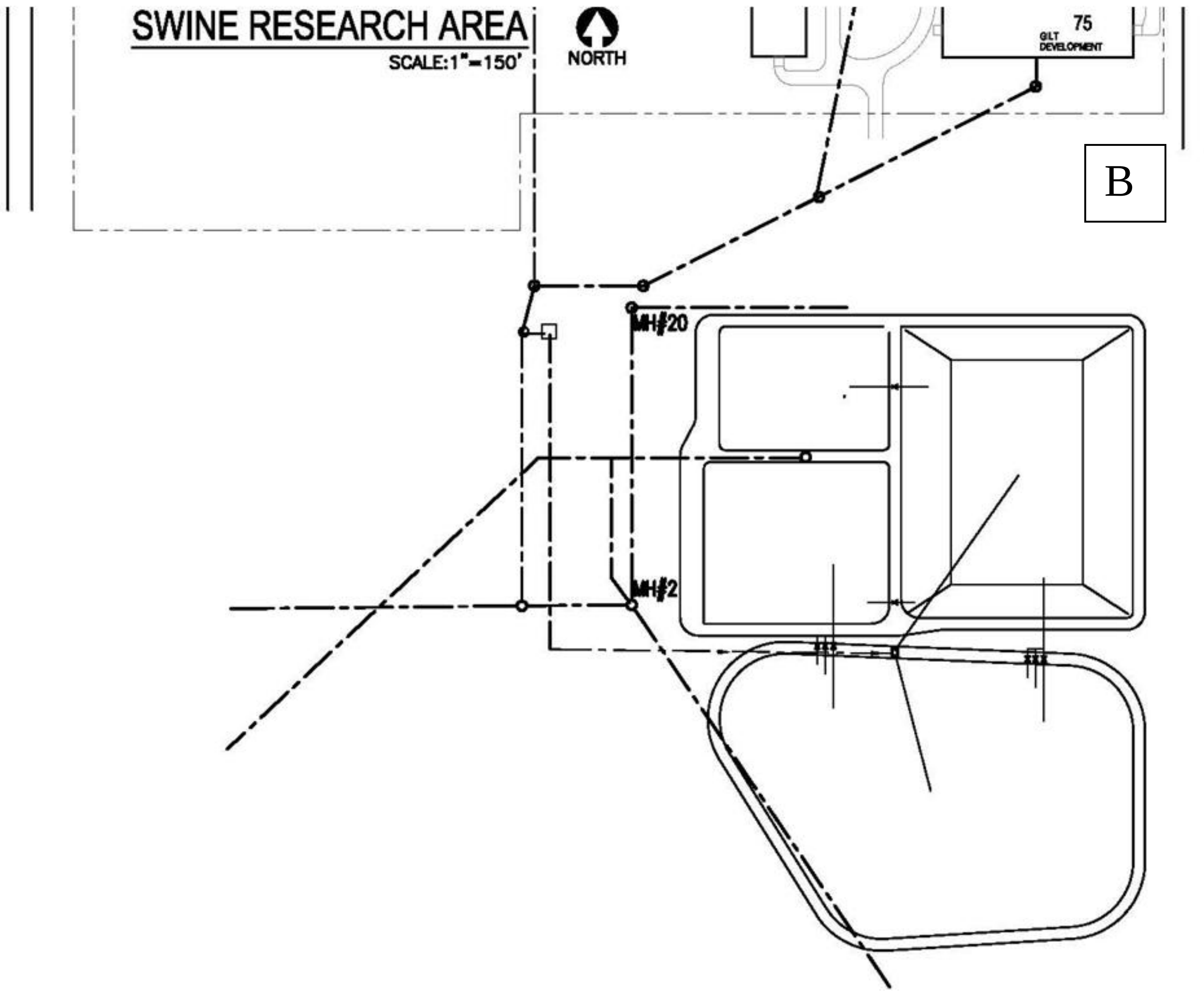


Figure S1. Map showing the locations of the barns and sampling points (A) and the lagoon systems (B) in the swine research area in the USDA Meat Animal Research Center.

Broadcast BAC	Broadcast CTC	Broadcast CONTROL	Broadcast TYL				
Incorporation CONTROL	Incorporation CTC	Incorporation TYL	Incorporation BAC	Injection TYL	Injection BAC	Injection CONTROL	Injection CTC
Injection CTC	Injection BAC	Injection TYL	Injection CONTROL	Incorporation TYL	Incorporation CTC	Incorporation CONTROL	Incorporation BAC
Broadcast BAC	Broadcast CTC	Broadcast CONTROL	Broadcast TYL	Incorporation BAC	Incorporation TYL	Incorporation CTC	Incorporation CONTROL
Broadcast TYL	Broadcast BAC	Broadcast CONTROL	Broadcast CTC	Injection TYL	Injection CONTROL	Injection BAC	Injection CTC

Figure S2. Randomized block design used in the field experiment. Plots in each row were constructed in the same week.

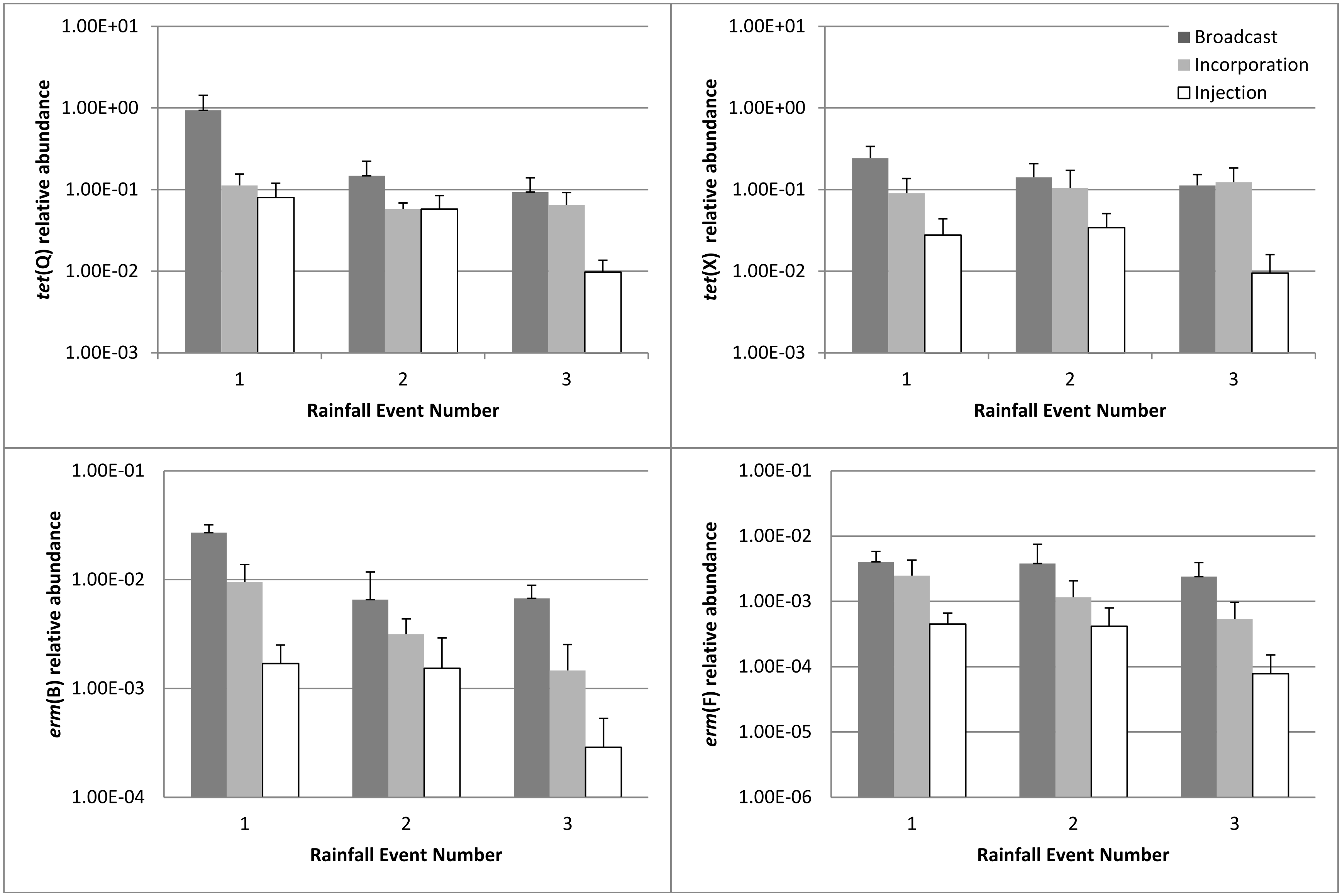


Figure S3. Relative abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in runoff from control and amended plots receiving broadcast, incorporation, or injection treatment. Error bars represent standard errors from triplicate field experiments.

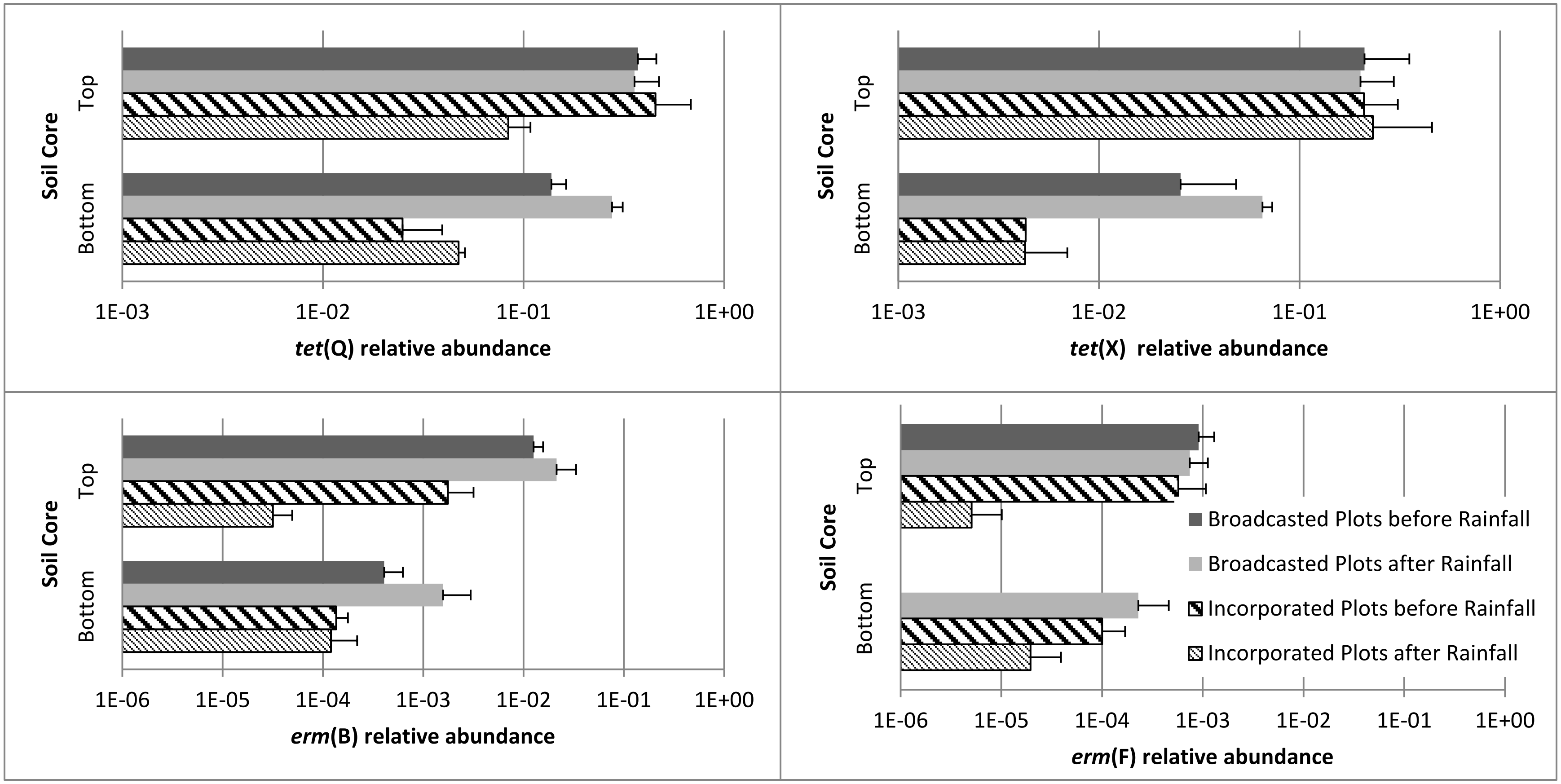


Figure S4. Relative abundance of *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in top and bottom soil in amended plots before and after three rainfall simulation tests. Error bars represent standard errors from triplicate field experiments.

Table S1. Properties of the antimicrobials used in this study.

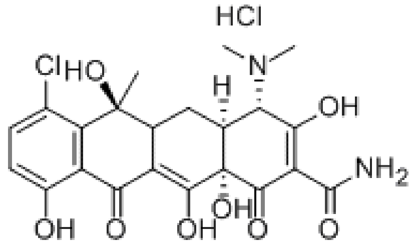
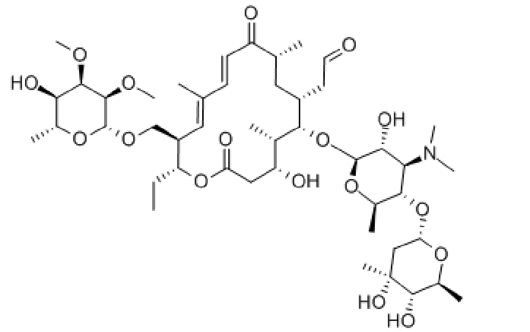
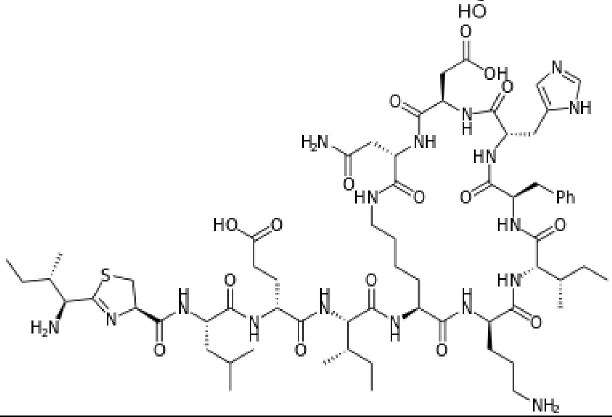
Antimicrobial	Chemical Structure	Properties
Chlortetracycline		K_d = 501-3715 L/kg [3] Solubility = 500 mg/L $t_{1/2}$ = 21 days [4]
Tylosin		K_d = 1,300 L/kg [5] Solubility = 6,000 mg/L $t_{1/2}$ = 6-8 days [4, 6]
Bacitracin (Bacitracin A)		Environmental fate data for Bacitracin A are not available in the literature

Table S2. Molecular weight, retention times, and MRM transition of antimicrobials, internal standards (IS), and surrogate (S) compound.

Analyte	Molecular weight	Retention time (min)	MRM Transition (m/z)
Bacitracin A	1422.7	9.82	712.10->86.20
Bacitracin F	1419.64	10.05	710.19->281.26
Chlortetracycline	478.88	8.71	478.90->444.00
Fenbendazole	299.35	10.63	300.20->268.20
Tylosin	916.10	10.40	916.9->174.2
Doxycycline (IS)	444.4	8.63	445.05->428.05
Oleandomycin (S)	687.86	10.51	688.35->544.10
Roxythromycin (IS)	837.05	11.58	837.55->679.50

Table S3. Relevant information of the qPCR and PCR reactions used in this study.

Target ARG	Primer	Sequence (5'-3')	Annealing Temp (°C)	Linear Range (copies/20μL)	R^2	Efficiency (%)	Reference
tet(Q)	TetQ-FW	AGAATCTGCTGTTTGCCAGTG					
	TetQ-RV	CGGAGTGTCAATGATATTGCA	63	10^2-10^9	0.996	104.4	[7]
tet(X)	TetX-FW	AGCCTTACCAATGGGTGTAAA					
	TetX-RV	TTCTTACCTTGGACATCCCG	70	10^1-10^9	0.997	81.8	[8]
erm(B)	ErmB-FW	GGTTGCTCTTGCACACTCAAG					
	ErmB-RV	CAGTTGACGATATTCTCGATTG	65	10^1-10^9	0.978	111.1	[9]
erm(F)	ErmF-FW	TCTGGGAGGTTCCATTGTCC					
	ErmF-RV	TTCAGGGACAACTTCCAGC	65	10^1-10^9	0.978	89.4	[9]
*bceA**	BceA-FW	GCTACGACAGCACTTAATCA					
	BceA-RV	CACCTTCAGTTAGTCCATCA	55				[10]
*bceR**	BceB-FW	TTAACCAACATCAACCTCAG					
	BceB-RV	CCCCATTTGTATTGCCAT	55				[10]
bcrA	BcrA-FW	AAGTGGCAAGGCTTTTGAGA					
	BcrA-RV	CTCAGGATCAATCGGCAAAT	60				[11]
bcrB	BcrB-FW	AAGTGGCAAGGCTTTTGAGA					
	BcrB-RV	AAATCACCGGGGGAATTAAG	60				[11]
bcrC	BcrC-FW	AAGTGGCAAGGCTTTTGAGA					
	BcrC-RV	CTCAAGTTCCCCAGTTTCCA	60				[11]

* Regular PCR reactions.

Table S4. Relative abundance (average ± standard error) of ARGs *tet*(Q), *tet*(X), *erm*(B), and *erm*(F) in manure slurry.

	ARG	Relative Abundance
CTC-Manure	*tet*(Q)	1.33±0.26
	tet(X)	0.078±0.018
TYL-Manure	*erm*(B)	0.12±0.024
	erm(F)	0.0022±0.0004

Table S5. Aqueous antimicrobial concentrations in runoff from control plots (average ± standard error). MDL was 0.005 ng/µL.

	Rainfall Event	CTC (ng/µL)	TYL (ng/µL)	BAC (ng/µL)
Broadcast	1	<MDL	0.006[*]	<MDL
	2	<MDL	0.011[*]	<MDL
	3	<MDL	<MDL	<MDL
Incorporation	1	<MDL	<MDL	<MDL
	2	<MDL	<MDL	<MDL
	3	<MDL	<MDL	<MDL
Injection	1	<MDL	0.007[a]	<MDL
	2	<MDL	<MDL	<MDL
	3	<MDL	<MDL	<MDL

[*]. Values are from one of the triplicate field experiments. No antimicrobials were detected in the other replicates.

Table S6. Concentrations of ARGs in runoff from control plots (average ± standard error). Standard errors were calculated based on triplicate field experiments. The MDL for each ARG is reported in Table S2.

		tet(Q) (copy/mL)	*tet*(X) (copy/mL)	*erm*(B) (copy/mL)	*erm*(F) (copy/mL)
Broadcast	Run 1	< MDL	< MDL	< MDL	< MDL
	Run 2	423 ± 416	233 ± 231	25 ± 25	16 ± 16
	Run 3	< MDL	< MDL	< MDL	< MDL
Incorporation	Run 1	< MDL	< MDL	< MDL	< MDL
	Run 2	< MDL	< MDL	< MDL	< MDL
	Run 3	< MDL	< MDL	< MDL	< MDL
Injection	Run 1	< MDL	< MDL	< MDL	< MDL
	Run 2	< MDL	< MDL	< MDL	< MDL
	Run 3	< MDL	< MDL	< MDL	< MDL

Table S7. No ARGs were detected in the top and bottom soil of control plots before and after the rainfall events in triplicate field experiments.

		Broadcast		Incorporation	
		Before Rainfalls	After Rainfalls	Before Rainfalls	After Rainfalls
tet(Q)	Top soil	<MDL	<MDL	<MDL	<MDL
	Bottom soil	<MDL	<MDL	<MDL	<MDL
tet(X)	Top soil	<MDL	<MDL	<MDL	<MDL
	Bottom soil	<MDL	<MDL	<MDL	<MDL
erm(B)	Top soil	<MDL	<MDL	<MDL	<MDL
	Bottom soil	<MDL	<MDL	<MDL	<MDL
erm(F)	Top soil	<MDL	<MDL	<MDL	<MDL
	Bottom soil	<MDL	<MDL	<MDL	<MDL

Table S8. Antimicrobial concentrations in swine manure slurry (average ± standard error). Standard errors were calculated based on five weekly manure slurry samples.

	Chlortetracyline	Tylosin (ng/g solid ww)	Bacitracin
CTC-manure	3,324 ± 1,560	2 ± 1	172 ± 164
TYL-manure	102 ± 40	287 ± 124	7 ± 7
BAC-manure	16 ± 9	124 ± 68	777±753

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