



Dissemination of veterinary antibiotics and corresponding resistance genes from a concentrated swine feedlot along the waste treatment paths



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ABSTRACT

Swine feedlots are an important pollution source of antibiotics and antibiotic resistance genes (ARGs) to the environment. This study investigated the dissemination of two classes of commonly-used veterinary antibiotics, namely, tetracyclines (TCs) and sulfonamides (SAs), and their corresponding ARGs along the waste treatment paths from a concentrated swine feedlot located in Beijing, China. The highest total TC and total SA concentrations detected were 166.7 mg kg^{-1} and $64.5 \mu\text{g kg}^{-1}$ in swine manure as well as 388.7 and $7.56 \mu\text{g L}^{-1}$ in swine wastewater, respectively. Fourteen tetracycline resistance genes (TRGs) encoding ribosomal protection proteins (RPP), efflux proteins (EFP) and enzymatic inactivation proteins, three sulfonamide resistance genes (SRGs), and two integrase genes were detected along the waste treatment paths with detection frequencies of 33.3–75.0%. The relative abundances of target ARGs ranged from 2.74×10^{-6} to 1.19. The antibiotics and ARGs generally declined along both waste treatment paths, but their degree of reduction was more significant along the manure treatment path. The RPP TRGs dominated in the upstream samples and then decreased continuously along both waste treatment paths, whilst the EFP TRGs and SRGs maintained relatively stable. Strong correlations between antibiotic concentrations and ARGs were observed among both manure and wastewater samples. In addition, seasonal temperature, and integrase genes, moisture content and nutrient level of tested samples could all impact the relative abundances of ARGs along the swine waste treatment paths. This study helps understand the evolution and spread of ARGs from swine feedlots to the environment as well as assess the environmental risk arising from swine waste treatment.

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1. Introduction

Antibiotics are widely used in livestock and poultry industries for disease control and growth promotion. It is estimated that 30–90% of the applied antibiotics cannot be digested or metabolized by animals, but excreted to the environment in their original forms with animal manure and urine (Sarmah et al., 2006). Tetracyclines (TCs) and sulfonamides (SAs) are two classes of the most commonly administrated veterinary antibiotics in concentrated swine feeding operations of China. In our previous studies, TCs and SAs were detected with high frequencies and concentrations in swine waste samples collected from 21 concentrated swine feedlots in Shandong province, China, with the highest concentration of 2.02 mg L^{-1} for oxytetracycline and $717 \mu\text{g L}^{-1}$ for sulfamethazine in swine wastewater (Ben et al., 2013), as well as 764.4 mg kg^{-1} for chlortetracycline and 28.7 mg kg^{-1} for sulfamethazine in swine manure (Pan et al., 2011a).

China has the largest pork production in the world, which produces large amounts of manure and wastewater containing eutrophication elements and residual antibiotics every year. The presence of residual antibiotics could enhance the resistance level of microbial communities and promote the proliferation of antibiotic resistance genes (ARGs) in swine manure (Loft et al., 2012), livestock and poultry wastewater (Mckinney et al., 2010; Peak et al., 2007), farmland soil (Huang et al., 2013; Wu et al., 2010), and surface water (Graham et al., 2010; Luo et al., 2010). The residual antibiotics could promote the accumulation of ARGs even at subtherapeutic levels (Ghosh and Lapara, 2007). Nevertheless, most concentrated swine feedlots in China are lack of sufficient waste treatment facilities (Tong et al., 2009), especially for wastewater treatment (Ben et al., 2013), which makes them important reservoirs of antibiotics and ARGs (Cheng et al., 2013; Zhao et al., 2010; Zhu et al., 2013). As a result, environmental contamination of antibiotics and ARGs through unregulated discharge or use of swine wastes has been frequently reported. For example, Hu et al. (2010) found that the antibiotic concentrations in soil were closely related to the manure fertilization events. Wei et al. (2011) revealed that livestock and poultry wastewater could contaminate surrounding water systems as a major pollution source of antibiotics. Likewise, ARGs could also be easily disseminated in the environment through manure application (Heuer

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and Smalla, 2007; Peng et al., 2015) and wastewater discharge (Koike et al., 2007). However, up to date, studies on the transport of ARGs along manure and wastewater treatment paths in swine feedlots are very limited, and the correlations between residual antibiotics and ARGs need to be clarified. Moreover, although many studies have reported that besides antibiotics, other influential factors such as temperature (Pei et al., 2007), and moisture content (Chee-Sanford et al., 2009) and nutrient level (Séveno et al., 2002) of samples could also affect the behavior of ARGs, the relationships between these factors and ARGs along the waste treatment paths were not examined.

Therefore, a field investigation was conducted on the dissemination of TCs, SAs and their corresponding ARGs along both wastewater and manure treatment paths from a typical concentrated swine feedlot located in Beijing, China. Polymerase chain reaction (PCR) was applied to detect the target ARGs in collected samples, including four mutant dihydropteroate synthase genes reflecting the primary mechanism of sulfonamide resistance (Sköld, 2000), and sixteen tetracycline resistance genes (TRGs) covering three main mechanisms of tetracycline resistance (ribosomal protection protein (RPP), efflux protein (EFP), and enzymatic inactivation) (Roberts, 2012; Thaker et al., 2010). Then, the frequently detected TRGs and sulfonamide resistance genes (SRGs) were further quantified by quantitative PCR (q-PCR). In addition, the effects of various influential factors (i.e., seasonal temperature, and integrase genes, moisture content and nutrient level of collected samples) on the dissemination of ARGs along the waste treatment paths were also evaluated.

2. Materials and methods

2.1. Swine feedlot and sample collection

A concentrated swine feedlot located in Tongzhou district of Beijing, representative of swine feedlots in Northern China, was selected as the studied site with its waste (manure and wastewater) treatment illustrated in Fig. 1. The feedlot covered an area of 66,000 m² and kept about 6,000 pigs inside. TCs and SAs were two major classes of

antibiotics used in this feedlot as feed additives and injectable drugs. Swine wastewater, mainly composed of swine urine, swine bathing water and piggery manure washing water, was successively discharged to a collection channel, a settling pond, and finally a storage lagoon. As the frequencies of swine bathing and piggery washing were reduced in winter, much less wastewater was discharged into the storage lagoon than in summer. Swine manure and the settled solids in the settling pond were periodically gathered and transported to an adjacent composting plant located across the road. A cropland located to the east of the composting plant was fertilized with the mature compost. In this study, the following samples were collected along the manure treatment path: piglet manure (M1), sow manure (M2) and fattening pig manure (M3) from the swine houses; settled solids (WS1) from the settling pond; mature compost from the composting plant (S1); fertilized soil from the cropland (S2); and unfertilized soil from the road-side adjacent to the cropland (S3). Samples collected along the wastewater treatment path included: wastewater from the collection channel (W1); wastewater from the settling pond (W2); and wastewater (W3) and sediment (WS2) from the storage lagoon. The samples were collected in winter (February) and summer (August) of 2013, and at each sampling site, four subsamples were collected and then mixed into one composite sample. All samples were stored in ice-packed coolers and transported immediately to laboratory for pretreatment and analysis.

2.2. Chemical analysis

For the analysis of target antibiotics, the wastewater samples (i.e., W1, W2 and W3) were adjusted to pH 2.5–3.0 and centrifuged at 5000 ×g for 15 min to separate the supernatant and suspended solids. Afterward, the liquid phase was subjected to solid phase extraction (SPE) (Ben et al., 2008), and the solid phase at the bottom of centrifuge tubes was collected and subjected to ultrasonic solvent extraction (USE) and SPE (Pan et al., 2011b). The detected concentrations in both liquid and solid phases were summed up as the total antibiotic concentration in a wastewater sample. The solid samples (i.e., M1, M2, M3, S1, S2, S3,

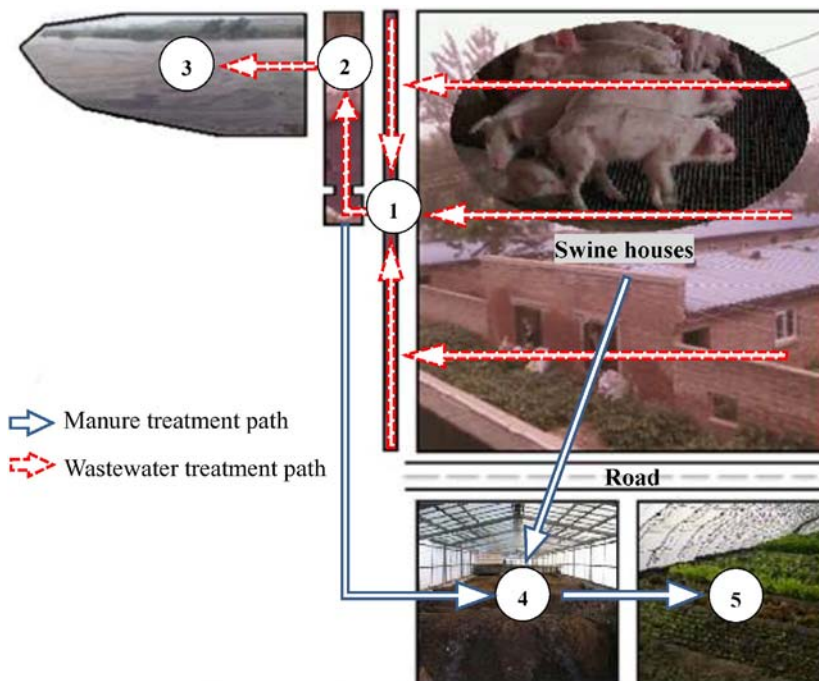


Fig. 1. Schematic diagram of waste treatment in the studied swine feedlot: wastewater collection channel (1); settling pond (2); storage lagoon (3); composting plant (4); and compost fertilized cropland (5).

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