Occurrence, Distribution and Risk Assessment of Five Kinds of Antibiotics in Mattress on Swine Farm use Ectopic Fermentation Systems in Zhejiang Province

Zhou wei
zhengjiang center for animal disease prevention and control

suo decheng (suodecheng@caas.cn)
Chinese Academy of Agricultural Sciences

https://orcid.org/0000-0001-9463-0297

xia fan
CAAS: Chinese Academy of Agricultural Sciences

zhiming xiao
ciaas

hangjun Zhang
zhengjiang center for animal and disease prevention and control

zhijin Zhou
zhengjiang center for animal disease prevention and control

xuan Huo
zhengjiang center for animal disease prevetion and control

yang Chong
zhengjiang center for animal disease prevention and control

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Abstract

Mattress is among the main products of ectopic fermentation system (EFS), however, the research on the data of antibiotic residues in the mattress of EFS and risk assessments of mattress have not been conducted. This study involved a scale survey to assess the levels and distributions of 54 antibiotics residues, including 4 tetracyclines, 19 quinolones, 22 sulfonamides, 3 amphenicols, and 6 macrolides in mattress on 12 swine farm that uses ectopic fermentation systems (EFS) in Zhejiang Province. A total of 25 antibiotics were detected in mattress, and the total residue amount of antibiotics in mattress samples of each farm was 0.77–28.2 g/T. Chlortetracycline had the highest contribution rate, and the residue amount of antibiotics in mattress is not entirely determined by the start-up time of EFS but is related to the use of feed containing antibiotics, medication habits, the level of mattress management, and maintenance methods of EFS. The risk assessments of antibiotics in the mattress were carried out. The results show low risk for soil on swine farm that uses EFS.

1 Introduction

Considering the low utilization rate of swine waste resources, their proper handling has become difficult during swine breeding. The development of scale swine breeding industry experiences important issues on environmental pollution (Oliveira C, 2001; Won S G, 2016). In recent years, ectopic fermentation system (EFS), as a new swine waste treatment technology, was developed for waste treatment by using a complex microbial preparation of functional thermophilic microbes mixed with straw as carrier prior to fermentation (Shen, 2021; Jiang, 2020, Yang, 2018, Guo, 2015). Figure 1 shows the working process of EFS. After dynamic spraying, ploughing, and fermentation, the odor of raw swine waste can be effectively controlled and treated. At present, with the prevention and control of African swine fever, EFS has been developed for waste treatment in a swine farm in Zhejiang Province, and this method has the advantage of separating from the wastes core area, satisfying the requirements of biological protection and convenient maintenance (Guo, 2015; Jiang, 2020).

Antibiotics are used for livestock, and approximately 46.1% of antibiotics produced (~90,000 tons) in China every year are used in animal husbandry (Shen, 2014, Zhang, 2015). In the United States, nearly 50% of antibiotics (~22,700 tons) are used in animals and aquaculture every year (Kümmerer, 2009). Van Boecke et al. conservatively estimated that the total consumption antibiotics in animal production in 2010 is 63,151 ± 1,560 tons, and the antibiotic consumption is expected to increase to 67% by 2030 (Van Boeckel, 2015). Serious dependence or even overuse of antibiotics will lead to two major problems in safety, namely, antibiotic residues and bacterial resistance, which not only do harm to the sustainable development of animal husbandry, but also seriously threaten food safety, human health, and ecological environment (Ramawamy, 2010; Hamscher, 2018).

Mattress is among the main products of EFS. In ectopic fermentation, high-temperature-resistant microorganisms are used to degrade feces and urine by aerobic fermentation (Jiang, 2020; Yang, 2018; Shen, 2019). Then, mattresses are used as organic fertilizer for agricultural soil. Antibiotics used in swine farm are mostly excreted in the form of prototypes through feces and urine (Rasschaert, 2020; Wohde, 2016). In the process of EFS, these antibiotics continuously accumulate in the mattress through the spray of feces and urine with prolonged fermentation accumulation time. If the amount exceeds the acceptable range, it may have adverse effects on the living environment of the normal soil and the horizontal transmission of drug resistance genes in the digestion area. Moreover, antibiotics in mattress have a negative effect on the microbial growth of mattress (Shen 2019). Efficient and low-cost treatment of mattress, the environmental safety of antibiotic consumption area, and the quality of mattress products can be ensured by solving the problems on antibiotic residues in matters of EFS and the safety evaluation of mattress.

Previous studies have investigated the ability of thermophiles and improve the fermentation performance in EFS (Shen, 2019; Jiang, 2020; Yang, 2018; Guo 2015). However, the research on the data of antibiotic residues in the mattress of EFS and risk assessments of mattress have not been conducted. Accordingly, the present study aimed to (1) determine the concentrations of 54 common antibiotics, including 4 tetracyclines (TCs), 19 quinolones (QNs), 22 sulfonamides (SAs), 3 amphenicols (APs), and 6 macrolides (MCs) in the mattress of Wenzhou, Lishui, and Quzhou region, Zhejiang Province; (2) analyze the occurrence and distribution of antibiotic residues in mattress of 12 typical farms at 2019; and (3) conduct risk assessments of antibiotic from mattress into absorptive soil along with the guidelines for environmental risk assessment of veterinary drugs. These results provide reliable data support for the safety problem of mattress in EFS.

2 Experiment

2.1. Study area of swine farm

The study was conducted in Southern Zhejiang Province. Twelve selected swine farms that use EFS were located in Wenzhou, Lishui and Quzhou region, from which mattress samples were collected in 2019 during the autumn. The locations of 12 swine farm are in Figure 2, and detailed information are shown in Table 1.
Table 1
informations of swine farm using ectopic fermentation systems

| Farm No. | Production of fattening pigs/year | excretion of mattress/2019 | start used time | number of beds |
|----------|----------------------------------|---------------------------|-----------------|---------------|
| 1        | 3000-4000                        | 3500                      | 2015            | 6             |
| 2        | 5000+                            | 4500                      | 2009            | 6             |
| 3        | 1000-2000                        | 1500                      | 2015            | 3             |
| 4        | 3400+                            | 2700                      | 2017            | 5             |
| 5        | 500-1000                         | 940                       | 2018            | 3             |
| 6        | 3000+                            | 2400                      | 2015            | 3             |
| 7        | 500-3000                         | 1590                      | 2016            | 4             |
| 8        | 2000+                            | 1700                      | 2015            | 5             |
| 9        | 1000-3000                        | 1680                      | 2015            | 5             |
| 10       | 1000-2000                        | 1300                      | 2015            | 4             |
| 11       | 500-1000                         | 700                       | 2019            | 3             |
| 12       | 500-1000                         | 800                       | 2015            | 4             |

2.2. Sample collection

Every fermentation bed is divided into nine zones, and the sample has been collected on the center of each area from the top of the bed with a pair of shovels. Then, nine mattress have been mixed evenly. Approximately 1 kg mattress was obtained from the sealed bag by quartering method. After rapid cooling with liquid nitrogen, the sample was stored at −20 °C until analysis.

2.3. Instruments and reagents

Intelligent sample grinder (Beijing Hede Technology Co., Ltd.), ultrasonic cleaner (Fungilab Company), high-speed desktop centrifuge (Sigma Company), and QL-901 automatic vortex mixer (Haimen Qilinbeier Instrument Manufacturing Co., Ltd.) were used in the experiment. HPLC-grade formic acid, methanol, and acetonitrile were obtained from Fisher Scientific. Prime HLB (60 mg, 3 mL) solid-phase extraction (SPE) column was obtained from Waters, USA. Chemically pure sodium hydroxide, hydrochloric acid, Na$_2$EDTA, disodium hydrogen phosphate, citric acid, and sodium hydroxide were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. Concentrated ammonia and acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. Water samples were purified by Mill-Q ultrapure water (>18 mΩ). Standard solution of 22 SAs, 19 QNs, 3 Aps, and 6 MCs were obtained from Alta Technology Co. Ltd. (Tianjin, China). TC, chlortetracycline (CTC), oxytetracycline (OTC), and doxycycline (DOX) were obtained from DR.E (Germany). Detailed information about these standards, CAS, concentration, and dissolving solution are provided in the supplementary information Table S1.

2.4. Sample treatment

Approximately 10 g of the sample was evenly divide into two ball milling tanks, and one steel ball was placed and then precooled with liquid nitrogen for 5 min and smashed with a ball mill at 30 Hz. The sample was placed in a plastic bag at −4 °C prior to analysis.

Approximately 2.0 g of the sample was accurately weighed into a 50 mL polypropylene copolymer centrifuge ask. Subsequently, 10 ml of McIlvaine-Na$_2$EDTA buffer (pH 4.5) was added to the sample and swirled for 30 s. The mixture was sonically oscillated for 20 min and then centrifuged for 5 min at 10,000 rpm and −4°C. Thereafter, the supernatant was directly loaded onto anPrime HLB SPE cartridge, and then Prime HLB was eluted using 8 ml of eluent (mixing with 150 ml of methanol, 150 ml of ethyl acetate, and 6 ml of concentrated ammonia). Then, the surplus residues extracted using McIlvaine-Na$_2$EDTA was ultrasonically extracted with 10 ml of acetonitrile repeatedly, and then subjected to acetonitrile extraction. Thereafter, the eluent was collected and evaporated to dryness under N$_2$ in 40 °C. The residue was reconstituted in 0.5 mL of starting mobile phase, filtered using a 0.22 µm nylon filter membrane, and transferred into vials until HPLC–MS/MS analysis.

2.5. LC–MS/MS Analysis

The samples were detected using Agilent 1290 Liquid chromatography coupled with AB 5500 QTrap mass spectrometry. Chromatographic separation was performed with a Waters Atlantis dC18 column (100 mm x 3.0 mm i.d.; 3.0 µm particle size). The mobile phase consisted of solvents A (0.2% formic acid in waters) and B (0.2% formic acid in methanol). The mobile phase flow rate was 0.50 mL/min with 20 min total runtime and a linear gradient under the following conditions: 0 min 95% A–0.5min 95% A, 1 min 95% A–16 min 2% A, 16 min 2% A–18 min 2% A, 18.1 min 95% A–20min 95% A for balance. The injection volume was 5 µL. The mass spectrometer parameters are as follows: ion source, electrospray ion source (ESI); scanning mode, positive and negative ion scanning and segmented scanning; monitoring mode, multi reaction monitoring (MRM); ion source temperature, 550°C; air curtain gas flow rate, 40 L h$^{-1}$; nebulized gas flow rate, 50 L h$^{-1}$; auxiliary gas flow rate, 35 L h$^{-1}$; and dwell time, 20 ms. The peak time and MRM conditions are shown in Table S2. Analyst software 1.7.1 and Multi Quant 3.0.3(AB SCIEX) were used for instrument control and data processing. All results on antibiotics in samples were performed using the Microsoft Office Excel.

2.6. Environmental risk assessment
2.6.1 Contribution rate

The contribution rate of each antibiotics \((x_i)\) was calculated as follows:

\[
x_i = \frac{c_i \times p_i}{\sum c_i \times p_i} \times 100\%
\]

where \(c_i\) is the mean detectable concentration of each antibiotic, and \(p_i\) is the detection frequency of each antibiotic.

2.6.2 Risk assessment

Reference guidelines for environmental risk assessment of veterinary drugs No. 89 and No. 166 (VICH, 2000; VICH 2004) and previous studies (Zhou, 2020; Li, 2013; Zhang, 2015) were used as reference. The potential risk posed by antibiotic on environmental species was assessed by calculating the predicted environmental concentration (PEC), and \(PEC_i\) is the predicted environmental concentration in soil for each antibiotic (mg/kg\(^{-1}\)). This value was calculated using a primary exposure model as follows:

\[
PEC_i = \frac{C_i \times M}{A \times H \times P}
\]

where \(C_i\) is the maximum antibiotic concentration in mattress (mg kg\(^{-1}\)), \(M\) is the excretion of mattress each year (kg), \(A\) is the area where mattress to soil was applied (ha), \(H\) is the soil layer (cm), and \(P\) is the density of the soil (kg m\(^{-3}\)). \(M/A\) was set to 3,000–5,000 kg/ha for the investigation of each farm about mattress to soil as fertilizers in agriculture in the three region. \(H\) and \(P\) were calculated as 20 cm and 1,500 kg/m\(^3\), respectively (Zhou, 2020; Zhang et al., 2015).

For the overall evaluation of total antibiotic risk of each farm, the \(PEC_{total}\) for each farm was calculated using the equation:

\[
PEC_{total} = \sum (PEC_i)
\]

3 Results

3.1 Distribution and occurrence of antibiotics in mattress of 12 swine farms

All collected mattresses from the 12 farms that use EFS contained 10 or more antibiotic residues. A total of 25 kinds of antibiotic residues in 54 monitored drugs were detected in mattresses, including 4 TCs, 10 QNs, 5 SAs, 2 Aps, and 4 MCs. The detection frequency of antibiotic residues in the 56 mattresses are shown in Figure S1. The frequency percentage of each antibiotics found in different mattress samples is in the following order: DOX>CTC>ENR>TC>OTC>CIP>OFL>IM>FF>SDP>SCP>OXO>LOM>TYL>SAR>SN>ENO>TAP>NOR>CTM. DOX is the most command antibiotic in mattress (98.4%), and the antibiotics with a detection frequency higher than 90% were OTC, TC, CTC, DOC, and ENR, while CTM was only found once in one mattress; Three antibiotics (SAR,NOR,ENO) were found on only one farm.

The ranges and averages of antibiotic in mattress from the 12 farms that use EFS are presented in Table 2. The concentrations of the antibiotics in all samples were extremely variable, ranging from ND to 30,579 µg/kg. The order of the total average accumulation of 25 antibiotics is as follows: CTC>DOC>TC>OTC>ENR>FF>IM>FLU>NOR>TAP>SCP>SN>SMZ>ENOS>CL>LOM>TYL>CTM. CTC had been highest concentration in most farms, except for farm 11, in which the highest concentration is DOX (1,090.6 µg/kg). Nine of the 25 antibiotics showed mean concentrations above 100 µg/kg with a highest concentration of more than 25,243 µg/kg for CTC in farm 8, while other antibiotics had a concentration below 100 µg/kg, and the lowest detectable concentration of antibiotics of 0.63 µg/kg was recorded on CTM in farm 4.
The detection mean range of four tetracycline were 5.3–921.2 µg/kg for OTC, 9.7–1,699.5 µg/kg for TC, 50.5–25,243.6 µg/kg for CTC, and 121.7–1,090.5 µg/kg for DOX. DOX had the highest detection rate for mattress, but its residue values are significantly lower than those of the other TCs in farm except for the mattress of farm 11. The total of TCs was the highest and was nearly 10–100 times of the other four kinds of antibiotic residues. The highest mean concentration (25,243 µg/kg) was recorded on farm 8 (CTC), whereas the lowest mean concentration (6.0 µg/kg) was recorded on farm 7 for OTC. For QNs, the detection rates of enrofloxacin and ciprofloxacin in farm were 100%, followed by oxolinic (91.7%) and flumequine (41.7%), and the six other QNs were
only sporadic. Enrofloxacin contained high residue (15.2–1,404 µg/kg) for 11 farms, except farm 4 (FLU, 82.6 µg/kg). Another interesting phenomenon is that the ciprofloxacin residue in the mattress of other farms was 1/3–1/7 of enrofloxacin residue. The highest mean concentration (1,404.5 µg/kg) was recorded on farm 8 (Enr), while the lowest mean concentration (0.9 µg/kg) was recorded on farm for OFL. Florfenicol was detected in 10 of 12 farms. The residues in the nine other samples were less than 60 µg/kg. The highest residue of florfenicol in the mattress of farm 1 was 582.8 µg/kg. TAP was detected in farms 1 and 2 with mean concentrations of 36.8 and 2.4 µg/kg, respectively. For MCs, TIM was detected in 9 bedding samples of 12 farms, and the contribution rate of TIM to the total residue of MCs was 58–100%. Although the detection rate of lincomycin was 83.3%, the residue of LIN (26.3 µg/kg) was lower than TIM. The highest mean concentration (170.4 µg/kg) was recorded on farm 11 (TIM), while the lowest mean concentration (0.6 µg/kg) was recorded on farm for LIN. The detection rates of SCR SMM, SDP, and TMP were higher in mattresses in all SAs. The highest mean concentration (33.6 µg/kg) was recorded on farm 9 (SDP), while the lowest mean concentration (1.0 µg/kg) was recorded on farm 11 for TMP.

In general, the total amount of five kinds of antibiotic residues in the bedding samples of 12 farms ranged from 0.77 g/T to 28 g/T. TCs were the highest among the five kinds of drugs, and the average order of the total accumulation of these five kinds of antibiotics is as follows: TCs (81,568.60 µg/kg) > QNs (3,785.08 µg/kg) > APs (811.67 µg/kg) > MCs (607.26 µg/kg) > SAs (229.44 µg/kg).

Figure 3 shows the total amount of mean concentration and the number of antibiotic residues in the mattress from the 12 farms that use EFS. The distributions of the total concentrations of antibiotic in the mattress from different feedlots varied greatly. The total mean concentrations of antibiotics were in the following order: Jinhua>qu zhou >Wen zhou. Among the farms, the total amount of antibiotics in the bedding samples at farm 2, which was first used ectopic fermentation mattress (2009), was not the highest. The total amount of antibiotics in farm 8 (29,668.9 µg/kg) is the highest, whereas the lowest total amount of antibiotics was recorded on farm 12 (979.1 µg/kg). The farm with the highest antibiotic residue did not have the highest number of antibiotics, Farm 3 had the highest number of antibiotics (16), whereas farms 3, 9, and 10 had the lowest (11).

3.2 Risk assessment

3.2.1 Contribution rate

The overall contribution rate of antibiotics is shown in Figure 4. CTC has provided approximately 80.5% of all antibiotics, other antibiotics, such as TYL and CTM blow 0.005%.

3.2.2 Risk assessment

The PEC_{tatal} and PEC_i of each farm is shown in Table S2. PEC_{cct} had the highest value (0.03756–22.83358) in all farms, except farm 11 (PEC_{doc} =0.72353). The highest PEC_{tatal} is 27.38 in farm 8, followed by farm 10 (19.28) and farm 1 (13.29). The PEC_{tatal} of the nine other swine farms is below 5. The lowest value of 0.77690 was observed in farm 2.

4 Discussion

4.1 Source of antibiotics in mattress

The extensive use of antibiotics in animal husbandry is a global concern. The farms in the study area use a complex mixture of antibiotics through feeding or medicated. Under the original Announcement No. 168 of the Ministry of Agriculture in China, OTC and CTC not only improve antibacterial effect for illness, but also as act as a growth-promoting additive for swine and poultry. In my survey, OTC and CTC was detected in 20 of the 24 batches of fattening swine feed used in 12 farms, while other antibiotics were not found in all feeds. This finding mainly explains the high residue rate and values of OTC and CTC. Moreover, DOX is mainly used in veterinary clinical treatment, and DOC may be deoxygenated by OTC and CTC, making its detection rate significantly higher than and its concentration lower than those of other TCs. ENR is a widely effective antibacterial agent, which can be used for the prevention of animal diseases with long half-life and good distribution. Therefore, ENR had the highest frequency and concentration in all QNs. CIP residue in mattress was 1/3–1/7 of ENR residue possibly because of the metabolism of ENR to CIP in swine. Other QNs such as Oxo, FLU, SAR and ENO have been found for therapeutic use for animal, and a small amount is detected in the mattress. LOM, OFL, and NOR have been banned in China in 2016, and they have been detected at 2.0–21.2 µg/kg possibly because of persistent environmental pollution. FF, which has a better antibacterial activity than CAP or TAP, is the top choice for the treatment of respiratory diseases in swine. Although TAP and FF have a good synergistic effect, the low concentrations of thiampenicol residues cannot rule out the possibility of being introduced as related substances in florfenicol preparations. TIM was detected in bedding samples of 9 farms, and this finding is related to the good effect of tilmicosin for the treatment of pleuropneumonia, Pasteurella infection, and mycoplasma infection. Although the detection rate of LIN was 83.3%, the residue of LIN was lower than 26.3 µg/kg, which may be related to the small using of lincomycin hydrochloride soluble powder for used in these farms.

The difference of antibiotic dose level in feed and the frequency of use of antibiotics as veterinary drugs are the main sources of difference in residual antibiotic level in the mattress, as determined by the varied demands for growth promotion and disease prevention. In addition, the lowest digestive and absorptive capacity for antibiotics could lead to the highest residual levels found in the piglet manure (Pan et al., 2019). The results obtained in the present study might have been influenced by different sampling seasons, pig types, feed sources, or other factors (Wang, 2017). Other antibiotics used in swine were not found because of the change of form in metabolic process in swine and the degradation during the fermentation of EFS.

4.2 Antibiotics in mattress, manure, and fertilizer base on swine

Although no relevant data are available to support the detection of antibiotic residues in mattress, the findings are consistent with the detection results of antibiotic residues in some livestock manure and fertilizers. Table 3 summarizes data on the maximum concentrations and mean concentrations of five kinds
of antibiotics on previous studies. The result shows that five antibiotics were found in farm of all world. Wang et al. studied occurrence and distribution of SAs and TCs in nine different swine manure from six representative large-scale feedlots in Zhejiang Province in 2017. The maximum concentration of residual antibiotic reached 57.95 mg/kg (CTC). SAs in animals were SDZ and SM with mean concentrations were 1.79–17.50 and 3.54–11.52 mg/kg. Chen et al. investigated the occurrence of 14 selected antibiotics in manures collected from four swine farms in Hangzhou. TCs and SAs were the most prominent contaminants in the manure samples, with a maximum concentration of 139.4 mg/kg CTC (Chen, 2012). Berendsen et al. used a comprehensive method for the analysis of trace levels of 44 antibiotic in animal feces and applied it to monitor swine and cattle feces in Dutch. In 55% of the swine, originating from 80% of the swine farms, antibiotics were detected. OTC, DOX, and SD were the most detected antibiotics. The analysis of feces is a simple and non-invasive strategy to successfully monitor the antibiotic use in animal breeding and to obtain knowledge on the dissemination of antibiotic residues and resistant bacteria throughout the environment (Berendsen, 2015). Li et al. studied six antibiotics in animal manure collected from 71 animal feedlots in Northern China. Swine (83,177 µg/kg) had the highest amount of antibiotics in manures among the animals. The highest detection frequency of each antibiotic was 97.1% for TC, 67.1% for MC, 64.7% for Lin, 61.9% for QN, and 50.7% for SA. The average antibiotic levels of each category decreased in the following order: TCs > QNs > AMs > Lin > SAs > MCs. TCs and QNs were the predominant antibiotics in the manure samples, accounting for 95.7% of the total concentration (Li, 2020). The manure in other regions in China and other countries have two or more kinds of antibiotics in manure the range of mg/kg.
| Region                  | Sample Type                     | TCs                       | QNs                       | SAs                        | APs                        | MCs                        |
|------------------------|---------------------------------|---------------------------|---------------------------|----------------------------|----------------------------|-----------------------------|
| Zhejiang, China        | 54 manure from 6 farm           | OTC ND~5.51(2.7)          | NM                        | SD 0.68~46.37(7.4)          | SMM 2.58~16.50(7.7)         | NM                          |
|                        |                                 | TC 0.43~40.39(6.3)        |                           |                            |                            |                             |
|                        |                                 | CTC 0.36~57.95(13.4)      |                           |                            |                            |                             |
|                        |                                 | QNs ND~9.7(6)             |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)         |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Zhejiang, China        | 4 manures form 4 farm           | TE 10~198.2(10)           | NOR ND~9.7(6)             | SD ND~7.1(0.1)              | SMM 6.3~16.2(9.1)           | ND                          |
|                        |                                 | OTC 0.354.0(215)         |                           |                            |                            |                             |
|                        |                                 | CTC 0.36~1394(900)       |                           |                            |                            |                             |
|                        |                                 | DOX 0.1~137.2(90)        |                           |                            |                            |                             |
|                        |                                 | QNs ND~9.2(3)            |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)        |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Zhejiang, China        | 116 fertilizers from pig manure | TC 0.59~4.1(2.4)         | ENR ND~6.4~4091(310)     | SMM 3.1~613(93.8)           | ND                          | ND                          |
|                        |                                 | OTC 0.52~16.2(5.3)       |                           |                            |                            |                             |
|                        |                                 | CTC 0.34~15.8(4.3)       |                           |                            |                            |                             |
|                        |                                 | DOX 0.178~14.6(3.4)      |                           |                            |                            |                             |
|                        |                                 | QNs ND~14.8              |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)        |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Hebei and Beijing, China | 23 manure from 23 farm       | CTC13.5~1048(182.9)      | NOR ND~1646(14.4)        | SMM ND~54(2.7)              | FF ND~17277(1828)           | ND                          |
|                        |                                 | OTC ND~2858(148.3)       |                           |                            |                            |                             |
|                        |                                 | CTC854~17500(2946)       |                           |                            |                            |                             |
|                        |                                 | DOX 454.5~294100(41462)  |                           |                            |                            |                             |
|                        |                                 | QNs ND~14.8              |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)        |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Denmark                | Liquid manureFrom 6 farm       | TE ND~1.9 (NM)            |                           |                            |                            |                             |
|                        |                                 | OTC ND~1.6 (NM)           |                           |                            |                            |                             |
|                        |                                 | CTC ND~24.4 (NM)          |                           |                            |                            |                             |
|                        |                                 | DOX 0.29~3.5 (NM)         |                           |                            |                            |                             |
|                        |                                 | QNs ND~14.8              |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)        |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Dutch                  | 340 manure from 23 farm        | OTC ND~1.5 (NM)           |                           |                            |                            |                             |
|                        |                                 | DOX ND~95(NM)            |                           |                            |                            |                             |
|                        |                                 | QNs ND~14.8              |                           |                            |                            |                             |
|                        |                                 | SAs ND~5.87(13.4)        |                           |                            |                            |                             |
|                        |                                 | APs ND~3.2~5.1(1.4)       |                           |                            |                            |                             |
| Northrhine-Westfalia, Germany | 34 manure       | TE ND~2.45 (NM)           |                           |                            |                            |                             |
|                        |                                 | OTC ND~1.49 (NM)          |                           |                            |                            |                             |
|                        |                                 | CTC ND~3.60 (NM)          |                           |                            |                            |                             |
|                        |                                 | ENR ND~0.55 (NM)          |                           |                            |                            |                             |
|                        |                                 | CIO ND~0.07 (NM)          |                           |                            |                            |                             |
|                        |                                 | MAR ND~0.05 (NM)          |                           |                            |                            |                             |
|                        |                                 | SAR ND~0.06 (NM)          |                           |                            |                            |                             |
|                        |                                 | SD ND~0.65 (NM)           |                           |                            |                            |                             |
|                        |                                 | SM ND~7.04 (NM)           |                           |                            |                            |                             |
|                        |                                 | SMP ND~0.02(NM)           |                           |                            |                            |                             |
|                        |                                 | SDZ 0.0194~0.04           |                           |                            |                            |                             |
|                        |                                 | STZ 0.11~0.11             |                           |                            |                            |                             |
| Albania and Kosovo.    | 22 manure                       | OTC ND~0.47CTC           |                           |                            |                            |                             |
|                        |                                 | ND~0.68D0XY              |                           |                            |                            |                             |
|                        |                                 | 0.30~0.30                |                           |                            |                            |                             |
|                        |                                 | SDZ 0.0194~0.04           |                           |                            |                            |                             |
|                        |                                 | STZ 0.11~0.11             |                           |                            |                            |                             |
| Jiangsu, China         | 9 manure                        | TC ND~187.7 (21.60)       |                           |                            |                            |                             |
|                        |                                 | OTC ND~108.7 (34.74)      |                           |                            |                            |                             |
|                        |                                 | CTC ND~84.60 (20)         |                           |                            |                            |                             |
|                        |                                 | DOX ND~2.93(1.04)         |                           |                            |                            |                             |
|                        |                                 | NOR ND~27.53(5.19)       |                           |                            |                            |                             |
|                        |                                 | ENR ND~57.62 (15.14)      |                           |                            |                            |                             |
|                        |                                 | CIP ND~63.63(14.39)       |                           |                            |                            |                             |
|                        |                                 | OFL ND~24.62(4.15)        |                           |                            |                            |                             |
|                        |                                 | SD ND~136.3 (15.48)       |                           |                            |                            |                             |
|                        |                                 | SPD ND~4960 (553.1)       |                           |                            |                            |                             |
|                        |                                 | SMZ ND~157.33 (17.82)     |                           |                            |                            |                             |
|                        |                                 | SMM ND~3490 (441.23)      |                           |                            |                            |                             |

**ND**: Below detection limit, **NM**: Not mentioned, **a**: only 1 sample have been found, **b**: number of farm and sample have not mentioned in party study, **c**: The unit indicates that all unit is µg/kg
| region                      | Sample type      | TCs                        | QNs            | SAs     | APs    | MCs    |
|----------------------------|------------------|----------------------------|----------------|---------|--------|--------|
|                           | 4 fertilizers    | TC ND~678.76 (1922)        | NOR ND~11.57 (35.92) | SM ND~2.843 (30.34) | NM     | NM     |
| from swine manure         |                  | OTC ND~28.17 (81.80)       | ENR ND~13.65 (43.35) | SMM ND~12.49 (44.98)  |        |        |
|                           |                  | CTC ND~381.48 (773.5)      | PEFND~9.26 (29.68)  | ST ND~0.74 (2.96)     |        |        |
|                           |                  | DOC ND~43.69 (85.40)       | CIP ND~8.39 (23.74)  | SG ND~0.65 (2.59)     |        |        |
| Beijing, China*           | 17 Manure from 11 farms | TC 29~3580(413)            | NFX24~4187 (692)   | SDZ ND~22 (3.9)       | NM     | ROX N  |
|                           |                  | OTC 187                   | CIP 76~9342(1313)   | SMZ 1.2~61 (7.5)      |        |        |
|                           |                  | CTC 107~26218 (2504)      | ENR 89~8684(1248)   | SM ND~1.8 (0.18)      |        |        |
|                           |                  |                            | LOM ND~38 (7.5)     | SMX ND~102(13)        |        |        |
| Shanghai, China           | 1 swine breeding| TC 12.27                  | NOR ND~11.57 (35.92) | SD 4.87  | CAP 11.01 | NM     |
|                           |                  | OTC 18.70                 | ENR ND~13.65 (43.35) | SMZ 7.56  |        |        |
|                           |                  |                            | PEFND~9.26 (29.68)  | SM 6.17   |        |        |
| Heilongjiang, Jilin, Liaoning, China | 18 faeces from 18 farms | TC 0.32~30.55(5.29)        | NFX24~4187 (692)   | SG 0.15~1.90 (0.63)  | NM     | TYL 0. (0.69) |
|                           |                  | OTC 0.73~56.81 (11.81)    | CIP0.31~0.96 (0.49) | SMZ 0.21~2.16 (1.07)  |        |        |
|                           |                  | CTC0.68~22.34 (3.19)      | ENR0.36~2.22 (0.87) | SMM 0.12~4.84 (1.14)  |        |        |
|                           |                  |                            | DIF 0.14           | SCP 0.13~2.13 (0.85)  |        |        |
| Japan                     | 5 manure         | TC ND~15 (NM)             | NFX24~4187 (692)   | SMM 210<sup>a</sup>  | NM     | NM     |
|                           |                  | OTC 13<sup>a</sup>        | CIP 76~9342(1313)   | SMX ND~35 (NM)        |        |        |
|                           |                  | CTC ND~280 ((NM).         | ENR 89~8684(1248)   |        |        |        |
|                           |                  |                            | LOM ND~38 (7.5)     |        |        |        |
| Austria                   | 30 manure from 6 farm | TC 0.36~23(NM)            | FQS 0.13~0.75 (NM). | SDM 20 (NM) | NM     | NM     |
|                           |                  | OTC 0.21~29(NM)           |                            | MP 17 (NM) |        |        |
|                           |                  | CTC 0.1~46 ((NM).         |                            |        |        |        |
| China                     | 31 faeces from 17 farms | TC 0.15~59.06(2.69)       | FIO 1.08~7.46(2.23) | SD 0.09~0.80 (0.21)  | NM     | NM     |
|                           |                  | OTC 0.16~21.06 (1.15)     | NRO 0.56~5.50 (2.09) | SMM 0.07~4.08 (0.20)  |        |        |
|                           |                  | CTC 0.16~21.06 (1.15)     | LOM 0.77~44.1(2.02)  | SCP 0.09~3.51 (0.82)  |        |        |
|                           |                  | DOX 0.23~13.50 (0.79)     | ENO 0.48~33.26(2.09) | SMZ 0.23~0.84 (0.51)  |        |        |
| East and West Flanders, Belgium | 7 farms | OTC ND~2.09 (NM)          | SD 0.7<sup>a</sup> | SDP 0.8~23.0 (2.2)    | NM     | ND     |
|                           |                  | DOX ND~22.7 (NM)          | SDM 0.6            | SMX 0.5<sup>a</sup>   |        |        |
| Lower Saxony, Germany     | 8 fattening farms| TC 1.5~300(152)           | ENR 1.3<sup>a</sup> | SDP 0.8~23.0 (2.2)    | NM     | ND     |
|                           |                  | OTC 6<sup>a</sup>         |                            | SMX 0.5<sup>a</sup>   |        |        |
|                           |                  | CTC 1.7~46.3 (26.9)       |                            | TMP 0.2<sup>a</sup>   |        |        |
|                           |                  | DOX11.0~28.9 (20.3)       |                            |        |        |        |
|                           | 8 breeding farms  | TC 1.5~227(16.5)          |                            |        |        |        |
|                           |                  | OTC 0.6~211 (13.6)        |                            |        |        |        |
|                           |                  | CTC 15.8~55.1 (37.4)      |                            |        |        |        |
|                           |                  | DOX5.0~101 (19.8)         |                            |        |        |        |

<sup>*ND**: Below detection limit, NM: Not mentioned, a: only 1 sample have been found, b: numer of farm and sample have not mentioned in pary study, c: The unit indicates that all unit is µg/kg

Manure-based fertilizers have been studied. Qian studied 43 antibiotics in manure-based fertilizers from the Zhejiang Province of China. Approximately 64% of manure-based fertilizers were positively detected with at least one drug. Seventeen antibiotics were detected from swine. ENR was the most frequently detected compound with concentrations ranging from 6.4 µg/kg to 4,091 µg/kg. Nine of the 17 antibiotics showed maximum concentrations above 1,000 µg/kg, with a highest concentration of more than 16,280 µg/kg for DOX. TCs were dominant antibiotic in fertilizers, with concentration reaching 1,920 µg/kg.
In total, TCs, QNS, and SAs were the most frequently detected antibiotics and exhibited a broad concentration range in animal manure and manure-based fertilizers. However, in our study, TCs and QNS had the highest mattress, which is consistent with the results of manure and fertilizer. However, SAs had the lowest detection rate and concentration. This finding was obtained possibly because of the strong water solubility of SAs, which will be discharged with wastewater in the process of treatment, or the degradation of SAs in the process of EFS. Considering the different degrees of pollution in the treatment process, the actual removal capacity and the removal mechanism of these ESF should be further studied for selected antibiotics.

These results are consistent with the results of feces and fertilizer samples containing 10 or more different antibiotics. The number of antibiotics in mattress is much higher than that in manure and fertilizer. This finding was obtained because the time of treatment cycle for mattress is much longer than manure, and the antibiotics continuously accumulated in the mattress through the spray of feces and urine. Moreover, the amount of antibiotics in mattress in most farms is lower than the amount of manure, possibly because antibiotics may be degraded by microorganisms during EFS or as an effect of prolonged accumulation.

The distribution of antibiotics in the mattress of 12 swine farms has many similarities and differences. Based on our study, the results show that the residue amount of antibiotics in the mattress is not entirely determined by the start-up time of EFS but is related to the use of feed containing antibiotics, medication habits, and the level of management and maintenance methods of mattress in EFS.

### 4.3 Risk assessment

Based on the contribution rate, CTC has the highest contribution to antibiotics of mattress. Therefore, CTC and TCs are the most noteworthy drug in mattress. Although the contribution rate of other drugs is very low, the toxicity and drug resistance of other antibiotics still need to be focused on.

For maintaining the vitality of the fermentation bed, each farm will take out part of the ripening mattress and supplement new raw material periodically (1 year or half a year). The mattress obtained in EFS was allowed to be applied with organic fertilizer for plant and same vegetable cultivation in Zhejiang with prescribed dosage. Therefore, the use of mattress may introduce antibiotics into agricultural soil. The application of mattress to organic fertilizer may cause some ecological risks. The International Coordinating Committee on technical requirements for veterinary drug registration (VICH) formulated guidelines for environmental risk assessment of veterinary drugs No. 89 and No. 166 respectively (VICH, 2000; VICH, 2004). PEC in soil (based on the exposure assessment model, the value calculated by substituting the drug administration scheme, the excretion rate of drug and its active ingredients from of the collected soil) was assessed in terms of the presence of high ecological risk.

In comparison with previous studies (Li, 2013; Zhang, 2015; Li, 2003), the risk of each antibiotic in manure and manure-based fertilizer was assessed separately. However, the mattress of each farm contains 1 or more antibiotics. Therefore, we used the PEC_{total} of each farm to evaluate the risk of mattress. Based on VICH, when PEC is greater than or equal to 100 µg/kg, the risk assessment should be started (VICH, 2000). Considering the difference in fertility, the amount of mattress used as fertilizer in soil per unit area is lower than that of manure. Therefore, the ratio of M/A does not refer to the values of relevant studies (6,000 kg/0.67 ha), but uses a permissible level of mattress of each region as fertilizers to soil. According to the calculation results, the PEC_{total} of all farms did not exceed 100 and poses low risk for soil as fertilizers. However, the economic risk of farms with high PEC_{total} value such as farms 8, 10, and 1 (27.38, 19.28, and 13.29, respectively) cannot be ignored, and some measures to control antibiotic residue should be taken.

The results showed that the effect of antibiotic residues in mattress on the environment was acceptable on the premise of supplementing other nutrients and the maximum single use of ectopic fermentation mattress. EFS can effectively solve the problem of fecal antibiotic treatment. Therefore, before applying organic fertilizer, the method of determination of antibiotic content in mattress and the risk assessment of antibiotic for soil can be used as reference standards to ensure the benefit of agricultural production and minimize the ecological risk.

### 5 Conclusions

This study involved a scale survey to assess the levels and distributions of antibiotics residues in mattress on 12 swine farms that use EFS in Zhejiang Province. A total of 25 kinds of antibiotics were detected in the mattress of 12 farms. The total residues of bedding samples in each farm ranged from 0.77 g/T to 28 g/T, and CTC had the highest contribution rate among the antibiotics. The amount of antibiotic residues in the mattress material of EFS was not entirely determined by the start-up time of EFS, related to the level of breeding management, medication habits, and maintenance methods of EFS. The results of data and risk show that the effect of antibiotic residues in the mattress on the environment was acceptable on the premise of the prescribed amount of mattress in the planting land. EFS can effectively solve the problem of fecal antibiotic treatment. However, the application of a large number of antibiotics leads to antibiotic residues in the mattress. Therefore, in scale breeding industry, we should strengthen feed management and strictly control the amount of antibiotics to reduce the residues of antibiotics in the environment.

### Declarations

-Ethical Approval

There are not Ethical problem.

-Consent to Participate
All authors have been indicated

- Consent to Publish

All authors agree to publish

- Authors Contributions

Zhouwei: Writing-original draft, Writing review & editing. Suodecheng: Project administration, Conceptualization, Writing - review & editing. Fan Xia: test design, Investigation. Xiaozhiming: Methodology study. Zhang Hangjun: Sample collection and analysis, Zhou Zhijin: Validation and Supervision. Chong Yang: Data curation.

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- Competing Interests

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.

- Availability of data and materials

Not applicable

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Figures

![Diagram of ectopic fermentation systems](image)

**Figure 1**: Schematic diagram of ectopic fermentation systems (EFS)
Figure 2

Location of 12 farm use EFS

Figure 3

Total amount and king number of antibiotics on 12 farm 1
Figure 4

Contribution rate of antibiotics in mattress

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