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Effect of temperature on sulfonamide antibiotics degradation, and on antibiotic resistance determinants and hosts in animal manures

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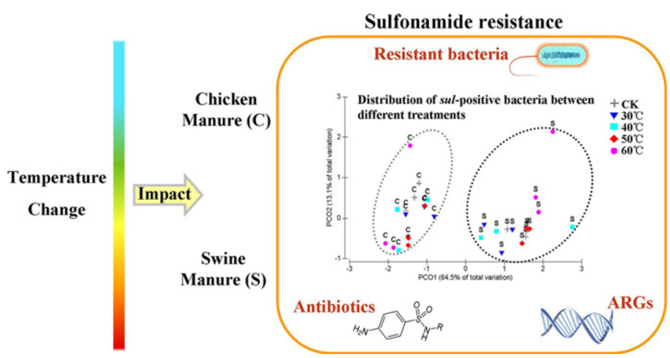
HIGHLIGHTS

- Temperature response of sulfonamide resistance in manure during aerobic incubation
- The effect of temperature on SAs and SR gene/bacteria vary with manure types
- Thermophilic removal of SAs and SR genes/bacteria is only evident in swine manure
- The antibiotic residual profile affects the prevalence of SR genes/bacteria in manure
- Differences in SR bacterial communities influence temperature responses of ARGs

GRAPHICAL ABSTRACT



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ABSTRACT

Animal manure is a main reservoir of antibiotic residues and antibiotic resistance. Here, the effect of temperature on sulfonamide antibiotics (SAs), sulfonamide-resistant (SR) genes/bacteria was investigated by aerobically incubating swine and chicken manures at different temperatures. In swine manure, the SAs concentration declined with increasing temperature, with a minimum at 60 °C. In chicken manure, the greatest degradation of SAs was noted at 30 °C. The reduction of relative abundance of antibiotic resistance genes (ARGs) and *sul*-positive hosts in swine manure was more pronounced during thermophilic than mesospheric incubation; neither temperature conditions effectively reduced these parameters in chicken manure. The relationship between the residual levels/distribution profiles of SAs, ARGs (*sul1*, *sul2* and *intI1*), cultivable SR bacteria and *sul*-positive hosts was further established. The antibiotic residual profile, rather than antibiotic concentration, acted as an important factor in the prevalence of ARGs and *sul*-positive hosts in manure. *Corynebacterium* and *Leucobacter* from the phylum Actinobacteria tend to be main carriers of *sul1* and *intI1*; the relative abundance of *sul2* was significantly correlated with the relative abundance of cultivable SR bacteria. Overall, differences in resistant bacterial communities also constitute a dominant factor affecting ARG variation. This study contributes to management options for reducing the pollution of antibiotics and antibiotic resistance within manure.

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1. Introduction

Sulfonamide antibiotics (SAs) are routinely used to treat a variety of bacterial and protozoan infections in cattle, swine, and poultry, and have been classed as 'high priority' veterinary medicines (Boxall et al., 2003). SAs, either as metabolites or parent compounds, are frequently detected in animal dung or derived products, sometimes at high concentrations. In a study monitoring veterinary antibiotics in animal dung (Martínez-Carballo et al., 2007), sulfamethazine (sulfadimidine, SMZ) was found in 60% of pig manure samples (up to 20 mg kg^{-1}) and sulfadiazine (SDZ) was present in turkey manure even at 91 mg kg^{-1}. Qian et al. (2016) reported that up to 40% of pig manure-based fertilizers from the Zhejiang province of China contain SA residues, and the maximum concentration of SMZ and sulfamonomethoxine (SMM) in these manure-based fertilizers can reach 4.18 mg kg^{-1} and 2.57 mg kg^{-1}, respectively. Accordingly, animal manure serves as a reservoir of antibiotic resistance genes (ARGs) and resistant bacteria (Zhu et al., 2013; Wichmann et al., 2014). The sulfonamide ARGs are one of the most frequently detected ARGs in the environment (Munir and Xagoraraki, 2011; Ji et al., 2012; Cheng et al., 2013; Lin et al., 2016); in chicken-manure based fertilizer, the absolute copy number of sulfonamide ARG and *intI1* can be 10^1- to 10^5-fold higher than that of tetracycline ARG (Lin et al., 2016). Antibiotics and antibiotic resistance genes/bacteria appear in the environment following the land application of manure, and can pose health and environmental problems (Sarmah et al., 2006). Mounting evidence suggests that the release of anthropogenic antibiotic residues, ARGs, and resistant bacteria into the environment increases the risk of emergence of multi-resistant animal pathogens, ultimately reducing the efficiency of antibiotic therapies (Martinez, 2008; Pruden et al., 2012). Particularly, the ecological risk of SAs is high owing to their high biological toxicity (Wang et al., 2015b) and recalcitrance (Ma et al., 2014).

Composting can be an efficient, simple, and economically feasible manure management for preventing the release of antibiotics, ARGs, and resistant bacteria from manures into the farmland soil (Storteboom et al., 2007; Selvam et al., 2012a; Selvam et al., 2012b; Zhu et al., 2013). However, the removal efficiencies of residual antibiotics, and particularly ARGs, by some composting technologies are still low and require further improvement. Zhu et al. (2013) observed that the relative abundance of sulfonamide ARGs in composted pig manure of Futian (China) were higher than before composting, whereas the trend was reversed in samples from Beijing (China). Temperature should be a critical variable affecting the fate of antibiotics and resistant gene/bacteria in many systems including composting. Not only the degradation of SAs (Wang et al., 2006; Srinivasan and Sarmah, 2014) but also the prevalence of some ARGs (also *sul1* and *sul2*) (Pei et al., 2007) have been previously reported to be temperature dependent. It is generally considered that thermophilic stage is optimal for antibiotic degradation (Selvam et al., 2012b) and the inhibition of resistant bacteria/genes (Selvam et al., 2012a) during composting; however, little research has been explicitly directed to determine how antibiotics and the corresponding resistance determinants within manure respond to temperature changes, and at which composting temperature the antibiotic concentration greatly decreases and the inhibition of antibiotic resistance spread is initiated. In anaerobic digestion, several works (Diehl and LaPara, 2010; Sun et al., 2016) indicated that thermophilic condition reduced the abundance of some ARGs (also *sul1* and *sul2*) more effectively than mesophilic condition, but others (Zhang et al., 2015) detected no obvious differences. Diehl and LaPara (2010) showed that the temperature responses of tetracycline ARGs and *intI1* within wastewater solids can be different between anaerobic and aerobic incubation. Since the types and abundances of ARGs in manure often differ greatly from those found in wastewater solids/sludge, is manure antibiotic and resistant determinants more dramatically reduced by thermophilic conditions than mesophilic conditions during aerobic incubation?

In this study, we compared the changes in SAs, sulfonamide-resistant (SR) bacteria, *sul* ARGs and *intI1* content within two different manure types in aerobic incubation at different temperatures. We evaluated the temperature responses of SAs and their resistance determinants, and delineated the relationship between SAs, SR bacteria, and target ARGs.

2. Materials and methods

2.1. Raw materials and reagents

Fresh swine manure was collected from backyard pig farms for pork supply located in Yiwu City (Zhejiang Province, China) in August, which was black-brown, humid, and with a strong odour. Fresh chicken manure was sampled at a broiler poultry farm in Yiwu City in August. Since rice husk litter was used during breeding, the chicken manure sample contained a large proportion of rice husk, and was yellow-brown, dry, and with a mild odour. Fresh swine and chicken manures had 68.7% or 60.5% water content, pH 7.4 or 8.8, 33.8% or 38.1% total carbon content (dry weight basis), and 2.6% or 2.9% total nitrogen content (dry weight basis), respectively. All the fresh manure samples were stored at 4–6 °C after collection.

2.2. Bench-scale study

Both fresh swine and chicken manures were subjected to solid-state incubation at different temperatures (30 °C, 40 °C, 50 °C and 60 °C) in incubators for 5 d. Four temperature treatments were designed for each manure type. For each treatment, 15 g of dry–weight equivalent fresh manure were laid flat in a Petri dish (9 cm diameter). Three biological replicates were performed for each treatment. The manure sample in each dish after incubation was divided into two parts: one was used for cultivable SR bacteria analysis and water content determination immediately after collection; another was stored at −20 °C or below for antibiotic concentration determination and genomic DNA extraction. The background level (BL) of residual antibiotics, ARGs and SR bacteria in original manure samples before incubation were also determined.

2.3. Determination of SAs concentrations in manure

SA residues were extracted from manure samples, purified, and subjected to high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis following the procedures described by Qian et al. (Qian et al., 2016), with a brief introduction of procedure shown in Supplementary materials. Twenty different SAs were analysed, including sulfacetamide, sulfadiazine (SDZ), sulfathiazole, sulfapyridine, sulfamerazine, sulfamoxol, sulfamethizole, sulfamethazine (SMZ), sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine (SMM), sulfadoxine, sulfabenzamide, sulfadimethoxine (SDM), sulfaquinoxaline (SQ), sulfanilamide, sulfameter, sulfamethoxazole (SMX), sulfisoxazole and sulfaphenazole. The recoveries of the twenty SAs were in the range of 71.6% to 110.7% in manure samples. Limit of detection (LOD) and Limit of quantification (LOQ) were in the range of 1.6–3.6 μg kg^{-1} and 5.3–12.0 μg kg^{-1}, respectively. All the data of antibiotic concentrations were expressed on a dry weight basis.

2.4. Enumeration of cultivable SR bacteria

Cultivable SR bacteria in manure were quantified by growing on nutrient broth agar plates containing SAs, followed by spread plating technique as previously described (Wang et al., 2014). In this work, the mixture of SDZ and SDM was used as the representative SA to prepare SR nutrient broth agar plates because they occurred in both swine and chicken manures herein at relatively high concentrations. Briefly, manure samples (5 g) were placed in 45 mL of sterilized water, in a flask,