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Research article

Dynamics of oxytetracycline, sulfamerazine, and ciprofloxacin and related antibiotic resistance genes during swine manure composting

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ABSTRACT

Understanding the dynamics of veterinary antibiotic and related antibiotic resistance genes (ARGs) during swine manure composting is crucial in assessing the environmental risk of antibiotics, which could effectively reduce their impact in natural environments. This study investigated the dissipation of oxytetracycline (OTC), sulfamerazine (SM1) and ciprofloxacin (CIP) as well as the behaviour of their corresponding ARGs during swine manure composting. These antibiotics were added at two concentration levels and two different methods of addition (single/mixture). The results indicated that the removal efficiency of antibiotics by composting were $\geq 85\%$, except for the single-SM1 treatment. The tetracycline resistance genes (TRGs) encoding ribosomal protection proteins (RPP) and efflux pump (EFP) and fluoroquinolone resistance genes (FRGs) could be effectively removed after 42 days. On the contrary, the TRGs encoding enzymatic inactivation (EI) and sulfonamide resistance genes (SRGs) were enriched up to 31-fold (*sul* 2 in single-low-SM1). Statistical analyses indicated that the behaviour of these class antibiotics and ARGs were controlled by microbial activity and significantly influenced by environmental factors (mainly C/N, moisture and pH) throughout the composting process.

1. Introduction

In recent years, the environmental pollution of antibiotics and antibiotic resistance genes (ARGs) has become a serious concern worldwide, due to the extensive use of veterinary antibiotics in livestock farming to control diseases and promote growth. As the largest antibiotic-consuming country in the world, it is estimated that more than half of the total usage (over 84,000 tons) was applied in the livestock and poultry industry in China for 2013 (Zhang et al., 2015). However, as much as 30–90% of the applied antibiotics are excreted in their parent compounds or primary metabolites through manure and urine (Sarmah et al., 2006). Those non-metabolized antibiotic residues in the animal manure become a significant source of antibiotics and induce the development of antibiotic resistant microbes in the environment (Guo et al., 2018).

Tetracyclines (TCs), fluoroquinolones (FQs) and sulfonamides (SAs) are three commonly used and frequently detected classes of veterinary antibiotics in China. According to the previous reports, the highest detected concentrations of ciprofloxacin (CIP), sulfamerazine (SM1) and oxytetracycline (OTC) in swine manure from different regions in China reached as high as 10, 17 and 354 mg kg^{-1}, respectively (Chen et al., 2012; Hou et al., 2015; Wang et al., 2017). The presence of residual antibiotics in manures can produce selective pressure for antibiotic resistant bacteria and ARGs that can enter the environment through a variety of pathways, especially land application of animal manure (He et al., 2016; Joy et al., 2013; Tang et al., 2015). The spread of these corresponding ARGs caused by discharge of swine waste has been frequently reported (Brooks et al., 2014; He et al., 2016). The tetracycline resistance genes (TRGs, including encoding ribosomal protection proteins (RPP), efflux pump (EFP), and enzymatic inactivation (EI) proteins), the sulfonamide resistance genes (SRGs) and fluoroquinolone resistance genes (FRGs) are commonly present in swine manure and manure-amended soil (Tang et al., 2015; Wang et al., 2015a, 2016a). These ARGs can be easily disseminated by means of bacterial reproduction and horizontal gene transfer (HGT) among bacteria in the environment. The transfer of ARGs, especially through HGT, may confer a novel antibiotic resistance in another organism, which could be a source of potential harm to the health of people and/or the environment (Keese, 2008).

Due to the production of a large amount of swine manure every year

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in China, it can be assured that swine manure is an important source of antibiotics and ARGs in elevated levels (Cheng et al., 2013; Hou et al., 2015). Moreover, because of the direct application of swine manure as fertilizer by local farmers, farmland is vulnerable to contamination by antibiotics and ARGs through direct application of swine manure (Joy et al., 2013; Tang et al., 2015). Therefore, it is urgent to significantly reduce or cut-off antibiotics and ARGs applications into the environment from swine manure. Previous studies have shown that aerobic composting could effectively remove residual antibiotics in swine manure (Arikan et al., 2009; Selvam et al., 2012b). Selvam et al. (2012b) found that chlortetracycline and sulfadiazine were completely removed from the composting mass within 21 and 3 d, respectively; whereas, 17–31% of the spiked ciprofloxacin remained in the composting mass. However, norfloxacin and ofloxacin were detected as the dominant antibiotics in swine thermophilic compost samples with concentrations of 1192 and 1802 μg kg^{-1}, respectively, which were significantly higher than those in the corresponding manures, due to the high adsorption of fluoroquinolones by organic matter (Xie et al., 2016). The presence of antibiotics can generate a selective pressure for ARGs, and thus there must be certain relationships between some ARGs and concentrations of antibiotics (Chao et al., 2016). Several studies have shown that the composting process is effective for reducing the abundance of several ARGs (Selvam et al., 2012a; Wallace et al., 2018; Wang et al., 2012), but other results suggested that the thermophilic composting of swine manure failed to prevent the proliferation of ARGs (Wang et al., 2015b). However, little information is available regarding the fate of oxytetracycline, sulfamerazine and ciprofloxacin and related ARGs when present during aerobic swine manure composting. Exploring the dynamic relationship between those antibiotics and related ARGs is important to effectively reduce the residual antibiotics and ARGs during composting.

In the present study, we hypothesized that antibiotic concentrations would have an important influence on the abundance of ARGs during composting. Therefore, the major objectives of this study were (1) to quantify the dynamic changes of three antibiotics and their corresponding ARGs and (2) to determine the relationships between residual antibiotics, their corresponding ARGs and physicochemical conditions during thermophilic aerobic composting of swine manure. Three typical antibiotics, including OTC, SM1 and CIP were selected for this study because they are commonly used in swine production in China.

2. Materials and methods

2.1. Reagents and chemicals

OTC, SM1 and CIP were obtained from Dr. Ehrenstorfer (Augsburg, Germany) (Table S1). The isotopes of norfloxacin-d5, sulfadiazine-d4 and tetracycline-d6 were purchased from WITEGA Laboratories (Berlin, Germany), C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada) and TRC, Toronto Research Chemicals (Toronto, Canada), which were used as the internal standards of CIP, SM1 and OTC, respectively. Methanol (MeOH) and acetonitrile (ACN) (HPLC grade) were acquired from Fisher Science Co. Milli-Q water from a Milli-Q Advantage A10 system (Millipore, USA) was used when ultrapure water was required.

2.2. Composting operation

The swine manure was collected from a large-scale farm in Daxing District of Beijing, China. The fresh manure was spread, and air dried (15–20 °C) to a water content < 30%, crushed, and sieved through a 5-mm mesh, then swine samples were analyzed for physicochemical properties including antibiotics. The manure had a pH of 7.57, total organic carbon (TOC) of 29.62%, total nitrogen (TN) of 2.50%. The concentrations of CIP and SM1 in the collected swine manures were not detected and OTC was below the method quantification limit (MQL) according to the limits described in subsection 2.3. The wheat straw and saw dust had total organic carbon contents of 44.63% and 49.27%, and total nitrogen contents of 1.13% and 0.09%, respectively.

The composting experiment was performed in an organic fertilizer plant at Daxing district of Beijing, China. Bubble boxes were used as composting units (Fig. S1). The swine manure with a moisture content of less than 30% was pre-spiked with OTC, SM1 and CIP separately (single-compound treatments) and together (mixture-compounds treatments) at two concentration levels: high (200 mg kg^{-1} OTC, 10 mg kg^{-1} SM1, 10 mg kg^{-1} CIP or 200 mg kg^{-1} OTC + 10 mg kg^{-1} SM1 + 10 mg kg^{-1} CIP) and low (20 mg kg^{-1} OTC, 1 mg kg^{-1} SM1, 1 mg kg^{-1} CIP or 20 mg kg^{-1} OTC + 1 mg kg^{-1} SM1 + 1 mg kg^{-1} CIP). To achieve the most homogeneous distribution, the target antibiotics in aqueous solution were sprinkled over the swine manures through manual sprayer, stirring constantly. Then spiked swine manures were allowed to equilibrate for 2 days and stirred regularly during this period. Meanwhile, the initial concentrations of target antibiotics in the composting were confirmed by the measured value after this period. A control treatment was also prepared without the addition of antibiotics. There were 9 treatments in total. For each treatment, wheat straw (8.76 kg, dw) and saw dust (3.75 kg, dw) were mixed with swine manure (15 kg, dw) to adjust the C/N to ~25 and moisture content to ~55%. The mixture of these raw materials was then put into a bubble box and the volume of mixtures was about 137 L (Fig. S1). Each treatment was repeated in triplicate. Considering the future use of the compost, the moisture was not re-adjusted during the composting process. The composts were allowed to process for 42 days, and turned every day during the first week and every two days after the first week to maintain aerobic conditions and homogeneity of the materials. During the composting period, a temperature detector continuously sensed the temperature in the centre of composting pile continuously. As shown in Fig. S1, composite samples of compost were prepared by mixing three subsamples, respectively, taken from the bottom, middle and top layers of each pile on days 0, 3, 5, 7, 17, 21, 28, and 42. One part of each composite sample was used for the measurement of moisture, wheat seed germination index (GI) immediately after preparation, one part was freeze-dried for the analysis of the pH, TOC, TN and targeted antibiotics, and the rest of the part was stored at −80 °C for later ARGs abundance analyses. The detailed processes of physicochemical and GI analysis of compost are summarized in the Supporting Information.

2.3. Determination of antibiotics

The targeted antibiotics were extracted from freeze-dried composite samples (Ho et al., 2013) and determined by an Agilent 1200 series HPLC equipped with a Sunfire C18 column (150 × 4.6 mm, 3.5 μm, Waters, USA) and an Agilent 6410 Triple Quadrupole mass spectrometer. Ultrapure water containing 0.1% formic acid (v/v) (A) and ACN (B) were used as the mobile phase at a total flow rate of 0.4 mL min^{-1}. A linear gradient elution was conducted as follows: 0 min, 8% B; 0–12 min, linear increase to 55% B; 12–13 min, linear decrease to 8% B; 13–16 min, 8% B. The MS parameters were listed in Table S2. The recovery and method quantification limit (MQL) of target antibiotics ranged from 62% to 89%, and 7.6 to 13.7 μg kg^{-1} (dw), respectively (Table S3). The detailed procedures of antibiotic analysis were described in the Supporting Information.

All the samples were analyzed in triplicate and procedural blanks (prepared without the targeted antibiotics or without the compost samples) were run to detect the possible sources of interference, and the relative standard deviation was less than 10%. Dissipation kinetics of the three antibiotics was calculated using the first-order kinetic model:

$$\frac{dC}{dt} = -kC \quad (1)$$

where C represents the concentration of target antibiotic at the time t, and k represents the rate constant. The half-life ($t_{1/2}$) of the antibiotics