Front. Environ. Sci. Eng. 2022, 16(3): 32
https://doi.org/10.1007/s11783-021-1466-7

RESEARCH ARTICLE

Antibiotic resistome mostly relates to bacterial taxonomy along a suburban transmission chain

Ziyan Qin[1], Qun Gao (✉)[1], Qiang Dong[2], Joy D. Van Nostrand[3], Qi Qi[1], Yifan Su[1], Suo Liu[1], Tianjiao Dai[1], Jingmin Cheng[1], Jizhong Zhou[3,4,5], Yunfeng Yang (✉)[1]

1 State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China
2 Institute of Chemical Defense, Beijing 102205, China
3 Institute for Environmental Genomics, and Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK 73019, USA
4 School of Civil Engineering and Environmental Sciences, University of Oklahoma, Norman, OK 73019, USA
5 Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

HIGHLIGHTS

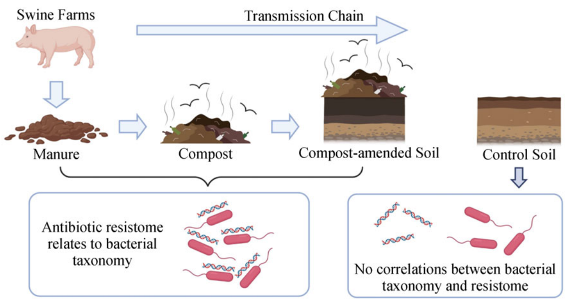
- The α-diversities of resistome were lower in manure and compost than in soils.
- There were significant correlations between the resistome and bacterial taxonomy.
- Bacterial taxonomy was the highest in explaining resistome variances.

ARTICLE INFO

Article history:
Received 5 February 2021
Revised 17 May 2021
Accepted 26 May 2021
Available online 25 June 2021

Keywords:
Antibiotic resistance genes
Resistome
Bacterial taxonomy
Transmission chain

GRAPHIC ABSTRACT



ABSTRACT

Antibiotic resistance genes comprising antibiotic resistome are of great concern due to their increase in the environment. Recent evidence of shared resistomes between soils and animal husbandry has imposed potential risks to human health. However, the correlation between a given community's resistome and bacterial taxonomic composition is controversial. Here, a transmission chain of resistomes from swine manure to compost and compost-amended soil were analyzed in five suburban areas of Beijing, China, with unamended agricultural soils as control soils. Antibiotic resistomes and bacterial taxonomic compositions were distinct between (I) manure and compost; and (II) compost-amended and control soils. In manure, compost, and compost-amended soils, the β-diversity of the resistome and bacterial taxonomic composition was significantly correlated, while no correlation was detected in control soils. Bacterial taxonomic composition explained 36.0% of total variations of the resistome composition, much higher than environmental factors. Together, those results demonstrated that antibiotic resistome was closely related to bacterial taxonomic composition along the suburban transmission chain.

1 Introduction

Bacterial resistance to antibiotics is a huge threat to human health, undermining medical treatments for pathogen-based infections worldwide. An increasing body of evidence illustrates that the widespread presence of antibiotic resistance genes (ARGs), termed the resistome when referring to all of detected ARGs, in human pathogens is associated with antibiotic usage (Marshall and Levy, 2011; Smillie et al., 2011; Forsberg et al., 2012). Therefore, resistome is regarded as an emerging biological hazard in various ecosystems (Smillie et al., 2011; Zhu et al., 2013; Su et al., 2017; Peng et al., 2020), whose dissemination is accelerated by human activities (Pruden et al., 2012; Chen et al., 2019).

✉ Corresponding authors
E-mail: gaoqun998@163.com (Q. Gao); yangyf@tsinghua.edu.cn (Y. Yang)

In modern societies, antibiotics are routinely used in livestock farming, which heightens the environmental burden of resistome (Zhu et al., 2013). China has become the largest producer and consumer of antibiotics in recent decades (Zhu et al., 2013). Until recently, unmonitored use of antibiotics to treat animal disease and improve growth has caused high concentrations of antibiotic residues in the environment (Qiao et al., 2012). Animal manure is a major route of antibiotic contamination because of poor absorption of veterinary antibiotics by the animals and common practice to use manure as organic fertilizer, leading to further diffusion (Alcock et al., 1999; Leclercq et al., 2016; Wu et al., 2020). Furthermore, the composting process of manure could be a hotbed causing enrichment of resistome and animal-associated pathogens. As a result, an increase in resistome is found in manure (Looft et al., 2012), compost (Liao et al., 2019), manure-amended soils (Ghosh and LaPara, 2007), riverine water, and sediments (Pruden et al., 2012).

Massive antibiotic use could escalate horizontal gene transfer (HGT) (Smillie et al., 2011; Forsberg et al., 2012; Gillings and Stokes, 2012; Ji et al., 2020). Accordingly, transferable, ARG-carrying plasmids were shown to be more abundant and persistent in manure-amended soils than in control soils (Jechalke et al., 2013; Jechalke et al., 2014). Because resistome is mobile, resistome may not be related to bacterial taxonomic composition (Smillie et al., 2011; Stokes and Gillings, 2011). However, the possibility was denied by observations that soil resistome composition was correlated with the microbial community composition across and within soil types, suggesting that bacterial taxonomy was influential for soil resistome (Forsberg et al., 2014). Similarly, *Clostridium, Acinetobacter, and Pseudomonas* were related to ARG enrichment in manure-amended soils (Leclercq et al., 2016). A possible explanation is that resistome originating from manure amendments to soils promotes the growth of its bacterial hosts, resulting in significant correlations (Udikovic-Kolic et al., 2014).

As most studies have focused on a single ecosystem, it remains unclear how possible correlations between resistome and bacterial taxonomy vary with human-induced dissemination of antibiotics. To this end, samples in manure, compost, compost-amended agricultural soil, and nearby unamended agricultural soil (control soil) were collected from five swine farms in Beijing's suburbs. Using the techniques of functional gene microarray named GeoChip and 16S rRNA gene amplicon sequencing, correlations between resistome and bacterial taxonomy were examined along the transmission chain. High concentrations of antibiotics impose environmental selection or pressure, potentially reducing the diversities of the resistome or associated microorganisms. Therefore, the resistome and microbial community profiles could vary with concentrations of antibiotics. Here, three hypotheses were aimed: 1) there are low diversities of resistome in manure and compost samples because of high concentrations of antibiotics; 2) both resistome and bacterial taxonomy are distinct in different samples; and 3) resistome is correlated with the bacterial taxonomy, showing stronger correlations in manure and compost samples.

2 Materials and methods

2.1 Sample collection and environmental factor measurements

Four manure and four aerobic compost samples were collected in April 2015 from each of the five swine farms (i.e., CP in Changping district, DX in Daxing district, HZ in Haidian district, JG and ZZ in Shunyi district) within the suburbs of Beijing, China. Four samples of surface soil (0–15 cm) in agricultural fields receiving compost from each farm (i.e., compost-amended soil) for one month were collected, together with four control soil samples nearby (~1 km) unamended agricultural fields, resulting in 80 samples in total. Each soil sample is a composite by mixing three soil cores (5 cm diameter) for compost-amended soils and unamended agricultural soils.

Thermometers were used to measure temperature on-site, and hygrometers were used to measure water content, which was performed in triplicates and averaged. Commonly used antibiotics containing three categories of tetracyclines, sulfonamides, and quinolones in livestock practices, including chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TCN), norfloxacin (NOR), ofloxacin (OFL), sulfamerazine (SMR), sulfadimidine (SMN), sulfamethoxazole (SMX), and sulfadiazine (SDZ), were measured in the laboratory (Gao et al., 2020). In brief, those antibiotics were extracted by 10 mL of a phosphate buffer ($pH = 3.0$) with 0.1 g of Na_2EDTA and acetonitrile (C_2H_3N) (1:1 vol/vol). All samples were sonicated for 30 min before centrifugation at 7000 × g for 10 min. The procedure was repeated three times to ensure efficient extraction. Three supernatants were mixed before dilution to 500 mL using deionized water, followed by filtering through 0.45 μm filters and pH adjustment to 3.0. Subsequently, samples were then extracted with 6 mL of Oasis HLB extraction cartridges and used for analyses with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Applied Biosystems, Foster City, CA, USA). The average recovery rate of antibiotics across samples was 90%.

2.2 DNA extraction from samples

For each sample, 1.5 g of sample was used to extract and purify DNA by the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA). A NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies

Inc., Wilmington, DE, USA) was used to evaluate DNA quality based on the absorbance ratios of 260/280 nm and 260/230 nm. DNA concentrations were determined by PicoGreen with a FLUOstar Optima fluorescence plate reader (BMG Labtech, Jena, Germany). DNAs were then stored in freezers at −80°C for subsequent analysis.

2.3 Bacterial 16S rRNA gene amplicon sequencing and data processing

Sequencing and library construction were performed as previously described (Wu et al., 2017). The primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) was used to amplify the V4 hypervariable region of bacterial 16S rRNA genes. A MiSeq benchtop sequencer (Illumina, San Diego, CA, USA) was used to perform sequencing in a 2 × 250 pair-end manner. Raw sequences were uploaded to the online Galaxy sequence analysis pipeline. Then, raw sequences were assembled with FLASH (Magoč and Salzberg, 2011). The Joined sequences with vague bases or lengths less than 245 bp were discarded. Bacterial operational taxonomic units (OTUs) were clustered by UPARSE7 at a threshold of 97% identity. For the remaining sequences, singletons and chimeras were also discarded, and all sequences were then resampled to a depth of 31109 sequences for each sample. Representative sequences were used to annotate OTU from the Ribosomal Database Project (RDP) Classifier with a 50% confidence evaluation (Wang et al., 2007).

2.4 GeoChip hybridization experiments and data processing

GeoChip 5.0M, a functional gene array targeting genes associated with biogeochemical cyclings, was utilized to examine functional genes present in microbial communities (Zhang et al., 2017), with 11 ARG types including *ABC transporter* (ATP binding cassette transporter), *MATE transporter* (Multi-antimicrobial extrusion protein), *Mex* (Mex proteins in the RND family), *MFS transporter* (Major facilitator superfamily), *SMR* (small multidrug resistance), *tet* (tetracycline resistance), *Fosfomycin Modifying Enzymes*, *vgb* (virginiamycin B Lyase), *β-lactamase*, *qnr* (quinolone resistance), and *van* (vancomycin resistance). Briefly, the DNA of each sample was labeled by a fluorescent dye named Cy-3 dUTP, then hybridized to GeoChip at 67°C in a rotator/incubator with 20 r/min for 24 h. The arrays were washed after hybridization and then scanned by a NimbleGen MS200 Microarray Scanner (Roche, San Francisco, CA, USA). Data were extracted from the array images using Agilent Feature Extraction software version 11.5 and uploaded to the online Microarray Data Manager, as previously described (Ma et al., 2019). Detected signals with the signal-to-noise ratios < 2 were removed, and signals among arrays were normalized by the universal standard of spot intensities.

2.5 Statistical analyses

The α-diversity includes richness, Simpson index, and Shannon's index. The β-diversity was calculated based on Bray-Curtis dissimilarity. Tukey HSD test was used to determine the differences in α-diversity. PERMANOVA (permutational multivariate analysis of variance) (Anderson, 2001), MRPP (multi-response permutation procedure), and ANOSIM (analysis of similarities), all based on Bray-Curtis dissimilarity, were used to determine the differences of the resistome or bacterial taxonomic compositions between sample types or between sampling sites. Heatmaps were generated based on the Z scores of the relative abundances. For each ARG or bacterial phylum, a Z score was calculated as its average relative abundance in a type of samples (e.g., control soil) subtracted by the mean and then divided by the standard deviation of the four average relative abundances in four sample types. Pearson correlation was used to examine the relationships between resistome and bacterial taxonomic composition. Mantel tests were used to examine correlational relationships between the resistome and bacterial taxonomic community based on Bray-Curtis dissimilarity (Legendre and Legendre, 2012). The multiple regression on distance matrices (MRM) analysis was carried out to investigate relationships among the bacterial taxonomic composition of the community, the resistome present, and environmental factors (Lichstein, 2007). All analyses were performed in R.

3 Results

3.1 The resistome composition

There are 9277 ARG probes in the GeoChip microarray, which can be divided into 34 ARG subtypes belonging to 11 ARG types (Figs. S1(a) and S2). All of the 34 ARG subtypes were detected across samples. Those ARGs represent classical mechanisms of antibiotic resistance, including antibiotic efflux, antibiotic inactivation, and target protection/redundancy. *MFS* transporter, *Van*, and *Mex* were the most abundant, followed by *ABC* and *MATE* transporters (Fig. S1(b)). In contrast to the observation that antibiotics levels were much higher in manure and compost samples than in compost-amended soils and unamended soils (control soils) (Table S1), the α-diversities of resistome were significantly lower in manure and compost than in soils ($P < 0.050$ by Tukey HSD test, Fig. S1(c)). *Van*, *MFS,* and *MATE* transporters were the most abundant in manure and compost samples, while *MFS*, *Mex,* and *ABC* transporters were the most abundant in compost-amended soil and unamended soil (Fig. S1(b)). In contrast, the α-diversities and abundance of resistome were not significantly different among CP, DX, HZ, JG, and ZZ sites, wherein each of the five dissemination chains of animal farm-compost-compost amended soil was established, as well as control soils ($P>0.050$ by Tukey HSD test, Fig. S1(d)).

The overall composition of resistome was distinct among manure, compost, compost-amended, and control soils, as revealed by three non-parametric dissimilarity tests (MRPP, ANOSIM, and PERMANOVA, Table 1 & Fig. 1(a)). In compost-amended soil and control soil samples, *B_lactamase_C*, *MFS_antibiotic*, *ABC_antibiotic_transporter_2*, *B_lactamase_5f0_D*, *ABC_antibiotic_transporter*, *b_lactamase*, *Mex_5f0_RND_antibiotic*, *fosA*, *MFS_antibiotic_5f0_other*, *B_lactamase_B_2*, *Van*, *MATE_antibiotic_5f0_gammaproteo*, and *SMR_antibiotics_2* showed relatively high abundances (Fig. 1(a)). In contrast, manure and compost samples had relatively high abundances of *B_lactamase_A_5f0_2*, *MFS_antibiotic_5f0_entero*, *fosB*, *Van_2*, *B_lactamase_A_5f0_firmicutes*, *MFS_antibiotic_5f0_Firmicutes*, *Tet*, *vgb*, *MFS_antibiotic_5f0_gammaproteo*, *MATE_antibiotic_5f0_other*, and *fosX* (Fig. 1(a)).

3.2 The bacterial taxonomic composition

A total of 74676 bacterial OTUs were assigned to 54 phyla. Consistent with observations in resistome, the bacterial taxonomic composition was distinct among manure, compost, compost-amended, and control soils (MRPP, ANOSIM, and PERMANOVA, Table 1 & Fig. 1(b)). Also, the bacterial taxonomic composition was distinct among the five sites. At the phylum level, *Diapherotrites*, *Thaumarchaeota*, *Woesearchaeota*, *Acidobacteria*, *Armatimonadetes*, *BRC1*, *candidate division WPS-1*, *candidate division WPS-2*, *candidate division ZB3*, *Candidatus Saccharibacteria*, *Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Cyanobacteria*, *Deferribacteres*, *Deinococcus-Thermus*, *Elusimicrobia*, *Fibrobacteres*, *Gemmatimonadetes*, *Hydrogenedentes*, *Ignavibacteriae*, *Latescibacteria*, *Marinimicrobia*, *Microgenomates*, *Nitrospirae*, *Omnitrophica*, *Parcubacteria*, *Planctomycetes*, *Synergistetes*, *Thermodesulfobacteria*, and *Thermotogae* were more abundant in soils than in manure and compost (Fig. 1(b)). In contrast, *SR1* and *Tenericutes* bacterial phyla were more abundant in manure and compost than in soils, consistent with a previous study showing *Tenericutes* as one of the dominant bacterial phyla in the manure composting process (Liu et al., 2020).

3.3 Linkages between resistome and bacterial taxonomy

Correlations between resistome and bacterial taxonomic

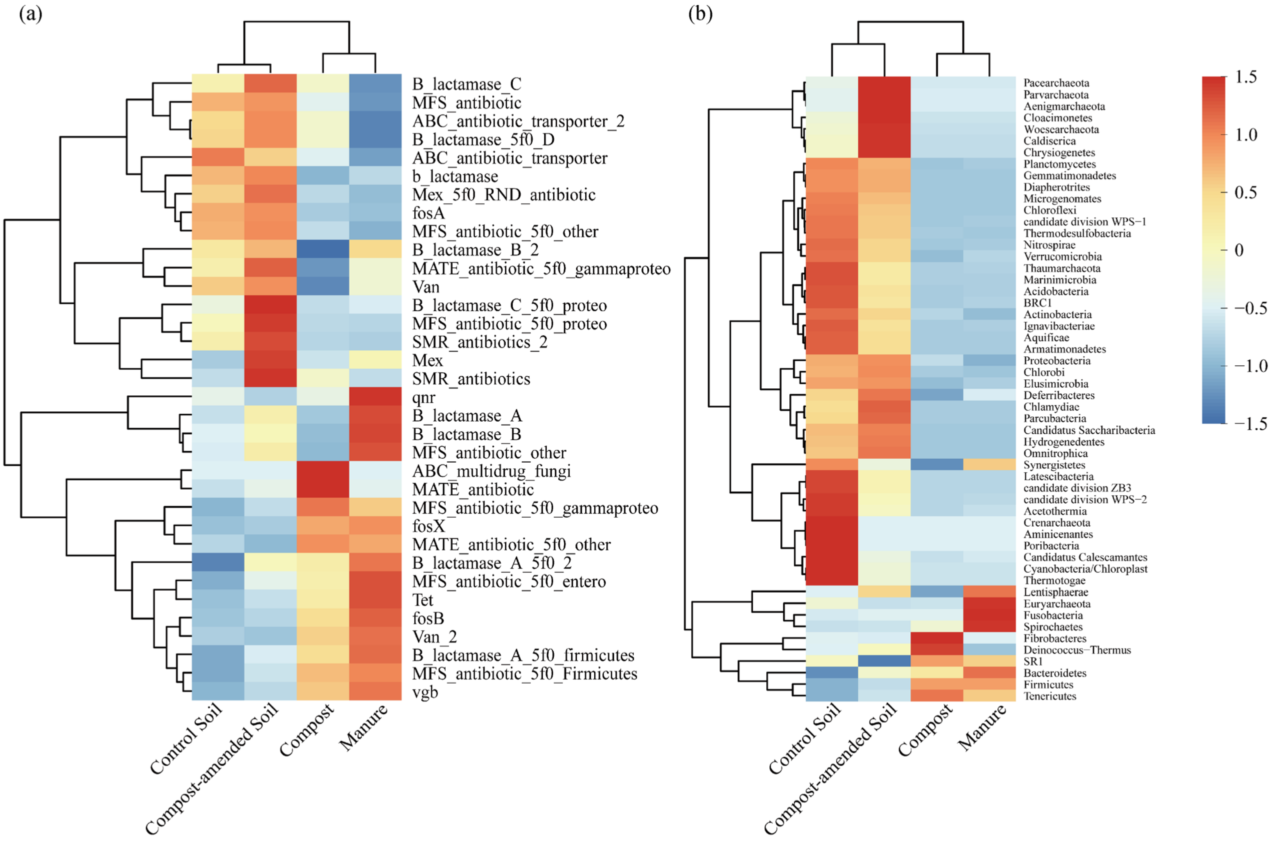


Fig. 1 Heatmaps of the (a) resistome and (b) bacterial phyla in manure, compost, compost-amended soil, and control soil, with 80 samples in total. The scale represents the relative abundance of each ARG or each bacterial phylum, which was normalized as a Z score detailed in the method.

composition were tested in all samples. The richness of resistome and bacterial OTUs were significantly correlated (R = 0.486, $P < 0.001$ by Pearson correlation, Fig. 2(a)). Similar results were observed when the Shannon index (R = 0.596, $P < 0.001$, Fig. 2(b)) and the Simpson index (R = 0.471, $P < 0.001$, Fig. 2(c)) were used as alternative indices of α-diversities. A strong, significant correlation was also observed for β-diversity between resistome and bacterial taxonomic composition (Bray-Curtis dissimilarity, R = 0.600, $P < 0.001$, Fig. 2(d)). Within each of five sampling sites, significant ($P < 0.050$) correlations between β-diversity of resistome and bacterial OTUs were observed (Table S2). However, when we divided samples into manure, compost, compost-amended, and control soils, there was no significant (P>0.050) correlation for α-diversities (Table 2). In contrast, there were significant ($P < 0.050$) correlations for β-diversity in manure, compost, compost-amended soils, but not control soils (Table 2), which was verified by Mantel tests (Table S3). Since antibiotics concentrations were the lowest in control soils (Table S1), it was likely that its resistome was least selected by antibiotics, resulting in a largely stochastic resistome unlinked to bacterial taxonomy.

We examined correlations between individual bacterial genera and resistome to reveal bacteria with the strongest linkages to resistome, similar to earlier studies (Tao et al., 2016; Zhang et al., 2019). A total of 15 pairs of bacterial genera and ARGs showed positive correlations ($P < 0.05$) in manure samples and also in compost samples (Figs. 3(a) and 3(b), & Table S4). The ARGs are β-*lactamase*, fosfomycin modifying enzyme, *MFS transporter*, *tet*, *Van*, and *vgb*. Eight out of 13 bacterial genera were affiliated to *Firmicutes*, four were affiliated to *Proteobacteria*, and one was affiliated to *Actinobacteria*. Only two bacterial genera were associated with more than one ARGs (genus *Acinetobacter* ~ *B_lactamase_A_5f0_firmicutes*, genus *Acinetobacter* ~ *tet*; genus *Alkalibaculum* ~ *fosX*, genus *Alkalibaculum* ~ *vgb*), suggesting a minor to modest risk to form "super-bugs" (i.e., bacteria with multiple resistance to

Table 1 Non-parametric multivariate dissimilarity analyses of resistome and bacterial taxonomic composition

Group	Treatment	PERMANOVA[a)		MRPP		ANOSIM	
		R^2	P	δ	P	R	P
Resistome[c)	Sample types	0.425	**0.001**[b)	0.169	**0.001**	0.549	**0.001**
	Sample sites	0.074	**0.001**	0.216	0.067	0.032	0.082
Bacterial taxonomic composition	Sample types	0.321	**0.001**	0.715	**0.001**	0.735	**0.001**
	Sample sites	0.106	**0.001**	0.821	**0.001**	0.065	**0.022**

Notes: a) All three tests of the permutational multivariate analysis of variance (PERMANOVA), multiple response permutation procedures (MRPP), and analysis of similarity (ANOSIM) are based on Bray–Curtis distance. b) Significant P-values less than 0.050 are shown in bold. c) There are 80 samples in total.

Table 2 Pearson correlations between richness, Shannon index, Simpson index, and β-diversity (based on Bray-Curtis dissimilarity) of ARGs and bacterial OTUs in manure, compost, compost-amended soil, and control soil

Types	Indices	*r*	*P*
Manure	Richness	0.145	0.543
	Shannon index	0.211	0.372
	Simpson index	0.215	0.363
	β-diversity	0.346	**<0.001**[a)
Compost	Richness	0.441	0.059
	Shannon index	0.284	0.238
	Simpson index	0.107	0.663
	β-diversity	0.430	**<0.001**
Compost-amended Soil	Richness	−0.163	0.493
	Shannon index	−0.069	0.773
	Simpson index	−0.164	0.489
	β-diversity	0.318	**<0.001**
Control Soil	Richness	−0.029	0.902
	Shannon index	−0.146	0.540
	Simpson index	−0.050	0.835
	β-diversity	−0.018	0.808

Notes: a) Significant P-values less than 0.050 are shown in bold.

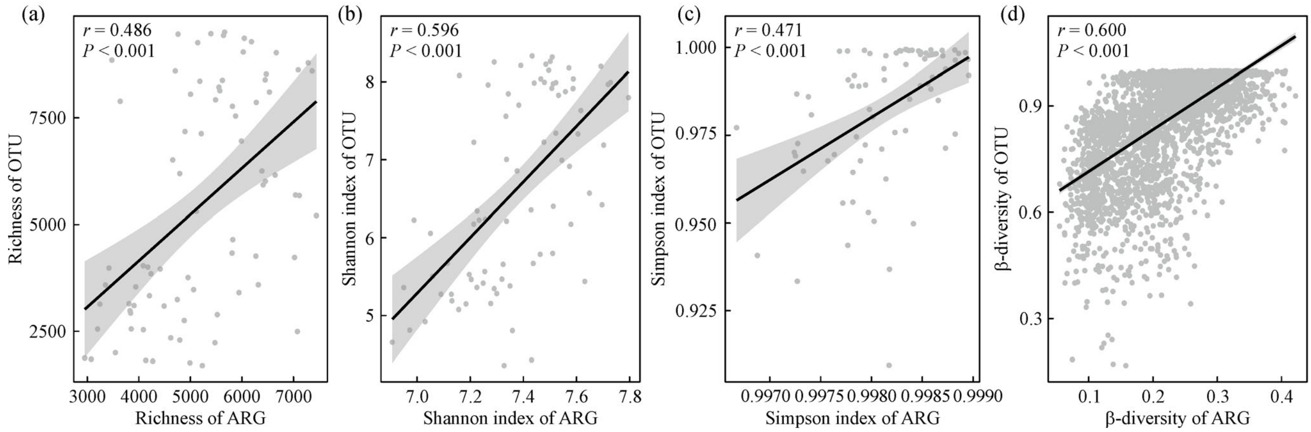


Fig. 2 Pearson correlations between richness (a), Shannon index (b), Simpson index (c), and β-diversity (based on Bray-Curtis dissimilarity) (d) of ARGs and bacterial OTUs. The significance of the correlation was determined by $P < 0.050$. There are 80 samples in total.

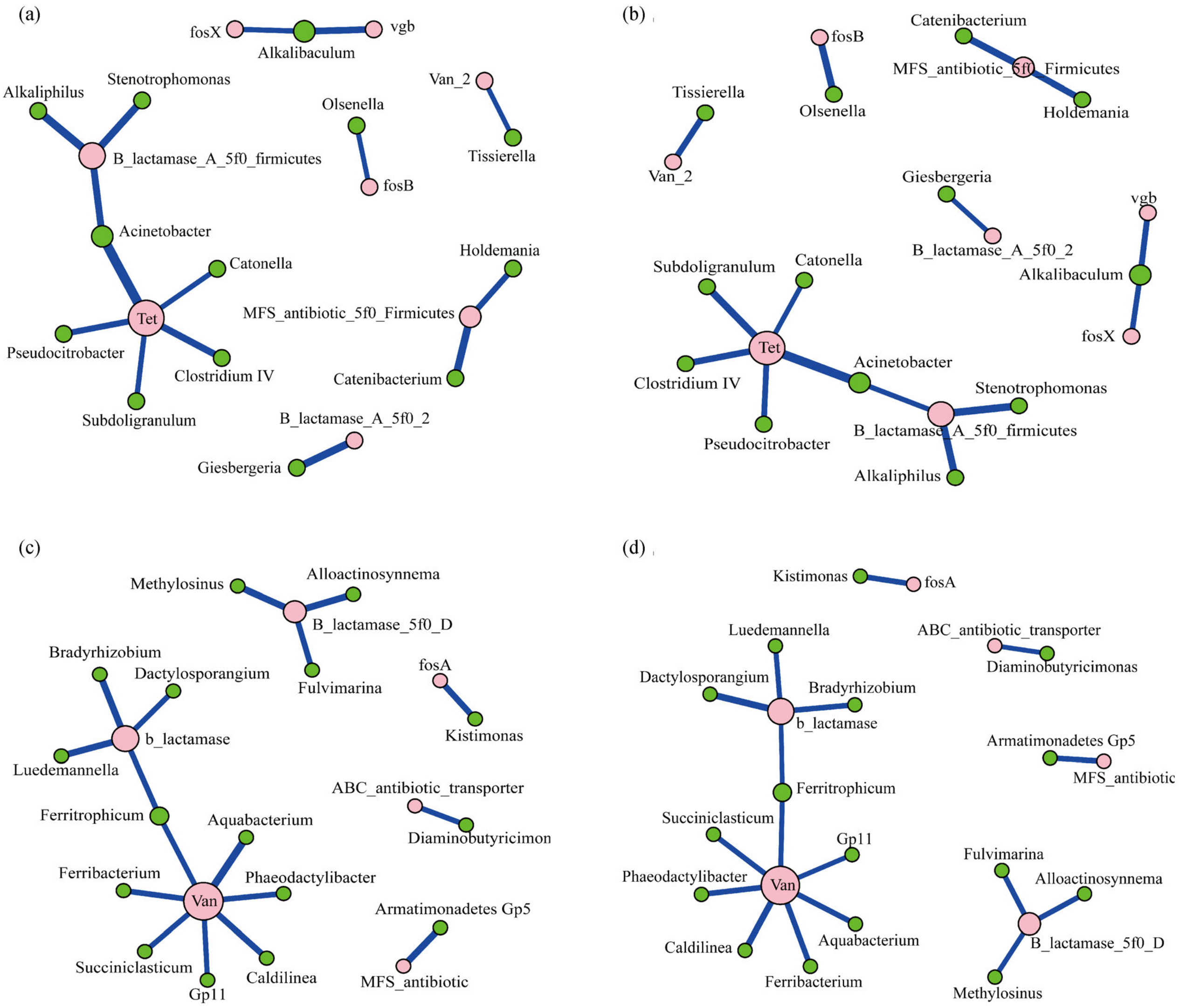


Fig. 3 Significant positive Pearson correlation pairs between bacterial genera and resistome in the group of (a) manure, (b) compost, (c) compost-amended soil, and (d) control soil samples. The thickness of the edge represents correlation values. Green circles represent bacterial genus. Pink circles represent resistome. The size of the circle represents the degree of connectedness. The significance of the correlation was determined by $P < 0.050$.

different antibiotics). However, *fosB* genes were correlated with *Olsenella*, which is involved in endodontic infections in humans (Siqueira and Rôças, 2008). *Van* genes were correlated with *Tissierella*, which can cause chronic osteomyelitis (Schweizer et al., 2016) and prosthetic knee joint and bloodstream infections (Caméléna et al., 2016). Some *Acinetobacter* spp. are multidrug-resistant and can act as major infectious agents in debilitated patients (Towner, 2009). Similarly, *Stenotrophomonas* caused opportunistic infections in patients with cystic fibrosis, cancer, and HIV (de Oliveira-Garcia et al., 2002). Those results suggest that these bacterial species may act as hubs for HGT of resistome and increase potential public health risks.

A total of 17 pairs of bacterial genera and ARGs showed positive correlations ($P < 0.05$) in compost-amended soils and also in control soils (Figs. 3(c) and 3(d), & Table S5), which did not overlap with 15 pairs in manure and compost samples. The ARGs are *ABC transporter*, *β-lactamase*, *MFS transporter*, *fosA*, and *Van*. Twelve out of 16 bacterial genera were affiliated to the phyla *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, while others were affiliated to *Bacteroidetes*, *Acidobacteria*, *Armatimonadetes*, and *Chloroflexi*. One bacterial genus was associated with both *b_lactamase* and *Van*. In contrast with the situation of multiple pathogens detected in manure and compost samples, only *van* gene was related to *Succiniclasticum*, an opportunistic pathogen and endotoxin producer (Neubauer et al., 2019), and *Aquabacterium*, in compost-amended and control soils, indicating a relatively less risk to public health than manure and compost samples.

3.4 Factors influencing the resistome

The multiple regression on distance matrices (MRM) analysis was performed to identify major factors influencing environmental resistome. The bacterial taxonomic composition explained 36.0% ($P = 0.001$) of the resistome (Table 3), much higher than the explanatory power of soil moisture (19.5%, $P = 0.001$) and the sample type (2.5%, $P = 0.001$). In contrast, the sample site and temperature were not important in shaping the resistome.

When samples were divided into manure, compost, compost-amended, and control soils, the bacterial taxonomic composition explained the resistome in manure (12.0%, $P = 0.003$), compost (18.5%, $P = 0.001$), and compost-amended soil (10.1%, $P = 0.001$). The sampling site explained 14.5% of the resistome ($P = 0.001$) in manure samples and 7.5% of the resistome ($P = 0.005$) in compost samples. These five different sample sites contained various antibiotics concentrations, which could be important in shaping the resistome. Soil moisture explained only 6.4% of the resistome ($P = 0.040$) in compost samples.

4 Discussion

In this study, significant correlations between resistome and bacterial taxonomy were detected in manure, compost, and compost-amended soil, but not controls soils, suggesting that interconnection between the resistome and bacterial taxonomy was environment-dependent. The results conflict with a previous study showing that bacterial phylogeny structures resistome in soils (Forsberg et al., 2014). The inconsistency may result from different soil types or technologies used across various studies. In addition, the results showed that control soils contained a large diversity of resistome and low antibiotic concentration settings (Fig. S1(c) & Table S1). As a result, high functional redundancy with a negligible environmental selection in control soils could obscure correlations with bacterial taxonomy.

Distinct profiles of ARGs regardless of similar geographical locations were observed in urban wastewater treatment plants (Fernandes et al., 2019). Similarly, variations of resistome and bacterial communities throughout the composting process were divided into two distinct stages, and total ARGs increased during composting (Cao

Table 3 Contribution of bacterial taxonomic composition and environmental factors in shaping the resistome by multiple regression on distance matrices (MRM) in all samples, manure, compost, compost-amended soil, and control soil

Influence factors	All Samples		Manure		Compost		Compost-amended Soil		Control Soil	
	R^2 a)	coef ($R^2 = 0.445$)	R^2	coef ($R^2 = 0.230$)	R^2	coef ($R^2 = 0.228$)	R^2	coef ($R^2 = 0.118$)	R^2	coef ($R^2 = 0.027$)
Bacterial taxonomic composition	0.360*** b)	0.286***	0.120**	0.064*	0.185***	0.086*	0.101***	0.107***	0.0003	0.026
Sample Type	0.025***	−0.004	–	–	–	–	–	–	–	–
Sample Site	0.002	−0.002	0.145***	0.027**	0.075**	0.006	0.006	−0.003	0.008	−0.007
Temperature	0.003	−0.010**	0.022	0.002	0.057	0.005	0.001	−0.004	0.0004	0.003
Moisture	0.195***	0.018***	0.026	−0.016	0.064*	0.011	0.004	0.0001	0.014	−0.007

Notes: a) R^2 represents explained variance in the distance matrix regression model for each influence factor; and coefficient (coef) estimates the correlation of each factor in multivariate regression model of distance matrices. b) Significant P-values indicated as $*P \leqslant 0.050$, $**P \leqslant 0.010$, and $***P \leqslant 0.001$.

et al., 2020). In this study, manure and compost samples were distinct from compost-amended and control soils (Fig. 1). *Van* genes encoding vancomycin resistance genes are often found in human pathogens, such as glycopeptide-producing *Actinomycetes* and nonglycopeptide-producing *Actinomycetes* (Hong et al., 2008). *MATE* (multidrug and toxic compound extrusion) transporters are also responsible for antibiotics uptake, conferring multidrug resistance to pathogens (Kusakizako et al., 2020). Both genes were among the most abundant in manure and compost samples (Fig. S1(b)), revealing potential health risks of human pathogens in manure and compost. In contrast, *MFS* (major facilitator superfamily) and *ABC* (ATP binding cassette) transporters are the most abundant in compost-amended and control soils (Fig. S1(b)). These two kinds of transporters can transport a wide spectrum of compounds in addition to antibiotics (Pao et al., 1998; Yan, 2013).

Firmicutes was the most abundant phylum in manure and compost samples (Fig. 1(b)). As well-known producers of antibiotics (Mannanov and Sattarova, 2001), *Firmicutes* was related to resistome (Huerta et al., 2013), leading to ARGs' dissemination as carriers. High abundances of *Firmicutes* in manure and compost coincided with the high antibiotic concentration (Table S1), imposing potential risks of resistome's dissemination. As the most abundant phylum in soil samples (Fig. 1(b)), *Proteobacteria* contain many multidrug-resistant strains (Forsberg et al., 2014) and are common carriers of class 1 integrons-integrase (Martinez et al., 2013). Feeding antibiotic-laced feed to pigs increased the abundance of *Proteobacteria* in the pig gut (Looft et al., 2012). The higher abundance of *Proteobacteria* increases antibiotic resistance in soils (Forsberg et al., 2012), representing a major conduit through which resistome is disseminated in the environment. It also increased the abundance of resistome for antibiotics classes not added to the feeding stock, promoting HGT of resistome within *Proteobacteria* or among different phyla (Klümper et al., 2015).

In manure and compost samples, tetracycline-resistance genes (*tet*) were correlated with several pathogenic bacterial genera such as *Pseudocitrobacter*, *Subdoligranulum*, *Catonella* associated with endodontic infections (Siqueira and Rôças, 2006), *Clostridium IV,* and *Acinetobacter* (Figs. 3(c) and 3(d)). *Pseudocitrobacter* is a novel genus isolated from hospitalized patients, while *Subdoligranulum* and *Catonella* are affiliated to the order *Clostridiales*, which includes several significant human pathogens. *Clostridium perfringens*, belonging to *Clostridium IV,* is a gastrointestinal disease pathogen that produces severe histotoxic hypoxia (Mendez et al., 2008). Tetracycline has been extensively used for treatment in various clinical conditions of humans and animals. In past decades, tetracycline resistance emerges due to the wide application of tetracycline for clinical, veterinary, and agricultural purposes, becoming a rising concern for clinical treatments. However, the correlations suggest that these pathogenic taxa mentioned above could be potential hosts for tetracycline-resistance genes, differing from previous studies showing that the distribution of some tetracycline-resistance genes was limited to a few genera such as *Sphingobium* and *Enterobacteriaceae* (Ghosh et al., 2009; Zhang et al., 2009). Therefore, more alternative bacterial carriers of tetracycline-resistance genes remain to be discovered, including pathogens and bacteriophages.

Consistent with previous studies showing that bacterial community shaped the antibiotic resistome (Liao et al., 2019; Zhu et al., 2019; Zhang et al., 2020), there were significant correlations between resistome and bacteria colonizing in manure, compost, and compost-amended soils (Table 2). MRM also indicated that bacterial taxonomy was the primary factor influencing resistome in manure, compost, and compost-amended soil (Table 3). Those results suggest that the spread of resistome by potentially pathogenic bacteria could impose a challenge to medical treatments.

These findings have important implications because animal husbandry and manure composting are mature industries in modern society. As incremental anthropogenic activities could impose pathogenic threats, interconnected resistome and microbiome suggest that bacteria could concurrently cause the spread of resistome. Our finding is an important step toward understanding how resistome is disseminated in our environments and assessing the ecological impact of manure composting for potential pathogenicity, which is indispensable for knowledge-based policy management to control the environmental dissemination of resistome.

5 Conclusions

In a nutshell, the resistome and bacterial community were distinct among samples, with more potential pathogens related to ARGs identified in manure and compost. The resistome was significantly related to bacterial taxonomy in swine manure, compost, and compost-amended soils, but not in unamended agricultural soils. Therefore, bacterial taxonomy was the primary determinant of the resistome in the transmission chain of antibiotics.

Acknowledgements This work was sponsored by the National Key R&D Program of China (No. 2019YFC1806204) and the National Natural Science Foundation of China (Grant Nos. 41825016 and 41430856).

Authorship Contribution Statement Ziyan Qin: Conceptualization, Methodology, Investigation, Visualization, Writing- original draft, Writing- review and editing. Qun Gao: Conceptualization, Investigation, Methodology, Writing- original draft, Writing- review and editing. Qiang Dong: Investigation, Formal analysis. Joy D. Van Nostrand: Writing- review and editing. Qi Qi: Writing- review and editing. Tianjiao Dai: Writing- review and editing. Jingmin Cheng: Writing- review and editing. Jizhong Zhou: Resources, Supervision, Funding acquisition. Yunfeng Yang: Validation, Writing- review and editing, Supervision, Funding acquisition.

Declaration of Competing Interest The authors declare no conflicts of interest.

Data Availability GeoChip data are available in the NCBI GEO database under project no. GSE132839. DNA sequences of the 16S rRNA gene are available in the NCBI Sequence Read Archive under project no. PRJNA516026.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11783-021-1466-7org/?? and is accessible for authorized users.

References

Alcock R E, Sweetman A, Jones K C (1999). Assessment of organic contanhnant fate in waste water treatment plants I: Selected compounds and physicochemical properties. Chemosphere, 38(10): 2247–2262

Anderson M J (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26(1): 32–46

Caméléna F, Pilmis B, Mollo B, Hadj A, Le Monnier A, Mizrahi A (2016). Infections caused by Tissierella praeacuta: A report of two cases and literature review. Anaerobe, 40: 15–17

Cao R, Wang J, Ben W, Qiang Z (2020). The profile of antibiotic resistance genes in pig manure composting shaped by composting stage: Mesophilic-thermophilic and cooling-maturation stages. Chemosphere, 250: 126181

Chen Q L, An X L, Zheng B X, Gillings M, Peñuelas J, Cui L, Su J Q, Zhu Y G (2019). Loss of soil microbial diversity exacerbates spread of antibiotic resistance. Soil Ecology Letters, 1(1–2): 3–13

de Oliveira-Garcia D, Dall'agnol M, Rosales M, Azzuz A C, Martinez M B, Giron J A (2002). Characterization of flagella produced by clinical strains of *Stenotrophomonas maltophilia*. Emerging Infectious Diseases, 8(9): 918–923

Fernandes T, Vaz-Moreira I, Manaia C M (2019). Neighbor urban wastewater treatment plants display distinct profiles of bacterial community and antibiotic resistance genes. Environmental Science and Pollution Research International, 26(11): 11269–11278

Forsberg K J, Patel S, Gibson M K, Lauber C L, Knight R, Fierer N, Dantas G (2014). Bacterial phylogeny structures soil resistomes across habitats. Nature, 509(7502): 612–616

Forsberg K J, Reyes A, Wang B, Selleck E M, Sommer M O, Dantas G (2012). The shared antibiotic resistome of soil bacteria and human pathogens. Science, 337(6098): 1107–1111

Gao Q, Dong Q, Wu L, Yang Y, Hale L, Qin Z, Xie C, Zhang Q, Van Nostrand J D, Zhou J (2020). Environmental antibiotics drives the genetic functions of resistome dynamics. Environment International, 135: 105398

Ghosh S, LaPara T M (2007). The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME Journal, 1(3): 191–203

Ghosh S, Sadowsky M, Roberts M, Gralnick J, LaPara T (2009). Sphingobacterium sp. strain PM2 - P1 - 29 harbours a functional *tet (X)* gene encoding for the degradation of tetracycline. Journal of Applied Microbiology, 106(4): 1336–1342

Gillings M R, Stokes H W (2012). Are humans increasing bacterial evolvability? Trends in Ecology & Evolution, 27(6): 346–352

Hong H J, Hutchings M I, Buttner M J (2008).Vancomycin resistance VanS/VanR two-component systems. In: Utsumi R, ed. Bacterial Signal Transduction: Networks and Drug Targets. New York: Springer New York, 200–213

Huerta B, Marti E, Gros M, Lopez P, Pompeo M, Armengol J, Barcelo D, Balcazar J L, Rodriguez-Mozaz S, Marce R (2013). Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. Science of the Total Environment, 456–457: 161–170

Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014). Fate and effects of veterinary antibiotics in soil. Trends in Microbiology, 22 (9): 536–545

Jechalke S, Kopmann C, Rosendahl I, Groeneweg J, Weichelt V, Krogerrecklenfort E, Brandes N, Nordwig M, Ding G C, Siemens J, Heuer H, Smalla K (2013). Increased abundance and transferability of resistance genes after field application of manure from sulfadiazine-treated pigs. Applied and Environmental Microbiology, 79(5): 1704–1711

Ji Q K, Zhang C H, Li D (2020). Influences and mechanisms of nanofullerene on the horizontal transfer of plasmid-encoded antibiotic resistance genes between *E. coli* strains. Frontiers of Environmental Science & Engineering, 14(6): 108

Klümper U, Riber L, Dechesne A, Sannazzarro A, Hansen L H, Sorensen S J, Smets B F (2015). Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. ISME Journal, 9(4): 934–945

Kusakizako T, Miyauchi H, Ishitani R, Nureki O (2020). Structural biology of the multidrug and toxic compound extrusion superfamily transporters. Biochimica et Biophysica Acta, 1862(12): 183154

Leclercq S O, Wang C, Sui Z, Wu H, Zhu B, Deng Y, Feng J (2016). A multiplayer game: Species of Clostridium, Acinetobacter, and Pseudomonas are responsible for the persistence of antibiotic resistance genes in manure-treated soils. Environmental Microbiology, 18(10): 3494–3508

Legendre P, Legendre L (2012). Numerical Ecology. Amsterdam,AE: Elsevier

Liao H, Friman V P, Geisen S, Zhao Q, Cui P, Lu X, Chen Z, Yu Z, Zhou S (2019). Horizontal gene transfer and shifts in linked bacterial community composition are associated with maintenance of antibiotic resistance genes during food waste composting. Science of the Total Environment, 660: 841–850

Lichstein J W (2007). Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecology, 188(2): 117–131

Liu T, Awasthi S K, Duan Y, Zhang Z, Awasthi M K (2020). Effect of fine coal gasification slag on improvement of bacterial diversity community during the pig manure composting. Bioresource Technology, 304: 123024

Looft T, Johnson T A, Allen H K, Bayles D O, Alt D P, Stedtfeld R D, Sul W J, Stedtfeld T M, Chai B, Cole J R, Hashsham S A, Tiedje J M, Stanton T B (2012). In-feed antibiotic effects on the swine intestinal microbiome. Proceedings of the National Academy of Sciences of the United States of America, 109(5): 1691–1696

Ma X, Zhang Q, Zheng M, Gao Y, Yuan T, Hale L, Van Nostrand J D, Zhou J, Wan S, Yang Y (2019). Microbial functional traits are sensitive indicators of mild disturbance by lamb grazing. ISME

Journal, 13(5): 1370–1373

Magoč T, Salzberg S L (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics (Oxford, England), 27(21): 2957–2963

Mannanov R N, Sattarova R K (2001). Antibiotics produced by Bacillus bacteria. Chemistry of Natural Compounds, 37(2): 117–123

Marshall B M, Levy S B (2011). Food animals and antimicrobials: impacts on human health. Clinical Microbiology Reviews, 24(4): 718–733

Martinez E, Djordjevic S, Stokes H, Chowdhury P R (2013).Lateral Gene Transfer in Evolution. RamatAviv: Springer, 79–103

Mendez M, Huang I H, Ohtani K, Grau R, Shimizu T, Sarker M R (2008). Carbon catabolite repression of type IV pilus-dependent gliding motility in the anaerobic pathogen Clostridium perfringens. Journal of Bacteriology, 190(1): 48–60

Neubauer V, Humer E, Mann E, Kroger I, Reisinger N, Wagner M, Zebeli Q, Petri R M (2019). Effects of clay mineral supplementation on particle-associated and epimural microbiota, and gene expression in the rumen of cows fed high-concentrate diet. Anaerobe, 59: 38–48

Pao S S, Paulsen I T, Saier M H Jr (1998). Major facilitator superfamily. Microbiology and Molecular Biology Reviews, 62(1): 1–34

Peng F, Guo Y, Isabwe A, Chen H, Wang Y, Zhang Y, Zhu Z, Yang J (2020). Urbanization drives riverine bacterial antibiotic resistome more than taxonomic community at watershed scale. Environment International, 137: 105524

Pruden A, Arabi M, Storteboom H N (2012). Correlation between upstream human activities and riverine antibiotic resistance genes. Environmental Science & Technology, 46(21): 11541–11549

Qiao M, Chen W, Su J, Zhang B, Zhang C (2012). Fate of tetracyclines in swine manure of three selected swine farms in China. Journal of Environmental Sciences-China, 24(6): 1047–1052

Schweizer M, Bloemberg G V, Graf C, Falkowski A L, Ochsner P, Graber P, Urffer S, Goldenberger D, Hinic V, Graf S, Tarr P E (2016). Chronic osteomyelitis due to *Tissierella carlieri*: First case. Open Forum Infectious Diseases, 3(1): ofw012

Siqueira J F Jr, Rôças I N (2006).Catonella morbi and *Granulicatella adiacens*: new species in endodontic infections. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 102(2): 259–264

Siqueira J F Jr, Rôças I N (2008). Update on endodontic microbiology: candidate pathogens and patterns of colonisation. Endodontic Practice Today, 2(1): 7–20

Smillie C S, Smith M B, Friedman J, Cordero O X, David L A, Alm E J (2011). Ecology drives a global network of gene exchange connecting the human microbiome. Nature, 480(7376): 241–244

Stokes H W, Gillings M R (2011). Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiology Reviews, 35(5): 790–819

Su J Q, An X L, Li B, Chen Q L, Gillings M R, Chen H, Zhang T, Zhu Y G (2017). Metagenomics of urban sewage identifies an extensively shared antibiotic resistome in China. Microbiome, 5(1): 1–15

Tao W, Zhang X X, Zhao F, Huang K, Ma H, Wang Z, Ye L, Ren H (2016). High levels of antibiotic resistance genes and their correlations with bacterial community and mobile genetic elements in pharmaceutical wastewater treatment bioreactors. PLoS One, 11 (6): e0156854

Towner K J (2009). Acinetobacter: an old friend, but a new enemy. Journal of Hospital Infection, 73(4): 355–363

Udikovic-Kolic N, Wichmann F, Broderick N A, Handelsman J (2014). Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. Proceedings of the National Academy of Sciences of the United States of America, 111(42): 15202–15207

Wang Q, Garrity G M, Tiedje J M, Cole J R (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73 (16): 5261–5267

Wu L, Yang Y, Chen S, Jason Shi Z, Zhao M, Zhu Z, Yang S, Qu Y, Ma Q, He Z, Zhou J, He Q (2017). Microbial functional trait of rRNA operon copy numbers increases with organic levels in anaerobic digesters. ISME Journal, 11(12): 2874–2878

Wu N, Zhang W Y, Xie S Y, Zeng M, Liu H X, Yang J H, Liu X Y, Yang F (2020). Increasing prevalence of antibiotic resistance genes in manured agricultural soils in northern China. Frontiers of Environmental Science & Engineering, 14(1): 1

Yan N (2013). Structural advances for the major facilitator superfamily (MFS) transporters. Trends in Biochemical Sciences, 38(3): 151–159

Zhang H, He H, Chen S, Huang T, Lu K, Zhang Z, Wang R, Zhang X, Li H (2019). Abundance of antibiotic resistance genes and their association with bacterial communities in activated sludge of wastewater treatment plants: Geographical distribution and network analysis. Journal of Environmental Sciences-China, 82: 24–38

Zhang J, Gao Q, Zhang Q, Wang T, Yue H, Wu L, Shi J, Qin Z, Zhou J, Zuo J, Yang Y (2017). Bacteriophage–prokaryote dynamics and interaction within anaerobic digestion processes across time and space. Microbiome, 5(1): 1–10

Zhang M, He L Y, Liu Y S, Zhao J L, Zhang J N, Chen J, Zhang Q Q, Ying G G (2020). Variation of antibiotic resistome during commercial livestock manure composting. Environment International, 136: 105458

Zhang T, Zhang M, Zhang X, Fang H H (2009). Tetracycline resistance genes and tetracycline resistant lactose-fermenting Enterobacteriaceae in activated sludge of sewage treatment plants. Environmental Science & Technology, 43(10): 3455–3460

Zhu D, Wang H T, Zheng F, Yang X R, Christie P, Zhu Y G (2019). Collembolans accelerate the dispersal of antibiotic resistance genes in the soil ecosystem. Soil Ecology Letters, 1(1–2): 14–21

Zhu Y G, Johnson T A, Su J Q, Qiao M, Guo G X, Stedtfeld R D, Hashsham S A, Tiedje J M (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proceedings of the National Academy of Sciences of the United States of America, 110(9): 3435–3440