

Distribution and risk assessment of antibiotic resistance genes of swine farm wastewater and surrounding environments: from soil to water

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This file contains: Table S1-S4 with the attached table and Figure S1-6.

The detailed information wastewater treatment processes is as followed.

Initially, pig urine and cleaning wastewater were collected centrally into a sewage ditch and then directed into a manure collection tank via piping. The wastewater in the manure collection tank was then diverted: one portion enters an anaerobic tower (800 m³) for fermentation, while the other undergoes solid-liquid separation. The separated solid manure residue was stored alongside fresh manure in a manure composting shed, where it was mixed with wood chips or straw and subjected to high-temperature composting (at 65-70 °C), air-drying, grinding, and screening to produce organic fertilizer. The liquid portion after solid-liquid separation was also diverted, with one stream entering an anaerobic storage pond for continued fermentation, and the other entering a biochemical regulation tank, where it converged with the effluent from solid-liquid separation, the effluent from the anaerobic tower, and the biogas slurry from the anaerobic storage tank. The biochemical system primarily treated the biogas slurry, and the treated tail-water was partially used directly for farmland irrigation, while the remaining portion was returned to the anaerobic storage pond for mixing and subsequent reuse.

Table S1. Information on samples

Sample Time	Sample Location	Sample Types	Sample Name
March, 2021	swine farm	biogas slurry	BS1
	agricultural land	soil	ES
	agricultural land	control soil	CS
August, 2021	swine farm	biogas slurry	BS2
	river	surface water	SW
	well about 100 m away from the swine farm	groundwater	GW1
	well about 800 m away from the swine farm	groundwater	GW2

Each sample has biological replicates.

Table S2. Information on the 47 target genes with 100% detection frequencies

Gene name	class	mechanism
<i>int1-a-marko</i>	Integrase	
<i>sul1</i>	Sulfonamide	protection
<i>int11</i>	Integrase	
<i>qacEΔ1</i>	MDR	efflux
IS6100	Insertional	
<i>oprJ</i>	MDR	efflux
<i>qacEΔ1</i>	MDR	efflux
<i>disul</i>	Sulfonamide	protection
<i>int13</i>	Integrase	
<i>tetG</i>	Tetracycline	efflux
<i>rpoB</i>	housekeeping	
IS26	Insertional	
<i>tetX</i>	Tetracycline	deactivate
<i>aadA17</i>	Aminoglycoside	deactivate
<i>ermF</i>	MLSB	protection
<i>aadA2</i>	Aminoglycoside	deactivate
<i>cefa_qacelta</i>	MDR	unknown
<i>tetG</i>	Tetracycline	efflux
<i>aac(6')-Ib</i>	Aminoglycoside	deactivate
<i>cmlA1</i>	Phenicol	efflux
<i>tetM</i>	Tetracycline	protection
<i>aadA5</i>	Aminoglycoside	deactivate
<i>floR</i>	Amphenicol	efflux
<i>repA</i>	Plasmid -ep	
<i>cmlA1</i>	Amphenicol	efflux
<i>strB</i>	Aminoglycoside	protection
ISSm2	Insertional	
<i>erm(42)</i>	MLSB	protection
<i>dfra14</i>	Trimethoprim	protection
<i>pica</i>	MLSB	protection
<i>tnpA</i>	Transposase	
<i>aph(3'')-ia</i>	Aminoglycoside	deactivate
<i>lnu(F)</i>	MLSB	deactivate
<i>dfra1</i>	Trimethoprim	protection
<i>tetR</i>	Tetracycline	regulator
<i>dfra17</i>	Trimethoprim	protection
<i>aac(3)</i>	Aminoglycoside	deactivate
<i>aac(6')-II</i>	Aminoglycoside	deactivate
<i>erm(O)</i>	MLSB	protection
<i>aphA1</i>	Aminoglycoside	deactivate
IS613	Transposase	
<i>blaOXA</i>	Beta Lactam	deactivate
<i>ereA</i>	MLSB	deactivate
<i>qacH</i>	MDR	efflux
<i>dfra1</i>	Trimethoprim	deactivate
<i>tetA</i>	Tetracycline	efflux
<i>tetR</i>	Tetracycline	unknown

Table S3. The Pearson correlation between the most abundant MGE and ARGs

Genes	IS6100	
	R²	P
<i>tetG</i>	0.98771	***
<i>sulI</i>	0.99801	***
<i>ermF</i>	0.99384	***
<i>qacEΔ1</i>	0.99662	***
<i>floR</i>	0.97061	***

Table S4. Information on high-risk ARGs

Gene	ARGs type	Relative Abundance	movability	Correlation with MGEs	Host Pathogenicity
<i>sulI</i>	Sulfonamide	1.31×10^{-1}	Yes	Yes	Acinetobacter baumannii Brandenburg
<i>oprJ</i>	MDR	8.51×10^{-2}	Yes	Yes	Pseudomonas aeruginosa
<i>tetX</i>	Tetracycline	4.27×10^{-2}	Yes	Yes	Bacteroides
<i>aadA17</i>	Aminoglycoside	3.46×10^{-2}	Yes	Yes	Acinetobacter baumannii
<i>ermF</i>	MLSB	3.46×10^{-2}	Yes	Yes	Riemerella anatipestifer
<i>aadA2</i>	Aminoglycoside	2.14×10^{-2}	Yes	Yes	Acinetobacter baumanniig
<i>tetG</i>	Tetracycline	1.97×10^{-2}	Yes	Yes	Pseudomonas aeruginosa
<i>aac(6')-Ib</i>	Aminoglycoside	1.92×10^{-2}	Yes	Yes	Mycobacteroides abscessus
<i>tetM</i>	Tetracycline	1.20×10^{-2}	Yes	Yes	Salmonella enterica

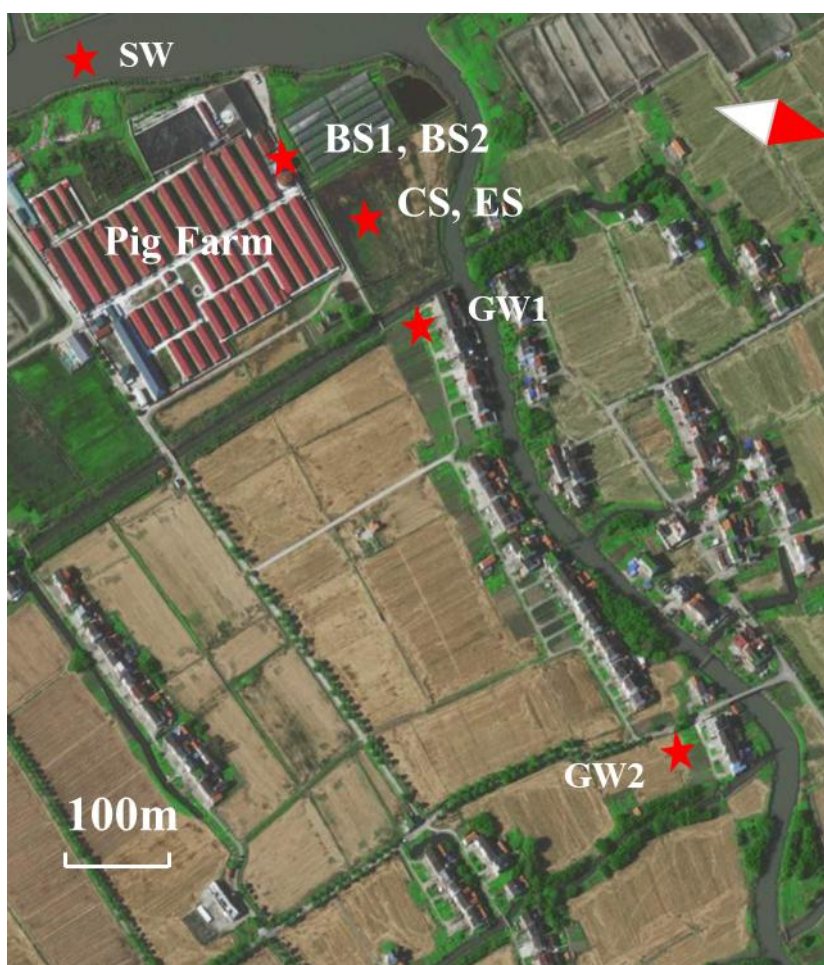


Fig. S1. The sampling sites in Jinshan district, Shanghai. BS1, BS2, CS, ES, SW, GW1, and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively.

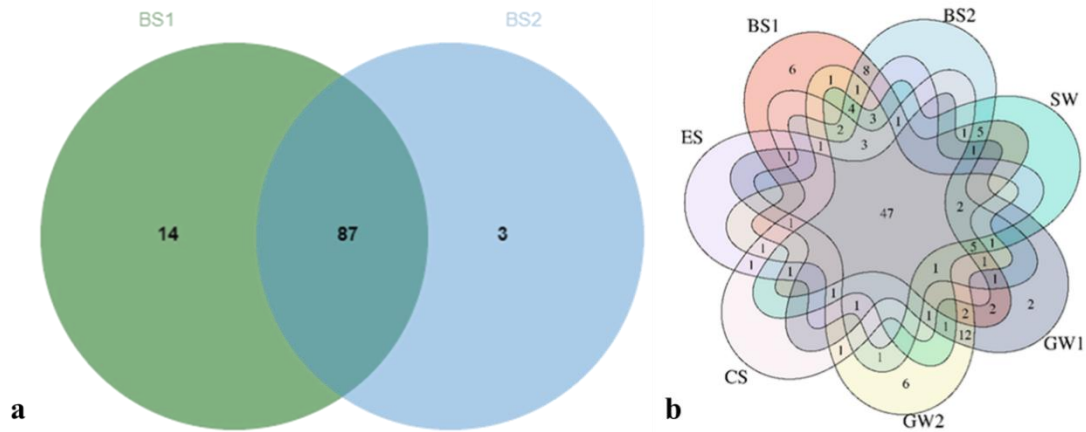


Fig. S2. Different Venn diagram analysis with BS1 and BS2 (a), BS1, BS2, CS, ES, SW, GW1, and GW2 (b) of 216 target genes. BS1, BS2, CS, ES, SW, GW1, and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively. Venn diagram analysis was plotted by <https://www.bioinformatics.com.cn> (last accessed on 20 Feb 2024), an online platform for data analysis and visualization.

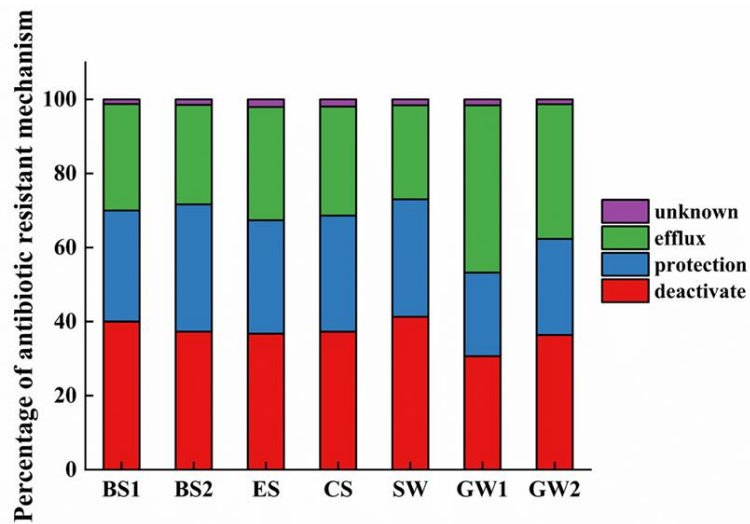


Fig. S3. Types of ARGs subtypes with different resistance mechanisms. BS1, BS2, CS, ES, SW, GW1 and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively.

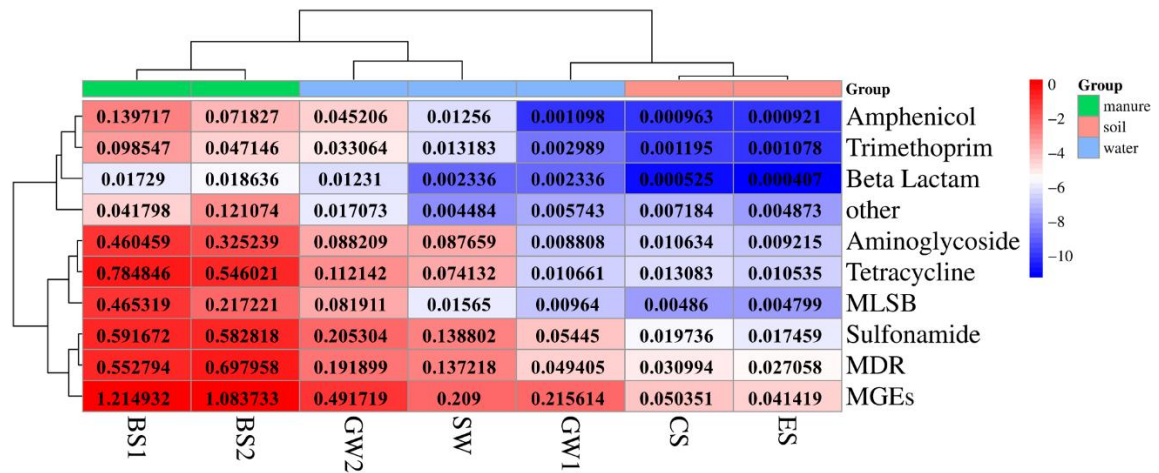


Fig. S4. The total relative abundance of various types of ARGs and MGEs in different samples. BS1, BS2, CS, ES, SW, GW1, and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively. The above relative abundances have been taken as \log_2 . Heatmap was plotted by <https://www.bioinformatics.com.cn> (last accessed on 20 Feb 2024), an online platform for data analysis and visualization.

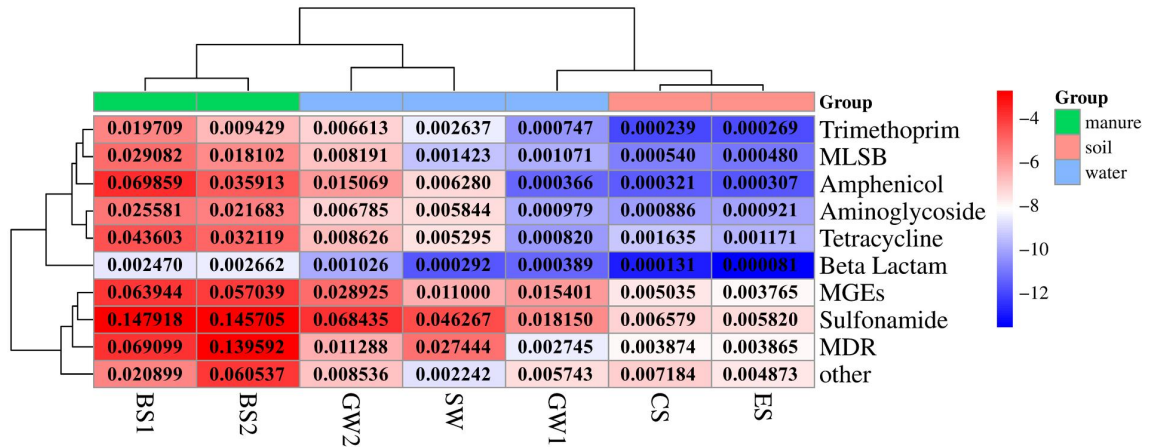


Fig. S5. The average relative abundance of various types of ARGs in different samples. BS1, BS2, CS, ES, SW, GW1, and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively. The above relative abundances have been taken as log2. Heatmap was plotted by <https://www.bioinformatics.com.cn> (last accessed on 20 Feb 2024), an online platform for data analysis and visualization.

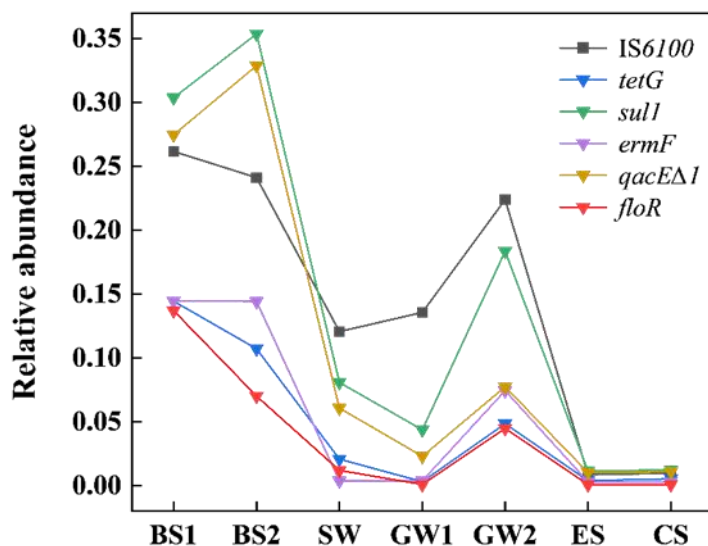


Fig. S6. The relative abundance of the most abundant MGE and ARGs in different samples. BS1, BS2, CS, ES, SW, GW1, and GW2 represent the biogas slurry of the swine farm in March 2021, the biogas slurry of the swine farm in August 2021, the agricultural soils not irrigated with biogas slurry, the agricultural soils after 4 days of irrigating with biogas, surface water from the river north of the swine farm, and groundwater about 100 m and 800 m away from the swine farm, respectively.