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Seasonal variation and removal efficiency of antibiotic resistance genes during wastewater treatment of swine farms

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Abstract The seasonal variation and removal efficiency of antibiotic resistance genes (ARGs), including tetracycline resistance genes (*tet*G, *tet*M, and *tet*X) and macrolide (*erm*B, *erm*F, *ere*A, and *mef*A), were investigated in two typical swine wastewater treatment systems in both winter and summer. ARGs, class 1 integron gene, and 16S rRNA gene were quantified using real-time polymerase chain reaction assays. There was a 0.31–3.52 log variation in ARGs in raw swine wastewater, and the abundance of ARGs in winter was higher than in summer. *tet*M, *tet*X, *erm*B, *erm*F, and *mef*A were highly abundant. The abundance of ARGs was effectively reduced by most individual treatment process and the removal efficiencies of ARGs were higher in winter than in summer. However, when examining relative abundance, the fate of ARGs was quite variable. Anaerobic digestion reduced the relative abundance of *tet*X, *erm*B, *erm*F, and *mef*A, while lagoon treatment decreased *tet*M, *erm*B, *erm*F, and *mef*A. Sequencing batch reactor (SBR) decreased *tet*M, *erm*B, and *erm*F, but biofilters and wetlands did not display consistent removal efficiency on ARGs in two sampling seasons. As far as the entire treatment system is concerned, *erm*B and *mef*A were effectively reduced in both winter and summer in both total and relative abundance. The relative abundances of *tet*G and *ere*A were significantly correlated with *int*I1 ($p<0.01$), and both *tet*G and *ere*A increased after wastewater treatment. This may pose a great threat to public health.

Keywords Antibiotic resistance genes · Swine wastewater · Biological wastewater treatment · Class 1 integron · Antibiotic resistant bacteria · Real-time polymerase chain reaction

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Introduction

Antibiotic resistance is an increasing threat to global public health, not only increasing the difficulty in infection control but also reaching alarming levels worldwide (WHO 2014). Antibiotic resistance genes (ARGs) were indicated as emerging contaminants by Pruden et al. (2006) and play an important role in antibiotic resistance. According to WHO and several recent studies, many gaps exist in our knowledge about the spread of ARGs, especially regarding animal production and related environments (WHO 2014; Berendonk et al. 2015). Extensive information is thus required to reduce the risk posed by environmental ARGs (Berendonk et al. 2015).

Animal production extensively utilizes antibiotics, especially in developing countries; for example, 52 % of total antibiotic use (162,000 tons) was due to animal production in China in 2013 (Zhang et al. 2015). with growth promotion, prophylactic, and therapeutic uses. Tetracyclines and macrolides are the most commonly used antibiotics for these

purposes (Barton 2000; Apley et al. 2012). Chantziaras et al. (2014) reported that the use of specific antibiotics on swine farms strongly correlated to the prevalence of antibiotic resistant bacteria. Zhu et al. (2013) found that ARGs were enriched 192 to 28,000-fold in antibiotic-treated feces compared with antibiotic-free swine manure or soil controls.

Swine wastewater is an important ARGs reservoir. Koike et al. (2007) detected *tet*M with a relative abundance of 1.38E −02 copies/16S rRNA in swine wastewater. Cheng et al. (2013) detected *tet* genes with different resistance mechanisms, showing that the abundance of ribosomal protection protein genes (*tet*Q, *tet*M, *tet*W, and *tet*O) in swine wastewater was higher than the efflux pump gene (*tet*G) and enzymatic modification gene (*tet*X). Jindal et al. (2006) found a high level of resistant rRNA (approximately 50 %) with methylation of a specific nucleotide encoded by the *erm* gene causing resistance to macrolides, lincosamides, and streptogramin B (MLS_B) in swine wastewater. However, most studies on ARGs abundance were focused on a single season, and few studies have examined the seasonal variation of ARGs.

Animal wastewater is typically initially treated with anaerobic digestion, aerobic biological treatment, constructed wetlands, or lagoons (Nasir et al. 2012; Deng et al. 2008; Cronk 1996). Tao et al. (2014) evaluated the removal of ARGs from swine wastewater using successive anaerobic and aerobic treatments, with a mean removal efficiency of *tet*A, *tet*W, *sul*I and *sul*II, and bla_{TEM} in the range of 33.30–97.56 %. Diehl and Lapara (2010) reported that removal of *tet*A, *tet*O, *tet*W, and *tet*X by anaerobic digestion fit a first-order kinetic model, while the abundance of *tet*L did not show appreciable decline. Joy et al. (2013) indicated that abundance of *erm*B and *erm*F were reduced by 50–60 % and 80–90 %, respectively, by simulated lagoon storage. However, the ARGs removal efficiency was different even with similar treatment processes. Tao et al. (2014) reported that *tet*W increased by 0.78 log after treatment by two-stage anaerobic digestion, while decreasing by 1.66 log on another swine farm with similar anaerobic process. Chen et al. (2010) indicated that the rate of ARGs removal was influenced by seasonal variation, as *erm*B was reduced by 1.9 log in a biofilter in winter while only decreasing 0.88 log in summer. Resende et al. (2014a) evaluated the dynamics of *erm*B and bla_{TEM-1} by anaerobic digestion at ambient temperature as a pilot scale in summer and winter. The removal efficiency of *erm*B was slightly higher in summer (93.9 %) than in winter (84.0 %), but the removal efficiency of bla_{TEM-1} was much higher in summer (2.2 log) than in winter (0.84 log). However, the seasonal variation of tetracycline ARGs has not, to out knowledge, been reported. Thus, the abundance and removal efficiency of ARGs may be impacted by seasonal factors; however, these variation patterns are unclear and needs further study.

Integrons are mobile genetic elements that could facilitate ARG prevalence, as some bacteria can capture ARGs housed on mobile genetic elements via horizontal transfer (Martínez 2008; Shi et al. 2013). Martínez et al. (2014) ranked the ARGs on mobile genetic elements harbored by human bacterial pathogens at the highest risk level. Amos et al. (2015) identified the class 1 integron as a molecular marker of antibiotic resistance, which has helped to better understand the transfer of ARGs in the environment.

Therefore two different swine wastewater treatment systems were chosen in this study, i.e., an "anaerobic digestion-lagoon" system and an "anaerobic digestion-aerobic biological treatment-wetland." The objective of this study was to evaluate ARGs, class 1 integron gene, and 16S rRNA gene variations between summer and winter both before and after wastewater treatment, as well as to determine the removal efficiencies of different tetracycline and macrolide resistance genes with real-time quantitative polymerase chain reaction (qPCR) assays.

Materials and methods

Description of sampling sites

Wastewater samples were collected from two confined swine farms. Farm 1 is located in Beijing, China (116°E, 40°N) and farm 2 is located in Jiangxi Province, China (117°E, 28°N). The swine wastewater treatment flowchart of these two farms is shown in Fig. 1. More detailed information about the treatment processes of the two systems is given in Table 1.

Wastewater samples of 500 mL were collected using sterile containers at influent and effluent of each treatment unit in August 2014 and February 2015. Grab samples were collected and stored at 4 °C before transferred to the laboratory.

DNA extraction

Wastewater samples were first filtered through 0.22-μm filters. For wastewater with high suspended solid (SS higher than 5000 mg/L), 10–20 mL of raw wastewater was filtered; for relatively low SS wastewater, 40–100 mL was filtered for DNA extraction. Then total DNA was extracted from the filter using the FAST DNA extraction Kit (MP Biomedicals, USA) according to the manufacturer's instructions. Extracted DNA was visualized and quantified using 1 % agarose gel electrophoresis and a NanoDrop 2000 (Thermo Scientific, USA), and then stored at −20 °C until use. Gene concentration was recalculated based on the volume of wastewater used for DNA extraction.

Quantification of ARGs by real-time qPCR

Tetracycline (*tet*G, *tet*M, *tet*X) and macrolide (*erm*B, *erm*F, *ere*A, and *mef*A) resistance genes, as well as a class 1 integron gene (*int*I1) and bacterial 16S rRNA genes were quantified

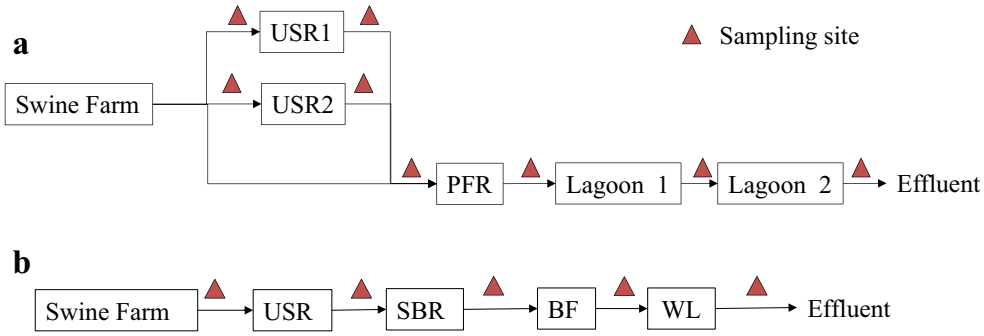

Fig. 1 Flowchart of the wastewater treatment systems in two swine farms (**a** farm 1; **b** farm 2; *USR* upflow solid reactor, *PFR* plug-flow reactor, *SBR* sequencing batch reactor, *BF* biofilter, *WL* wetland; *triangles* represent sampling points)

using qPCR. The resistance mechanisms of the selected ARGs and the primers used in qPCR are listed in Table 2. The plasmids containing these specific genes were manufactured by Zhejiang Tianke Biotechnology Company (Zhejiang, China), as shown in Table S1. The standard samples were diluted to yield a series of decreasing 10-fold concentrations and subsequently used for generating standard curves. The 25 μL PCR reaction mixtures contained 12.5 μL SYBR Green qPCR Super-Mix-UDG with Rox (Invitrogen, USA), 0.5 μL each of 10 μM forward and reverse primers, 10.5 μL DNA-free water, and 1.0 μL standard plasmid or DNA extract. The cycling conditions for qPCR amplification were as follows: 50 °C for 2 min, 95 °C for 5 min; followed by 40 cycles of 95 °C for 20 s, annealing temperature for 30 s, 72 °C for 31 s, and plate reading. Melt-curve analyses were performed from 60 to 95 °C, with 0.2 °C/read. Reaction was performed using an ABI Real-time PCR system 7500 (ABI, USA). Melting curve analysis and gel electrophoresis ensured specificity. Each gene was quantified in triplicate using a standard curve and a negative control.

Statistical analysis

Independent *t* tests were conducted to detect significant differences between the target genes. Differences with a *p* value <0.05 were considered statistically significant. The Spearman rank correlation was used to assess the association between ARGs and *int*I1. Statistical analyses were performed using SPSS 20.0 (IBM, USA).

Results and discussion

Seasonal variation of ARGs abundance

Target genes were detected in almost all wastewater samples, as shown in Fig. 2. The abundance of each ARG and 16S rRNA gene in the influent (raw swine wastewater) of the two swine farms was similar for each sampling season. The abundance of *tet*M, *tet*X, *erm*B, *erm*F, and *mef*A was relatively higher than that of other detected ARGs, with mean abundances of 6.91E+10, 2.43E+11, 1.07E+11, 1.45E+11, and 7.16E+10 copies/mL in raw swine wastewater, respectively. The mean abundance of *tet*G and *ere*A was at least 2 logs lower, at 6.02E+09 and 8.27E+08 copies/mL, respectively. The reported abundance of tetracycline and macrolide resistance genes was lower than this study, including *tet*A of 9.1E+03, *tet*W of 8.0E+04 (Tao et al. 2014). *tet*G of 2.0E+07 copies/mL (Chen et al. 2010). *erm*B of 6.3E+08, *erm*F of 1.0E+08, and *erm*X of 7.9E+05 copies/mL (Chen et al.

Table 1 Detailed information of the two swine wastewater treatment systems

Swine farm	Treatment process	Flow (m^3/day)	Working temperature (°C)	Individual flow (m^3/day)	Working volume (m^3)	HRT (h)
Farm 1	USR1	60	30	28	400	16
	USR2		35	26	525	20
	PFR		N.C.	60	500	8
	Lagoon1		N.C.	60	1394	23
	Lagoon2		N.C.	60	967	16
Farm 2	USR	150	30	150	1650	11
	SBR		N.C.	150	360	2.4
	BF		N.C.	150	360	2.4
	WL		N.C.	150	150	1.0

N.C. not controlled, *USR* upflow solid reactor, *PFR* plug-flow reactor, *SBR* sequencing batch reactor, *BF* biofilter, *WL* constructed wetland

Table 2 Resistance mechanisms, primer sequences, expected amplicon size, and annealing temperature for each target gene

Gene	Resistance mechanism	Primer sequence (5′-3′)	Size (bp)	Annealing temp. (°C)	Reference
*tet*G	Efflux pump	TTATCGCCGCCGCCCTTCT TCATCCAGCCGTAACAGAAC	134	64.2	Apley et al. (2012a)
*tet*M	Ribosomal protection protein	ACAGAAAGCTTATTATATAAC TGGCGTGTCTATGATGTTCAC	171	55	Apley et al. (2012a)
*tet*X	Tetracycline inactivation enzyme	CAATAATTGGTGGTGGACCC TTCTTACCTTGGACATCCCG	468	64.5	Ng et al. (2001)
*erm*B	23S rRNA methyltransferase	GATACCGTTTACGAAATTGG GAATCGAGACTTGAGTGTGC	364	58	Chen et al. (2007)
*erm*F	23S rRNA methyltransferase	TCTAGCAATGAGAATGAAGGT ACTATAACGTGATGGTTGGGAGGGA	309	56	Sutcliffe et al. (1996)
*ere*A	Macrolide inactivation enzyme	AACACCCTGA ACCCAAGGGACG CTTCACATCCGGATTCGCTCGA	420	60	Volokhov et al. (2003)
*mef*A	Efflux pump	AGTATC ATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	348	52	Sutcliffe et al. (1996)
*int*I1		CTGGATTTCGATCACGGCACG ACATGCGTGTAAATCATCGTCG	473	60	Stokes et al. (2006)
16S rRNA		CGGTGAATACGTTCYCGG GGWTACCTTGTTACGACTT	128	55	Suzuki et al. (2000)

2010). In this study, filtration of samples prior to DNA extraction may have increased the DNA extraction efficiency compared with the wet material centrifugation method adopted by other studies (Tao et al. 2014; Chen et al. 2010). In general, the abundance of ARGs in the raw swine wastewater may reflect the feeding status, antibiotic consumption, and state of antibiotic resistance contamination. The abundance of ARGs detected in these samples confirms that a more rational use of antibiotics in swine farming is essential, which is reiterated by WHO (2014).

The abundance of all the genes quantified in summer was significantly different ($p<0.05$) from winter, indicating that season plays an important role in ARGs abundance. In most cases, the ARGs and 16S rRNA genes of samples were more abundant in winter than in summer. The mean difference and range of variation in target genes were 1.19 log (0.09–2.35 log) for *tet*G, 1.14 log (0.47–3.40 log) for *tet*M, 1.10 log (0.25–5.35 log) for *tet*X, 0.83 log (0.32–2.48 log) for *erm*B, 1.34 log (0.21–5.87 log) for *erm*F, 1.16 log (0.47–3.24 log) for *ere*A, 1.16 log (0.41–3.11 log) for *mef*A, and 1.09 log (0.29–2.34 log) for 16S rRNA gene, respectively, with the range of average variation of 0.31–3.52 log in summer and winter.

However, all detected genes in the lagoon 2 effluent from farm 1 were more abundant in summer, which was similar to the BF effluent from farm 2. Chen et al. (2010) evaluated the seasonal variation of macrolide resistance genes, including *erm*B and *erm*F, and showed that these are more abundant in winter than in summer. However, after anaerobic digestion and BF treatments, the abundance of *erm*B and *erm*F was higher in summer than in winter. These variations in *erm*B and *erm*F were inconsistent with our findings which suggested other currently unknown mechanisms may be at work in causing variation in these ARGs levels.

Veterinary antibiotics are used in many forms, including oral solutions, oral powders, premixed with feed, injections, etc. In addition, the impact on ARGs abundance in manure of antibiotics administered in feed is quite different than those administered by injection (Bibbal et al. 2007). The higher abundance of ARGs in winter than in summer may be due to increased antibiotic administration during the cold season to prevent disease (Awad et al. 2014). And the antibiotics administered between summer and winter greatly impacted the seasonal ARGs abundance. Additionally, as shown in Fig. 2, higher population of total bacteria (copies of 16S rRNA gene) was in winter. Given that animals are kept inside barns for heat preservation during the winter, decreased ventilation and less frequent cleaning may result in discharging less wastewater with a higher concentration of pollutants, such as higher concentrations of COD, ammonia, nitrogen, and bacteria.

ARGs removal efficiencies by different treatment processes

As shown in Fig. 2, in most cases, the abundance of ARGs was effectively reduced by most treatment processes, including anaerobic digestion, sequencing batch reactor (SBR), and wetland. Removal of ARGs was more efficient in winter than in summer; however, the lagoons and the biofilter increased the abundance of ARGs, especially in summer. Therefore, further research is needed, as this may be due to the proliferation of ARG carriers in summer.

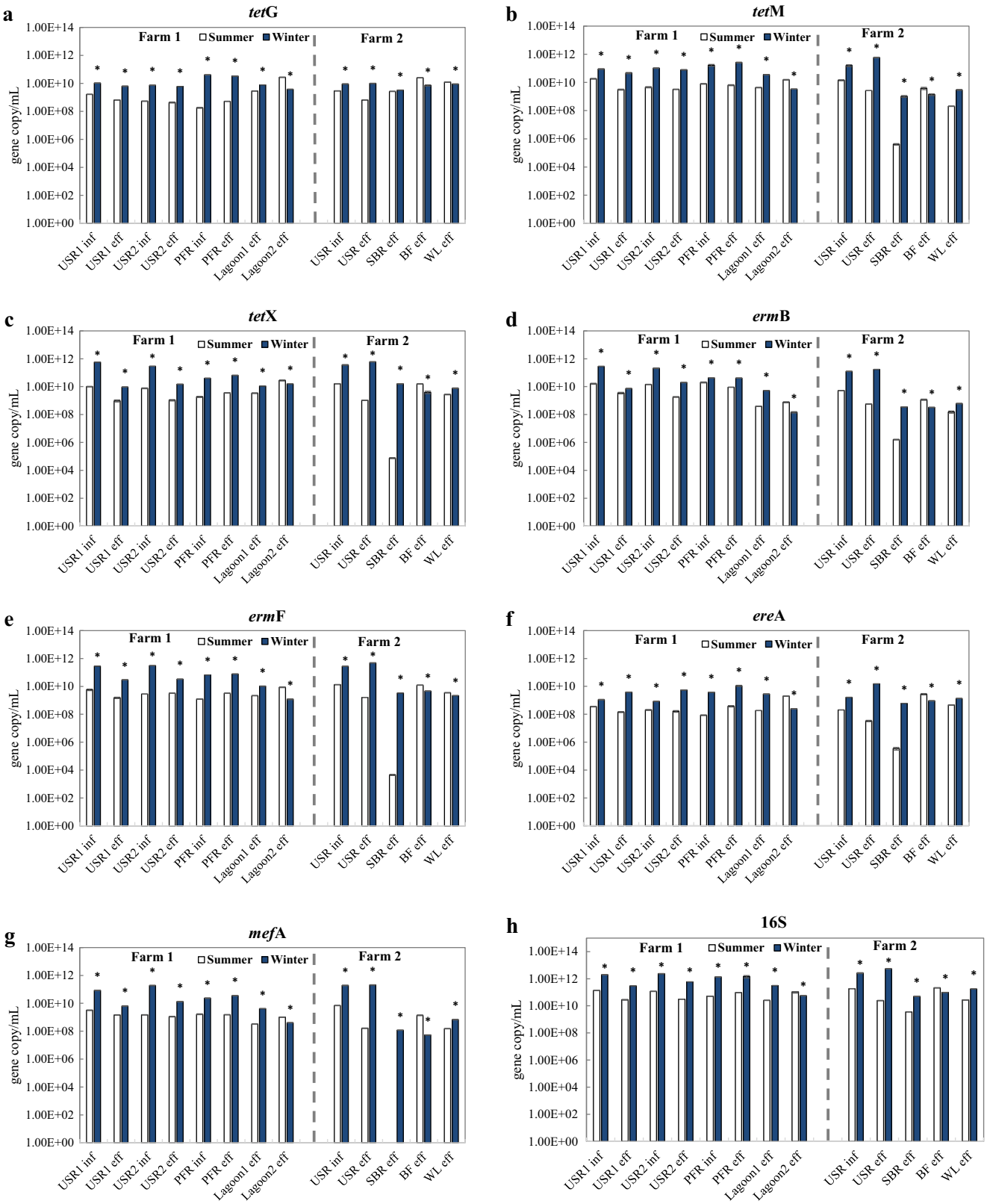

Fig. 2 Abundance of ARGs and 16S rRNA gene in swine wastewater treatment systems (**a** *tet*G; **b** *tet*M; **c** *tet*X; **d** *erm*B; **e** *erm*F; **f** *ere*A; **g** *mef*A; **h** 16S rRNA gene; *$p<0.05$, significant difference between values of summer and winter; *USR* upflow solid reactor, *PFR* plug-flow reactor, *SBR* sequencing batch reactor, *BF* biofilter, *WL* wetland, *inf* influent sample, *eff* effluent sample)

The abundance of ARGs was significantly correlated with the 16S copy number ($p<0.01$), and the efficiency of ARG removal was greatly influenced by the effects of treatments on total bacterial numbers. To observe effects on ARGs specifically in each treatment process, the relative abundance of ARGs in the swine wastewater treatment systems, normalized to 16S rRNA gene, is shown in Fig. 3. In raw swine wastewater, *tet*M and *tet*X were the highly abundant tetracycline resistance genes in this study. Cheng et al. (2013) reported that ribosomal protection genes (*tet*Q, *tet*M, *tet*W, *tet*O) were more abundant than other tetracycline resistance genes in swine wastewater, with 5.53×10^{-2} copies of *tet*M/16S rRNA. Li et al. (2015) proposed that *tet*M has a strong correlation with other ARGs and has been proposed as an indicator of co-occurring ARGs in environment. In this study, the relative abundance of *tet*M was high in raw swine wastewater and was correlated with the abundance of macrolide resistance genes, including *erm*B, *erm*F, and *mef*A ($p<0.05$). The *tet*X system, encoding tetracycline inactivation enzyme, had a similar magnitude to *tet*M in raw swine wastewater on the two investigated farms. After anaerobic digestion, the relative abundance of tetracycline resistance genes shifted; while *tet*M and *tet*X still dominated, with *tet*X slightly decreased and *tet*M increased in most cases, the relative abundance of *tet*G constantly increased. Unlike the changes seen in absolute abundance of ARGs, the relative abundance of ARGs after biological treatments did not show consistent seasonal trend.

After anaerobic digestion, the relative abundance of *tet*M was increased by 0.14–0.54 log and *tet*X reduced by 0.11–0.96 log, while *tet*M in the lagoon system was reduced by 0.06–0.27 log. In most cases, the relative abundance of *tet*X was increased by 0.28–0.89 log after lagoon treatment. The relative abundance of *tet*G was constantly increased by 0.22–0.60 log and 0.03–1.31 log after anaerobic digestion and lagoon treatments, respectively. But for SBR, BF, and WL systems on farm 2, the variations of *tet*M, *tet*X, and *tet*G were inconsistent in summer and winter. Diehl and Lapara (2010) reported that the reductions of *tet*A, *tet*O, *tet*W, and *tet*X over a 5-day anaerobic digestion fit a first-order kinetic model, but aerobic processes had a less substantial effect on removal of *tet* genes compared with the anaerobic process. The increase in relative abundance of *tet*G after biological treatment of swine wastewater was also reported by Cheng et al. (2013) and Chen et al. (2015). Interestingly, *tet*G was not the dominant ARGs in raw swine wastewater, but increased in relative abundance after biological treatment, which could be due to an increase in abundance of the microbial community harboring the *tetG* gene. *Salmonella* spp. is the primary potential host of *tet*G, which poses a high risk to the environment in effluent discharged from swine farms (Adesiji et al. 2014). Although *Salmonella* is vulnerable to biological treatment, *tet*G still increased; therefore, *Salmonella* alone cannot explain the occurrence of *tet*G, and further study is needed into *tet*G carriers and antibiotic-resistant pathogens. For the total tetracycline resistance genes detected (*tet*G, *tet*M,

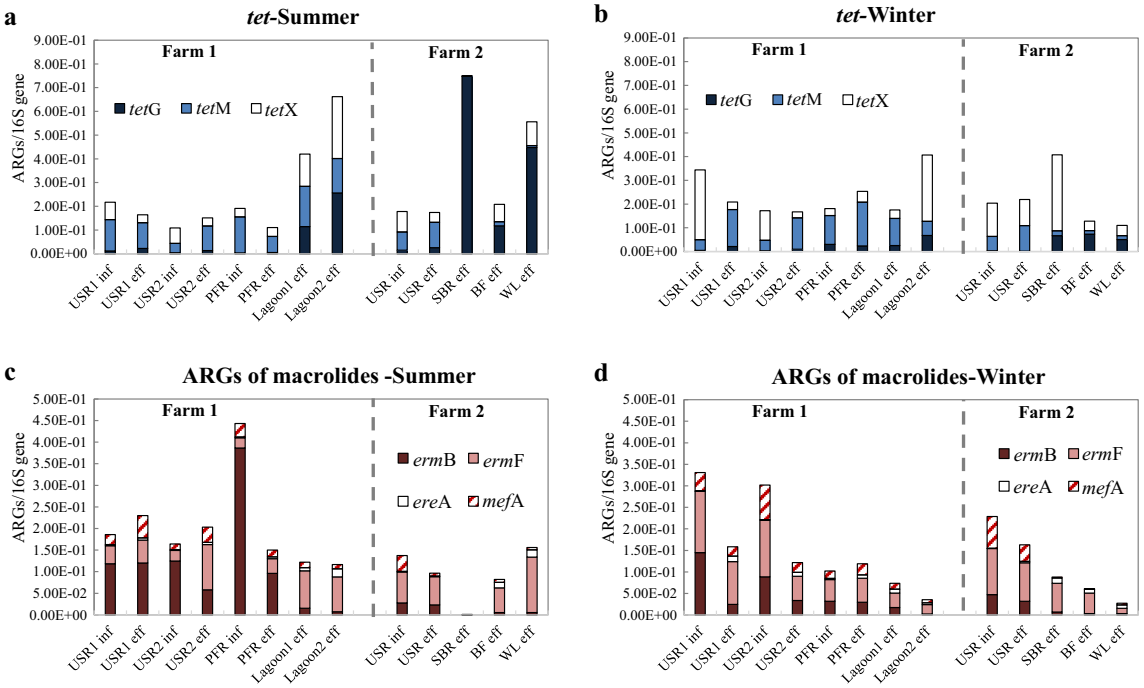

Fig. 3 Relative abundance of ARGs (normalized to 16S rRNA gene) in swine wastewater treatment systems (**a** *tet* in summer; **b** *tet* in winter; **c** ARGs of macrolide in summer; **d** ARGs of macrolide in winter; *USR* upflow solid reactor, *PFR* plug-flow reactor, *SBR* sequencing batch reactor, *BF* biofilter, *WL* wetland, *inf* influent sample, *eff* effluent sample)

and *tet*X), the effluents of the two wastewater treatment systems showed an increase in relative abundance, comparing with raw swine wastewater, especially in summer. The normalized abundance of total *tet* genes decreased only in the effluent sample of farm 2 during in winter.

For macrolide resistance genes as shown in Fig. 3, *erm*B, *erm*F, and *mef*A were the dominant ARGs in raw swine wastewater, whereas *ere*A showed the lowest relative abundance. *erm*B and *mef*A, encoding 23S rRNA methyltransferase and an efflux protein, respectively, showed a decline in relative abundance during the anaerobic, lagoon, and aerobic treatments on the two swine farms, both in summer and winter. *erm*B is one of the most common macrolide resistance genes detected in swine wastewater, with relatively high abundance of 1.2E−01 copies/16S rRNA (Joy et al. 2013; Joy et al. 2014). *mef*A was always detected in *Streptococci* and *Enterococci* isolated from pig carcasses (Martel et al. 2003). but its abundance has only been rarely quantified in swine wastewater. *erm*F is another 23S rRNA methylation gene and was always detected with high relative abundance of 2.2E−03 (Joy et al. 2014) and 6.04E−04 copies/16S rRNA (Brooks et al. 2014) in swine wastewater. *ere*A is not the dominant macrolide resistance genes in wastewater; Pei et al. (2007) detected *ere*A in dairy wastewater with a low relative abundance of 5.95E−6 copies/16S rRNA (Pei et al. 2007).

In this study, the relative abundance of *erm*B was effectively reduced after anaerobic digestion, lagoon, and SBR treatments, by 0.08–0.76 log, 0.24–0.83 log, and 0.65–1.71 log, respectively. In most cases, *erm*F was reduced after anaerobic digestion, lagoon, SBR, and constructed wetland treatments, by 0.57–0.98 log, 0.16–0.94 log, 2.16–5.56 log, and 0.32–0.55 log, respectively. Joy et al. (2014) reported that the relative abundance of *erm*B and *erm*F decreased by 0.5 log and 1.5 log, respectively, at 37 °C in a 100-mL storage reactor after 40 days. These results suggest that the variation of ARGs under real environmental conditions can be quite different from those in a controlled experiment.

The relative abundance of *ere*A increased after anaerobic digestion and lagoon treatment by 0.09–1.41 log and 0.06–0.40 log, respectively, but did not show a constant trend in the "SBR+BF+WL" system. While *ere*A may not be a prevailing ARG in animal wastewater, its relative abundance increased after biological treatment observed by Pei et al. (2007) in a study with aerobic or anaerobic incubation of dairy wastewater for 10–20 days. In most cases, *mef*A was effectively removed after anaerobic digestion and lagoon treatments, with reduction of 0.28–0.74 log and 0.10–0.29 log. The total relative abundance of all the macrolide resistance genes (*erm*B, *erm*F, *ere*A and *mef*A) in the effluent was lower in winter than that of the influent. However, removal of macrolide resistance genes in summer was not obvious.

Removal of ARGs by the entire treatment system

Swine wastewater is generally treated by combinations of technologies, including anaerobic and aerobic processes. The occurrence of ARGs in the two biological wastewater treatment systems tested is shown in Fig. 4a. Treatment removed more ARGs in winter than in summer in most cases; in both systems, some ARGs were only reduced in winter, including *tet*G and *ere*A. Interestingly, these two ARGs proliferated in relative abundance after biological wastewater treatment. Some ARGs may have differences in variation of abundance and relative abundance after wastewater treatment, which was also reported by Li et al. (2014). In these two wastewater treatment systems, *tet*M, *erm*B, and *mef*A were all effectively removed, with 0.95, 1.43, and 1.08 log removed in summer, and 1.43, 2.01, and 1.82 log removed in winter, respectively. If we compare the two wastewater treatment systems in this study, the system of farm 2 was more effective than that of farm 1, except for *tet*G and *ere*A. However, these were not the predominant ARGs for tetracycline and macrolide resistance. *tet*X was only removed in the wastewater treatment system of farm 2 and

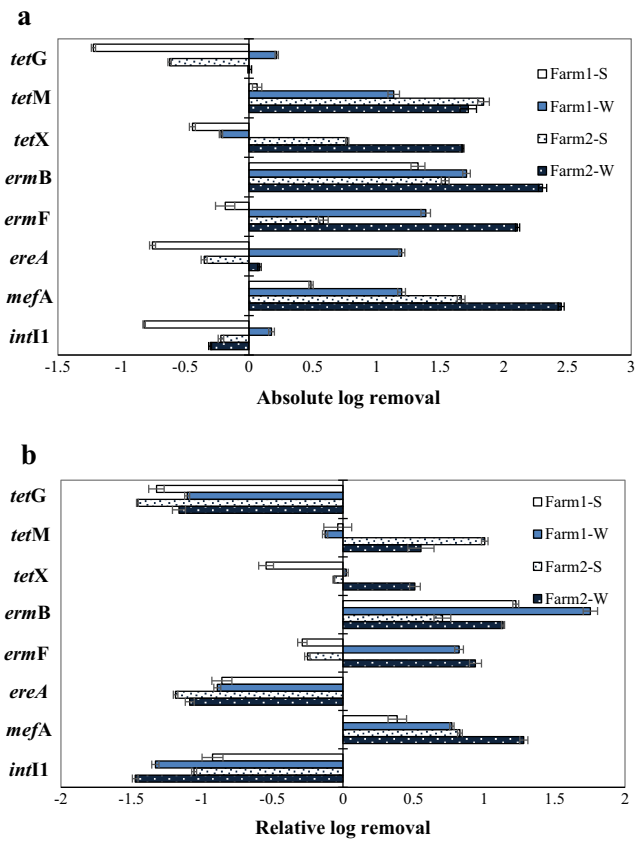

Fig. 4 Absolute and relative logarithmic removal of target genes in swine wastewater treatment systems (**a** absolute abundance removal; **b** relative abundance removal; *Farm1-S* summer samples from Farm 1, *Farm1-W* winter samples from Farm 1, *Farm2-S* summer samples from Farm 2, *Farm2-W* winter samples from Farm 2)

not affected by the lagoon system of farm 1. Barkovskii et al. (2012) indicated that *tet*X was a persistent ARG in swine wastewater lagoons with an abundance of 10^6–10^7 copies/mL. This study indicated that a combination of anaerobic digestion with aerobic biological and constructed wetland processes may be effective for *tet*X removal.

The decrease in relative abundance of ARGs (normalized to 16S rRNA copy number) is shown in Fig. 4b. In most cases, the relative log removal of ARGs during the entire treatment process was higher in winter than in summer. The relative abundance of *tet*M was reduced only by the wastewater treatment system of farm 2. The relative abundance of *tet*M increased after lagoon treatment on farm 1, indicating swine wastewater stored in lagoons may increase bacterial proliferation resulting in amplification of the *tet*M gene, which was also reported by Barkovskii et al. (2012). The declining trend of relative abundance of *tet*X and *erm*F was greatly influenced by the season. The relative abundance of *tet*X and *erm*F was only reduced in winter, whereas their relative abundance in summer increased. The *erm*F variation trend between seasons was consistent with the results of Chen et al. (2010). indicating *erm*F could be effectively reduced in winter. Higher ambient temperatures in summer may make the removal of some kinds of antibiotic resistant bacteria (ARB) more difficult; however, this is another field that requires further research.

The abundance of *erm*B and *mef*A consistently declined in two swine wastewater treatment systems, with an average log removal of 0.97 and 0.61 in summer and 1.44 and 1.03 in winter, respectively. However, the relative abundances of *tet*G and *ere*A increased after wastewater treatment in both seasons. This could be due to proliferation of the ARB carrying *tet*G and *ere*A during the wastewater treatment process or horizontal transfer of the ARGs to other species (Li et al. 2014). The relative abundance of *tet*G and *ere*A was significantly correlated with the relative abundance of *int*I1 ($p<0.01$), and there was also a significant correlation between the relative abundance of *tet*G and *ere*A ($p<0.01$). Du et al. (2014) reported a consistent variation trend between *tet*G and *int*I1 during wastewater treatment. Integrons could facilitate the horizontal transfer of ARGs (Martínez 2008; Shi et al. 2013). Previous studies have indicated that *tet*G and *ere*A are all inducible ARGs co-occurring with the class 1 integron (Cheng et al. 2013; Shahada et al. 2007; Sung and Oh 2014). Shahada et al. (2007) indicated that *tet*G and class 1 integron were simultaneously harbored by *Salmonella enterica* serotype Typhimurium isolated from bovine. Sung and Oh (2014) reported that class 1 integron carrying cassette arrays of *dfr*A32-*ere*A-*aad*A2 were harbored by *Proteus mirabilis* and *Proteus penneri* belonging to Enterobacteriaceae isolated from chickens. Class 1 integron is capable of transferring resistance by conjugation between different species of Enterobacteriaceae, which have been identified in isolates from municipal wastewater treatment plant (Mokracka et al. 2012). In addition, Enterobacteriaceae are at high prevalence in animal wastewater and some species of Enterobacteriaceae are important human pathogens (Resende et al. 2014b). The antibiotic resistance of Enterobacteriaceae and the horizontal transfer of ARGs could pose public health risks and needs further study.

Conclusions

ARGs for tetracycline (*tet*G, *tet*M, and *tet*X) and macrolide (*erm*B, *erm*F, *ere*A, and *mef*A) were quantified in two typical swine wastewater treatment systems, i.e., an "anaerobic digestion + lagoon" system and an "anaerobic digestion + aerobic biological treatment+ wetland" system in both winter and summer. The abundance of ARGs in winter was higher than in summer, with an average variation of 0.31–3.52 log between summer and winter. *tet*M, *tet*X, *erm*B, *erm*F, and *mef*A were highly abundant in raw swine wastewater. ARGs abundance was effectively reduced by most of the individual treatment processes, including anaerobic digestion, SBR, and wetlands, with more efficient removal in winter than summer; however, the relative abundance of ARGs was quite variable. Anaerobic digestion decreased the relative abundance of *tet*X, *erm*B, *erm*F, and *mef*A; lagoon treatment decreased *tet*M, *erm*B, *erm*F, and *mef*A; and SBR decreased *tet*M, *erm*B, and *erm*F. However, biofilter and constructed wetland did not display consistent removal efficacy on ARGs over two sampling seasons. *erm*B and *mef*A were effectively reduced during both winter and summer, not only in abundance but also in relative abundance. *tet*G and *ere*A were not the dominant ARGs in raw swine wastewater but all increased after wastewater treatment. The relative abundance of *tet*G and *ere*A was significantly correlated with *int*I1 ($p<0.01$) implying horizontal transfer of ARGs, which poses a great threat to public health. Further research is needed to determine the effective method for controlling ARGs.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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