



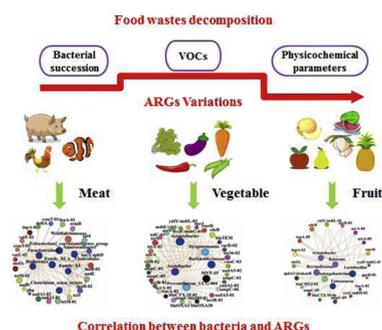
The formation mechanism of antibiotic-resistance genes associated with bacterial communities during biological decomposition of household garbage



Zhishu Liang, Yuna Zhang, Tao He, Yun Yu, Wen Liao, Guiying Li*, Taicheng An

Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Guangzhou Key Laboratory Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: D. Aga

Keywords:

Food wastes decomposition
Antibiotic resistance genes
Microbial community
Mobile genetic elements
Volatile organic compounds

ABSTRACT

Food wastes are significant reservoir of antibiotic-resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) available for exchange with clinical pathogens. However, food wastes-related changes of antibiotic resistance in long-period decomposition have been overlooked. Here, we evaluated the comprehensive ARG profile and its association with microbial communities, explained how this might vary with household garbage decomposition. Average of 128, 150 and 91 ARGs were detected in meat, vegetable and fruit wastes, respectively, with multidrug and tetracycline as the predominant ARG types. ARG abundance significantly increased at initial stage of waste fermentation and then decreased. High abundance of *Eubacterium-coprostanoligenes*, *Sporanaerobacter*, *Peptoniphilus*, *Peptostreptococcus* might be explained for the high relative abundance of ARGs in meat, while high abundance of *Advenella*, *Prevotella*, *Solobacterium* was attributed to the high diversity of ARGs in vegetables. Significant correlations were observed among volatile organic compounds, mobile genetic elements and ARGs, implying that they might contribute to transfer and transport of ARGs. Network analysis revealed that *aph(2')-Id-01*, *acrA-05*, *tetO-1* were potential ARG indicators, while *Hathewayia*, *Paraclostridium* and *Prevotellaceae* were possible hosts of ARGs. Our work might unveil underlining mechanism of the effects of food wastes decomposition on development and spread of ARGs in environment and also clues to ARG mitigation.

* Corresponding author.

E-mail address: ligy1999@gdut.edu.cn (G. Li).

<https://doi.org/10.1016/j.jhazmat.2020.122973>

Received 8 March 2020; Received in revised form 17 May 2020; Accepted 17 May 2020

Available online 21 May 2020

0304-3894/ © 2020 Elsevier B.V. All rights reserved.

1. Introduction

The increasing antibiotic-resistant bacteria (ARB) infections and emerging spread of antibiotic-resistance genes (ARGs) through horizontal gene transfer (HGT) from selective pressures such as antibiotics (Beaber et al., 2004; Jutkina et al., 2016) or others including heavy metals (Hu et al., 2016; Li et al., 2017), light irradiations (Chen et al., 2019), or minerals (Li et al., 2020) have attracted great worldwide attention. They threatened the achievements of modern medicine as well as posed a dangerous health hazard to people. This is because the ARGs could horizontal transfer through mobile genetic elements (MGEs) including transposons, plasmids, integrons as well as insertion sequences, or intrinsically exist in microorganisms (Lin et al., 2018). It has been estimated that antibiotic resistance has caused more than 2 million illnesses and 700,000 deaths per year, and the death toll will increase to 10 million globally by 2050 under the most alarming scenario if no action is taken (Wu et al., 2017a; IACG, 2019). Besides, the economic impact of uncontrolled antibiotic resistance would also be catastrophic as a result of dramatically increased health care expenditures. The evaluation of ARG reservoirs and environmental persistence are thus vital for executing strategies to reduce ARG spread.

Until now, therefore, lots of researches have been carried out to discover occurrence and abundance of ARGs in landfill, wastewater treatment plants as well as the recipient environments (aquatic bodies, livestock farms, soils and aerosols) (Zhu et al., 2018; Liu et al., 2018). Among them, municipal solid waste landfills with numerous unused/expired antibiotics and microbial flora especially pathogens, are normally considered as the reservoirs of ARGs and ARB and have caused increasing concern (Chen et al., 2017). Some works have demonstrated the release of ARGs, MGEs, ARB and antibiotics from municipal solid wastes landfills (Liu et al., 2018; Zhao et al., 2018; You et al., 2018). Significant correlations have been found among ARGs and humid acids, antibiotics and heavy metals in landfill (Yu et al., 2016; Wu et al., 2015). Besides, microbial communities were closely correlated with ARGs in refuse, whereas ARGs were more related to MGEs in leachates (Wu et al., 2017a).

Nevertheless, there is little knowledge about antibiotic resistance in food wastes, especially during their decomposition process, even though food wastes have currently contributed to more than 51% of municipal solid wastes landfill in most large cities in China (Yang et al., 2018). So far, food wastes were suggested as ARG reservoir used to transfer with clinical biohazards (Founou et al., 2016; Eckstrom and Barlow, 2019). For example, the bacteria related to food waste decomposition and those employed for starter cultures might form ARG reservoirs (Garofalo et al., 2007). Previous study also found that saprophytic bacteria may possibly act as ARG reservoirs in causative agents (van den Bogaard and Stobberingh, 2000). High abundance of beta-lactam resistance genes was detected in food wastes-recycling wastewater, demonstrating the ARG pollution from food wastes (Lee et al., 2017). The *blaOXA-20* also presented on radish, carrot, and lettuce, which were grown in sludge-amended soil (Rahube et al., 2014). Retail vegetables (e.g., tomatoes, celery), fruits (pears, strawberries), and meat (chicken, beef, pork, shrimps) were also reported to have antimicrobial resistance risks (Garofalo et al., 2007; He et al., 2019; Zhou et al., 2019; Ruimy et al., 2010). This means that fresh and fermented foods may be possible vehicles for dissemination of ARB and/or antibiotic resistance through food chain to the users (Ruimy et al., 2010), which has attracted people's particular attention. However, the dynamic of ARGs and fundamental mechanisms in the course of food wastes composting are not completely investigated, and we have no idea whether meat wastes harbored different ARGs abundance from that of fruit and vegetable wastes.

To address this knowledge gap of antibiotic resistance and discover the key factors affecting ARG abundance, household garbage was decomposed in lab-scale bioreactors. Using high-throughput qPCR with 295 proven primers (including 284 ARGs, 8 transposase genes, 2

integrase genes, and one 16S rRNA gene) and Illumina sequencing, the distributions and similarity/difference of ARG compositions and microbial communities' dynamics in three kinds of food wastes were investigated. In an attempt to draw public attention to underlying risk of ARG contamination and dissemination, the relationships between environmental factors, released odorous volatile organic compounds (VOCs) as well as ARGs and microbial community were all investigated. Furthermore, to provide potential indicators and hosts information for the ARGs, we investigated the co-occurrence patterns among ARG subtypes and microbial taxa in food wastes using network analysis. These results would offer valuable clues for ARG removal from major components of landfill and clarify micro-ecological mechanism by which antibiotic resistome are generated.

2. Materials and methods

2.1. Experimental design and sample collection

Three cylindrical Perspex bioreactors (56.5 L) were assembled to simulate the decomposition processes of meat, vegetable and fruit wastes in lab. The design parameters, composition of the household food wastes and operation conditions of decomposition are provided in our recent work (Zhang et al., 2020) and in SI. In brief, a total of 10 kg raw meat (including fish, pork and chicken), 10 kg raw vegetables (including spinach, bitter melon, bean, cabbage, radish, eggplant, lettuce and pepper) and 20 kg fruit (including pear, pineapple skin, apple, hami melon, watermelon, muskmelon and peach) were collected from Tangde food markets in Guangzhou, China. After shredded into approximately 2 cm pieces in size and well-mixed, these food wastes were loaded into bioreactors to initiate the decomposition at room temperature (24–29 °C). Ambient air (except during sampling) was constantly fed into the reactors at an aeration rate of 0.2–0.5 L min⁻¹ kg⁻¹ to ensure the aerobic fermentation and leachates were mixed thoroughly by re-circulating with peristaltic pump before sampling. Then the leachates (50 mL) and refuse samples (10 g) are sampled on days 1, 15, 30, 45 and 60 for the detection of physicochemical properties and isolation of genomic DNA using the methods described in SI. After sample collection, 100 mL deionized H₂O was supplied to the reactor to make up the loss of leachate.

2.2. High-throughput quantitative PCR (HT-qPCR)

To assess the composition and ARG abundance in food wastes, HT-qPCR was performed using the above extracted DNA as the template. The validated primers in this study including 285 primer sets for ARGs with resistance to antibiotics (e.g. aminoglycoside, macrolide–lincosamide–streptogramin B (MLS_B), beta-lactam, chloramphenicol, sulfonamide, tetracycline, multidrug and vancomycin), 10 primer sets for MGEs encoding transposase and integrase, and 1 16S rRNA gene could be found in the reference Zhu et al. (2013). The detailed PCR reaction mixture and procedure for amplification are described in SI. Amplification wells with several melting peaks, or amplification efficiency out-of-range (90%–110%), or C_T > 31 were discarded (Wang et al., 2014). Gene relative copy number can be evaluated with formula described in SI. The normalized copy number of ARGs was determined by dividing the relative gene copy number of ARG by that of reference gene (16S rRNA).

2.3. 16S rRNA gene amplification, sequencing and data processing

The extracted genomic DNA was also used to amplify the bacterial 16S rRNA (V4 region) with primers F515: 5'-GTGCCAGCMGCCGCGG-3' and R907: 5'-GGACTACHVGGGTWTCTAAT-3'. PCR reactions were carried out in accordance with reference Bates et al. (2011). The detailed information is provided in SI.

2.4. Statistical test

All statistical analysis was regarded significant for $p < 0.05$. Principal component analysis (PCA) was used to assess the bacterial community and ARG profiles among different samples. Using mantel test with Canoco 5.0 software (Microcomputer Power, NY, USA), correlation between ARGs and bacterial community structure, chemical parameters were performed. The correlation-based network between the bacterial community and ARG subtypes was analyzed in R (V 3.5.3). If Spearman's correlation coefficient (R^2) and P were more than 0.6 and less than 0.01, respectively (Huang et al., 2015), the correlation between two items is statistically reliable. Using software of Cytoscape 3.5.1, the network graphs were visualized with algorithm of circular layout (Yin et al., 2015).

3. Results

3.1. ARGs evolution profiles during decomposition of different food wastes

A total of 114 shared ARGs contributed to 87.2–98.1% of total ARG abundances were found in all samples (Fig. S1), indicating their ubiquity in food wastes. The ARG detection frequency increased at the initial phase of decomposition and then gradually decreased after day 15, suggesting that complete decomposition of food wastes will result in the reduction of ARGs. Vegetable wastes have higher average ARG diversity (150) than meat (128) and fruit (91) wastes (Fig. 1a), while meat wastes have higher average abundance of ARGs (13.45) than vegetable (4.29) and fruit (2.65 copies/cell). The ARG abundances increased from 5.30–34.46 copies/cell (meat), 3.05–6.02 copies/cell (vegetable), and 0.52–5.9 copies/cell (fruit) from days 1–30 decomposition time. However, further prolonging decomposition time to day 60 resulted in decreased ARG abundances of meat (1.54) and fruit (1.43 copies/cell). Interestingly, the ARG abundance of vegetables did not decrease at day 60, which was probably due to higher enrichment of some special ARGs like tetracycline resistance genes.

Further analysis the distribution of ARGs and MGEs, we found that multidrug, tetracycline, aminoglycoside, MLSB and beta-lactam ARGs were the 5 top abundant genes, followed by the MGEs; with chloramphenicol, sulfonamide and vancomycin ARGs as the least (Fig. S2). Antibiotic deactivation as well as efflux pump are two predominant resistance mechanisms, with cellular protection mechanism followed (Fig. 1b). PCA analysis using Bray-Curtis distance based on relative abundance of ARGs showed that ARGs in same kind of wastes clustered together and were separated from the other two wastes (Fig. 2). More than half variance (55%) of ARGs could be explained by the first two

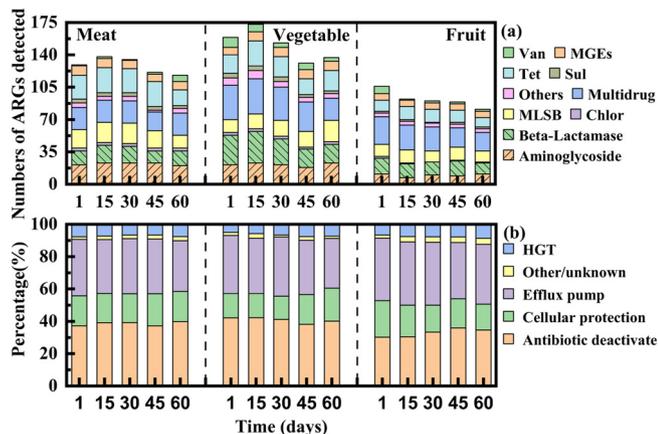


Fig. 1. (a) The number of unique resistance genes detected during the decomposition of food wastes. (b) Percentage of antibiotic resistance mechanism in all samples. Vancomycin (Van), tetracycline (Tet), sulfonamide (Sul) and chloramphenicol (Chlor).

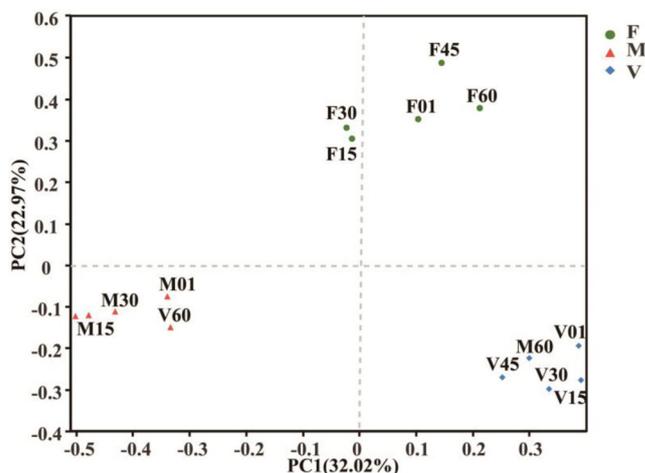


Fig. 2. Principal component analysis (PCA) based on the Bray-Curtis distance showing the overall distribution pattern of ARGs during the decomposition of meat (M), vegetable (V) and fruit (F). ARGs data was based on HT-qPCR.

principal components. Specifically, the meat samples' ARGs were separated from vegetables mainly along PC1 and fruit along PC2, which explained 32.02% and 22.97% of variance, respectively, indicating that meat wastes caused larger shift in ARG pattern than vegetable and fruit wastes.

As raw food wastes decomposition progressed, ARGs behaved differently with some ARGs (such as tetracycline) increased, while others decreased. Overall, as Fig. 3 shows, the decomposition of meat markedly decreased the tetracycline, MLSB, aminoglycoside resistance genes as well as MGEs. Additional decrease in multidrug, beta-lactam resistance genes and MGEs was also observed during vegetable decomposition. However, the tetracycline and MLSB resistance genes increased at the late phase of vegetable decomposition. Similarly, increased multidrug and tetracycline resistance genes were also detected at later stage of fruit samples, indicating that vegetable and fruit decomposition will increase the abundance of certain ARGs. Besides, the detailed variation trends of each ARG type during food waste decomposition (Fig. S3) indicated that the maximum abundance of each ARG type occurred primarily in meat wastes, followed by vegetable and fruit.

3.2. Co-occurrence patterns among ARG subtypes

The co-occurrence patterns among ARG subtypes were investigated using network analysis on the basis of the strong ($r > 0.6$) and significant ($P \leq 0.01$) correlations. As Fig. 4a shows, the co-occurrence ARGs conferred resistance to main classes of antibiotics in meat such as

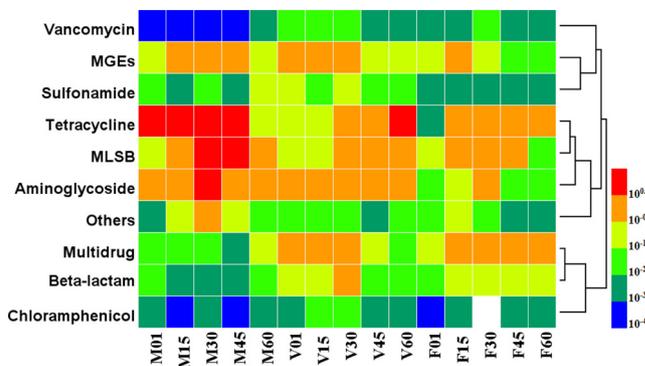


Fig. 3. Heat map showing the distribution of different types of ARG (classified by the classes of antibiotics they resisted) during the decomposition of raw meat, vegetable and fruit samples of 1, 15, 30, 45 and 60 days.

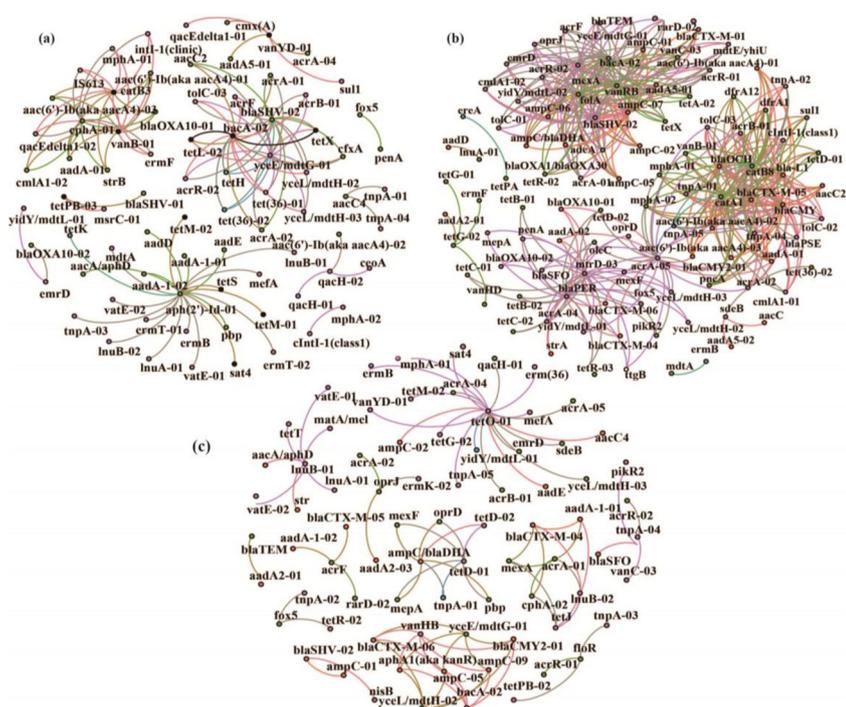


Fig. 4. The network analysis revealing the co-occurrence patterns among ARG subtypes during the decomposition of meat (a), vegetable (b) and fruit(c) samples. The nodes were colored according to ARGs types. A connection represents a strong (Spearman's correlation coefficient $R^2 > 0.6$) and significant (P -value < 0.01) correlation. Edges and node size weighted were based on the correlation coefficient and the relative abundance of ARGs, respectively.

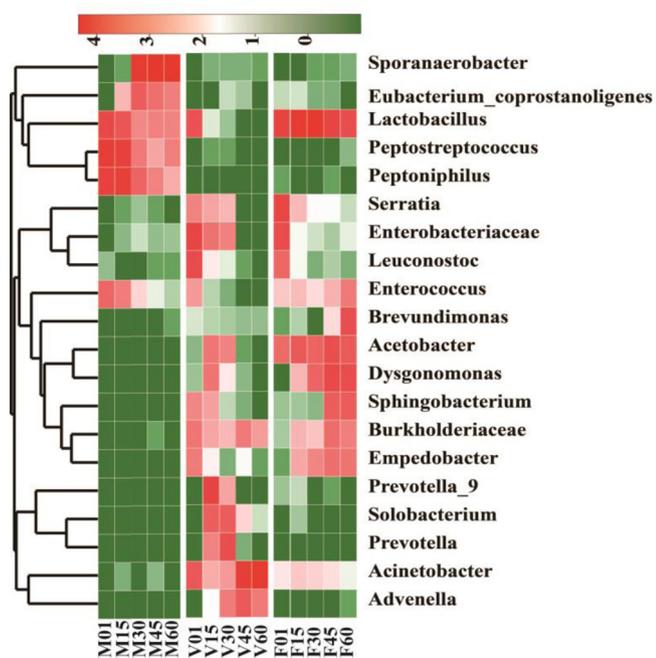


Fig. 5. Heatmap of the relative abundance of bacterial communities at the genus level during the decomposition of meat, vegetable and fruit wastes. The color transition from dark green to dark red represents relative abundances from low to high. The left neighbor-joining tree was clustered in accordance with the similarity of bacterial abundance and different genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

aminoglycoside, β -lactam, multidrug, and vancomycin. For instance, *aph(2)-Id-01* significantly correlated with 22 of other ARGs, while *yceE/mdtG-01* and *blaSHV-02* correlated with 15 of other genes. Besides, some genes such as *cphA-01* and *vanB-01* were also noticed to be associated with 11 (positive) and 17 (negative) of other ARGs.

In comparison, the co-occurrence patterns of vegetables consisted higher numbers of nodes (106) and edges (395) than that of meat and

fruit. In particular, up to 28 strong positive correlations between *acrA-05* and other ARGs were found, including 3 aminoglycosides, 7 β -lactams, 4 MLSBs, 10 multidrugs, 1 tetracycline, 1 vancomycin resistance genes and 2 MGEs (*tnpA-04* and *tnpA-05*) during vegetable fermentation (Fig. 4b). The *ampC-07*, *blaSHV-02*, *bla-L1*, *blaCMY*, *blaCTX-M-05*, *blaOCH* and *blaPER* are class of aminoglycoside resistance genes with the most densely connected nodes (≥ 25). Besides, *mexA*, *mtrD-03*, *vanRB* and *folA* were correlated with more than 25 ARGs, suggesting that these genes might be served as the indicators for unique ARGs. Moreover, MGEs were also positively correlated with *bla-L1*, *blaCMY*, *blaCTX-M-05* and *blaOCH*, while negatively related to *tetG-02*, *tetH* and *tetPA*, indicating that MGEs contributed significantly to various ARG transfer. In addition, a negative association of *bla-L1*, *blaCMY*, *blaCTX-M-05*, *blaOCH* with *ereA* was found. For fruit fermentation, *tetO-01* conferred resistance to tetracycline was in the center of the network and had a more complex correlation (20) than other ARGs (Fig. 4c). The genes conferred resistance to multidrug, β -lactam and MLSB occupied 22, 15, 12 nodes of 78 total nodes, respectively, and were the main ARG subtypes in the network. To summaries, the above complex patterns revealed that these ARGs can be used as indicators for qualitative and quantitative analysis of related ARG co-occurrence.

3.3. Correlation between bacterial communities

Microbes played key role in the biochemical conversion of food wastes by mediating the hydrolysis, acidogenesis, acetogenesis and methanogenesis (Meng et al., 2018), thus it is very necessary to explore the response of bacterial community to food wastes bio-decomposition. As compared to meat wastes, the shared genera between fruits and vegetables (180) were higher (Fig. S4a), indicating that they might have very similar community composition. However, their compositions exhibited different temporal variations in the course of decomposition at genus level as revealed by the heatmap based on phylogenetic classification (Fig. 5). That is, butyrate-producing, non-saccharolytic genera (Ezaki et al., 2001), *Peptoniphilus* and *Peptostreptococcus*, were the two most dominant genera at the initial phase of meat decomposition (from days 1–15) that used amino acids and peptone as main sources of energy. They accounted for 29–39% of total bacterial 16S rRNA gene sequences, but rapidly decreased with the

fermentation progress. In addition, *Lactobacillus* also reduced, while *Sporanaerobacter* increased at the late phase of meat decomposition.

For vegetable fermentation, the relative abundance of the enriched genera *Lactobacillus*, *Leuconostoc*, *Enterobacteriaceae* reduced, while dominant *Acinetobacter* first decreased from days 1 to 30 and then increased. As the largest group in fruit decomposition, *Lactobacillus* first increased from 27% (day 1) to 73% (day 30) then decreased to 16% (day 60). *Serratia* and *Enterobacteriaceae* belonging to phylum Proteobacteria were the other two dominant genera, but decreased during the same period. The other genera dominant at late stage of fermentation were *Dysgonomonas*, *Brevundimonas* and *Acetobacter*. On the basis of Bray-Curtis distance, PCA analysis also demonstrated that bacterial patterns in same kind of wastes was clustered together and separated from the other two wastes, suggesting that food wastes rather than decomposition shifted the pattern of bacteria (Fig. S4b).

Definitely, strong competition and interactions existed in bacterial communities for limited resources during decomposition. Network analysis therefore was employed to reveal co-occurrence between bacterial communities (Fig. S5). Among 30 enriched genera in meat, *Fusobacterium* and *Clostridiaceae-1* were negatively related to *Gallicola*, *Peptostreptococcus*, *Peptoniphilus*, *Lactococcus*, *Streptococcus*, *Enterococcus* and *Veillonellaceae*, while *Eubacterium-coprostanoligenes* were positively associated with *Family_XI*, *Hathewayia*, *Anaerobaculum*, *Erysipelothrix* and *Caldicoprobacter*. Among the visualized linkages in vegetables, the dominant genera like *Proteiniphilum*, *Petrimonas* and *Advenella* have the highest connection with other genera, including 6 positive and 6 negative genera. Besides, 3 positive and negative correlations were detected between *Lactobacillus*, *Leuconostoc* and *Enterobacteriaceae* with other genera. *Acinetobacter* was dominated at later stage of vegetable fermentation but was negatively correlated with *Acetobacter*, *Prevotella-9* and *Dysgonomonas*. This indicated that *Acinetobacter* would probably impede these genera reproduction in vegetable fermentation. Conversely, more significant negative correlation between the microbial taxa was observed during fruit fermentation. For example, *Clostridiaceae-1* and *Serratia* were negatively correlated with 9 genera. Besides, enriched *Enterobacteriaceae* at initial stage and dominant *Lactobacillus* in the whole decomposition process were negatively correlated with 4 and 3 genera, respectively. But, bacterium like *Brevundimonas* was beneficial for the growth of other genera, as revealed by its positive correlation with 7 genera.

3.4. Correlation between bacterial community and ARGs

On the basis of Bray-Curtis distance ($r = 0.3321$, $P = 0.007$), mantel test demonstrated that ARG profile was significantly correlated with microorganism compositions and structures. Co-occurrence patterns between bacterial taxa and ARGs were therefore investigated by network analysis (Fig. 6). A total of 74 nodes (23 microbial taxa and 51 ARG subtypes) and 304 edges, 60 nodes (20 microbial taxa and 40 ARG subtypes) and 462 edges, 65 nodes (25 microbial taxa and 40 ARG subtypes) and 310 edges were detected in meat, vegetable and fruit wastes, respectively. We supposed that co-occurrence patterns might reveal potential host of ARGs if there existed a strong ($r > 0.65$) and significant ($P < 0.01$) positive correlation between microbial taxa and ARGs. Considering *Hathewayia* and *Paraclostridium* possessed positive correlation with ARGs conferring resistance to different classes of antibiotics, they may be served as the possible hosts of these ARGs during meat decomposition. Besides, *Enterococcus*, *Streptococcus*, *Gallicola* and *Peptostreptococcus* were the possible hosts of ARGs conferring resistance to tetracycline, multidrug, β -lactam (*cfxA*) and aminoglycoside (*aacC2*).

Comparatively, the correlation profile between ARGs and bacterial taxa during vegetable decomposition was more complex with a single bacterial taxon correlated with diverse ARGs, and a single ARG correlated with different bacterial taxa. For example, *Prevotellaceae-UCG-004* was the possible host of 30 ARGs (Table S3). *Acetobacter* and *Dysgonomonas* were correlated positively with 17 ARGs conferring

resistance to multidrug, β -lactams, tetracycline and vancomycin; while *Leuconostoc* co-occurred with *aadA-02*, *acrA-04*, *oprD*, *mexF*, *ttgB*, *yceL/mdtH-03*, *yidY/mdtL-01*, *blaCTX-M-04*, *blaCTX-M-06*, *blaOXA10-01*, *blaSFO*, *fox5* and *tetD-02* (Table S4), indicating that these genera may be the important ARG hosts in vegetables.

Even though the bacterial communities between vegetable and fruit wastes were very similar, their associations with ARGs were significantly different. For fruit wastes, *Lactobacillus* co-occurred with 16 ARGs, while other 9 genera only carried *mepA*, *mexF*, *oprD* and *pbb* (Table S5). To sum up, the correlation between bacterial communities and ARGs varied significantly responding to different selective pressures such as carbon source shortage and toxicity of harmful pollutants during the decomposition of food wastes.

4. Discussion

4.1. ARG diversity as well as abundance in food wastes

The evolution of ARGs in the municipal solid waste landfill has been studied widely, whereas the impacts of specific household food wastes on ARGs have long been overlooked. Here, by investigating the antibiotic resistance of food wastes, we found up to 138, 173 and 106 ARGs during decomposition of meat, vegetables and fruit, respectively, with MLSB, β -lactam, aminoglycoside, multidrug and tetracycline resistance genes as the predominant ARGs, highlighting that raw food wastes are also important reservoir of ARGs. Whereas, in the untreated meat, vegetable and fruit, the total ARG numbers detected were 95, 95 and 60 (Fig. S6) with the relative abundance of 0.115, 0.016, 0.015 copies/cell, respectively (Fig. S7), suggesting that ARGs were diverse in food wastes. This should not be a surprise because antibiotic resistance was proven as ancient and could naturally happen (D'Costa et al., 2011; Martinez, 2008).

Efflux pump and antibiotic deactivation are two predominant mechanisms of antibiotic resistance and this result was supported by previous reports that the deactivation to efflux pump genes ratio was shifted during the sewage sludge, manure and food waste decomposing process (Su et al., 2015; Wu et al., 2017b; Liao et al., 2019). High abundance of *tetM*, *tetL*, *tet(36)*, *tetT*, *tetO* and *tetX* suggested that tetracycline-resistance mechanisms include efflux pumping of tetracycline outside the cell, preventing contacts of cells from tetracycline by alteration of the ribosomal proteins, and activating tetracycline degrading enzymes (He et al., 2019; Aminov et al., 2001). Our result is similar to a previous study, which demonstrated that *ermB* and *tetM* were largely distributed in chicken, pork meat and their processing lines (Garofalo et al., 2007). The significant higher ARG abundances in meat wastes than those in fruits and vegetables may be because the meat wastes containing some fish and chicken gut, which were usually considered as ARG reservoirs and hotspots of ARG horizontal transfer and spread (Fu et al., 2017; Hu et al., 2013).

Comparatively, vegetable wastes with relatively low abundance but the highest diversity of ARG is likely due to two factors: (1) vegetables especially organic one usually exposed to animal feces, which were probably excreted by animals with antibiotics treatment, therefore certainly rise ARG diversity; (2) the inputs of chemical fertilizer, trace metals or pesticides in vegetable especially conventional one possibly reduced total bacterial number and resulted in reduced ARG abundance (Zhu et al., 2018). However, this is contrary to the result found by Chen et al. that the application of chemical fertilizer would cause the increased ARG abundance and diversity possibly due to change of bacterial community structure including indigenous ARB in soil (Chen et al., 2016). Previous reports have demonstrated that fertilization with manure or municipal sewage sludge led to ARG dissemination to farmlands, or even harvested vegetables (Rahube et al., 2014; Zhu et al., 2017). Researchers have also found ARGs on harvested vegetables cultured in soil amended with manure (Wang et al., 2015a). Besides, high diversity of ARGs in vegetables might be associated with

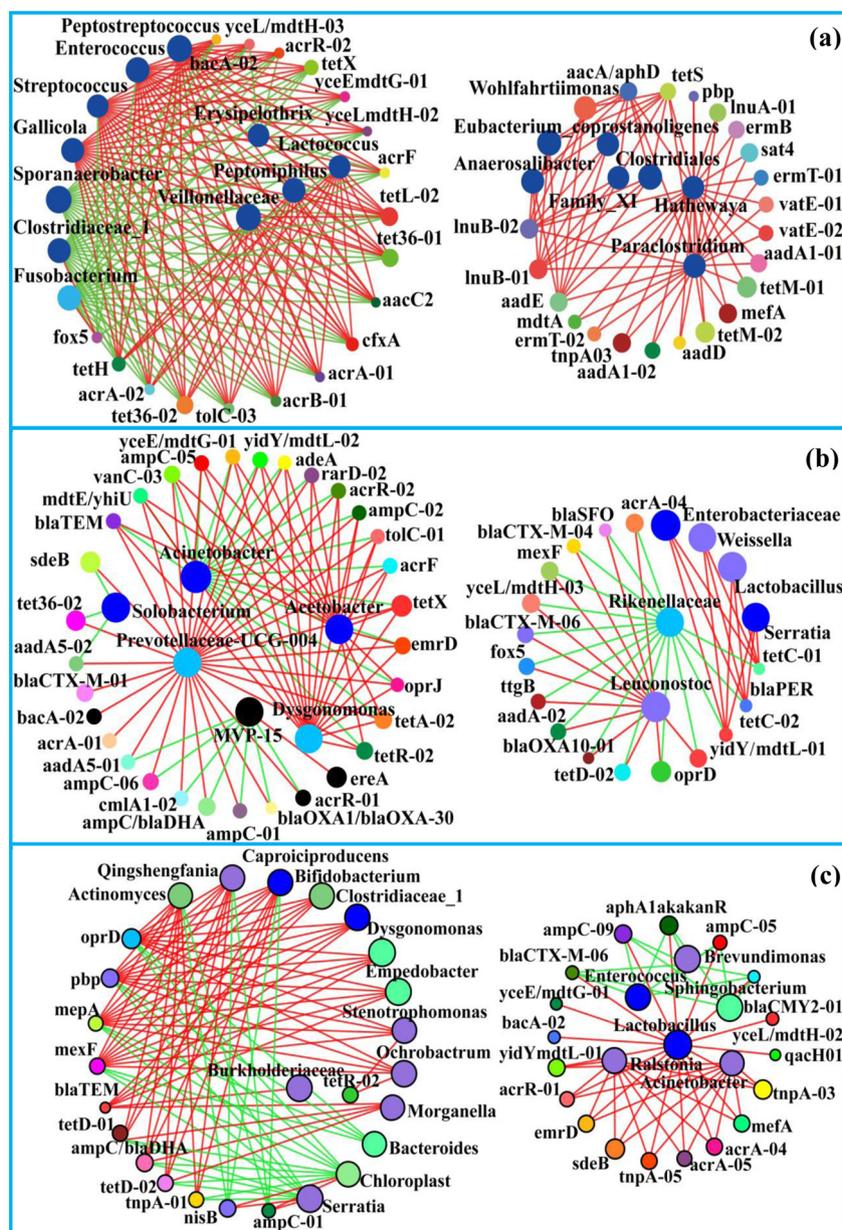


Fig. 6. The network analysis revealing the co-occurrence patterns among ARG and microbial taxa in meat (a), vegetable (b) and fruit (c) samples. The nodes were colored according to ARGs and bacterial types. Edges and node size weighted were based on the correlation coefficient and the relative abundance of ARGs and bacterial communities, respectively. Edge color represents positive (red) and negative (green) correlations. A more detailed correlation between ARG and bacterial taxa was provided in Table S2–S4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

soil bacterial colonization during farming (Zhu et al., 2017; Bulgarelli et al., 2013). However, for fruit wastes, the antibiotic resistance was significantly lower than that of meat and vegetables. This was probably related to the bacterial ARGs on fruit being less affected by anthropologic activity and therefore was not exposed to ARG selection stress as straightly as those in meat and vegetables.

4.2. ARG fate during the decomposition of food wastes

Most ARGs were recalcitrant during food waste decomposition processes. After 30 days of decomposition, the diversity and abundance of ARGs in meat significantly rose, particularly for tetracycline, MLSB and aminoglycoside resistance genes, indicating that meat decomposition could result in the ARG occurrence and dissemination. This might be because of the propagation of bacterial numbers regarding the increased abundance of bacterial 16S rRNA genes at initial stage of

fermentation. However, as the decomposition progress, the relative abundance of ARGs evidently decreased. This is probably attributed to the decreased relative abundance of the strict mesophilic and anaerobic bacteria in mesophilic phase (days 15–30), given composting is an aerobic process, while most animal gut bacteria are anaerobic. Therefore, the late (thermophilic) phase of decomposition (days 45–60) is not suitable for the survival and proliferation of the gut bacteria and ARG-carrying gram-negative bacteria (Selvam et al., 2012). This is agreed with previous study that decomposition process could reduce different groups of ARGs in management systems of livestock manure (Su et al., 2015). Similarly, ARG abundance in fruit also significantly decreased after 60-days decomposition, suggesting that this process can eliminate the health risk exposure to fruit wastes from the aspect of ARGs.

Surprisingly, the total abundances of ARGs significantly increased after 60-days decomposition of vegetables. Particularly, the

enrichments of *tetM-01* (951), *tetM-02* (881) and *tetL* (107 folds) (Fig. 3c) are in according to previous study that the abundances of ARGs including *tetL*, *sul2* and *ermB* increased after food wastes decomposition (Liao et al., 2019). The increased ARG abundance might be associated with HGT among bacterial cells through MGEs and succession of the associated bacterial communities (Su et al., 2015). More diverse MGEs and bacterial communities found in vegetables than those in fruit and meat further supported this hypothesis. Besides, positive correlation between ARGs and MGEs (*trpA-01*, *trpA-02*, *trpA-04*, *trpA-05*, *cInt1-1(class1)*) (Fig. 4) also demonstrated that MGEs might be one of critical origination of ARGs in vegetable wastes. This was coincided with previous studies that MGEs could potentially serve as a HGT route for ARGs during food manufacturing and consuming processes (Wang et al., 2012; Verraes et al., 2013).

4.3. Effects of bacterial community, environmental factors, released volatile organic compounds (VOCs) on the ARG prevalence during food waste decomposition

Early study has considered microbial communities as important factors to structure the target ARGs or-and local resistome (Forsberg et al., 2014). Consistent with higher ARG abundance in meat, the relative abundance of protein-decomposing *Eubacterium-coprostanoligenes*, *Sporanaerobacter*, *Peptoniphilus* and *Peptostreptococcus* were also significantly higher than that of fruit and vegetable, suggesting that these genera maybe the reason for high ARG enrichment. The fundamental mechanism might be the competition and interactions between species for limited resources (van Elsas et al., 2012). Furthermore, *Peptostreptococcus* has been demonstrated to become increasingly resistant to antibiotics (Kononen et al., 2007). Even though both vegetable and fruit wastes have low ARG abundance, ARG diversity in vegetables was significantly higher than that of fruit. This may be attributed to higher abundance of *Advenella*, *Solobacterium* and *Prevotella-9*, indicating that the prevalence of these genera may result in diversifying ARGs in vegetables. This result was in agreement with previous study, which proposed *Prevotella* species as the inhibitors of clindamycin, penicillin, beta-lactams, tetracycline and metronidazole (Boyanova et al., 2010).

The hydrolysis, acidogenesis, acetogenesis and methanogenesis of food wastes by microbes brought various intermediates or end products, including NH_3/NH_4 and carbonates, resulting in variation of carbon-related parameters and pH value. As Table S1 shows, the physicochemical parameters of food wastes held a different tendency with higher phosphorus (TP), TOC and BOD_5 detected in meat than fruit and vegetable wastes. As for pH value, a climbing curve was reported for vegetable and fruit fermentation, while initially increased and then decreased pH was detected during meat waste decomposition (Zhang et al., 2020). PCA also revealed that $\text{NH}_3\text{-N}$ was positively correlated ($P < 0.05$) to the ARGs conferring resistance to vancomycin, chloramphenicol, multidrug and β -lactam; while negative correlation ($P < 0.05$) was obtained between ARGs (sulfonamide and chloramphenicol resistance) and COD. BOD_5 and TOC were both negatively correlated with chloramphenicol and beta-lactam resistance genes, as supported by a previous conclusion (Yu et al., 2016). Besides, strong relationship was observed between ARGs (aminoglycoside) and pH as well as TP.

The correlation between ARGs and emitted VOCs was also analyzed based on our reported VOC concentrations (Zhang et al., 2020). As Fig. 7 shows, VOCs were correlated positively with ARGs conferring resistance to tetracycline, aminoglycoside, MLSB, and MGEs. Specifically, *trpA-03* and *trpA-07* were positively correlated with 9 and 3 VOCs, respectively, indicating that VOCs might promote the occurrence of HGT via MGEs. High abundances of tetracycline resistance genes such as *tetT*, *tetL*, *tetM* and *tet36* were related with acetone, styrene, dimethyl disulfide and methyl isobutyl ketone. Aminoglycoside (*aadA* and *aacA/aphD*) and MLSB (*inuB*, *matA/mel* and *mefA*) were also associated with dimethyl disulfide and methyl isobutyl ketone. Besides,

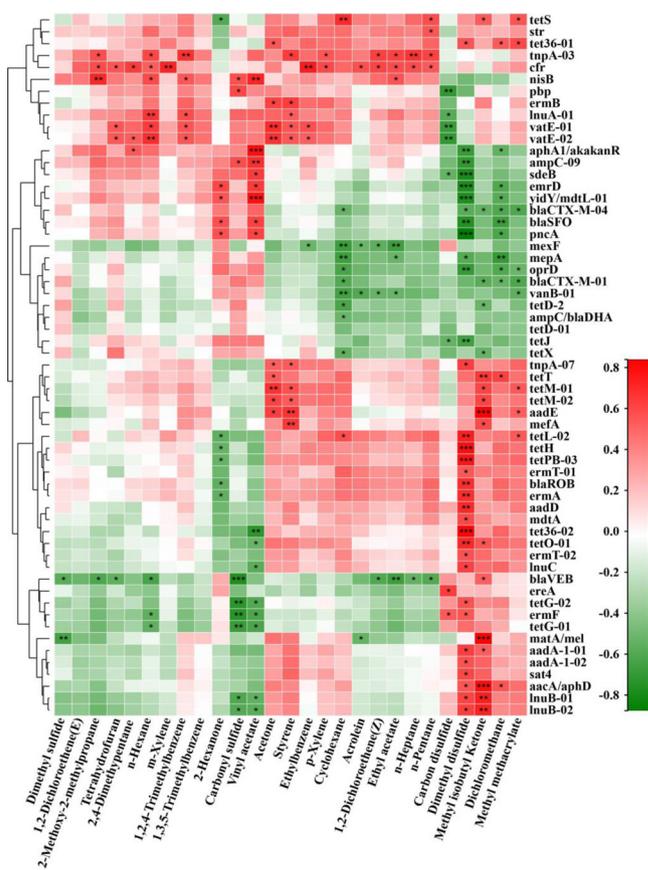


Fig. 7. Spearman correlations between ARGs and emitted VOCs from food wastes. The plot used asterisks as indicators for statistical significance (*). No symbol was used when the $P > 0.05$ (not significant). One, two and three asterisks were used when $0.01 < P^* \leq 0.05$ (relatively strong correlation), $0.001 < P^{**} \leq 0.01$ (strong correlation) and $P^{***} \leq 0.001$ (significantly strong correlation), respectively. The color transition from dark green to dark red represents relative abundances of ARGs from low to high. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

strong positive relations were obtained between vinyl acetate, 1,2,4-trimethylbenzene, n-hexane and the prominent ARGs. This indicated that these VOCs may contribute to the highly enriched ARGs in food wastes. Previous study also revealed that VOCs modify the antibiotic resistance phenotype of *Escherichia coli* (Kim et al., 2013). However, it has to be mentioned that dimethyl disulfide and vinyl acetate would also negatively correlated with ARGs, this may be due to the toxic effect of these compounds on the bacterial community (Kiragosyan et al., 2020). Besides, dichloromethane, cyclohexane and carbonyl sulfide would also negatively affect the distribution of ARGs. In general, close relationship between VOCs and highly enriched ARGs and MGEs reveals that VOCs can affect the transfer and dissemination of ARGs during food waste decomposition and this mechanism may deserve increasing attention in the near future.

Overall, the ARG evolution profiles and fate during the decomposition of food wastes might be associated with different factors, including type of food wastes, decomposition time, concentration of emitted VOCs as well as physicochemical properties of leachates (Wang et al., 2015b; Su et al., 2017).

4.3.1. Correlation between ARG subtypes

Considering network analysis could offer fresh idea about ARGs and their potential hosts (Zhang et al., 2016), it was employed to reveal co-occurrence patterns among subtypes of ARGs in this study (Fig. 4). ARG co-occurrence was observed within the same or different ARG types.

The co-occurrence between the same ARG types like *aph(2')-Id-01* with other aminoglycoside resistance genes, reflecting selective pressure exerted by antibiotics, may shape antibiotic resistance; while the intertype ARG co-occurrence, for instance, the correlation between *aph(2')-Id-01* with MLSB (*lnuB-01*) and tetracycline (*tetM-01*), indicated that they are under the impulse of same mechanism of resistance (Ju et al., 2016). This is accordant with previous studies, which published co-occurrence mechanisms of different subtypes of ARGs: (1) aminoglycoside (*aph(30)-Vtb*, *aph(300)-Ib*, *aph(6)-Id*) and tetracycline (*tetA*) resistance genes in *Aeromonas* sp. ARM81 (Adamczuk and Dziejewicz, 2017); (2) *sul2*, *dfrA1* and *cmlA1* with class 1 integrase and IS6100-type transposons in swine farms in China (Johnson et al., 2016). As the most connected nodes in meat, vegetable and fruit, *aph(2')-Id-01*, *acrA-05*, *tetO-1* can be served as the representatives of unique ARGs. That is, these genes can act as ARG indicators for qualitative and quantitative analysis the co-occurrence of ARGs during food waste decomposition (Zhu et al., 2017).

4.3.2. Correlation of microbial community with variations of ARGs

Being possible hosts of ARGs, microbes will reproduce or decay under variant situations. As such, microbial community was also deeply related to the persistence and propagation of ARGs (Wu et al., 2017b). Network analysis, enabling us to observe complex relationships between microbial taxa and ARG, was demonstrated as a credible method to explore possible ARG hosts in complex environments (Huang et al., 2015; Forsberg et al., 2012). In other words, the corresponding similar abundance trends could be obtained due to certain microbial taxa harboring specific ARGs (Forsberg et al., 2014). As Fig. 6 shows, 8 genera may be the potential hosts of 4 most abundant ARGs (*lnuB-01*, *lnuB-02*, *aadE* and *aacA/aphD*) in meat wastes, while 20 and 25 bacterial taxa may be possible hosts harboring ARGs in vegetable and fruit wastes, respectively. Among them, the dominant *Prevotellaceae*-UCG-004 and *Lactobacillus* co-occurred with more ARGs than other genera, suggesting that they may also be the dominant factors shaping ARG profiles.

The above correlation between ARGs and most aerobic or facultative anaerobic bacteria was further validated using culture-based method. *Enterobacter cloacae* isolated from vegetable wastes, showing association with *tetC* and *blaPER*, was determined to be resistant to ampicillin ($100 \mu\text{g mL}^{-1}$) and tetracycline ($50 \mu\text{g mL}^{-1}$) (data not shown). *Lactobacillus plantarum* isolated from fruit wastes can suffer ampicillin ($100 \mu\text{g mL}^{-1}$) and gentamycin ($30 \mu\text{g mL}^{-1}$) (data not shown). Similarly, *Acinetobacter* as a possible host of *adeK*, has been proven previously (Lopes and Amyes, 2013). *Lactococcus* usually employed for milk products fermentation in dairy industry was detected with the multi-antibiotic resistance plasmid carrying *tetQ*, *tetM* and *ermB* (Mathur and Singh, 2005). *Enterococcus* was the host of *ermB* during refuse decomposition (Liu et al., 2018). Overall, these results showed that microbes were an important source of ARGs, which might conduce to ARG enrichment during decomposition and may deserve increasing concern. Nevertheless, considering most bacterial communities are anaerobe, it is necessary to make further efforts to probe into the relationships between microbial communities and ARGs under anaerobic conditions and offer valuable facts for ARG control in food waste treatment processes.

5. Conclusions

The work documented high diversity and extraordinary enrichment of ARGs as well as high correlation between bacterial community compositions and ARG structures during the decomposition of raw food wastes in lab. ARG abundance significantly increased at initial stage of waste fermentation and then decreased. Vegetable wastes have higher ARG diversity, while meat wastes have higher ARGs abundance. Genes resistance to multidrug, β -lactam, aminoglycoside, MLSB and tetracycline were the five most abundant ARGs and efflux pump as well as

antibiotic deactivation were two the predominant resistance mechanisms. ARG structures were mainly driven by the bacterial community shift. Besides, the positive correlation between VOCs and ARGs as well as MGEs suggested that VOCs might promote the transfer of ARGs. Finally, the ARG potential indicators and hosts were evaluated on the basis of co-occurrence pattern of ARG subtypes and microbial taxa. Overall, this study provides insight for ARGs behavior in food waste decomposition and will be useful for supervision as well as risk management of ARGs in enriched food waste landfill environments.

CRedit authorship contribution statement

Zhishu Liang: Methodology, Investigation, Writing - original draft. **Yuna Zhang:** Investigation. **Tao He:** Data curation. **Yun Yu:** Validation. **Wen Liao:** Methodology. **Guiying Li:** Conceptualization, Supervision. **Taicheng An:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by Science and Technology Program of Guangzhou, China (201704020185), National Natural Science Foundation of China (41877363, U1901210 and 41807405), Natural Science Foundation of Guangdong Province (2019A1515010599), and Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Z032).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2020.122973>.

References

- Adamczuk, M., Dziejewicz, L., 2017. Genome-based insights into the resistome and mobilome of multidrug-resistant *Aeromonas* sp. ARM81 isolated from wastewater. Arch. Microbiol. 199, 177–183.
- Aminov, R.I., Garrigues-Jeanjean, N., Mackie, R.I., 2001. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl. Environ. Microbiol. 67, 22–32.
- Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. ISME J. 5, 908–917.
- Beaber, J.W., Hochhut, B., Waldor, M.K., 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature 427, 72–74.
- Boyanova, L., Kolarov, R., Gergova, G., Dimitrova, L., Mitov, I., 2010. Trends in antibiotic resistance in *Prevotella* species from patients of the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria, in 2003–2009. Anaerobe 16, 489–492.
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838.
- Chen, Q., An, X., Li, H., Su, J., Ma, Y., Zhu, Y.G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ. Int. 92–93, 1–10.
- Chen, Q.L., Li, H., Zhou, X.Y., Zhao, Y., Su, J.Q., Zhang, X., Huang, F.Y., 2017. An underappreciated hotspot of antibiotic resistance: the groundwater near the municipal solid waste landfill. Sci. Total Environ. 609, 966–973.
- Chen, X., Yin, H., Li, G., Wang, W., Wong, P.K., Zhao, H., An, T., 2019. Antibiotic-resistance gene transfer in antibiotic-resistance bacteria under different light irradiation: implications from oxidative stress and gene expression. Water Res. 149, 282–291.
- D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debryne, R., Golding, G.B., Poinar, H.N., Wright, G.D., 2011. Antibiotic resistance is ancient. Nature 477, 457–461.
- Eckstrom, K., Barlow, J.W., 2019. Resistome metagenomics from plate to farm: the resistome and microbial composition during food waste feeding and composting on a Vermont poultry farm. PLoS One 14, e0219807.

- Ezaki, T., Kawamura, Y., Li, N., Li, Z.Y., Zhao, L.C., Shu, S.E., 2001. Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. *Int. J. Syst. Evol. Microbiol.* 51, 1521–1528.
- Forsberg, K.J., Reyes, A., Bin, W., Selleck, E.M., Sommer, M.O.A., Dantas, G., 2012. The shared antibiotic resistome of soil bacteria and human pathogens. *Science* 337, 1107–1111.
- Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 509, 612–616.
- Founou, L.L., Founou, R.C., Essack, S.Y., 2016. Antibiotic resistance in the food chain: a developing country-perspective. *Front. Microbiol.* 7, 19.
- Fu, J.L., Yang, D., Jin, M., Liu, W.L., Zhao, X., Li, C.Y., Zhao, T.Y., Wang, J.F., Gao, Z.X., Shen, Z.Q., Qiu, Z.G., Li, J.W., 2017. Aquatic animals promote antibiotic resistance gene dissemination in water via conjugation: role of different regions within the zebra fish intestinal tract, and impact on fish intestinal microbiota. *Mol. Ecol.* 26, 5318–5333.
- Garofalo, C., Vignaroli, C., Zandri, G., Aquilanti, L., Bordoni, D., Osimani, A., Clementi, F., Biavasco, F., 2007. Direct detection of antibiotic resistance genes in specimens of chicken and pork meat. *Int. J. Food Microbiol.* 113, 75–83.
- He, P., Yu, Z., Shao, L., Zhou, Y., Lü, F., 2019. Fate of antibiotics and antibiotic resistance genes in a full-scale restaurant food waste treatment plant: implications of the roles beyond heavy metals and mobile genetic elements. *J. Environ. Sci. (China)* 85, 17–34.
- Hu, Y., Yang, X., Qin, J., Lu, N., Cheng, G., Wu, N., Pan, Y., Li, J., Zhu, L., Wang, X., Meng, Z., Zhao, F., Liu, D., Ma, J., Qin, N., Xiang, C., Xiao, Y., Li, L., Yang, H., Wang, J., Yang, R., Gao, G.F., Wang, J., Zhu, B., 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nat. Commun.* 4, 2151.
- Hu, H.W., Wang, J.T., Li, J., Li, J.J., Ma, Y.B., Chen, D., He, J.Z., 2016. Field-based evidence for copper contamination induced changes of antibiotic resistance in agricultural soils. *Environ. Microbiol.* 18, 3896–3909.
- Huang, G., Xia, D., An, T., Ng, T.W., Yip, H.Y., Li, G., Zhao, H., Wong, P.K., 2015. Dual roles of capsular extracellular polymeric substances in photocatalytic inactivation of *Escherichia coli*: comparison of *E. coli* BW25113 and isogenic mutants. *Appl. Environ. Microbiol.* 81, 5174–5183.
- IACG, 2019. No Time to Wait: Securing the Future From Drug-resistant Infections, Reports to the Secretary-general on the United Nations.
- Johnson, T.A., Stedtfeld, R.D., Wang, Q., Cole, J.R., Hashsham, S.A., Looft, T., Zhu, Y.G., Tiedje, J.M., 2016. Clusters of antibiotic resistance genes enriched together stay together in swine agriculture. *mBio* 7, 11.
- Ju, F., Li, B., Ma, L.P., Wang, Y.B., Huang, D.P., Zhang, T., 2016. Antibiotic resistance genes and human bacterial pathogens: co-occurrence, removal, and enrichment in municipal sewage sludge digesters. *Water Res.* 91, 1–10.
- Jutkina, J., Rutgersson, C., Flach, C.F., Joakim Larsson, D.G., 2016. An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. *Sci. Total Environ.* 548–549, 131–138.
- Kim, K.S., Lee, S., Ryu, C.M., 2013. Interspecific bacterial sensing through airborne signals modulates locomotion and drug resistance. *Nat. Commun.* 4, 1809.
- Kiragosyan, K., Picard, M., Sorokin, D.Y., Dijkstra, J., Klok, J.B.M., Roman, P., Janssen, A.J.H., 2020. Effect of dimethyl disulfide on the sulfur formation and microbial community composition during the biological H₂S removal from sour gas streams. *J. Hazard. Mater.* 386, 121916.
- Kononen, E., Bryk, A., Niemi, P., Kanervo-Nordstrom, A., 2007. Antimicrobial susceptibilities of *Peptostreptococcus anaerobius* and the newly described *Peptostreptococcus stomatis* isolated from various human sources. *Antimicrob. Agents Chemother.* 51, 2205–2207.
- Lee, J., Shin, S.G., Jang, H.M., Kim, Y.B., Lee, J., Kim, Y.M., 2017. Characterization of antibiotic resistance genes in representative organic solid wastes: food waste-recycling wastewater, manure, and sewage sludge. *Sci. Total Environ.* 579, 1692–1698.
- Li, L.G., Xia, Y., Zhang, T., 2017. Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection. *ISME J.* 11, 651–662.
- Li, G., Chen, X., Yin, H., Wang, W., Wong, P.K., An, T., 2020. Natural sphalerite nanoparticles can accelerate horizontal transfer of plasmid-mediated antibiotic-resistance genes. *Environ. Int.* 136, 105497.
- Liao, H., Friman, V.-P., Geisen, S., Zhao, Q., Cui, P., Lu, X., Chen, Z., Yu, Z., Zhou, S., 2019. Horizontal gene transfer and shifts in linked bacterial community composition are associated with maintenance of antibiotic resistance genes during food waste composting. *Sci. Total Environ.* 660, 841–850.
- Lin, W., Zeng, J., Wan, K., Lv, L., Guo, L., Li, X., Yu, X., 2018. Reduction of the fitness cost of antibiotic resistance caused by chromosomal mutations under poor nutrient conditions. *Environ. Int.* 120, 63–71.
- Liu, X., Yang, S., Wang, Y., Zhao, H.P., Song, L., 2018. Metagenomic analysis of antibiotic resistance genes (ARGs) during refuse decomposition. *Sci. Total Environ.* 634, 1231–1237.
- Lopes, B.S., Amyes, S.G.B., 2013. Insertion sequence disruption of *adeR* and ciprofloxacin resistance caused by efflux pumps and *gyrA* and *parC* mutations in *Acinetobacter baumannii*. *Int. J. Antimicrob. Agents* 41, 117–121.
- Martinez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. *Science* 321, 365–367.
- Mathur, S., Singh, R., 2005. Antibiotic resistance in food lactic acid bacteria—a review. *Int. J. Food Microbiol.* 105, 281–295.
- Meng, X.S., Zhang, Y.X., Sui, Q.W., Zhang, J.Y., Wang, R., Yu, D.W., Wang, Y.W., Wei, Y.S., 2018. Biochemical conversion and microbial community in response to ternary pH buffer system during anaerobic digestion of swine manure. *Energies* 11, 17.
- Rahube, T.O., Marti, R., Scott, A., Tien, Y.C., Murray, R., Sabourin, L., Zhang, Y., Duenk, P., Lapen, D.R., Topp, E., 2014. Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. *Appl. Environ. Microbiol.* 80, 6898–6907.
- Ruimy, R., Brisabois, A., Bernede, C., Skurnik, D., Barnat, S., Arlet, G., Momcilovic, S., Elbaz, S., Moury, F., Vibet, M.A., Courvalin, P., Guillemot, D., Andremont, A., 2010. Organic and conventional fruits and vegetables contain equivalent counts of Gram-negative bacteria expressing resistance to antibacterial agents. *Environ. Microbiol.* 12, 608–615.
- Selvam, A., Xu, D., Zhao, Z., Wong, J.W.C., 2012. Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresour. Technol.* 126, 383–390.
- Su, J.Q., Wei, B., Ou-Yang, W.Y., Huang, F.Y., Zhao, Y., Xu, H.J., Zhu, Y.G., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ. Sci. Technol.* 49, 7356–7363.
- Su, Y., Wang, J., Huang, Z., Xie, B., 2017. On-site removal of antibiotics and antibiotic resistance genes from leachate by aged refuse bioreactor: effects of microbial community and operational parameters. *Chemosphere* 178, 486–495.
- van den Bogaard, A.E., Stobberingh, E.E., 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. *Int. J. Antimicrob. Agents* 14, 327–335.
- van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1159–1164.
- Verraes, C., Van Boxtael, S., Van Meervenne, E., Van Coillie, E., Butaye, P., Catry, B., de Schaeften, M.A., Van Huffel, X., Imberechts, H., Dierick, K., Daube, G., Saegerman, C., De Block, J., Dewulf, J., Herman, L., 2013. Antimicrobial resistance in the food chain: a review. *Int. J. Environ. Res. Public Health* 10, 2643–2669.
- Wang, H., McEntire, J.C., Zhang, L., Li, X., Doyle, M., 2012. The transfer of antibiotic resistance from food to humans: facts, implications and future directions. *Rev. Sci. Tech.* 31, 249–260.
- Wang, F.H., Qiao, M., Su, J.Q., Chen, Z., Zhou, X., Zhu, Y.G., 2014. High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. *Environ. Sci. Technol.* 48, 9079–9085.
- Wang, F.H., Qiao, M., Chen, Z., Su, J.Q., Zhu, Y.G., 2015a. Antibiotic resistance genes in manure-amended soil and vegetables at harvest. *J. Hazard. Mater.* 299, 215–221.
- Wang, Y.Q., Tang, W., Qiao, J., Song, L.Y., 2015b. Occurrence and prevalence of antibiotic resistance in landfill leachate. *Environ. Sci. Pollut. Res.* 22, 12525–12533.
- Wu, D., Huang, Z., Yang, K., Graham, D., Xie, B., 2015. Relationships between antibiotics and antibiotic resistance gene levels in municipal solid waste leachates in Shanghai, China. *Environ. Sci. Technol.* 49, 4122–4128.
- Wu, D., Huang, X.H., Sun, J.Z., Graham, D.W., Xie, B., 2017a. Antibiotic resistance genes and associated microbial community conditions in aging landfill systems. *Environ. Sci. Technol.* 51, 12859–12867.
- Wu, D., Chen, G., Zhang, X., Yang, K., Xie, B., 2017b. Change in microbial community in landfill refuse contaminated with antibiotics facilitates denitrification more than the increase in ARG over long-term. *Sci. Rep.* 7, 41230.
- Yang, N., Damgaard, A., Scheutz, C., Shao, L.M., He, P.J., 2018. A comparison of chemical MSW compositional data between China and Denmark. *J. Environ. Sci. (China)* 74, 1–10.
- Yin, H., Niu, J., Ren, Y., Cong, J., Zhang, X., Fan, F., Xiao, Y., Zhang, X., Deng, J., Xie, M., He, Z., Zhou, J., Liang, Y., Liu, X., 2015. An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. *Sci. Rep.* 5, 14266–14266.
- You, X., Wu, D., Wei, H., Xie, B., Lu, J., 2018. Fluoroquinolones and beta-lactam antibiotics and antibiotic resistance genes in autumn leachates of seven major municipal solid waste landfills in China. *Environ. Int.* 113, 162–169.
- Yu, Z., He, P., Shao, L., Zhang, H., Lu, F., 2016. Co-occurrence of mobile genetic elements and antibiotic resistance genes in municipal solid waste landfill leachates: a preliminary insight into the role of landfill age. *Water Res.* 106, 583–592.
- Zhang, J.Y., Chen, M.X., Sui, Q.W., Tong, J., Jiang, C., Lu, X.T., Zhang, Y.X., Wei, Y.S., 2016. Impacts of addition of natural zeolite or a nitrification inhibitor on antibiotic resistance genes during sludge composting. *Water Res.* 91, 339–349.
- Zhang, Y., Liang, Z., Tang, C., Liao, W., Yu, Y., Li, G., Yang, Y., An, T., 2020. Malodorous gases production from food wastes decomposition by indigenous microorganisms. *Sci. Total Environ.* 717, 137175.
- Zhao, R., Feng, J., Yin, X., Liu, J., Fu, W., Berendonk, T.U., Zhang, T., Li, X., Li, B., 2018. Antibiotic resistome in landfill leachate from different cities of China deciphered by metagenomic analysis. *Water Res.* 134, 126–139.
- Zhou, R., Zeng, S., Hou, D., Liu, J., Weng, S., He, J., Huang, Z., 2019. Occurrence of human pathogenic bacteria carrying antibiotic resistance genes revealed by metagenomic approach: a case study from an aquatic environment. *J. Environ. Sci. (China)* 80, 248–256.
- Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3435–3440.
- Zhu, B., Chen, Q., Chen, S., Zhu, Y.G., 2017. Does organically produced lettuce harbor higher abundance of antibiotic resistance genes than conventionally produced? *Environ. Int.* 98, 152–159.
- Zhu, D., Chen, Q.L., Li, H., Yang, X.R., Christie, P., Ke, X., Zhu, Y.G., 2018. Land use influences antibiotic resistance in the microbiome of soil collembolans *Orchesellidius sinensis*. *Environ. Sci. Technol.* 52, 14088–14098.