Metagenomic Assembly Reveals the Fate of the Core Antibiotic Resistome, its Hosts and Mobility in Animal Manure and After Compost

Tianlei Qiu

Beijing Academy of Agriculture and Forestry Sciences

Linhe Huo

Beijing Academy of Agriculture and Forestry Sciences

Yajie Guo

Beijing Academy of Agriculture and Forestry Sciences

Min Gao

Beijing Academy of Agriculture and Forestry Sciences

Guoliang Wang

Beijing Academy of Agriculture and Forestry Sciences

Dong Hu

Hebei Academy of Agriculture and Forestry Sciences

Cheng Li

Beijing Academy of Agriculture and Forestry Sciences

Zhanwu Wang

Hebei Academy of Agriculture and Forestry Sciences

Guiming Liu

Beijing Academy of Agriculture and Forestry Sciences

Xuming Wang (✉ wangxuming@baafs.net.cn)

Beijing Academy of Agriculture and Forestry Sciences <https://orcid.org/0000-0003-3590-562X>

Research

Keywords: Metagenome, Antibiotic resistance genes, Resistome, Bacterial community, Manure

Posted Date: November 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1023999/v1>

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Abstract

Background

Antibiotics and antibiotic resistance genes (ARGs) used in intensive animal husbandry threaten human health worldwide; however, the core resistome, mobility of ARGs, and the composition of ARG hosts in animal manure and the following composts remain unclear. In the present study, metagenomic assembly was used to comprehensively decipher the core resistome and its potential mobility and hosts in animal manure and compost.

Results

In total, 201 ARGs were shared among different animal (layer, broiler, swine, beef cow, and dairy cow) manures and accounted for 86–99% of total relative abundance of ARGs, which mainly comprised multidrug, macrolide-lincosamide-streptogramin (M-L-S), tetracycline, beta-lactam, aminoglycoside, and sulfonamide resistance genes. Moreover, efficient composting reduced the total relative abundance of ARGs in manure from 0.938 to 0.405 copies per 16S rRNA gene; however, it did not have any remarkable effect on the multidrug, sulfonamide, and trimethoprim resistance genes. Procrustes analysis indicated that composting can reduce antibiotic residues and decrease the correlation between antibiotics and resistance genes. Furthermore, the ARG hosts included Proteobacteria (50.08%), Firmicutes (37.77%), Bacteroidetes (6.49%), and Actinobacteria (5.24%). In manure, aminoglycoside resistance genes were majorly found in *Enterococcus*, *Streptococcus*, and *Enterobacter*; tetracycline resistance genes (TRGs) were found in *Pseudomonas*, *Lactobacillus*, and *Streptococcus*; and multidrug resistance genes were mainly found in *Escherichia coli*. In our samples, ARGs were more prevalent in plasmids than in chromosomes. The broad host range and diverse mobile genetic elements may be two key factors for ARGs, such as *sul1* and *aadA*, which could survive during composting. The multidrug resistance genes represented the dominant ARGs in pathogenic antibiotic-resistant bacteria (PARB) in manure, and composting could effectively control PARB.

Conclusions

Our experiments revealed the core resistome in animal manure, classified and relative quantified the ARG hosts, and assessed the mobility of ARGs. Composting can mitigate ARGs in animal manure by altering the bacterial hosts; however, some ARGs can escape from the removal with the survivor heat-tolerant hosts or transfer to these hosts. These findings will help optimize composting strategies for the effective treatment of ARGs and their hosts in farms.

Background

Antibiotics have been widely used in livestock husbandry for growth promotion and prophylactic purposes or for treating animal diseases since they were initially approved as feed additives by the U.S. Food and Drug Administration (FDA) in 1950. Approximately, 162,000 tons of antibiotics are consumed by livestock and poultry per year in China, accounting for more than 52% of the total antibiotic usage^[1]. Most of these antibiotics are poorly metabolized by animals, resulting in abundant residues in feces leading to the development of antibiotic-resistant bacteria (ARB) and associated resistance genes (ARGs)^[2,3]. Increasing research reveals that livestock and poultry manure are reservoirs for the dissemination of ARB, ARGs, and potential human pathogenic bacteria. China is the largest poultry and pig producer worldwide^[4], with approximately 3.8 billion tons of livestock and poultry manure production per year^[5]. Aerobic composting of manure is a commonly employed technology to decompose complex organic compounds into steady end-products that can be used as organic fertilizers for agricultural soil amendment. In general, the total ARG abundance in raw manure can be decreased to a certain extent during composting; however, some ARG subtypes, such as sulfonamide resistance genes, may increase in abundance after composting, a phenomenon that may be related to their transferability and host bacterial resistance to high temperatures during composting^[6–8].

The enrichment of ARGs in the environment is widespread and mainly occurs through horizontal gene transfer (HGT) mediated by mobile genetic elements (MGEs), including plasmids, transposons, integrons, and integrative conjugative elements^[9–11]. Therefore, mobile ARGs linked with MGEs are a higher risk to human health than nonmobile ARGs located on the chromosomes. ARGs and MGEs in animal manure and composting processes have been well documented in terms of diversity and abundance^[7,12–15], however the core resistome and mobile ARGs of different manures and composts remain to be elucidated, which hinders the comprehensive understanding of the manure resistome and associated risk.

The bacterial community is a key factor affecting the ARG profiles in animal manure and compost. Therefore, clarifying the hosts of ARGs is conducive for controlling ARG dissemination during manure composting. In addition, mobile ARGs present among pathogens are considered to pose the highest risk, thereby indicating that determining the mobility and hosts of ARGs is crucial for their risk assessment. The identification of potential host bacteria and mobility of ARGs is often based on correlation analysis^[7,16–21]; moreover, direct evidence for the presence of ARG hosts in animal manure is lacking. Metagenomic analysis based on assembled contigs or binning bacterial genomes can directly link ARGs to specific bacterial hosts, thereby improving the ability to assess the risks of manure and compost resistomes.

We collected five types of food-producing animal manure (layer, broiler, swine, beef cow, and dairy cow) and their composting samples that were raised in large quantities in China, and employed high-throughput sequencing and metagenomic assembly to identify the contigs harboring ARGs. The present study aimed to (i) determine the core resistome of animal manure and its variation after composting, (ii) reveal mobile ARGs and their abundance, and (iii) determine the composition and abundance of bacterial hosts of ARGs. The results will improve our understanding of manure and compost resistomes, particularly mobile resistomes, in addition to assessing their risks.

Methods

Sample collection

Manure and compost samples were collected from 29 concentrated animal feeding operations farms and 13 commercial manure composting plants from Beijing and Hebei Province, Northern China (representative sites; including 8 broiler farms, 8 layer farms, 5 swine farms, 4 beef cow farms, and 4 dairy cow farms in northern China) (Fig. S1 and Table S1). Sampling campaigns were conducted between October 2017 and October 2018. Fresh manure samples (500 g) were collected by mixing subsamples directly from five different points of each farm to achieve uniform representativeness. Using the same sampling methods, 500 g of compost samples were collected from the finished compost warehouses. All samples were incubated on ice in coolers, transported immediately to the laboratory for pretreatment, and then stored at -80°C until further analysis.

Determination of antibiotic concentration

The antibiotics analyzed in this work were selected mainly from those used for livestock and poultry animals^[1]. The nineteen antibiotics were analyzed which belong to five categories. Four tetracyclines (TCs) included tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DC). Four sulfonamides (SAs) included sulfadiazine (SDZ), sulfamethoxazole (SMZ), sulfamethoxazole (SMX) and sulfachlorpyridazine (SCP). Four quinolones included norfloxacin (NFX), ciprofloxacin (CFX), enrofloxacin (EFX) and lomefloxacin (LFX). Three macrolides (MLs) included erythromycin (ETM), tylosin (TYL), roxithromycin (RTM). Two amphenicols (AMs) included florfenicol (FF) and chloramphenicol (CAP). Two others antibiotics including lincomycin (LC) and ceftiofur (CEFT). Antibiotics were pretreated and analysis according Li's study^[22].

DNA extraction, metagenomic sequencing, and quality analysis

DNA was extracted from three replicates of each manure or compost sample using the MoBio PowerSoil DNA isolation kit (Mobio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration and quality of the extracted DNA were determined using Nanodrop 2000c and Qubit 3.0 Fluorometer (Thermo Fisher Scientific). The DNA from the manure and compost samples was used for shotgun sequencing using the Illumina Novaseq 6000 and paired-end 150 bp reads were generated. The raw data obtained from the Illumina HiSeq sequencing platform using Readfq V8 (<https://github.com/cjfields/readfq>) was preprocessed to acquire clean data for subsequent analyses. The specific processing steps were as follows: a) removal of reads comprising low quality bases (the quality threshold value ≤ 38) >40 bp; b) removal of reads in which the N base reached 10 bp; c) removal of reads, which shared the overlap >15 bp with Adapter. On average, 23.36 M (SD $\pm$ 6.36 M) reads passed and were used in all further analyses. Sequencing data were deposited in the Genome Sequence Archive (GSA) repository under BioProject number CRA005191.

Calculating ARG abundance

ARG abundance was determined using ARGs-OAP v2.0, which integrates the detection of ARGs using the reference database SARG.2.2^[23]. Briefly, reads were annotated as ARG-like reads at the cutoff of E value of 10^{-7} , sequence identity of 90%, and alignment length of more than 25 amino acids. ARGs were quantified by normalizing ARG abundance to the copy number of the 16S rRNA gene using the following equation^[24] [15]:

$$\text{Abundance} = \sum_1^n \frac{N_{\text{ARG-like sequence}} \times L_{\text{reads}} / L_{\text{ARG reference sequence}}}{N_{16S \text{ sequence}} \times L_{\text{reads}} / L_{16S \text{ sequence}}}$$

where $N_{\text{ARG-like sequence}}$ is the number of ARG-like sequences annotated to one specific ARG reference sequence, N_{reads} represents the length of the reads, $L_{\text{ARG-reference sequence}}$ is the nucleotide sequence length of the corresponding ARG reference sequence, $N_{16 \text{ sequence}}$ is the number of the 16S rRNA gene sequences, $L_{16 \text{ sequence}}$ is the full length of the 16S rRNA gene, n is the number of mapped ARG reference sequences belonging to the ARG type or subtype.

Statistical analyses

The Tukey HSD test was conducted in R to analyze the statistical differences between different animal manure and composts. The t-test or wilcox.test in R were used for analyze the statistical differences between manure and compost. The correlation matrix was constructed with ARG and antibiotics by calculating all pairwise Spearman's rank correlations. A correlation between any two items was considered statistically significant if the Spearman's correlation coefficient (ρ) was > 0.5 and the P value was < 0.05 . The resulting correlation matrixes were translated into an association network using Gephi 0.9.2^[25]. The protest in vegan R package was conducted to Procrustes between antibiotics residue and ARGs in all samples.

Metagenomic data analysis

First, Kraken2 was used to obtain the taxonomic profile of each sample, and its relative abundance was estimated using Bracken based on filtered metagenomic data^[26, 27]. Principal coordinate analysis (PCoA) was used to estimate the community dissimilarities based on the bacterial community structure at the genus level using the vegan package in R.

Metagenomes were assembled de novo for each sample using MEGAHIT^[28], thereafter, all contigs were combined into a single file. All contigs over 500 bp in the combined file were retained and merged at 95% identity using CD-HIT-EST^[29]. An 8.11 M consensus contigs longer than 500 bp were obtained including 2.60 M contigs longer than 1000 bp. The N50 of the consensus contigs was 1723 bp and the L50 was 1150,602 bp. For identifying antibiotic resistance contigs (ARCs), the DIAMOND (v0.9.22.123) was used to search the protein sequences of the SARG.2.2 database using BLASTx algorithm with an e-value cutoff of $1e^{-5}$. The best hit results were filtered with an identity cutoff of 80% and a subject (ARGs) coverage

cutoff of 70%. For the multiple ARGs annotated, genes were predicted on the ARGs using Prokka^[30]. ARG-like open reading frames (ORFs) on ARCs were determined using BLASTP against the SARG.2.2 database with a minimum similarity of 80% over 70% of the query coverage^[31].

The occurrence of ARCs in chromosomes or plasmids was determined using BLAST + BLASTn against PLSDB^[32] and against a representative set of 1789 closed bacterial chromosomes from the PATRIC database^[33, 34]. Hit ARCs with >80% identity and >70% query coverage were retained.

For MGEs, one ORF was considered as a transposon or recombinase gene if one of the following keywords was in its best BLAST hit description: transposase, transposon, or recombinase^[35]. Integrons in ARCs were identified using the IntegronFinder^[36].

The virulence genes were identified using Abricate (<https://github.com/tseemann/abricate>) by comparing ARCs against VFdb^[37]. Only hits with sequence identity >70% and query coverage >90% were retained^[34].

The abundance of consensus contigs in each sample was determined using Salmon^[38]. The relative abundance of contigs was presented with Contigs per kilobase per million mapped reads (cpm) according to the following equation:

$$\text{cpm} = 1,000,000 * \frac{\text{reads mapped to contig/contig length}}{\text{sum}(\text{reads mapped to contig/contig length})}$$

The relative abundance of ARCs was extracted from the whole quantification table of all consensus contigs. The ARCs were first taxonomically classified using Kraken2 in metaWARP^[39], and the taxon description header of both plasmids and chromosomes was used for revision.

The relative abundance of ARG hosts at phylum or genus level were calculated by summing the cpm of ARCs under the specific level of taxonomy. PCoA based on the ARG hosts at the genus level was used to estimate the community dissimilarities using the vegan package in R. Bipartite network analysis to uncover the unique and shared ARGs subtypes in plasmid and chromosome. Bipartite network was also used to analyze ARGs subtype with integron, transposase and recombinase. The visualization of the bipartite network and ARG-host network were both conducted using Gephi software.

Results

ARG profile in manure and core resistance genes

In manure samples from 29 farms, 20 types of ARGs comprising 626 subtypes were detected; 545 in broiler manure, 427 in swine manure, 419 in layer manure, 288 in dairy cow manure, and 232 in cattle manure. Moreover, 86.58% of the ARGs belonged to the top five subtypes, i.e., beta-lactam, multidrug,

macrolide, aminoglycoside, and tetracycline resistance genes; these subtypes accounted for 74.29% of the total ARG abundance (Fig. 1a). The sulfonamide resistance genes (SRGs) comprised three subtypes; however, they accounted for 6.68% of the total ARG abundance. In contrast, the beta-lactam resistance genes accounted for 55.11% of the total ARGs, but they only accounted for 3.47% of the total ARG abundance. The average number (311) and relative abundance (1.59 per 16S rRNA gene) of total ARGs in broiler manure revealed the highest values in manure samples.

The PCoA-based relative abundances of the ARG subtypes revealed that the profile of ARGs in broiler manure was more similar to that in swine manure (Fig. 1b). As the dairy cow manure had the lowest antibiotic residue in all samples (Dataset 2), the profiles of ARGs in dairy cow manure differed from those in manure from other animals.

Manure core-resistome The 201 subtypes of ARGs, mainly comprising multidrug (46), M-L-S (30), tetracycline (28), beta-lactam (26), and aminoglycoside (25) resistance genes, were shared among different animal manures (Fig. 2). Moreover, these subtypes accounted for majority of the total ARG relative abundance in manure samples; layer manure (98–99%), broiler (89–99%), swine (86–99%), and beef and dairy cow (> 99%). The chloramphenicol exporter genes exhibited the highest average relative abundances (0.046 copies per 16S rRNA gene) in all samples, whereas the highest relative abundance (0.25 copies per 16S rRNA gene) of ARG type was found in sulfonamide resistance genes *su1* in a broiler manure sample (Fig. 2b). Furthermore, genes such as *tetW* (0.0017–0.085 copies per 16S rRNA gene) and *tetM* (0.001–0.082 copies per 16S rRNA gene) that encode the ribosomal protection proteins and the aminoglycoside inactivation gene (*aadE*, 0.0022–0.093 copies per 16S rRNA gene) in all manure samples (Fig.2 group A) were the most widely presented ARG subtypes. Aminoglycoside nucleotidyltransferase gene (*aadA*), streptomycin phosphotransferase genes (*AAC(6')-Ie/APH(2')-Ia*, *aph(3_-I*, *aph(3)-I*, and *aph(6)-I*), and the M-L-S resistance genes (*ermB* and *InuA*) were dominant in swine and chicken manure samples (group B). The broiler samples exhibited markedly higher relative abundances of multidrug efflux pump genes, such as *mdfA*, *mdtD*, *mdtE*, *mdtF*, *mdtG*, *mdtK*, *mdtL*, *mdtM*, *mdtN*, *mdtP*, and *mexX* (group C) than the other samples. In contrast, some tetracycline resistance genes (*tetO*, *tetQ*, *tet32*, and *tet40*) and beta-lactamase gene (*cfxA2*) were specific to beef cow manure (group D).

Changes in the manure core-resistome after composting

Composting reduced the relative abundance of the main ARGs in the manure core-resistome; the total relative abundance (0.938 copies per 16S rRNA gene) of ARGs in manure was significantly higher ($p=0.0016$) than that (0.405 copies per 16S rRNA gene) in the composts (Table S2). The relative abundances of 10/19 ARG types decreased significantly in composts than in the manure samples; however, six other ARG types increased significantly after composting, but they all exhibited low relative abundances (Table S2). Moreover, composting did not have any significant effect on three types (multidrug, sulfonamide, and trimethoprim resistance genes). The relative abundances of most main ARG types, such as aminoglycoside, chloramphenicol, M-L-S, tetracycline, and beta-lactam resistance genes,

were significantly decreased after composting (Fig. 2a), whereas that of vancomycin resistance genes increased significantly.

Furthermore, the relative abundances of 123 ARG subtypes significantly decreased after composting, whereas those of other 32 ARGs significantly increased among the 201 manure core-resistome ARGs. The relative abundances of the top20 ARGs subtypes decreased significantly after composting, except for two SRGs: *sul1* and *sul2* and one aminoglycoside *aadA* (Fig. 2b). Among all the ARG subtypes with average relative abundance >0.001, only eight ARGs, including *vanR*, *mutigrug_ABC_transporter*, and *fosB*, increased significantly in the compost samples (Fig. S3). PCoA revealed that the composition of ARGs in most composts differed from that in the manure samples (Fig. 3c).

Changes in relationship between antibiotic residue and ARGs after composting

Nineteen antibiotics were detected in the manure samples, and they were classified into five categories, i.e., tetracyclines (TCs), quinolones (QNs), macrolides (MLs), sulfonamides (SAs), amphenicols (AMs), and two others (lincomycin and ceftiofur) (Dataset 2). The average total concentrations of antibiotic residues were 5321 µg/kg (layer manure), 96,937 µg/kg (broiler manure), 50,248 µg/kg (swine manure), 858 µg/kg (Beef cow manure) and 295 µg/kg (Dairy cow manure). TCs were the dominant antibiotics in swine and broiler manure, and they contributed 96.28% and 93.77% of the total concentration of antibiotics, respectively. Moreover, TCs and QNs accounted for 55.99% and 24.3% of the total concentrations in the layer manure. QNs were the dominant antibiotics in beef cow manure, contributing 89.77% of the total concentration of antibiotics.

We evaluated the correlations between ARGs and antibiotics in the manure samples. Significant positive correlations ($p < 0.05$, $r > 0.5$) were observed between the 12 antibiotics and six ARGs (Fig. 4a). The network revealed that most antibiotic residues were related to multiple ARG types. The dominant antibiotics (TCs) in manure exhibited a significant positive correlation with all six ARG types, indicating that numerous antibiotic residues will directly lead to the development of antibiotic resistance in animal manure. Some low concentrations of antibiotics were also correlated with several ARGs. CEFT is a therapeutic antibiotic, which has a low concentration in manure (0–561.42 µg/kg); however, it revealed a significant correlation with 37 ARG subtypes. Procrustes analysis confirmed that manure resistomes were significantly correlated with antibiotic residues (Fig. 4b); however, they were not correlated with antibiotics after composting (Fig. 4c). These results indicate that composting can reduce the antibiotic residues and decrease the correlation between antibiotics and resistance genes in composts.

Changes in bacterial hosts of ARGs

In total, 1202 nonredundant ARCs were assembled from all metagenomes, including 224 ARG subtypes. Proteobacteria and Firmicutes were the dominant hosts, accounting for 50.08% and 37.77% of the ARCs, respectively, whereas Bacteroidetes and Actinobacteria accounted for 6.49% and 5.24% of the ARCs, respectively (Table S3). The host bacteria include pathogens, such as *Escherichia*, *Enterococcus*, *Klebsiella*, *Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Clostridium*, *Citrobacter*, *Streptococcus*, and

Enterobacter (Fig. 5). *Enterococcus* and *Escherichia* were the dominant hosts, accounting for 10.4% and 10.23% of the total 1202 ARCs, respectively, whereas *Enterococcus* and *Escherichia* carried 17.4% and 33.04% of all detected 224 ARG subtypes, respectively (Table S4). *Escherichia* carried the most diverse ARGs and it exhibited 74 ARG subtypes classified into 15 types of ARGs (Table S4). Although the relative abundance (cpm) of all the host ARCs in manure decreased after composting, the hosts of the top list ARGs in manure and compost differed (Fig. S4). In the composts, *Pseudomonas*, not *Enterococcus*, was the most abundant host; *Microbacterium* and *Riemerella* were included in the top ten list in the compost, but *Lactobacillus* and *Streptococcus* were not.

Moreover, based on the cpm of ARCs at genus level, the major hosts of the main ARG types, such as aminoglycoside, chloramphenicol, M-L-S, tetracycline, multidrug, and sulfonamide resistance genes were investigated (Fig. S5). The major hosts of aminoglycoside resistance genes were *Enterococcus*, *Streptococcus*, and *Enterobacter*, whereas those of TRGs were *Pseudomonas*, *Lactobacillus*, and *Streptococcus*. Compared to other samples, the cpm of hosts for the six major types of ARGs in broiler and swine manure were significantly higher. *Escherichia* was the dominant host for multidrug-resistance genes. The richness of ARG host in compost at genus level was significant lower than that in manure samples (Fig S6), while the Shannon and Simpson indices of ARG hosts did not exhibit significant difference between manure and composts.

To observe the changes in the composition of the ARG hosts in manure after composting, the percentage of hosts was calculated based on the relative abundance (cpm) (Fig. S7b). According to the results of taxon classification on ARCs, we found that the hosts could change associated with different ARG types at the phylum level. The aminoglycoside resistance genes (AMRGs) were mainly distributed in Firmicutes and Proteobacteria; however, the two phyla carrying AMRGs were affected by composting; the average percentage of Firmicutes and Proteobacteria with AMRGs decreased from 14.58% to 5.48% and increased from 14.73% to 18.55% after composting, respectively. TRGs were widely distributed in all four phyla. Additionally, the percentage of the other three phylum hosts with TRGs decreased after composting; however, those of Actinobacteria carrying TRGs did not. Firmicutes mainly carried the M-L-S resistance genes, whereas Proteobacteria carried the sulfonamide, multidrug, and trimethoprim resistance genes. Notably, the percentage of Proteobacteria carrying SRGs increased from 6.41% in manure to 17.28% in compost.

Based on the percentage of ARCs at the genus level, the dominant ARGs in animal manure and compost are illustrated in Fig. S8. The percentage of *Enterococcus* carrying AMRGs accounted for more than 6% in layer, broiler, swine, and dairy cow manure; however, after composting, this percentage decreased to 2.27%. *Staphylococcus* was the dominant ARG host in beef cow and dairy cow manure. it carried AMRGs accounting for 10.18% of all hosts in dairy cow manure and M-L-S resistance genes accounting for 16.32% and 13.47% in beef cow manure and dairy cow manure, respectively. *Pseudomonas* carried sulfonamide, accounting for 9.40% of the total ARG hosts in compost. Moreover, *Streptococcus* with TRGs was dominant, accounting for 9.79% of all hosts in swine manure. Furthermore, the dominant TRG hosts were *Lactobacillus* in layer (6.49%) and broiler (6.11%) manure, thereby exhibiting high

concentration of tetracycline residue, similar to that in swine manure. The results indicated that veterinary antibiotics could cause antibiotic resistance in animal guts; however, the ARGs in different animal guts may have their own dominant hosts.

Distribution of ARGs in mobile gene elements

In general, 531 (44.17%) of 1202 ARGs carried MEGs, including plasmids (31.49%), transposase (14.53%), integron (7.52%), and recombinase (2.73%); the detailed co-occurrence patterns of ARGs and MGEs are summarized in Table S6. Moreover, 525 ARGs were located on plasmids or chromosomes, carrying 181 ARG subtypes classified into 20 ARG types. In our samples, ARGs were more prevalent in the plasmids than in chromosomes, and all the top 20 high relative abundance ARG subtypes (Fig. 3) were carried by plasmids except for *bacA* (Fig. 6). Of the 181 ARGs, 44.2% were shared between plasmids and chromosomes. The 37.02% ARG subtypes comprising aminoglycoside, beta-lactam, tetracycline, and M-L-S resistance genes were only found in the plasmids, whereas 18.78% subtypes including multidrug, tetracycline, and M-L-S resistance genes were only found on the chromosomes. The TRGs encoded in chromosomes mainly comprised ribosomal protection protein genes and tetracycline efflux proteins. The circos diagram revealed that the plasmids had a higher relative abundance (cpm) of ARGs (Fig. 7a) than the chromosomes. AMRGs and TRGs were dominant in both plasmids and chromosomes. The chromosomes possessed more multidrug resistance genes, whereas plasmids carried more chloramphenicol and M-L-S resistance genes.

The heatmap reveals the changes in the relative abundance (cpm) of plasmid-associated or chromosome-associated ARGs in manure and compost (Fig. S9). The plasmid-only ARGs had higher relative abundance in composts and multiple ARGs carrying contigs than the chromosome-only ARGs (Fig. S9A). The dominant ARG subtypes in composts were mostly carried by the plasmids and chromosome-shared ARGs (Fig. S9B), including TRGs (*tetL* and *tetM*), SRGs (*sul1* and *sul2*), chloramphenicol resistance genes (chloramphenicol exporter), and AMRGs (*aadA*, *aad(9)*, *aadE*, *aph(3)*, and *aph(6)*). The chromosome-carrying multidrug resistance genes (Fig. S9C), which had a high relative abundance in meat animal (broiler and swine) manure, could be efficiently reduced by composting.

Of the 107 ARG subtypes, 85 subtypes were linked with transposase, 34 with integron, and 22 with recombinase (Fig. S10). *Sul1* and *aadA*, which had high relative abundance in all manure samples and did not significantly reduce after composting, were linked with all three MGEs in our samples. This result indicated that compared with “single” ARGs, the ARGs connected with MGEs could exhibit higher chance of survival during composting. The heatmaps based on relative abundance (cpm) revealed that integron-carried ARGs exhibited more multiple ARGs (30/100) than the transposase-carried (21/182) and recombinase-carried ARGs (4/37); furthermore, multiple ARGs revealed the highest relative abundance of integron-carried ARGs in composts (Figs. S14–S16). The AMRGs were dominant in all three MGEs carrying ARGs (Fig. 7b). The distribution of ARGs connected to the three MGEs was distinct. Transposase was associated with the highest number and relative abundance of ARGs in all three MGEs (Fig. S10 and S11b); moreover, the MLS-resistance genes, multidrug-resistance genes, and TRGs were more easily

found in transposase-related ARCs. The chloramphenicol resistance genes and trimethoprim resistance genes were two dominant ARGs in the integron-related ARCs. Among all the recombinase-carrying ARGs, SRGs had the highest relative abundance. The taxon circos diagram depicts that the potential hosts of transposase-related ARCs were included with all four phyla, and 98.9% of the integron-carrying ARCs belonged to Proteobacteria (Fig. S11b).

Discussion

Animal manure is an important source of ARGs in the environment, and 70% of the antibiotics are used in animals worldwide^[40]. Direct use of raw manure on soil could significantly increase the abundance of ARGs^[41]. Composting is one of the manure management practices, and its high temperature can effectively reduce the antibiotic residue in feces^[42], however, as ARGs could be duplicated or spread through HGTs, composting did not reveal consistent results with respect to reducing ARGs^[43]. During composting, the maintenance of ARGs is associated with HGT and shifts in related bacterial communities^[19]. Thus, it is important to analyze the core-resistome in animal manure and the changes in ARG-related hosts and MGEs after composting. In the present study, we investigated different livestock manure and their composts through metagenomic analysis to assess the composition of the core resistome and its potential hosts in manure, and to evaluate the potential hosts and mobility in the finished composts.

In general, 626 ARG subtypes were detected in our samples via metagenomic analysis, compared to the 109 ARG subtypes in different animal manure detected via high-throughput qPCR in a previous study^[3]. We found 201 ARG subtypes (core resistome) shared among all manure samples, and each exhibited special ARG subtypes (Fig. 2). The manure core-resistome, mainly including the genes resistant to aminoglycoside, tetracycline, M-L-S, chloramphenicol, sulfonamide, and multidrug were found in this study as well as in other niches, such as urban sewage, human feces, and farm soil^[44–46]. Aminoglycoside and tetracycline resistance genes are abundant in human feces and animal manure^[45]. Compared to urban sewage and human feces, animal manure had more multidrug resistance genes, accounting for 20.3% of the total ARGs, and a lower relative abundance of beta-lactam resistance genes (3.47%)^[44]. The intensification of livestock operations caused a rapid increase in ARGs in chickens and pigs. In low- and middle-income countries, from 2000 to 2018, the percentage of multidrug-resistant bacteria increased from 0.15 to 0.41 in chicken and from 0.13 to 0.34 in pigs^[47]. This indicates that the profiles of ARGs are often associated with selective pressure and spatiotemporal conditions^[48]. PCoA-based relative abundance of the total bacteria and ARG hosts revealed that the profile of total bacteria in layer and broiler manure are similar; however, the profile of ARG hosts in broiler manure was closer to that in swine manure (Fig. S12). Owing to the use of antimicrobial growth promoters in livestock, the long-term selection pressure of antibiotics has resulted in the emergence of multidrug-resistance bacteria; moreover, multidrug-resistance genes in animal guts are more abundant than in human guts, sewage, and soils.

In contrast to the fact that the concentration of antibiotics in swine manure is usually higher than that in chicken manure^[2, 3, 22], the average concentration of total antibiotics in broiler manure was higher than that in swine manure in our samples (Dataset 2); however, the concentration of total antibiotics in the layer manure was lower than that in the swine manure. In general, because the researchers did not distinguish between layer and broiler, the concentration of total antibiotics in chicken manure was lower than that in swine manure. Consistent with the antibiotics, the relative abundance of total ARGs in broiler manure was higher than that in swine or layer manure, which was similar to the previous research^[3, 49, 50], and particularly, the relative abundance of multidrug resistance genes in broiler manure was significantly higher than that in other manure. Thus, although layers and broilers are both chickens, because broilers are normally exposed to larger amounts of antibiotics for growth promotion than layers^[51], they possessed more antibiotic residues and ARGs in manure than the layers. Furthermore, the average relative abundance of total ARGs in beef cow manure was higher than that in dairy cow manure. This indicates that although the animal species are similar, compared with the functional animals (egg or milk production), the meat animals were associated with more serious ARG pollution in their manure.

The bacterial community can be controlled to limit the spread of ARGs by determining the ARG host information. Moreover, ARGs could be directly linked to specific bacterial hosts based on the assembled contigs or binning bacterial genomes via metagenomic analysis than that by correlation analysis^[52]. In contrast to the results of metatranscriptomic analysis during pig manure composting^[53], Ascomycota (fungus) related contigs were not found in our samples. The correlation analysis majorly revealed that the main host phylum in composts was Actinobacteria^[16, 54, 55], however, in our assembled contigs, the dominant ARG host phylum was Proteobacteria (55.24%) in composts. Consistent with other composts^[53], Actinobacteria also exhibited dominant relative abundance (51.71%) in our composts, however, the relative abundance of Actinobacteria hosts only accounted for 16.91% of all ARG hosts. This phenomenon indicated that most of the increased Actinobacteria did not carry ARGs after composting.

The antimicrobial growth promoters could exert pressure for selecting multidrug-resistant *E. coli* in the broiler guts^[56]; therefore, in our samples, *E. coli* carried the most diverse ARGs and it was the dominant host of multidrug resistance genes in broiler and swine manure (Table S4 and Fig. S8). Moreover, it was related to diverse emerging human ARGs in various agricultural systems, such as chicken farms^[45, 57, 58], swine farms^[59, 60], dairy cow manure^[61], and farming soils^[62]. Another important ARG host in manure is *Enterococcus*, which is a major host of AMRGs and M-L-S resistance genes in broiler and swine manure. Consistent with Xiong's research, *Enterococcus* was linked with vancomycin resistance genes (*vanR*, *vanS*)^[58]. After composting, the relative abundance of ARG-carrying *Enterococcus* decreased from 832 to 16 cpm; however, this strain exhibited a high abundance in the packaging area of a commercial composting plant; therefore, a suitable method should be followed for protecting workers from these potential ARGs pathogen^[63]. Although *Lactobacillus* are commonly used as probiotics and are considered to be beneficial in the gut microbiota of animals, the tetracycline resistance and M-L-S resistance gene hosts were dominant in our broiler manure samples, which is consistent with the findings

of studies on antibiotic susceptibility in *Lactobacillus* strains from poultry^[64, 65]. Consistent with Keenum's results, *Staphylococcus* and *Streptococcus* were the major hosts for M-L-S and aminoglycoside resistance genes in beef and dairy cow manure^[12]. Noteworthy, the relative abundance of *vanR* significantly increased after composting (Fig. S3), because *vanR* was linked with several Actinobacteria, including *Streptomyces*, *Glutamicibacter*, u_Micrococcales, and some heat-tolerant Firmicutes genus such as *Bacillus* (Fig. 5).

The constant or increased abundance of sulfonamide or trimethoprim resistance genes are often found during composting with various materials, such as swine manure^[6–8, 66], chicken manure^[67, 68], cow manure^[12, 17], sewage sludge^[16], and food waste^[19]. Previous correlation analysis showed that the increase in SRGs was positively correlated with strains that were privilege during the cooling-maturation stages^[7, 19], and the SRGs were also often found to be correlated with MGEs, such as *intI1* or *ISR1*^[7, 17, 67]. These results indicate that ARGs may be maintained in other strains due to HGTs. In our samples, based on the metagenomic analysis, we found two evidences that could be the reason for SRGs to escape composting removal. 1. SRGs had diverse hosts (a wide host range); *sul1* was carried by five genera belonging to Proteobacteria, Firmicutes, and Actinobacteria (Fig. 5), including *Escherichia*, *Pseudomonas*, *Micrococcales*, *Pseudonocardia*, *Citrobacter*, and *Corynebacterium*. The quantification of contigs showed that the *Pseudonocardia*-like contig (XT2k141_483370) increased in the composts. *Pseudonocardia thermophiles* are thermophilic actinomycetes that can produce spores to resist high temperatures^[69]. The major host analysis showed that *Pseudomonas*, instead of *Enterobacter* and *Escherichia*, became the dominant SRG hosts after composting. The metatranscriptomic sequence and culture-dependent analysis both found that *Pseudomonas* was stable throughout the whole process of composting^[13, 53, 70]. These results indicate that *sul1* could likely be maintained in thermotolerant bacteria during composting. 2. SRG-carrying contigs were harbored on all types of MGEs, including plasmids, integrons, transposons, and recombinase (Figs. 6 and S10). The cpm of the dominant *sul1*-carrying contig (J5k141_381193) was located on the *Pseudomonas aeruginosa* plasmid and harbored *intI1* and IS6100 (Fig. S16). The shared MGEs ensure that *sul1* has the potential to spread in multiple hosts by HGTs. Another ARG subtype, which may be maintained through HGTs in composts is the aminoglycoside nucleotidyltransferase gene (*aadA*). In our samples, *aadA* was also linked with all MGEs (Fig. S10), and its hosts were distributed across 20 genera belonging to the phyla Proteobacteria and Actinobacteria (Table S5).

In total, 22 assembled ARCs were identified as VG-carrying contigs, which could be the potential pathogenic antibiotic-resistant bacteria (PARB)^[71], and most of them belonged to Enterobacteriaceae, including *Citrobacter*, *Escherichia*, *Enterobacter*, and *Klebsiella*, except for *Enterococcus* (Fig. S17). *Enterobacter* were also found as ARG- and VG-carried pathogens in long-term manure-amended greenhouse soils^[72]. The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are responsible for the majority of nosocomial infections globally and can acquire antibiotic resistance^[73]. Although we found ARCs with virulence genes in *Enterococcus faecium*, *Klebsiella pneumoniae*, and

Enterobacter, it is worth noting that we did not find any thermotolerant human pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* relative ARCs with virulence genes in our composts. Most ARGs carried by PARB were multidrug resistance genes, including *mdfA*, *mdtG*, *acrA*, and *acrB*. *emrD*, *emrE*, *emrB-qacA*, and *TolC* belong to the antibiotic efflux mechanism^[74]. In contrast to Korin's results that virulence genes were associated with MGEs during food waste feeding and composting on poultry farms, most PARB contigs did not harbor MGEs, except for two contigs (*Enterococcus* and *Klebsiella*) linked with transposase genes and two contigs located on plasmids, so the PARB in our manure samples may not have wide HGT possibility. Most PARB were distributed in the high antibiotic residue manure, such as broilers, layers, and swine. Compared to that (31.13 cpm) in the manure samples, the average relative abundance (0.06 cpm) of PARB in composts decreased significantly. Thus, composting has a good effect on controlling PARB in manure.

HGT is an essential mechanism for the dissemination of ARGs in the environment via MGEs, such as plasmids, integrons, and transposons^[11, 75, 76]. The current correlation analysis revealed that MGEs are important factors for eliminating ARGs during composting^[15, 19, 21, 77]; however, there is a lack of direct links and evidence for the co-occurrence of ARGs and MGEs. In our samples, the top-20 high relative abundance ARGs were all harbored on the plasmids (except for *bacA*) and the plasmid-associated ARCs revealed higher subtype numbers and relative abundance (cpm) in composts than the chromosome-associated ARGs did (Figs. 6 and S9). The plasmids may help the resistant bacteria share their ARGs with other strains, and the mobile and conjugative plasmids are particularly important in spreading ARGs in bacterial communities^[11, 78]. Plasmids carrying shared ARGs were found in different genera in the same hospital^[79]. Furthermore, the plasmid-associated ARGs had a higher chance of being shared with the heat-tolerant hosts by HGTs than the chromosome-only ARGs; thus, they exhibited higher relative abundance in composts than the chromosome-only ARGs (Fig. S9). We found that most (82%) of 181 identified ARGs were related to plasmids in our samples (Fig. 6). This result differs from the results of the enrichment and metagenomic analyses of cow manure, which indicated that most ARGs were harbored on the chromosome^[80], but revealed similar results as the metagenomic analysis in sewage waters^[34] and wastewater treatment plants^[81]. The multidrug resistance genes, particularly the major facilitator superfamily (MFS) antibiotic efflux pump genes, were mostly harbored on the chromosome, which is consistent with the metagenomic results of extrachromosomal MGEs in the human gut, where cell wall/membrane/envelope biogenesis functions were more frequently harbored by chromosomes than by plasmids^[82]. The chloramphenicol exporter is a multidrug resistance gene; however, it is only found in plasmids in our samples. Although the relative abundance of chloramphenicol exporter decreased significantly after composting, it still revealed a high average relative abundance in all ARG subtypes. Nevertheless, the MGEs, not the only reason for ARGs to escape from composting, the relative abundance of a vancomycin-resistance gene (*vanR*), which was not linked to any MGEs and only harbored on chromosome, was significantly increased after composting, because it had 15 hosts, including thermophilic Actinobacteria (*Thermobispora*) and heat-tolerant Firmicutes (*Bacillus*) (Table S4).

Based on the taxon classification of the MGE-related ARCs, we found that the dominant hosts of transposase-, integron-, and recombinase-related ARCs belonged to different phyla (Fig. S11b). Most integron-related ARCs belonged to Proteobacteria, which indicates that integrons mainly contributed to shared ARGs in Proteobacteria^[36]. Transposase-related ARCs included all four phyla; Jiang et al. reported that Proteobacteria pathogens could acquire the ARGs from Actinobacteria with the transposon and conjugative plasmids^[83]. Metagenomic analysis revealed that a majority (63.2%) of the recently transferred ARGs between plasmids and bacterial chromosomes can be attributed to the insertion sequences (ISs)^[11]. Transposases are coded by ISs, and in our samples, 14.53% of the ARCs carried transposase (ISs) with 18 ARG types, whereas 7.52% carried integron elements with 13 ARG types. Thus, transposases (ISs) could play a pivotal role in mediating the transfer of ARGs between different phyla in animal manure and composting.

Compared to the read-based analysis, the assembly-based analysis may have lost some low-richness ARGs. In our samples, all the assembled 1202 ARCs (>500 bp) covered 224 ARG subtypes, which only accounted for 35.8% of all 626 ARG subtypes based on the read analysis; however, the assembled ARCs accounted for 72.6% of the ARG subtypes of all the manure core-resistome, which includes 201 ARG subtypes. Based on metagenomic analysis, we can directly obtain the mobility and host-related information of ARGs at the DNA level; however, further host tracking technologies (such as fluorescence-activated cell sorting or single cell fusion PCR) are required to verify the host changes of ARGs and HGTs during composting.

Conclusion

In this study, profiling of ARGs in manure and compost from 29 concentrated animal feedlots and 13 composting plants in Northern China was conducted using high-throughput sequencing technology and metagenomic analysis. The multidrug, M-L-S, tetracycline, beta-lactam, aminoglycoside, and sulfonamide resistance genes were the main ARGs in the manure core resistome. The relative abundance of ARGs among different animal manures was in the following order: broiler > swine > layer chicken > beef cow > dairy cow. After composting, the relative abundances of most ARG types in the manure core-resistome were significantly decreased, except for multidrug, sulfonamide, and trimethoprim resistance genes. Composting can reduce the correlation between antibiotics and resistance genes in compost. Several human pathogenic genera were observed in the hosts of ARGs in manure, and the gene linkage analysis of ARGs and VF revealed that the relative abundances of potential pathogenic bacteria carrying ARGs in composts were significantly decreased. At the phylum level, the ARGs hosts could change in association with different ARG types. In manure, *Enterococcus* and *Escherichia* were the dominant ARG hosts; however, *Pseudomonas* was abundant in the composts. Surviving ARGs, such as *sul1*, *sul2*, and *aadA*, usually have a broad range of bacterial hosts, including some heat-tolerant genera, and are also linked with transposase, integron, and combinase. Our research indicated that composting can mitigate core resistome in animal manure by changing the bacterial hosts; however, some ARGs can escape composting with the survivor heat-tolerant hosts or transfer to these hosts. Thus, it is necessary to

improve the traditional composting process to mitigate HGTs and curb the growth of heat-tolerant hosts in order to efficiently control the animal source ARGs.

List Of Abbreviations

ARGs antibiotic resistance genes

TRGs tetracycline resistance genes

M-L-S macrolide-lincosamide-streptogramin

PARB pathogenic antibiotic-resistant bacteria

ARB antibiotic-resistant bacteria

HGT horizontal gene transfer

MGEs mobile genetic elements

ARCs antibiotic resistance contigs

ORFs open reading frames

cpm Contigs per kilobase per million mapped reads

PCoA Principal coordinate analysis

SRGs sulfonamide resistance genes

AMRGs aminoglycoside resistance genes

Declarations

Acknowledgements

We thank Professor Tong Zhang and his students Xiaotao Jiang and Xiaole Yin at the University of Hong Kong for providing help on using ARGs-OAP pipeline. We thank Dr. Gregorio Iraola at the Institut Pasteur Montevideo for his advices to build the representative chromosomes database.

Availability of data and materials

All data generated during this study is available at the Genome Sequence Archive (GSA) repository under BioProject number CRA005191.

Author's contributions

XMW and GML conceived the idea. DH and ZWW collected the samples. TLQ and MG generated the data. TLQ, LHH and YJG analyzed and interpreted the data. TLQ, GML and XMW wrote the manuscript. All authors read and approved the final manuscript.

Funding

The authors are grateful for the financial support offered by the Special Program for Creative Ability Foundation of BAAFS (grant number KJCX20210424), and Beijing Agriculture Innovation Consortium (BAIC04-2019).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., and Zhao, J.L., Comprehensive evaluation of antibiotics emission and fate in the river basins of china: source analysis, multimedia modeling, and linkage to bacterial resistance. *Environ Sci Technol*, 2015. 49(11): 6772-82.
2. Zhao, L., Dong, Y.H., and Wang, H., Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of The Total Environment*, 2010. 408(5): 1069-1075.
3. Qian, X., Gu, J., Sun, W., Wang, X.-J., Su, J.-Q., and Stedfeld, R., Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. *Journal of Hazardous Materials*, 2018. 344: 716-722.
4. Zalewska, M., Błażejewska, A., Czapko, A., and Popowska, M., Antibiotics and Antibiotic Resistance Genes in Animal Manure – Consequences of Its Application in Agriculture. *Frontiers in Microbiology*, 2021. 12(640).
5. Xiaotang, N.X.J., Organic fertilizer resources and utilization in China. *J. Plant Nutr. Fertil.*, 2017. 23: 8.
6. Wang, J., Ben, W., Zhang, Y., Yang, M., and Qiang, Z., Effects of thermophilic composting on oxytetracycline, sulfamethazine, and their corresponding resistance genes in swine manure. *Environmental Science: Processes & Impacts*, 2015. 17(9): 1654-1660.
7. Cao, R., Wang, J., Ben, W., and Qiang, Z., The profile of antibiotic resistance genes in pig manure composting shaped by composting stage: Mesophilic-thermophilic and cooling-maturation stages. *Chemosphere*, 2020. 250: 126181.

8. Cheng, D., Feng, Y., Liu, Y., Xue, J., and Li, Z., Dynamics of oxytetracycline, sulfamerazine, and ciprofloxacin and related antibiotic resistance genes during swine manure composting. *Journal of Environmental Management*, 2019. 230: 102-109.
9. Durrant, M.G., Li, M.M., Siranosian, B.A., Montgomery, S.B., and Bhatt, A.S., A Bioinformatic Analysis of Integrative Mobile Genetic Elements Highlights Their Role in Bacterial Adaptation. *Cell Host Microbe*, 2020. 27(1): 140-153.e9.
10. Wolters, B., Widyasari-Mehta, A., Kreuzig, R., and Smalla, K., Contaminations of organic fertilizers with antibiotic residues, resistance genes, and mobile genetic elements mirroring antibiotic use in livestock? *Applied Microbiology and Biotechnology*, 2016. 100(21): 9343-9353.
11. Che, Y., Yang, Y., Xu, X., Břinda, K., Polz, M.F., Hanage, W.P., et al., Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proceedings of the National Academy of Sciences*, 2021. 118(6): e2008731118.
12. Keenum, I., Williams, R.K., Ray, P., Garner, E.D., Knowlton, K.F., and Pruden, A., Combined effects of composting and antibiotic administration on cattle manure–borne antibiotic resistance genes. *Microbiome*, 2021. 9(1): 81.
13. Guo, W., Huang, C., Xi, B., Tang, Z., Tan, W., Li, W., et al., The maturity period is the main stage of antibiotic resistance genes reduction in aerobic composting process of swine manure in sub-scale farms. *Bioresource Technology*, 2021. 319: 124139.
14. Ezugworie, F.N., Igbokwe, V.C., and Onwosi, C.O., Proliferation of antibiotic-resistant microorganisms and associated genes during composting: An overview of the potential impacts on public health, management and future. *Science of The Total Environment*, 2021. 784: 147191.
15. Tang, Z., Xi, B., Huang, C., Tan, W., Li, W., Zhao, X., et al., Mobile genetic elements in potential host microorganisms are the key hindrance for the removal of antibiotic resistance genes in industrial-scale composting with municipal solid waste. *Bioresource Technology*, 2020. 301: 122723.
16. Su, J.-Q., Wei, B., Ou-Yang, W.-Y., Huang, F.-Y., Zhao, Y., Xu, H.-J., et al., Antibiotic Resistome and Its Association with Bacterial Communities during Sewage Sludge Composting. *Environmental Science & Technology*, 2015. 49(12): 7356-7363.
17. Qian, X., Sun, W., Gu, J., Wang, X.-J., Sun, J.-J., Yin, Y.-N., et al., Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. *Journal of Hazardous Materials*, 2016. 315: 61-69.
18. Sardar, M.F., Zhu, C., Geng, B., Huang, Y., Abbasi, B., Zhang, Z., et al., Enhanced control of sulfonamide resistance genes and host bacteria during thermophilic aerobic composting of cow manure. *Environ Pollut*, 2021. 275: 116587.
19. Liao, H., Friman, V.-P., Geisen, S., Zhao, Q., Cui, P., Lu, X., et al., 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*, 2019. 660: 841-850.
20. Zhang, R., Gu, J., Wang, X., Li, Y., Zhang, K., Yin, Y., et al., Contributions of the microbial community and environmental variables to antibiotic resistance genes during co-composting with swine manure

- and cotton stalks. *Journal of Hazardous Materials*, 2018. 358: 82-91.
21. Zhu, L., Zhao, Y., Yang, K., Chen, J., Zhou, H., Chen, X., et al., Host bacterial community of MGEs determines the risk of horizontal gene transfer during composting of different animal manures. *Environmental Pollution*, 2019. 250: 166-174.
 22. Li, C., Li, Y., Li, X., Ma, X., Ru, S., Qiu, T., et al., Veterinary antibiotics and estrogen hormones in manures from concentrated animal feedlots and their potential ecological risks. *Environmental Research*, 2020: 110463.
 23. Yin, X., Jiang, X.-T., Chai, B., Li, L., Yang, Y., Cole, J.R., et al., ARGs-OAP v2. 0 with an expanded SARG database and Hidden Markov Models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes. *Bioinformatics*, 2018. 1: 8.
 24. Yang, Y., Jiang, X., Chai, B., Ma, L., Li, B., Zhang, A., et al., ARGs-OAP: online analysis pipeline for antibiotic resistance genes detection from metagenomic data using an integrated structured ARG-database. *Bioinformatics*, 2016. 32(15): 2346-2351.
 25. Bastian, M., Heymann, S., and Jacomy, M. Gephi: An Open Source Software for Exploring and Manipulating Networks. in *Proceedings of the Third International Conference on Weblogs and Social Media, ICWSM 2009, San Jose, California, USA, May 17-20, 2009*. 2009.
 26. Wood, D.E. and Salzberg, S.L., Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biology*, 2014. 15(3): R46.
 27. Lu, J., Breitwieser, F.P., Thielen, P., and Salzberg, S.L., Bracken: Estimating species abundance in metagenomics data. *bioRxiv*, 2016: 051813.
 28. Li, D., Liu, C.M., Luo, R., Sadakane, K., and Lam, T.W., MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, 2015. 31(10): 1674-6.
 29. Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W., CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics*, 2012. 28(23): 3150-2.
 30. Seemann, T., Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, 2014. 30(14): 2068-9.
 31. Ma, L., Xia, Y., Li, B., Yang, Y., Li, L.-G., Tiedje, J.M., et al., Metagenomic Assembly Reveals Hosts of Antibiotic Resistance Genes and the Shared Resistome in Pig, Chicken, and Human Feces. *Environmental science & technology*, 2016. 50(1): 420-427.
 32. Galata, V., Fehlmann, T., Backes, C., and Keller, A., PLSDB: a resource of complete bacterial plasmids. *Nucleic Acids Research*, 2018. 47(D1): D195-D202.
 33. Wattam, A.R., Abraham, D., Dalay, O., Disz, T.L., Driscoll, T., Gabbard, J.L., et al., PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Research*, 2013. 42(D1): D581-D591.
 34. Fresia, P., Antelo, V., Salazar, C., Giménez, M., D'Alessandro, B., Afshinnikoo, E., et al., Urban metagenomics uncover antibiotic resistance reservoirs in coastal beach and sewage waters. *Microbiome*, 2019. 7(1): 35.

35. Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., et al., Bacterial phylogeny structures soil resistomes across habitats. *Nature*, 2014. 509: 612.
36. Cury, J., Jové, T., Touchon, M., Néron, B., and Rocha, E.P., Identification and analysis of integrons and cassette arrays in bacterial genomes. *Nucleic acids research*, 2016. 44(10): 4539-4550.
37. Chen, L., Xiong, Z., Sun, L., Yang, J., and Jin, Q., VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors. *Nucleic Acids Research*, 2011. 40(D1): D641-D645.
38. Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C., Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods*, 2017. 14(4): 417-419.
39. Uritskiy, G.V., DiRuggiero, J., and Taylor, J., MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome*, 2018. 6(1): 158.
40. UNEP, *Frontiers 2017 Emerging Issues of Environmental Concern*. 2017, United Nations Environment Programme: Nairobi.
41. Gou, M., Hu, H.-W., Zhang, Y.-J., Wang, J.-T., Hayden, H., Tang, Y.-Q., et al., Aerobic composting reduces antibiotic resistance genes in cattle manure and the resistome dissemination in agricultural soils. *Science of The Total Environment*, 2018. 612: 1300-1310.
42. Mitchell, S.M., Ullman, J.L., Bary, A., Cogger, C.G., Teel, A.L., and Watts, R.J., Antibiotic Degradation During Thermophilic Composting. *Water, Air, & Soil Pollution*, 2015. 226(2): 13.
43. Youngquist, C.P., Mitchell, S.M., and Cogger, C.G., Fate of Antibiotics and Antibiotic Resistance during Digestion and Composting: A Review. *Journal of environmental quality*, 2016. 45(2): 537-545.
44. Su, J.-Q., An, X.-L., Li, B., Chen, Q.-L., Gillings, M.R., Chen, H., et al., Metagenomics of urban sewage identifies an extensively shared antibiotic resistome in China. *Microbiome*, 2017. 5(1): 84.
45. Zeng, J., Pan, Y., Yang, J., Hou, M., Zeng, Z., and Xiong, W., Metagenomic insights into the distribution of antibiotic resistome between the gut-associated environments and the pristine environments. *Environment International*, 2019. 126: 346-354.
46. Peng, S., Feng, Y., Wang, Y., Guo, X., Chu, H., and Lin, X., Prevalence of antibiotic resistance genes in soils after continually applied with different manure for 30 years. *Journal of Hazardous Materials*, 2017. 340: 16-25.
47. Van Boeckel, T.P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N.G., et al., Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science*, 2019. 365(6459): eaaw1944.
48. Li, L.-G., Huang, Q., Yin, X., and Zhang, T., Source tracking of antibiotic resistance genes in the environment — Challenges, progress, and prospects. *Water Research*, 2020. 185: 116127.
49. Cheng, W., Chen, H., Su, C., and Yan, S., Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China. *Environment International*, 2013. 61(0): 1-7.
50. Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J.M., et al., Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The Isme Journal*,

2015. 9: 2490.

51. Yoshimura, H., Ishimaru, M., Endoh, Y.S., and Kojima, A., Antimicrobial susceptibilities of enterococci isolated from faeces of broiler and layer chickens. *Letters in Applied Microbiology*, 2000. 31(6): 427-432.
52. Rice, E.W., Wang, P., Smith, A.L., and Stadler, L.B., Determining Hosts of Antibiotic Resistance Genes: A Review of Methodological Advances. *Environmental Science & Technology Letters*, 2020. 7(5): 282-291.
53. Wang, C., Dong, D., Strong, P.J., Zhu, W., Ma, Z., Qin, Y., et al., Microbial phylogeny determines transcriptional response of resistome to dynamic composting processes. *Microbiome*, 2017. 5(1): 103.
54. Zhang, M., He, L.-Y., Liu, Y.-S., Zhao, J.-L., Zhang, J.-N., Chen, J., et al., Variation of antibiotic resistome during commercial livestock manure composting. *Environment International*, 2020. 136: 105458.
55. Duan, M., Changes in antibiotic resistance genes and mobile genetic elements during cattle manure composting after inoculation with *Bacillus subtilis*. *Bioresource technology*, 2019: 2019.
56. Diarra, M.S., Silversides, F.G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., et al., Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. *Appl Environ Microbiol*, 2007. 73(20): 6566-76.
57. Baron, S., Jouy, E., Larvor, E., Eono, F., Bougeard, S., and Kempf, I., Impact of Third-Generation-Cephalosporin Administration in Hatcheries on Fecal *Escherichia coli* Antimicrobial Resistance in Broilers and Layers. *Antimicrobial Agents and Chemotherapy*, 2014. 58(9): 5428-5434.
58. Xiong, W., Wang, Y., Sun, Y., Ma, L., Zeng, Q., Jiang, X., et al., Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes. *Microbiome*, 2018. 6(1): 34.
59. Park, J.-H., Kim, Y.-J., Binn, K., and Seo, K.-H., Spread of multidrug-resistant *Escherichia coli* harboring integron via swine farm waste water treatment plant. *Ecotoxicology and Environmental Safety*, 2018. 149: 36-42.
60. von Salviati, C., Laube, H., Guerra, B., Roesler, U., and Friese, A., Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. *Veterinary Microbiology*, 2015. 175(1): 77-84.
61. Rehman, M., Yin, X., Lepp, D., Laing, C., Ziebell, K., Talbot, G., et al., Genomic Analysis of Third Generation Cephalosporin Resistant *Escherichia coli* from Dairy Cow Manure. *Veterinary Sciences*, 2017. 4(4): 57.
62. Zheng, B., Huang, C., Xu, H., Guo, L., Zhang, J., Wang, X., et al., Occurrence and Genomic Characterization of ESBL-Producing, MCR-1-Harboring *Escherichia coli* in Farming Soil. *Frontiers in Microbiology*, 2017. 8(2510).

63. Gao, M., Yang, Z., Guo, Y., Chen, M., Qiu, T., Sun, X., et al., The size distribution of airborne bacteria and human pathogenic bacteria in a commercial composting plant. *Frontiers of Environmental Science & Engineering*, 2020. 15(3): 39.
64. Dec, M., Urban-Chmiel, R., Stępień-Pyśniak, D., and Wernicki, A., Assessment of antibiotic susceptibility in *Lactobacillus* isolates from chickens. *Gut Pathogens*, 2017. 9(1): 54.
65. van Hoek, A.H.A.M., Mayrhofer, S., Domig, K.J., Flórez, A.B., Ammor, M.S., Mayo, B., et al., Mosaic Tetracycline Resistance Genes and Their Flanking Regions in *Bifidobacterium thermophilum* and *Lactobacillus johnsonii*. *Antimicrobial Agents and Chemotherapy*, 2008. 52(1): 248-252.
66. Chang, J., Jiang, T., Zhao, M., Yang, J., Wen, Z., Yang, F., et al., Variation pattern of antibiotic resistance genes and microbial community succession during swine manure composting under different aeration strategies. *Journal of Chemical Technology & Biotechnology*, 2020. 95(2): 466-473.
67. Qiu, T., Wu, D., Zhang, L., Zou, D., Sun, Y., Gao, M., et al., A comparison of antibiotics, antibiotic resistance genes, and bacterial community in broiler and layer manure following composting. *Environ Sci Pollut Res Int*, 2021. 28(12): 14707-14719.
68. Li, H., Duan, M., Gu, J., Zhang, Y., Qian, X., Ma, J., et al., Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting. *Ecotoxicology and environmental safety*, 2017. 140: 1-6.
69. Fergus, C.L., Resistance of spores of some thermophilic actinomycetes to high temperature. *Mycopathologia et mycologia applicata*, 1967. 32(3): 205-208.
70. Kaszab, E., Szoboszlay, S., Dobolyi, C., Háhn, J., Pék, N., and Kriszt, B., Antibiotic resistance profiles and virulence markers of *Pseudomonas aeruginosa* strains isolated from composts. *Bioresour Technol*, 2011. 102(2): 1543-8.
71. Liang, J., Mao, G., Yin, X., Ma, L., Liu, L., Bai, Y., et al., Identification and quantification of bacterial genomes carrying antibiotic resistance genes and virulence factor genes for aquatic microbiological risk assessment. *Water Research*, 2020. 168: 115160.
72. Zhang, H., Zhang, Q., Song, J., Zhang, Z., Chen, S., Long, Z., et al., Tracking resistomes, virulence genes, and bacterial pathogens in long-term manure-amended greenhouse soils. *Journal of Hazardous Materials*, 2020. 396: 122618.
73. Santajit, S. and Indrawattana, N., Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed Research International*, 2016. 2016: 2475067.
74. Alcock, B.P., Raphenya, A.R., Lau, T.T.Y., Tsang, K.K., Bouchard, M., Edalatmand, A., et al., CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res*, 2020. 48(D1): D517-d525.
75. Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A., and Dantas, G., The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens. *Science*, 2012. 337(6098): 1107-1111.
76. McInnes, R.S., McCallum, G.E., Lamberte, L.E., and van Schaik, W., Horizontal transfer of antibiotic resistance genes in the human gut microbiome. *Current Opinion in Microbiology*, 2020. 53: 35-43.

77. Cui, P., Bai, Y., Li, X., Peng, Z., Chen, D., Wu, Z., et al., Enhanced removal of antibiotic resistance genes and mobile genetic elements during sewage sludge composting covered with a semi-permeable membrane. *Journal of Hazardous Materials*, 2020. 396: 122738.

78. Tamminen, M., Virta, M., Fani, R., and Fondi, M., Large-Scale Analysis of Plasmid Relationships through Gene-Sharing Networks. *Molecular Biology and Evolution*, 2011. 29(4): 1225-1240.

79. Evans, D.R., Griffith, M.P., Sundermann, A.J., Shutt, K.A., and Tyne, D.V., Systematic detection of horizontal gene transfer across genera among multidrug-resistant bacteria in a single hospital. 2020.

80. Qian, X., Gunturu, S., Sun, W., Cole, J.R., Norby, B., Gu, J., et al., Long-read sequencing revealed cooccurrence, host range, and potential mobility of antibiotic resistome in cow feces. *Proceedings of the National Academy of Sciences*, 2021. 118(25): e2024464118.

81. Che, Y., Xia, Y., Liu, L., Li, A.D., Yang, Y., and Zhang, T., Mobile antibiotic resistome in wastewater treatment plants revealed by Nanopore metagenomic sequencing. 2019. 7(1): 44.

82. Suzuki, Y., Nishijima, S., Furuta, Y., Yoshimura, J., Suda, W., Oshima, K., et al., Long-read metagenomic exploration of extrachromosomal mobile genetic elements in the human gut. *Microbiome*, 2019. 7(1): 119.

83. Jiang, X., Ellabaan, M.M.H., Charusanti, P., Munck, C., Blin, K., Tong, Y., et al., Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. *Nature Communications*, 2017. 8(1): 15784.

Figures

Figure 1

Antibiotic resistance gene (ARG) profiles in manures from different animals. a. The average detected numbers and relative abundance (normalized by 16S rDNA) of ARGs subtype. b. Principal co-ordinates analysis (PCoA) depicting the overall pattern of ARGs.

Figure 2

Venn diagram and heatmap of ARG subtypes in manure core-resistome. a. Venn diagram depicts the numbers of shared ARGs subtype. b. The composition of core-resistome (201 ARGs subtypes); c. The heatmap of core-resistome ARGs (113 out of 201 ARGs subtypes) which average relative abundance was up than 0.001 copies per 16S rRNA gene. (A) dominant ARGs subtypes in all samples; (B) dominant ARGs subtypes in manure samples except cow manure; (C) dominant ARGs subtypes in broiler manure; (D) dominant ARGs subtypes in beef cow manure.

Figure 3

Manure core-resistome shifted after composting. a. Box-plot of ARG types between manure and compost. b. The average relative abundances of top 20 ARGs subtypes in manure and compost. The asterisk “*” and “**” denote the significance level of 0.05 and 0.01, respectively. c. Principal co-ordinates analysis (PCoA) depicts the shift pattern of ARG subtypes between manure and compost.

Figure 4

ARGs and antibiotic correlation analysis. a. Network analysis based on the co-occurrence of ARGs and antibiotics ($r > 0.5$, $p < 0.05$). b. Procrustes analysis indicating correlations between subtype ARG and antibiotics in manure. c. Procrustes analysis indicating correlations between subtype ARG and antibiotics in compost.

Figure 5

Network analysis based on the physical linkage pattern of ARG and hosts on the antibiotic resistance contigs (ARCs). The nodes (ARG or genus), which had at least two edges were retained. The nodes were colored according to ARG types or hosts phylum.

Figure 7

Circos representation showing the distribution of ARGs. a. the distribution of ARGs harbored on plasmids and chromosome. b. the distribution of ARGs linked with transposase, integron and recombinase. Figure produced using the circlize package in R (4.0.2).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Dataset1Antibioticresidue.xlsx](#)
- [Dataset2Bacterialcommunity.xlsx](#)
- [FigureS13integron.heatmap.tif](#)
- [FigureS14transposase.heatmap01.tif](#)
- [FigureS9Variationsofcontigscarriedcoreresistome.tif](#)
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