

Antibiotic Resistance Genes in Different Animal Manure and their Derived Organic Fertilizer

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Research

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Abstract

Background: The prevalence of antibiotic resistance genes (ARGs) in animal manure poses threats to the environmental safety. Organic fertilizers fermented by livestock and poultry manure are directly applied to farmland, which would cause the potential outbreak of bacterial resistance in agricultural environment. This study investigated the composition of ARGs in different animal manure and their derived organic fertilizers.

Results: Results showed that the abundance of several ARGs, such as sul 2, Tet B-01, Tet G-01 and Tet M-01 in organic fertilizer samples was 12%~96% lower than in animal manure. However, there was an increasing of Tet K and erm C abundance from animal manure to the organic fertilizers. No correlation between ARGs and environmental factors such as pH, TN, antibiotics was observed by Redundancy analysis (RDA). Procrustes analysis revealed the significant correlation between bacterial community structures and the ARGs abundance ($r=0.799$, $p<0.01$). Non-metric multidimensional scaling (NMDS) analysis suggested that microorganisms in organic fertilizer may be derived from animal manure. Additional, pathogenic bacteria (especially *Actinomadura*) would proliferate rather than decrease from manure to organic fertilizer.

Conclusion: Overall, this research suggests that the composting treatment of manure could effectively reduce these ARGs and pathogens, even cause partial ARGs and pathogens proliferation. It also shows that the microorganism might significantly influence ARGs profiles in composting.

Background

Antibiotics are a class of medicine extensively used for promoting growth and controlling diseases in the livestock and poultry cultivation farms [1]. However, up to 30~90% of the administered antibiotics are excreted through urine and feces, leading to the accumulation of residual antibiotics in the livestock environment [2–4]. Typical concentrations of antibiotics in livestock manure were generally in the range of 1~10 mg/kg and could be high up to 200 mg/kg [5]. The high residual antibiotics give the selective pressure to the native microbial communities after the application of the manure in soil [6–8], and the bacteria could acquire antibiotic resistance genes (ARGs) via horizontal gene transfer or spontaneous mutation, thereby causing a massive proliferation of bacteria resistance [9–10].

ARGs could enter the environment via discharge of animal manure, leading to the contamination of soil, water and crops [11]. Most of the recent research was focused on the quantitative detection of ARGs in manure and their surrounding environment [12]. In the soils around a pig farm, a total of 15 tetracycline resistance genes were detected [13]. The abundance of resistance genes was reported to be highly enriched in animal manures (121,000-fold in Beijing farm, 39,000-fold in Jiaying farm and 57,000-fold in Pu Tian farm) [14]. The continuous increase or high persistence of ARGs in livestock environment may pose potential threats to human health and the ecological environment [15, 16].

Composting is widely used to treat and re-utilize animal manures [17–19]. It has been reported that the fertilizer production by composting could not completely remove antibiotics, and even could cause the proliferation of resistance bacteria [20, 21]. Although there was a decrease on individual ARGs after composting, risks of ARGs propagation were still existing [22, 23]. To our best knowledge, no systematic evaluation is available on the comparison of ARGs composition both in the livestock manure and their derived organic fertilizers. The relationship of ARGs in different animal manures and their derived organic fertilizers is insufficient as well. This study is devoted to address this concern, with the following objectives: 1) investigating the ARGs composition in different animal manure and fermented organic fertilizer, 2) exploring the effect of bacterial communities on ARGs change, and 3) demonstrating the variations of antibiotic-resistant pathogens in manure and organic fertilizers.

Materials And Methods

Sample collection and analysis

The animal manure and organic fertilizers were obtained from different organic fertilizer enterprises in China. Three kinds of animal manure, including chicken manure (JF1, JF2, JF3, JF4, JF5), cow manure (NF1, NF2, NF3, NF4, NF5), and sheep manure (YF1, YF2, YF3), were collected from different organic fertilizer factories. These fertilizers were prepared by aerobic composting of the raw materials. Three types of organic fertilizers were derived from a single manure (JF1→JM1, JF3→JM3, JF4→JM4), (YF1→YM1, NF2→NM2). The following organic compound fertilizers were derived from a mixture of various animal manure (NF4+YF1+JF5→NYJM1, NF1+YF2→NYM1, YF2+JF3→YJM1, YF3+JF2→YJM2). At each enterprises site, three samples were collected from manure/fertilizer and combined to prepare one sample. The mixed sample was divided into two portions: one subsample was used for analyzing physicochemical properties, and the second subsample was freeze-dried and stored at -80°C for extracting DNA and analyzing microbial community structures. Antibiotic concentrations were determined according to the approach by Li et al. [24].

DNA extraction

DNA Extraction was carried out from 0.2 g animal manure and prepared organic fertilizer samples using Power Soil DNA isolation kit (Omega Bio-Tek, Norcross, GA, USA). Specific extraction steps followed the kit manual. The extracted DNA was quantified with a Nano Drop spectrophotometer (Thermo Scientific), and stored at -80°C till PCR analysis.

HT-qPCR

ARGs were quantitatively analyzed with the Smart Chip Real-Time PCR System (WaferGen Biosystems Inc, Fremont, CA, USA), equipped with a high throughput quantitative reaction platform (Qiyin Biological Technology Co. Ltd, Shanghai, China). A total of 51 resistance genes primers and a pair of bacterial

universal 16S rRNA gene primers were selected according to the literature [25], including five sulfonamide resistance genes (SRGs), sixteen tetracycline resistance genes (TRGs), two fluoroquinolone resistance genes (FRGs), five aminoglycoside resistance genes, eleven β -Lactamase resistance genes, seven macrolide lincomamide streptomycin B (MLSB) resistance genes, two vancomycin resistance genes, three other/efflux resistance genes, and 16S rRNA gene. The experiment condition and the data processing procedure were the same as the published literature [26].

16S high-throughput sequencing

The 16S V4 region was analyzed by the Illumina Hiseq 2500 platform to study bacterial community composition. The chimeras were filtered using USEARCH to cluster the remaining sequences into 97% similarity operational classification units (OTUs). Each OTU sequence was selected by default, and the ribosomal database item classifier was classified with the latest version of the green gene database with a confidence threshold of 80%.

Data analysis

The weighted and unweighted unifracs tests were performed using Mothur to determine the statistical significance of structural similarity among communities across sampling locations. Ordination plotting with nonmetric multidimensional scaling (NMDS) was employed to obtain the visualization of beta-diversity information. Principal component analysis (PCA, Bray–Curtis distance based) was used with STAMP software. R3.1.2 with Vegan 2.0 was used for Mantel test and Procrustes analysis determining the correlation between ARGs data and bacterial communities.

Results And Discussion

Diversity and abundance of ARGs in the animal manure and organic fertilizer

The abundance and diversity of 51 target ARGs in the animal manure and their prepared organic fertilizer were obtained through the HT-qPCR. The result showed that the ARGs diversity in the organic fertilizer samples was relatively lower than those in the animal manure ($p < 0.05$) (Fig. 1A). The absolute abundance of ARGs in the animal manure and organic fertilizer samples was detected in the range from 2.1×10^5 to 7.8×10^5 copies/g and from 1.6×10^5 to 7.3×10^5 copies/g, respectively, which was similar to the findings by Zhang et al. [27]. Previous study also found that the composting could reduce the abundance and diversity of ARGs in animal manure, likely due to the inactivation of some microorganisms by high temperature [28].

The types of ARGs among the animal manure from different breeds were significantly different, which was due to the differences in fecal properties and microbial composition (Fig. 1B) [29]. The detection

rates of ARG subtypes from a single kind of animal manure to manufactured organic fertilizer samples (JF1→JM1 and NF2→NM2) were reduced by 42% and 34.6%, respectively. However, the detection rates of ARG subtypes from multiple animal manure (JF, YF and NF) to compound organic fertilizer samples (NF1+YF2→NYM1, YF2+JF3→YJM1) increased by 28% and 24%, respectively (Fig. 1A), which was in line with previous observation [30]. These results indicated that composting process could reduce the release of partial ARG subtypes from single manure to organic fertilizer. It is noteworthy that the composite organic fertilizer fermented by multiple manure would increase the diversity of ARGs, which should draw much attention on the importance of manure management concerning the fate of ARGs [31].

The level of ARGs in chicken manure was detected with the highest absolute abundance ($2.8 \times 10^5 \sim 7.8 \times 10^5$ copies/g), which was 2~4 times higher than those in cow manure ($2.1 \times 10^5 \sim 3.3 \times 10^5$ copies/g) and sheep manure ($2.2 \times 10^5 \sim 5.1 \times 10^5$ copies/g) (Fig. 1C). Previous studies have shown that the difference of ARG levels between poultry manure and cattle manure may be related to the difference of antibiotic use patterns and fecal microorganisms among different species of livestock [32, 33]. The removal efficiencies of ARGs in different animal manure and/or different treatment processes were also reported [14, 34]. Most TRGs (*tetB*-01, *tetG*-01 and *tetM*-01) decreased by 12%~96% after composting (Fig. 1C), which was confirmed by previous findings [35, 36]. The abundance of *tetX* was higher than other ARGs in all samples, likely due to the broad range of potential hosts of *tetX* [31]. Some TRGs decreased, while others persisted or significantly increased (such as *tetK*, *tetX*) after thermophilic composting [37]. Compared with in response raw of animal manure, the abundance of individual ARGs (*tetK*) increased 2~216 times in their organic fertilizer. Ezzariai et al. reported that the abundance of *tetX* in the swine manure reduced exponentially under the anaerobic condition [34]. The reason for this opposite trend was being explored, since the resistance mechanism of *tetX* was still unknown [14, 38].

The SRGs were predominant in terms of absolute abundance in all samples. From JF1 to JM1, the level of *sul1* and *sul2* genes decreased from 1.1×10^5 copies/g to 10^0 copies/g and from 1.2×10^5 copies/g to 1.58×10^1 copies/g, respectively. The results were consistent with the previous study that the level of *sul2* dramatically decreased during composting [33]. The abundance of MLSB resistance genes (*ermA*, *ermB*, *ermF* and *ermX*) significantly decreased by 10%~98% from manure to organic fertilizer (Fig. 1C), lower than TRGs and SRGs. It was related to the low use of macrolides during feeding. The response of ARGs varied in composting due to the ecological complex microbial processes. It suggested that the composting process may cause individual ARGs proliferation.

Fresh manure composting is a feasible approach to decrease the level of certain ARGs before its application to farmland. However, most ARGs would still retain or even proliferate after composting, which may be caused by the difference in external conditions, such as raw materials, environmental factors or microbial community, etc. [39]. The recent study also showed that the variations in microbial communities may have an impact on ARGs in composting [28].

Microbial communities in the animal manure and organic fertilizer

Figure 2 showed that no significant correlation was observed between the ARGs and the environmental factors in this study, and we focused on the microbial community structures in animal manure and organic fertilizer samples. The results were shown in Fig. 3a, b. It demonstrated that the composition and abundance of microbial communities varied greatly in different sample classifications. The compound organic fertilizer NYJM had the highest microbial diversity (Fig. 3a), and the samples of different animal manure and organic fertilizer were clustered into different categories (Fig. 3b). From the sorted map of the microbial community in Fig. 3b, the fecal samples were partially overlapped with the aggregated organic fertilizer samples (such as NF and NM), suggesting that the bacterial community structures in organic fertilizer were similar with those in manure from the corresponding composting source.

Compared with their derived fertilizer, the abundance of microorganisms decreased in cow manure, conversely, an increasing of the abundance was observed in chicken manure, which might be related to composting conditions and manure nutritional structure (Fig. 3a) [40, 41]. Especially, the abundance of microorganisms varied dramatically among JM samples, which might originate from the condition of livestock farm as well as individual difference in animals (such as age, species) [42]. As shown in Fig. 3b, the composition of the microbial community was significantly different between animal manure and their derived organic fertilizer (e.g. YF→YM, and JF→JM). Interestingly, the Bray–Curtis distance between YF and YM and between JF and JM was closer than YF or JF samples to other organic fertilizer samples. The overlap between NF and NM indicated that the structures of microbial communities between NF and NM were more alike than the others. The intersections between YJM and JM, JF, YF samples suggested there was a correlation of microbial composition between animal manure and their derived organic fertilizer.

Relationship between the bacterial communities and ARGs

Result from Mantel test indicated that ARGs abundance was substantially correlated with bacterial community structures based on Bray–Curtis distance. Procrustes analysis showed the clustering based on the abundance of ARGs and 16S OTUs exhibited a goodness-of-fit test (sum of squares $M^2 = 0.476$, $r = 0.799$, $p < 0.01$, 999 permutations), indicating that the bacterial community structures exerted significant influence on ARGs abundance. This finding was consistent with the previous study that the changed structure of microbial communities was the major factor driving the variation of ARGs profile in the animal manure and organic fertilizer [43, 44]. PCA result also confirmed that ARGs within different animal manure and organic fertilizer showed a similar distribution pattern of the bacteria communities (Fig. 4). It was concluded that the ARGs variation from animal manure to organic fertilizer was strongly correlated with the microbial community. The shift of the bacterial communities played key roles in the direct change of the ARGs patterns [31]. It is noted that the presence of pathogens in the microbial community

would not only decrease the productivity of livestock and poultry breeding, but also increase the risk of ARGs spread from organic fertilizer to agricultural environment.

The fate of pathogens from animal manure to organic fertilizer

The relative abundance variability of the top 20 genera was selected to evaluate the risk of pathogens from manure to organic fertilizer (Fig. 5). It is worth noting that most of the top 20 genera were pathogens. *Corynebacterium*-1, *Virgibacillus*, *Streptomyces* and *Actinomadura* were the major genera in animal manure and organic fertilizer samples. After composting, the relative abundance of pathogens was altered, but the dominant genus was still *Corynebacterium*-1 which was usually a heterogeneous population composed of human and animal pathogens that could cause disease in livestock [45]. The relative abundance of *Virgibacillus* was significantly reduced from animal manure to prepared organic fertilizer (especially in JF-JM). Besides, the abundance of *Actinomadura* in organic fertilizer was 2~300 times higher than those in animal manure. These two genera were the carriers of antibiotic resistance genes (such as *ermX*, *tetPA*), and their abundances were found to have a similar reduction trend as ARGs after composting. The *Actinomadura* and *Virgibacillus* genera belong to the phylum Actinobacteria, which are opportunistic pathogens that would cause disease in animals and plants [46]. Lv et al. identified that Actinobacteria were prominent in the thermophilic stage and the groups probably could carry and disseminate ARGs [47]. The abundance of *Pseudomonas* (phylum Proteobacteria) which was found as opportunistic pathogen carrying most ARGs with multiple resistance was significantly increased from animal manure to organic fertilizer [27, 48]. The network analysis was conducted to determine the correlations between ARGs and the top bacterial genera, which may judge the potential host bacteria for ARGs. As illustrated in Fig. 6, there were significant correlations between ARGs and the potential host bacteria in the animal manure and their derived organic fertilizer samples ($p < 0.05$ and $R > 0.60$). In addition to the host bacteria obtained above, *Atopostipes* from Firmicutes were found to contain potential host bacteria for *ermA*, *ermB*, *ermX*, *tetK*, *tetM*-01.

Pathogens could not be completely removed from animal manure composting to organic fertilizer, but would cause partial proliferation. Significant correlations between pathogens and ARGs occurred, suggesting that the pathogens might become the important hosts of ARGs [46]. Therefore, once antibiotic-resistant pathogens are ubiquitous in organic fertilizer, they are bound to pose a threat to the health of farm land soil and crops.

Conclusions

This study investigated the composition of ARGs and bacteria community structure between different animal manure and their derived organic fertilizer. The diversity and abundance of most ARGs significantly decreased from animal manure to organic fertilizer. Microorganism in the prepared organic fertilizer may mainly inherit from the animal manure. Results also showed that the reduction of

pathogens would significantly affect their prepared organic fertilizer, even cause partial pathogens proliferation. It is urgent and necessary to explore the optimal fermentation processes to improve the removal efficiency of ARGs and pathogen in animal manure.

Abbreviations

ARGs: antibiotic resistance genes; NMDS: Non-metric multidimensional scaling; SRGs: sulfonamide resistance genes; TRGs: tetracycline resistance genes; FRGs: fluoroquinolone resistance genes; MLSB: macrolide lincomamide streptomycin B; OTUs: operational classification units; PCA: Principal component analysis; RDA: Redundancy analysis.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YX, HL and RS involved in the experiments and manuscript writing, JL, BL, FY and XZ were responsible for the data analysis. JX contributed to the study design and manuscript correction. All authors read and

approved the final manuscript.

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References

1. Done HY, Venkatesan AK, Halden RU. (2016) Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? *AAPS J* 17, 513-524.
2. Chiesa L, Nobile M, Arioli F, Britti D, Trutic N, Pavlovic R, Panseri S (2015) Determination of veterinary antibiotics in bovine urine by liquid chromatography-tandem mass spectrometry. *Food Chem* 185, 7-15.
3. Guo T, Lou CL, Zhai WW, Tang XJ, Hashmi MZ, Murtaza R, Li Y, Liu XM, Xu JM (2018) Increased occurrence of heavy metals, antibiotics and resistance genes in surface soil after long-term application of manure. *Sci Total Environ* 635, 995-1003.
4. Heuer H, Schmitt H, Smalla K (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol* 14, 236-243.
5. Liu YW, Feng Y, Cheng DM, Xue JM, Wakelin S, Li ZJ (2018) Dynamics of bacterial composition and the fate of antibiotic resistance genes and mobile genetic elements during the co-composting with gentamicin fermentation residue and lovastatin fermentation residue. *Bioresource Technol* 261, 249-256.
6. Cleary DW, Bishop AH, Zhang LH, Topp E, Wellington EMH, Gaze WH (2016) Long-term antibiotic exposure in soil is associated with changes in microbial community structure and prevalence of class 1 integrons. *FEMS Microbiol Ecol* 92, fiw159.
7. Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014) Fate and effects of veterinary antibiotics in soil. *Trends Microbiol* 22, 536-545.
8. Nordenholt RM, Goyne KW, Kremer RJ, Lin CH, Lerch RN, Veum KS (2016) Veterinary antibiotic effects on atrazine degradation and soil microorganisms. *J Environ Qual* 45, 565-575.
9. Peng S, Wang YM, Zhou BB, Lin XG (2015) Long-term application of fresh and composted manure increase tetracycline resistance in the arable soil of eastern China. *Sci Total Environ* 506-507, 279-286.
10. Cheng DM, Feng YY, Liu YW, Xue JM, Li JZ (2019) Dynamics of oxytetracycline, sulfamerazine, and ciprofloxacin and related antibiotic resistance genes during swine manure composting. *J Environ Manage* 274, 102-109.
11. He LY, Ying GG, Liu YS, Su HC, Chen J, Liu SS, Zhao JL (2016) Discharge of swine wastes risks water quality and food safety: antibiotics and antibiotic resistance genes from swine sources to the

- receiving environments. *Environ Int* 92-93, 210-219.
12. Sui QW, Meng XS, Wang R, Zhang JY, Yu DW, Chen MX, Wang YW, Wei YS (1028) Effects of endogenous inhibitors on the evolution of antibiotic resistance genes during high solid anaerobic digestion of swine manure. *Bioresource Technol* 270, 328-336.
 13. Wu N, Qiao M, Zhang B, Cheng WD, Zhu YG (2010) Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in china. *Environ Technol* 44, 6933-6939.
 14. Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. *P Natl Acad Sci USA* 110, 3435-3440.
 15. Negreanu Y, Pasternak Z, Jurkevitch E, Cytryn E (2012) Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils. *Envir Sci Technol* 46, 4800-4808.
 16. Wang BY, Guo XH, Wang C, Ma JY, Niu FF, Zhang HF, Yang B, Liang WW, Han F, Jiang YQ (2015) Identification and characterization of plant-specific NAC gene family in canola (*Brassica napus*L.) reveal novel members involved in cell death. *Plant Mol Biol* 87, 395-411.
 17. Wang J, Ben WW, Zhang Y, Yang M, Qiang ZM (2015) Effects of thermophilic composting on oxytetracycline, sulfamethazine, and their corresponding resistance genes in swine manure. *Environ Sci Proc Imp* 17, 1654-1660.
 18. Wu XF, Wei YS, Zheng JX, Zhao X, Zhong WK (2011) The behavior of tetracyclines and their degradation products during swine manure composting. *Bioresource Technol* 102, 5924-5931.
 19. Zhao JC, Sun XN, Awasthi MK, Wang Q, Ren XN, Li RH, Chen HY, Wang MJ, Liu T, Zhang ZQ (2018) Performance evaluation of gaseous emissions and Zn speciation during Zn-rich antibiotic manufacturing wastes and pig manure composting. *Bioresource Technol* 267, 688-695.
 20. Ray P, Chen CQ, Knowlton KF, Pruden A, Xia K (2017) Fate and effect of antibiotics in beef and dairy manure during static and turned composting. *J Environ Qual* 46, 45-54.
 21. Chen ZQ, Wang Y, Qin X (2018) Effects of chlortetracycline on the fate of multi-antibiotic resistance genes and the microbial community during swine manure composting. *Environ Pollut* 237, 977-987
 22. Wallace JS, Garner E, Pruden A, Aga DS (2018) Occurrence and transformation of veterinary antibiotics and antibiotic resistance genes in dairy manure treated by advanced anaerobic digestion and conventional treatment methods. *Environ Pollut* 236, 764-772.
 23. Selvam A, Xu DL, Zhao ZY, Wong JWC (2012) Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresource Technol* 126, 383-390.
 24. Li LX, Guo CS, Fan SS, Lv JP, Zhang Y, Xu Y, Xu J (2018) Dynamic transport of antibiotics and antibiotic resistance genes under different treatment processes in a typical pharmaceutical wastewater treatment plant. *Environ Sci Pollut* 25:30191–30198
 25. Luo Y, Mao DQ, Rysz M, Zhou DX, Zhang HJ, Xu L, Alvarez PJJ (2010) Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environ Sci Technol* 44, 7220-7225.

26. Su JQ, Wei B, Ou-Yang WY, Huang FY, Zhao Y, Xu HJ, Zhu YG (2015) Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ Sci Technol* 49, 7356-7363.
27. Zhang RR, Gu J, Wang XJ, Li Y, Zhang KY, Yin YN, Zhang X (2018) Contributions of the microbial community and environmental variables to antibiotic resistance genes during co-composting with swine manure and cotton stalks. *J Hazard Mater* 358, 82-91.
28. Sun W, Gu J, Wang XJ, Qian X, Peng HL (2019) Solid-state anaerobic digestion facilitates the removal of antibiotic resistance genes and mobile genetic elements from cattle manure. *Bioresour Technol* 274, 287-295.
29. Ji XL, Shen QH, Liu F, Ma J, Xu G, Wang YL, Wu MH (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai, China. *J Hazard Mater* 235-236, 178-185.
30. Wolters B, Ding GC, Kreuzig R, Smalla K (2016) Full-scale mesophilic biogas plants using manure as C-source: bacterial community shifts along the process cause changes in the abundance of resistance genes and mobile genetic elements. *FEMS Microbiol Ecol* 92, fiv163.
31. Ezzariai A, Hafidi M, Khadra A, Aemig Q, El Felsa L, Barret M, Merlina G, Patureau D, Pinelli E (2018) Human and veterinary antibiotics during composting of sludge or manure: Global perspectives on persistence, degradation, and resistance genes. *J Hazard Mater* 359, 465-481.
32. Cheng GY, Hao HH, Xie SY, Wang X, Dai MH, Huang LL, Yuan ZH (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Front Microbiol* 5, 217.
33. Han XM, Hu HW, Chen QL, Yang LY, Li HL, Zhu YG, Li XZ, Ma YB (2018) Antibiotic resistance genes and associated bacterial communities in agricultural soils amended with different sources of animal manures. *Soil Biol Biochem* 126, 91-102.
34. Ling Z, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Sci Total Environ* 408, 1069-1075.
35. Yu ZT, Michel FC, Hansen G, Wittum T, Morrison M (2005) Development and application of real-time PCR assays for quantification of genes encoding tetracycline resistance. *Appl Environ Microbiol* 71, 6926-6933.
36. Storteboom HN, Kim SC, Doesken KC, Carlson KH, Davis JG, Pruden A (2007) Response of antibiotics and resistance genes to high-intensity and low-intensity manure management. *J Environ Qual* 36, 1695-1703.
37. Hang W, Li HY, Gilbert JA, Li HB, Wu LH, Liu M, Wang LL, Zhou Q.S, Yuan JX, Zhang ZZ (2015) Housefly larva vermicomposting efficiently attenuates antibiotic resistance genes in swine manure, with concomitant bacterial population changes. *Appl Environ Microbiol* 81, 7668-7679.
38. Wang FH, Qiao M, Su JQ, Chen Z, Zhou X, Zhu YG (2014) High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. *Environ Sci Technol* 48, 9079-9085.

39. Tien YC, Li B, Zhang T, Scott A, Murray R, Sabourin L, Marti R, Topp E (2017) Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest. *Sci Total Environ* 581, 32-39.
40. Chen QL, An, XL, Li, H, Su, JQ, Ma, YB, Zhu, YG (2016) Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. *Environ Int* 92-93, 1-10.
41. Zhao X, Wang JH, Zhu LS, Ge WL, Wang J (2017) Environmental analysis of typical antibiotic-resistant bacteria and ARGs in farmland soil chronically fertilized with chicken manure. *Sci Total Environ* 593–594, 10-17.
42. Huang P, Zhang Y, Xiao KP, Jiang F, Wang HC, Tang DZ, Liu D, Liu B, Liu YS, He X (2018) The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids. *Microbiome* 6, 211
43. Zhang YJ, Li HC, Gu J, Qian X, Yin YN, Li Y, Zhang RR, Wang XJ (2016) Effects of adding different surfactants on antibiotic resistance genes and intl1 during chicken manure composting. *Bioresource Technol* 219, 545-551.
44. Sun W, Qian X, Gu J, Wang XJ, Zhang L, Guo AY (2017) Mechanisms and effects of arsanilic acid on antibiotic resistance genes and microbial communities during pig manure digestion. *Bioresource Technol* 234, 217-223.
45. Mohammadi NS, Mafakheri S, Abdali N, Barcena-Uribarri I, Tauch A, Benz R (2013) Identification and characterization of the channel-forming protein in the cell wall of *Corynebacterium amycolatum*. *BBA-Biomembranes* 1828, 2574-2582.
46. Lu LH, Zeng G, Fan CZ, Zhang JC, Chen AW, Chen M, Jiang M, Yuan YJ, Wu HP, Lai MY, He YB (2014) Diversity of Two-Domain Laccase-Like Multicopper Oxidase Genes in *Streptomyces* spp.: Identification of Genes Potentially Involved in Extracellular Activities and Lignocellulose Degradation during Composting of Agricultural Waste. *Appl Environ Microbiol* 80, 3305-3314.
47. Lv BY, Xing MY, Yang J, Zhang LB (2015) Pyrosequencing reveals bacterial community differences in composting and vermicomposting on the stabilization of mixed sewage sludge and cattle dung. *Appl Microbiol Biot* 99, 10703-10712.
48. Wang HX, Wang N, Wang B, Zhao Q, Fang H, Fu CW, Tang CX, Jiang F, Zhou Y, Chen Y, Jiang QW (2016) Antibiotics in drinking water in shanghai and their contribution to antibiotic exposure of school children. *Environ Sci Technol* 50, 2692-2699.

Figures

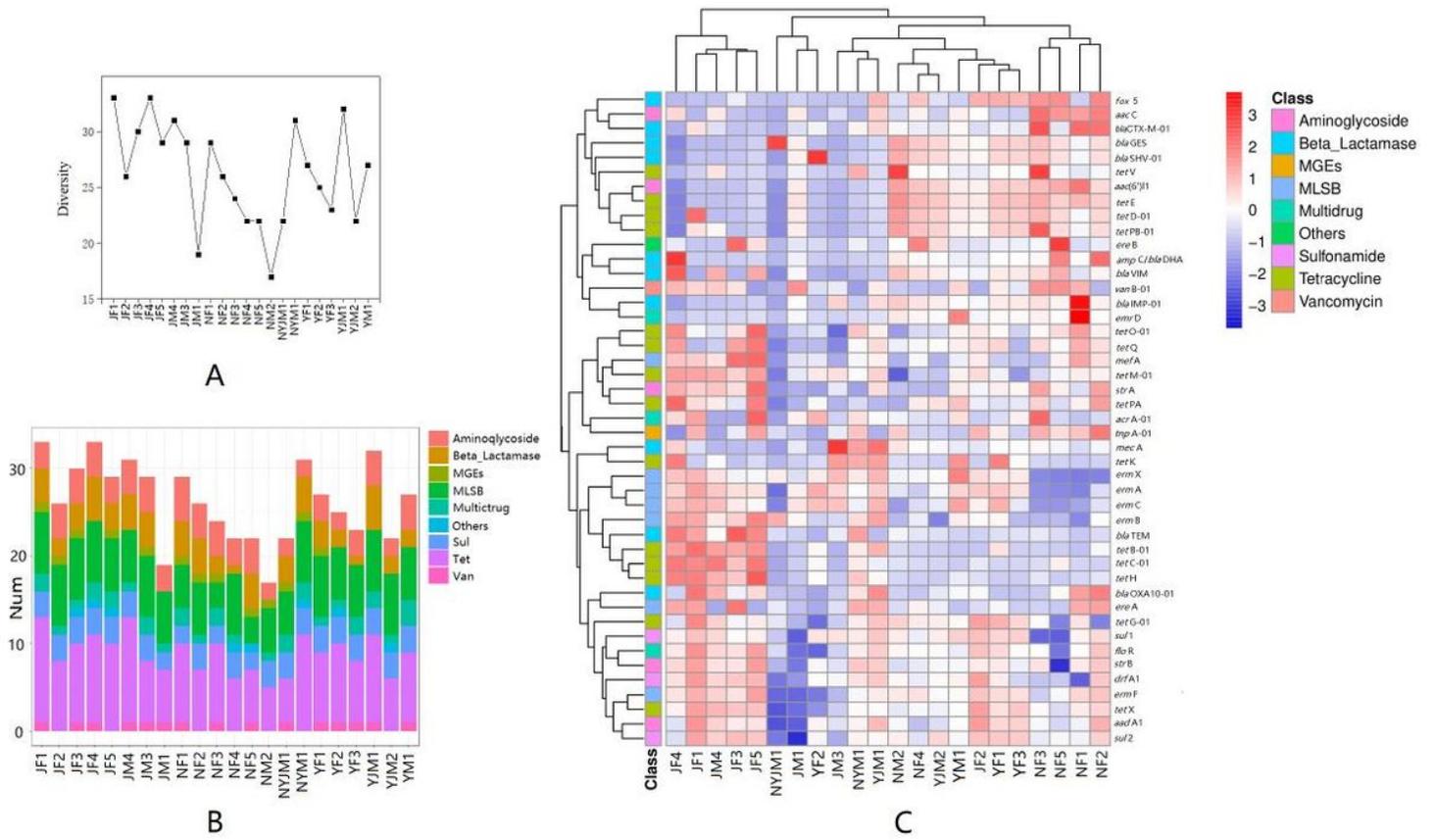


Figure 1

A: The diversity of ARGs in different animal manure and organic fertilizer; B: The diversity of ARGs subtypes in different animal manure and organic fertilizer; C: Heatmap of the relative abundance of ARGs in animal manure and organic fertilizer.

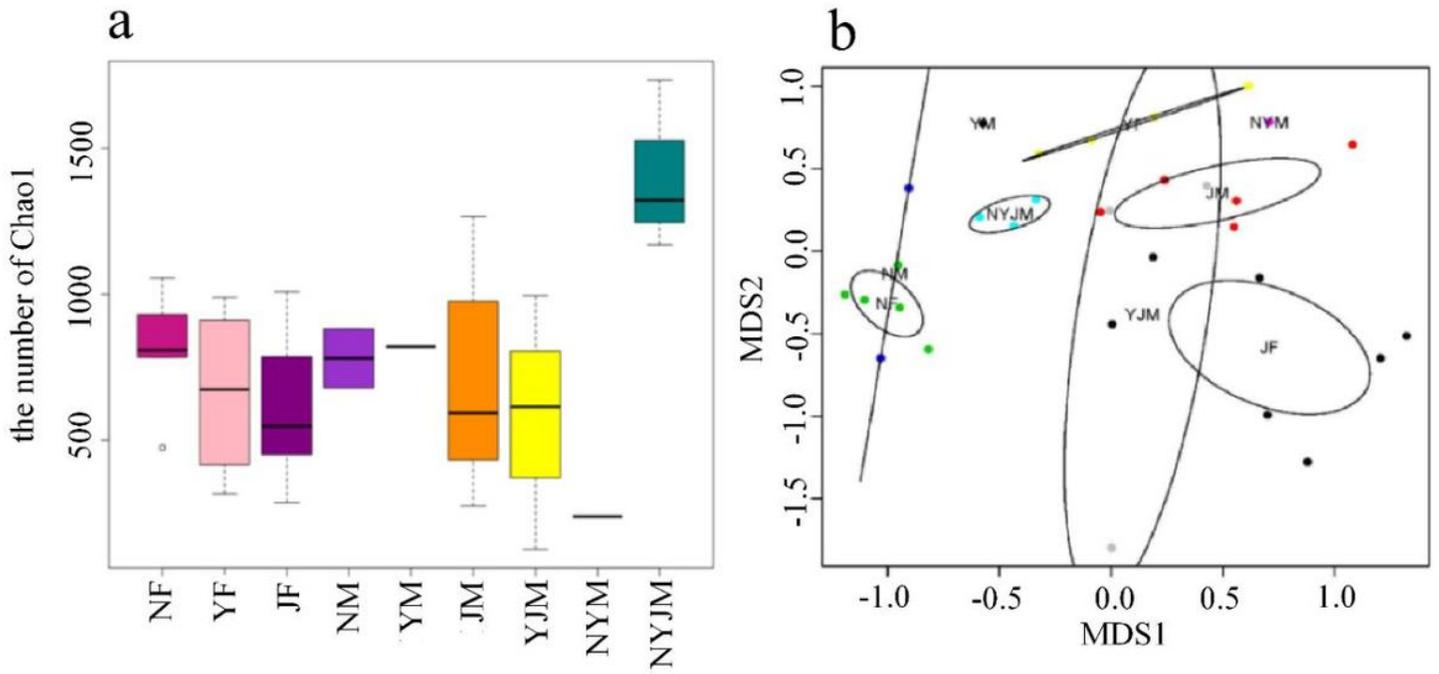


Figure 3

a. Alpha diversity of Chao 1 for each group sample; b. Nonmetric multidimensional scaling (NMDS) plot of all samples.

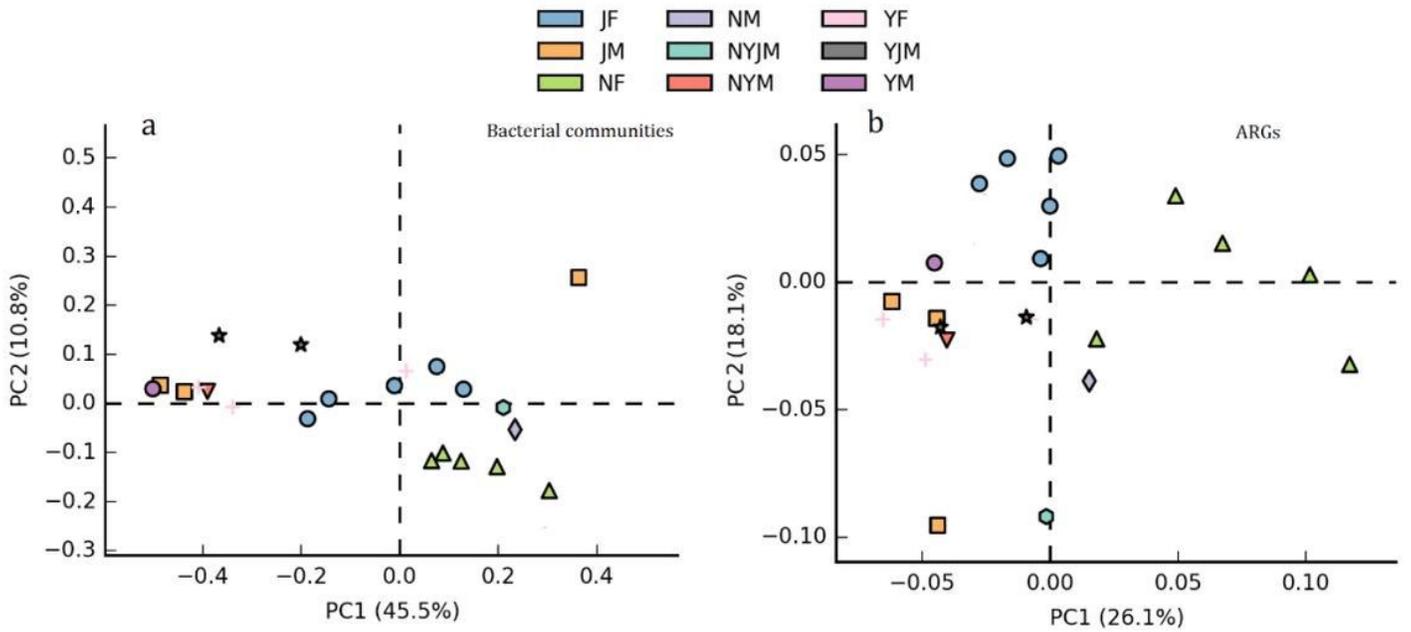


Figure 4

Principal component analysis (PCA) showing the bacterial communities.

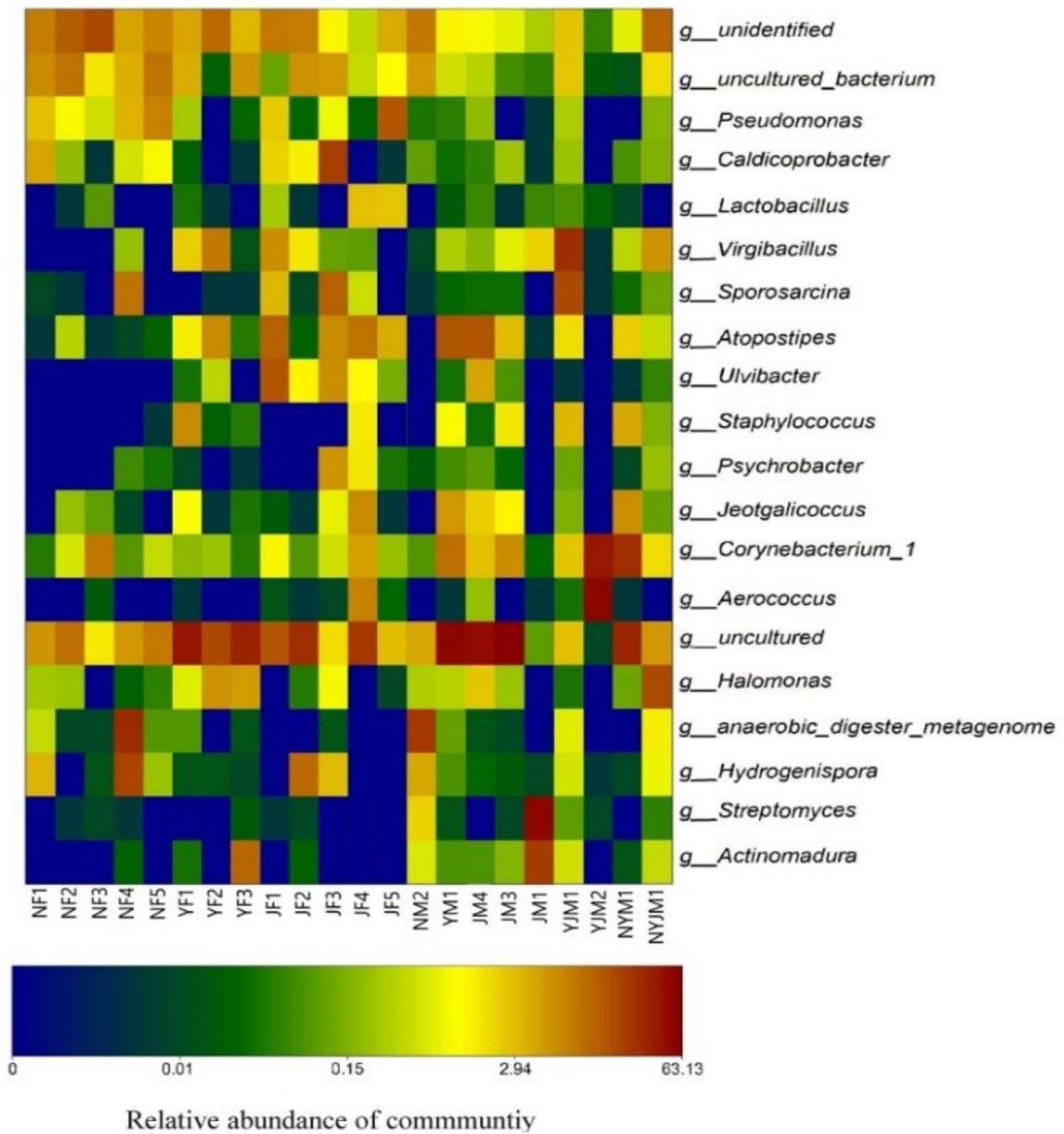


Figure 5

Heatmap of the relative abundance of top 20 genera in animal manure and organic fertilizer.

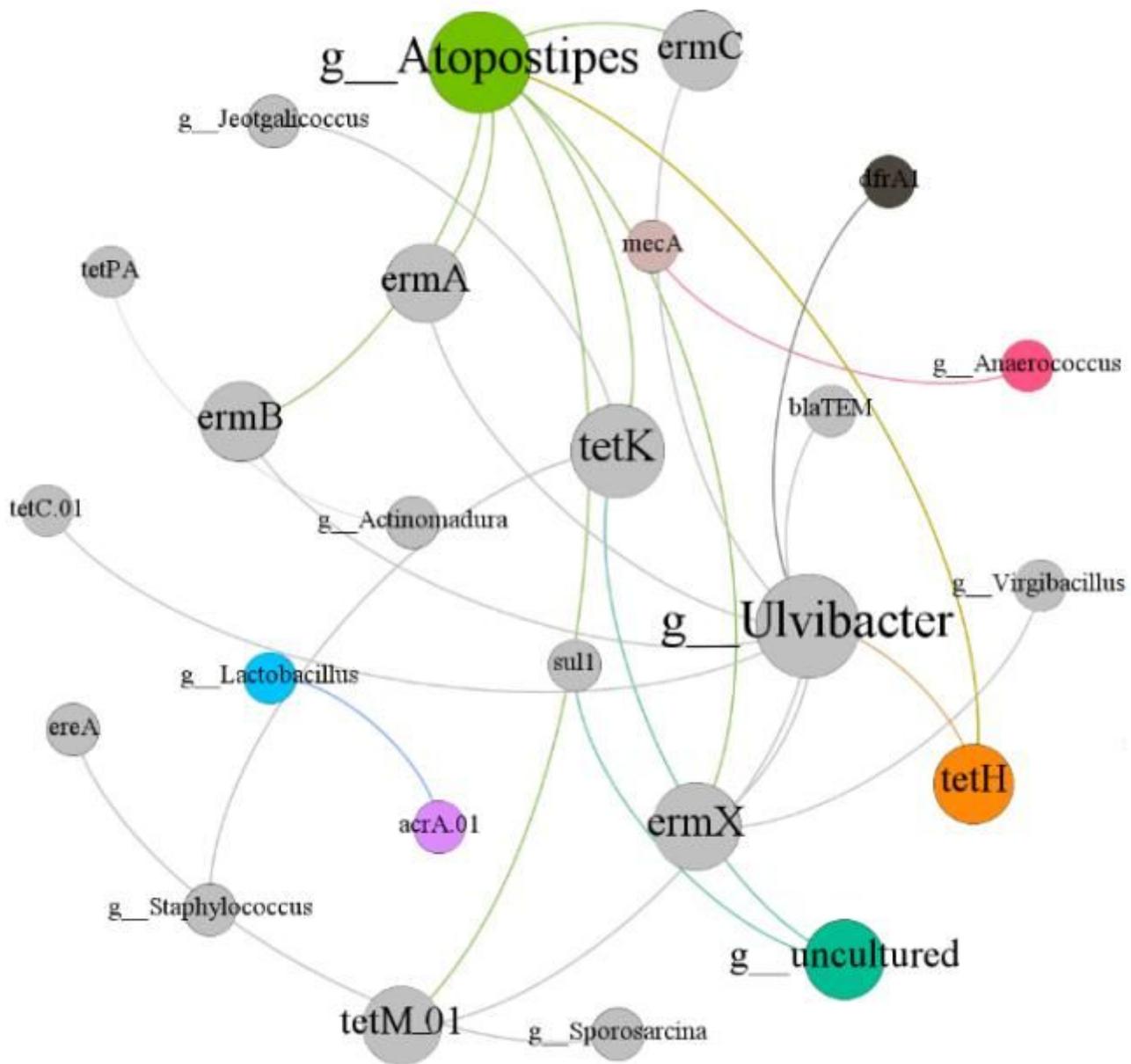


Figure 6

Network analysis of ARGs and potential host bacteria (top 20 genera) ($p < 0.05$, $R > 0.60$).

Supplementary Files

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

- [Supplementarymaterial.docx](#)