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RESEARCH ARTICLE

Occurrence of sulfonamide-, tetracycline-, plasmid-mediated quinolone- and macrolide-resistance genes in livestock feedlots in Northern China

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Abstract Antibiotic resistance genes (ARGs) in livestock feedlots deserve attention because they are prone to transfer to human pathogens and thus pose threats to human health. In this study, the occurrence of 21 ARGs, including tetracycline (*tet*)-, sulfonamide (*sul*)-, plasmid-mediated quinolone (PMQR)- and macrolide-resistance (*erm*) genes were investigated in feces and adjacent soils from chicken, swine, and cattle feedlots in Northern China. PMQR and *sul* ARGs were the most prevalent and account for over 90.0 % of the total ARGs in fecal samples. Specifically, PMQR genes were the most prevalent, accounting for 59.6 % of the total ARGs, followed by *sul* ARGs (34.2 %). The percentage of *tet* ARGs was 3.4 %, and *erm* ARGs accounted for only 1.9 %. Prevalence of PMQR and *sul* ARGs was also found in swine and cattle feces. The overall trend of ARG concentrations in feces of different feeding animals was chicken>swine>beef cattle in the studied area. In soils, *sul* ARGs had the highest concentration and account for 71.1 to 80.2 % of the total ARGs, which is possibly due to the widely distributed molecular carriers (i.e., class one integrons), facilitating *sul* ARG propagation. Overall, this study provides integrated profiles of various types of ARGs in livestock feedlots and thus provides a reference for the management of antibiotic use in livestock farming.

Keywords Antibiotic resistance genes · Livestock farming · Antibiotics

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Introduction

Antibiotics have been routinely utilized in livestock farming to control diseases caused by various bacterial pathogens and at a sub-therapeutic dose to improve feed efficiency (Aarestrup 2012; Khan et al. 2008). The great boom in the number of confined animal feeding operations for poultry, swine, and dairy production contributed to a large increase in demand for antibiotics. Antibiotics are fed continuously to animals for almost a whole growth life, which results in a maintained concentration of these reagents in the gastrointestinal tracts. Under the selection pressure imposed by antibiotics, resistance in the gut microbiota of animals increases owing to the acquisition of antibiotic resistance genes (ARGs). Antibiotic resistance among bacteria in soil may also increase due to the use of livestock slurry (Agersø et al. 2006; Sengeløv et al. 2003). Even ARGs themselves have been considered as emerging environmental pollutants recently (Pruden et al. 2006). Therefore, studies on the occurrence and distribution of ARGs in animal farming environments are critically important.

Sulfonamides, tetracyclines, quinolones, and macrolides are widely used in modern livestock farming industries. Previous studies have reported the occurrence of their corresponding resistance genes in water, soil, and sediment (Chen et al. 2010; Cheng et al. 2013; Li et al. 2012; Luo et al. 2010; Peak et al. 2007; Wu et al. 2010; Zhang and Zhang 2011). Our previous study found that sulfonamide resistance genes were prevalent in sediments in the Haihe River basin, with concentrations ranging from $(7.8\pm1.0)\times10^{9}$ to $(1.7\pm0.2)\times10^{11}$ copies/g (Luo et al.

2010). Macrolides are often applied together with lincosamides and streptogramins in livestock usage. Only very limited investigations have shed light on macrolide resistance genes in livestock farming, except for one study from livestock farm reported that macrolide resistance genes were prevalent but in low abundance in livestock manure (Chen et al. 2007). Quinolones are a family of pure synthetic antibiotics and were originally used in human clinical medicine. Nevertheless, quinolone-resistant bacteria and the resistance genes have been prevalent in livestock feedlots (Price et al. 2007), which is most likely due to their heavy use in livestock farming in recent years (Zhao et al. 2010b; Zhou et al. 2011). In a recent study, the concentration of *oqx*B was tenfold higher than 16S ribosomal RNA (rRNA) gene in swine feedlot wastewater and soil (Li et al. 2012). To date, there has been a lack of systematic studies of the overall occurrence of the ARGs selected by commonly utilized antibiotics including sulfonamides, tetracyclines, quinolones, and macrolides in animal farming environments.

In China, the average annual usage of veterinary antibiotics has reached approximately 6000 tons (Zhao et al. 2010b). Liaoning Province, an important part of northeastern China, is located in the Liaohe River basin, with a population of approximately 44 million people. The annual livestock production was 1.2×10^8 in head of pigs (the number of animals other than pigs was converted into the number of pigs according to the volume of waste generated by each animal). Tianjin City is located on the estuary between the Haihe River and Bohai Sea, and the number of livestock is 2.0×10^8 head of pigs (converted as above) (Zhou et al. 2011). Livestock and aquaculture account for 57 % of Tianjin's gross agricultural output, whereas the national average is 30 %. Therefore, investigation of the antibiotic resistance pollution profile in livestock farming in these areas is especially necessary.

The objective of this study is to investigate the occurrence of ARGs, i.e., sulfonamide-, tetracycline-, and plasmid-mediated quinolone- and macrolide-resistance genes, in feces of chicken, swine, and beef cattle in feedlots and adjacent soils in Northern China. Culture-independent methods, including PCR and qPCR, were used for detecting 21 selected ARGs. The study provides integrated profiles of various types of antibiotic resistance genes (sulfonamides, tetracyclines, quinolones, and macrolides) in livestock farming feedlots and thus provides a reference for the management of veterinary antibiotic usage in livestock farming.

Materials and methods

Sampling sites and sample collection

Multiple livestock feedlots were selected to investigate the occurrence of antibiotic resistance genes in livestock farming in northern China (Fig. 1). These include three chicken feedlots (designated as C1a-C3a), six swine feedlots (S1a-S6a), and seven beef cattle feedlots (B1a-B7a) in Liaoning Province and three chicken feedlots (C1b-C3b) and three swine feedlots (S1b-S3b) in Tianjin City. All of these feedlots were concentrated animal feeding operations. Scales of the feedlots were summarized in Supplementary Information, Table S1. The soil sampling sites were selected in arable lands (used for crop and/or vegetable planting) adjacent (distance of about 100–500 m) to the feedlots which are amended with the animal manure from the feedlots. Meanwhile, soil from a Forest Park in Tianjin (point R in Fig. 1), which has no history of antibiotic or manure application, was sampled as the soil reference. More detailed information about soil sampling is provided in Supplementary Information.

Feces and soil samples from the feedlots were collected in August 2011. Fresh feces were collected within minutes after defecation and kept sterile to avoid cross-contamination. Soil samples were collected on that same day as the collection of feces. For each sampling site, samples were collected from the top 10 cm of the surface soil using a multiple-point sampling method and then mixed together to obtain one composite sample. All samples were immediately stored in dark and maintained with ice packets until returned to the laboratory, where they were stored at −20 °C before DNA extraction (within 24 h). Water content of each sample was measured by weighing an aliquot of the sample before and after lyophilization for at least 48 h. Physiochemical properties including pH and total organic matter of the samples were also measured. Soil pH was determined with a sample (feces or soil) to water ratio of 1:10. Total organic matter was determined by the $K_2Cr_2O_7$ oxidation method.

DNA extraction

Soil DNA was extracted using the Soil DNA Isolation Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocol. For feces, an extraction method established previously was followed (Yu and Morrison 2004). The product of DNA extraction was further purified using the DNA Pure-spin Kit (Vigorousbio, Beijing, China) to minimize PCR inhibition. The product DNA was checked for integrity by agarose gel electrophoresis with Lambda DNA HindIII Digest standards (Transgen, Beijing, China). DNA extraction was conducted in triplicate, and the extracted DNA samples were stored at −20 °C until subsequent PCR analyses.

Qualitative PCR

Qualitative PCR assays were conducted in a Biometra T100 PCR machine (Biometra, Goettingen, Germany) targeting at *Bacteria* 16S rRNA gene and 21 selected ARGs. Information of the primers is listed in Table 1. The PCR mixture (25 μL total volume) consisted of 2.5 μL of Taq reaction buffer,

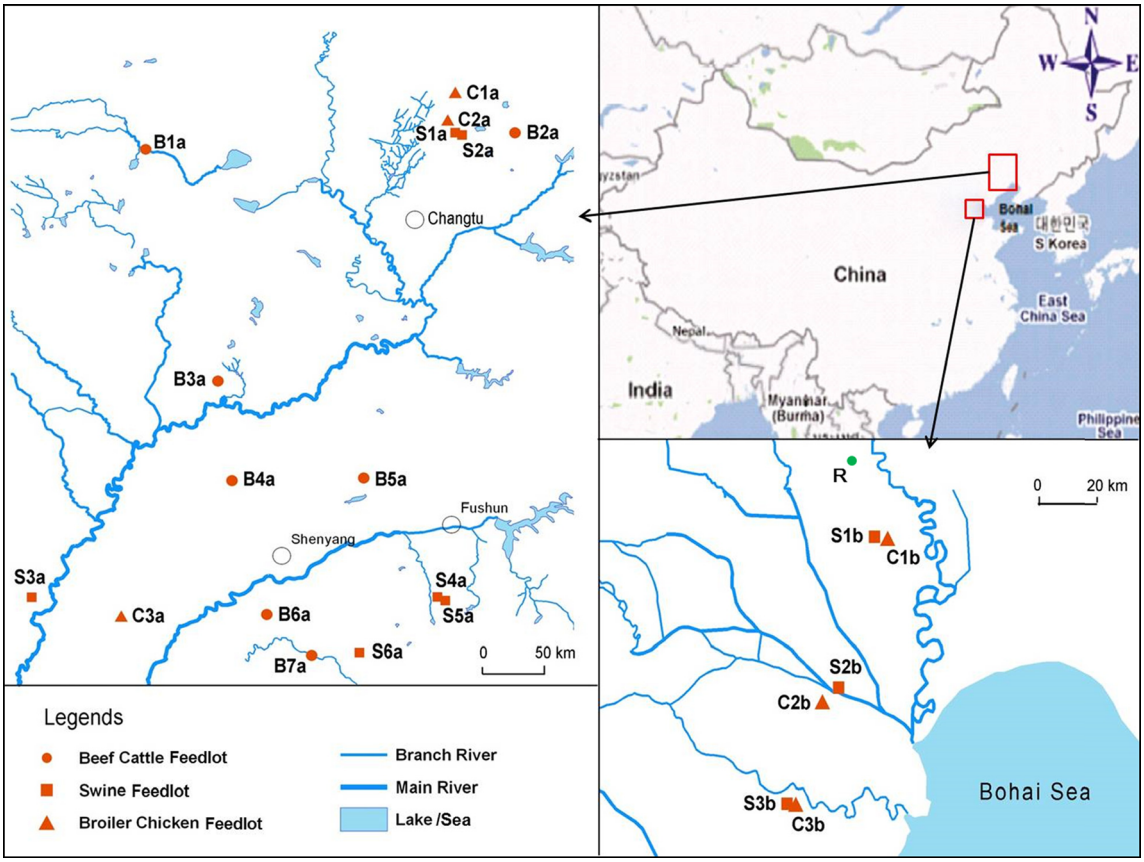

Fig. 1 Geographical locations of sampled cattle feedlots (*B*), swine feedlots (*S*), chicken feedlots (*C*), and reference (*R*). Two areas were included: Liaoning Province (**a**), located in the Liaohe River basin and Tianjin City (**b**), located in the Haihe River basin

0.2 mM dNTPs, 0.2 μM primers, 1.75 units of TaqDNA polymerase (EasyTaq, Transgene, Beijing, China), and 1 μL of template DNA. The PCR product of each gene was ligated to plasmid pEasy-T1 (Transgen, Beijing, China) and used as positive control when detecting ARGs in samples. For the negative control, sterile-deionized water was added instead of DNA samples. The plasmids containing target genes were further used to prepare the standardized product for real-time qPCR.

Quantitative PCR

Quantitative PCR analyses were performed on a Bio-Rad iQ5 instrument (Bio-Rad, CA, USA) to quantify *sul*1, *sul*2, *tet*M, *tet*O, *tet*Q, *tet*W, *qnr*S, *oqx*B, *erm*B, *erm*C, and 16S rRNA gene. The qPCR reactions were conducted in 96-well plates. DNA extracted from the samples was amplified against the tenfold serially diluted calibration curve over eight orders of magnitude on the same real-time PCR plate in triplicate. The reactions were performed at a final volume of 25 μL of reaction mixture (SYBR® Premix Ex Taq™ II, Takara, Japan), including 0.25 μM of each primer and 1 μL of template DNA. The real-time qPCR program was as follows: initial denaturing for 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 1 min at annealing temperatures, 30 s at 72 °C, and a final melt curve stage with temperature ramping from 55 °C to 95 °C (0.5 °C per read, 30-s hold).

Statistical analysis

All statistical analysis was conducted using SPSS Statistics version 20.0 (IBM, NY, USA). *T*-tests were used for comparisons of concentrations of ARGs between samples. The level for statistical significance was set at $p<0.05$.

Results and discussion

Occurrence of ARGs in livestock feces

Twenty-one ARGs among tetracycline-, sulfonamide-, and plasmid-mediated quinolone- and macrolide- resistance genes were investigated in this study. Detection frequencies (DFs) of the targeted ARGs in samples are summarized in Table 1. *sul*1, *sul*2, *tet*M, *tet*O, *tet*Q, *tet*W, *oqx*B, *qnr*S, *erm*B, and *erm*C were detected in all feces and soil samples (Table 2, or Tables S2 and S3 in Supplementary Information for details),

Table 1 PCR (qPCR) primers and the PCR parameters used in this study

Target Gene	Primer sequence (5′–3′)	Product Size (bp)	Annealing temperature (°C)	Reference
sul1	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	163	56	Pruden et al. 2006
sul2	TCCGGTGGAGGCCGGTATCTGG CGGGAATGCCATCTGCCTTGAG	191	60	Pruden et al. 2006
sul3	TCCGTTCAGCGAATTGGTGCAG TTCGTTCACGCCTTACACCAGC	128	60	Pruden et al. 2006
tetA	GCGCGATCTGGTTCACTCG AGTCGACAGYRGCGCCGGC	164	61	Aminov et al. 2002
tetB/P	AAAACTTATTATATTATAGTG TGGAGTATCAATAATATTCAC	169	46	Aminov et al. 2001
tetC	CTTGAGAGCCTTCAACCCAG ATGGTCGTCATCTACCTGCC	418	55	Aminov et al. 2002
tetG	GTCGATTACACGATTATGGC CACTTGGCCGATCAGTTGA	468	55	Aminov et al. 2002
tetH	CAGTGAAAATTCACTGGCAAC ATCCAAAGTGTGGTTGAGAAT	185	61	Aminov et al. 2002
tetM	ACAGAAAGCTTATTATATAAC GGCGTGTCTATGATGTTCAC	171	45	Aminov et al. 2001
tetO	ACGGARAGTTTATTGTATACC TGGCGTATCTATAATGTTGAC	171	46	Aminov et al. 2001
tetQ	AGAATCTGCTGTTTGCCAGTG CGGAGTGTCAATGATATTGCA	169	60	Aminov et al. 2001
tetS	GGTCAACGGCTTGTCTATGTA CCAGGCTCTCATACTGAATGC	667	56	Aminov et al. 2001
tetT	AAGGTTTATTATATAAAAGTG AGGTGTATCTATGATATTTAC	169	46	Aminov et al. 2001
tetW	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	168	60	Aminov et al. 2001
tetX	CAATAATTGGTGGTGGACCC TTCTTACCTTGGACATCCCG	468	55	Zhang and Zhang 2011
qnrD	ACGACAGGAATAGCTTGGAAGG TCAGCCAAAGACCAATCAAACG	465	50	Cavaco et al. 2009
qnrS	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	54	Cattoir et al. 2007
oqxB	TCCTGATCTCCATTAACGCCCA ACCGGAACCCATCTCGATGC	131	64	Kim et al. 2009
qepA	CCAGCTCGGCAACTTGATAC ATGCTCGCCTTCCAGAAAA	570	60	Xia et al. 2010
ermB	CCGATACCGTTTACGAAATTGG TACTTTGGCGTGTTTCATTGC	190	57	This study
ermC	GAAATCGGCTCAGGAAAAGG TAGCAAACCCGTATTCCACG	293	57	This study
16S	CGAATATGGAATCCCTAGTAACT GCCCACTCAGTTCGATACGC	140	58	Luo et al. 2010

and they were further quantified using real-time qPCR. DFs of *tet*C, *tet*G, *tet*H, *tetS*, *tetT*, and *tet*X ranged from 66.7 to 97.4 % (Table 2). *tet*A, *tet*B/P, as well as *sul*3, *qnr*D, and *qep*A were detected in less than 50 % of the samples. The two selected macrolide resistance genes (*erm*B and *erm*C) were detected in all samples (Table 2).

Concentration of each quantified ARG in feces of chicken, swine, and cattle is presented in Fig. 2, a, c, e. Among all the quantified ARGs, quinolone resistance gene *oqx*B and sulfonamide resistance genes *sul*1 and *sul*2 had higher concentrations than other ARGs in feces. For instance, in feces of chicken, the concentration of *oqx*B was (3.48±2.48)×

Table 2 Detection frequency (DF) of the 21 targeted ARGs in feces (n=22) and soil samples (n=17). All data are shown in percentage (%)

ARG type	DF in feces	DF in soil	ARG type	DF in feces	DF in soil
*tet*A	50.00	41.18	*sul*1	100.0	100.0
*tet*B/P	45.45	5.88	*sul*2	100.0	100.0
*tet*C	77.27	58.82	*sul*3	40.91	0
*tet*G	95.45	70.59			
*tet*H	95.45	100.0	*qnr*D	40.91	23.53
*tet*M	100.0	100.0	*qnr*S	100.0	100.0
*tet*O	100.0	100.0	*qep*A	22.73	58.82
*tet*Q	100.0	100.0	*oqx*B	100.0	100.0
*tet*S	59.09	88.23			
*tet*T	100.0	88.23	*erm*B	100.0	100.0
*tet*W	100.0	100.0	*erm*C	100.0	100.0
*tet*X	54.55	82.35			

10^{10} copies/g dry weight, $(1.39\pm0.92)\times10^{10}$ copies/g dry weight for *sul*1, and $(7.53\pm4.26)\times10^{9}$ copies/g dry weight for *sul*2 (Detailed data in Tables S4 and S5). Prevalence of *oqx*B, *sul*1, and *sul*2 genes was also observed in swine and cattle feces. In cattle feces, the concentrations of these genes followed the order of *sul*2>*sul*1>*oqx*B (*t*-test, $p<0.05$). As for *tet* genes, their detected concentrations were lower than those of the *oqx*B, *sul*1, and *sul*2 genes, and the four subtypes (*tet*M, *tet*O, *tet*Q, and *tet*W) had similar concentrations (Fig. 2c). The concentration of the macrolide resistance gene *erm*B was comparable to those of *tet* genes. *qnr*S and *erm*C had the lowest concentrations.

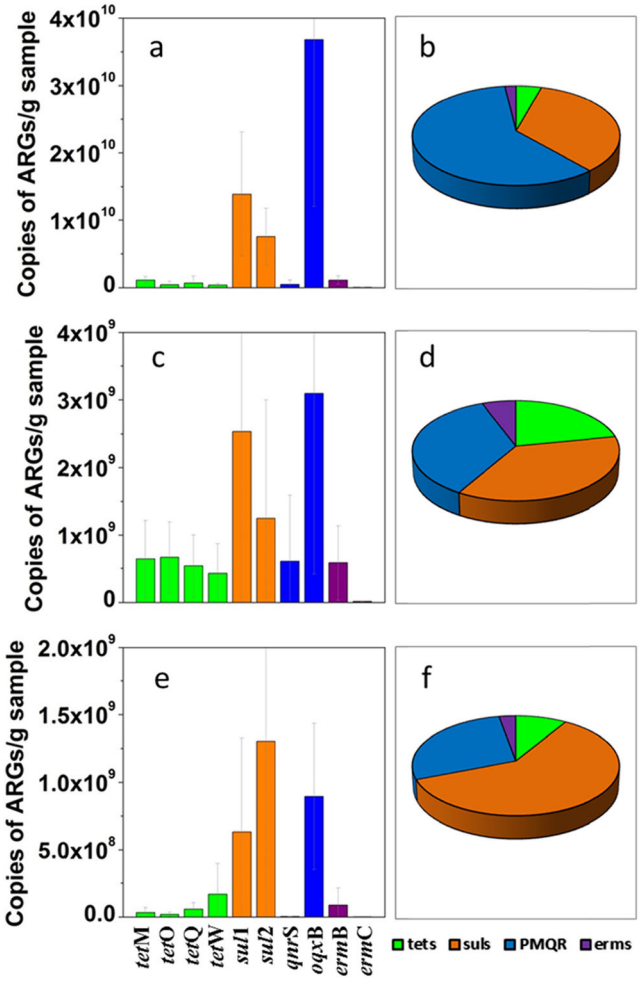

Fig. 2 Concentrations of ARGs in feces from chicken (**a**), swine (**c**), and cattle (**e**) feedlots, and percentages of *tet*, *sul*, PMQR, and *erm* ARGs accounting for the total amount of ARGs in chicken (**b**), swine (**d**), and cattle animal feedlots (**f**)

To quantitatively compare the *sul*, *tet*, plasmid-mediated quinolone (PMQR), and *erm* ARGs in livestock feces, the index of concentrations of a particular type of ARG (i.e., *sul*, *tet*, *PMQR*, or *erm*) over the overall concentrations of all ARGs was obtained (Fig. 2b, d, f). In chicken feces, PMQR and *sul* ARGs accounted for over 90 % of the total ARGs in fecal samples (Fig. 2b). Specifically, PMQR are the most prevalent, and they account for 59.6 % of the total ARGs, followed by *sul* ARGs (34.2 %). Compared to PMQR and *sul* ARGs, *tet* and *erm* ARGs account for lower percentages of the total ARGs in fecal samples. The percentage of *tet* ARGs was 3.4 %, and *erm* ARGs accounted for only 1.9 % (Fig. 2b). Similar to chicken feedlot samples, PMQR and *sul* ARGs were the most prevalent, while *tet* and *erm* were the least prevalent ARGs in swine and cattle samples (Fig. 2d, f). Unlike chicken samples, the percentages of *sul* ARGs are higher than those of PMQR ARGs in swine and cattle samples. The difference in the dominant ARGs in chicken, swine, and cattle samples may reflect the priority of different antibiotic usage in different feeding animals.

The high concentration of PMQR gene *oqx*B in fecal samples in this study is noticeable. This gene was first found on plasmid pOLA52 in *Escherichia coli* isolated from swine manure by Sørensen et al. (2003), and it is proved to participate in encoding OqxAB, a multidrug efflux pump, resulting in reduced susceptibility towards chloramphenicol,

ciprofloxacin, and olaquindox (Hansen et al. 2007). Recently, this gene was proved to be prevalent (328 out of 696 isolates were positive) among *E. coli* isolates from food-producing animals in South China (Liu et al. 2013). Besides, the plasmid carrying *oqx*B have been demonstrated to be highly transferable, facilitating its dissemination to other bacteria in the environment (Li et al. 2013; Zhao et al. 2010a). Another study from Li et al. (2012) also reported the abundance of *oqx*B to be up to 10 times of 16S rRNA gene in swine feedlot wastewater in Beijing, China. Meanwhile, high levels of quinolones in sediments from the Haihe River (Luo et al. 2011) and the Liaohe River (Zhou et al. 2011), the study area, have been previously reported. The prevalence of PMQR genes in animal feces in this study also reflects the heavy use of quinolone antibiotics in livestock farming in the studied area. The prevalence of sulfonamide resistance genes *sul*1 and *sul*2 in feces in the present study agreed with previous research which observed the prevalence of *sul*1 and *sul*2 in the surface water and sediment of the Haihe River, which is located in the same area as this study (Luo et al. 2010).

To compare results of this study with previous studies, relative abundance of each ARG was calculated by normalizing the copies of each ARG to 16S rRNA gene. Relative abundance of ARGs in feces/manure and soil from previous studies is summarized in Table 3. Generally, the relative abundance of *tet*, *sul* and PMQR genes are comparable to prior studies on ARGs in livestock farming environments (Table 3), indicating similar antibiotic use in different areas.

ARGs in feces of different feeding animals

The overall concentrations of all quantified ARGs (*tet*, *sul*, PMQR, and *erm*) in different feeding animals including swine, chicken, and beef cattle was chicken>swine>beef cattle (Fig. 2). The concentration of total ARGs in chicken feces were slightly higher (*t*-test, $p=0.03$) than that of swine (detailed data are shown in Supplementary Material, Tables S4–S5), and the concentration of ARGs in swine feces was higher than that in beef cattle (*t*-test, $p<0.01$). The high concentrations of ARGs in chicken feedlots deserve attention. Very few studies have ever investigated ARG abundance in chicken feces previously (Table 3). Though it is fairly difficult to collect the exact data of antibiotic usage in feeding animals in the studied area, the generally higher antibiotic residues found in the feces of chicken and swine than in those of beef cattle were also reported previously (Campagnolo et al. 2002; Zhao et al. 2010b). Compared with swine and cattle, larger doses of antibiotics are permitted in broiler chicken feeding

Table 3 Abundance of *tet*, *sul*, and PMQR genes in feces and soil from feedlots of chicken, swine, and cattle in prior reports and this study. The unit is gene copies/copies of 16S rRNA

Animal type	Matrix	ARGs	Abundance	Reference
Chicken	Manure	*sul*1, 2	$(0.41\sim2.06)\times10^{-2}$/16S[a]	Ji et al. 2012
	Manure	*tet*M, O, W	$(0.07\sim3.92)\times10^{-3}$/16S[a]	
	Manure	*sul*1, 2,3, A	$(0.11\sim1.32)\times10^{-1}$/16S	Cheng et al. 2013
	Manure	*tet*Q	$(7.12\pm2.99)\times10^{-2}$/16S	
	Feces	*tet*M, O, Q, W	$(0.24\sim6.66)\times10^{-2}$/16S	This study
	Feces	*sul*1, 2	$(0.22\sim8.12)\times10^{-1}$/16S	
	Soil	*tet*M, O, Q, W	$(0.04\sim1.19)\times10^{-2}$/16S	
	Soil	*sul*1, 2	$(0.17\sim6.20)\times10^{-1}$/16S	
Swine	Manure	*tet*M, O, W	$(0.05\sim1.21)\times10^{-4}$/16S[a]	Ji et al. 2012
	Manure	*sul*1, 2	$(0.25\sim5.97)\times10^{-4}$/16S	Ji et al. 2012
	Soil	*tet*M, O, Q, W	$(0.02\sim4.41)\times10^{-2}$/16S	Wu et al. 2010
	Soil	*qnr*D, *qep*A, *oqx*B	$(0.22\sim8.19)\times10^{0}$/16S	Li et al. 2012
	Feces	*tet*M, O, Q, W	$(0.30\sim2.10)\times10^{-2}$/16S	This study
	Feces	*sul*1, 2	$(0.06\sim6.80)\times10^{-1}$/16S	
	Soil	*tet*M, O, Q, W	$(0.04\sim1.73)\times10^{-2}$/16S	
	Soil	*sul*1, 2	$(0.27\sim6.90)\times10^{-1}$/16S	
	Soil	*oqx*B, *qnr*S	$(1.31\sim7.16)\times10^{-2}$/16S	
Cattle	Manure	*sul*1,2, 3, A	$(0.33\sim2.81)\times10^{-3}$/16S	Chen et al., 2013
	Manure	*tet*M, O, W	$(0.07–2.46)\times10^{-4}$/16S[a]	Ji et al. 2012
	Manure	*sul*1, 2	$(0.49\sim3.13)\times10^{-2}$/16S[a]	
	Feces	*tet*M, O, Q, W	$(0.005\sim9.08)\times10^{-2}$/16S	This study
	Feces	*sul*1, 2	$(0.10\sim6.69)\times10^{-2}$/16S	

[a] Data were extracted from the figures using Engauge Digitalizer 4.1 (http://digitizer.sourceforge.net)

(Ministry of Agriculture 2003). In addition, breeding density in chicken feedlots is generally higher than that in swine and beef cattle feedlots, leaving the chickens more vulnerable to infectious diseases. For the purpose of preventing and treating diseases, the farm owners are likely to use more antibiotics than permitted. A previous study also reported that enrofloxacin residues in chicken feces in China reached as high as 1420.76 mg/kg (Zhao et al. 2010b). Such high residual quinolone antibiotics likely exerted selective pressure for ARG maintenance and proliferation in the animal feces (Knapp et al. 2008; Li et al. 2012; Luo et al. 2010).

In addition to antibiotic use, the highly transferability of ARGs in chicken microbiome may also contribute to the prevalence of ARGs in chicken feces. In an early study by Levy et al. (1976), chickens were fed tetracycline-supplemented feed, and within 1 week, their intestinal flora contained almost entirely tetracycline-resistant organisms. Increased numbers of resistant intestinal bacteria also appeared in farm members, suggesting that antibiotic resistance is easy to transfer within the intestinal flora of one chicken and also to other chickens. Using metagenomics approaches, Qu et al. (2008) found that mobile DNA elements are a major functional component of chicken cecal microbiomes, thus contributing to horizontal gene transfer. Similar to this study, their results also revealed that in the chicken cecum metagenomes, resistance to antibiotics and other toxic compounds dominated (55–57 %) in the SEED Virulence Subsystem, with tetracycline and fluoroquinolone resistance genes being the most abundant.

Previous studies in swine feedlots were mostly focused on *tet* and *sul* genes (Heuer et al. 2008, 2011; Koike et al. 2007; Wu et al. 2010; Zhu et al. 2013). This study provided pollution profile of the overall range of *sul*, *tet*, PMQR, and *erm* ARGs in the feces of swine production feedlots. In swine feces, *sul* genes accounted for 36.6 %, PMQR 35.8 %, and *tet* 21.9 %. PMQR, *sul*, and *tet* ARGs took up 95.2 % of the total amount of ARGs in swine feces. A previous study also found the prevalence of *oqx*B in wastewater and soil from swine feedlots. The positive correlation between the abundance of ARGs and the concentrations of quinolone antibiotics was obtained in a previous study (Li et al. 2012) and thus confirmed that a high abundance of ARGs was caused by heavy usage of veterinary antibiotics in the studied area.

ARGs in soil adjacent to livestock feedlots

The percentage of the sum of each *tet* (*tet*M, *tet*O, *tet*Q, *tet*W), PMQR (*qnr*S, *oqx*B), and *erm* (*erm*B, *erm*C) account for in the total amount of *sul*, *tet*, PMQR, and *erm* ARGs are quite different in soil compared to feces (Fig. 3). *sul* ARGs remain the most prevalent, and they account for over 70 % of the total ARGs in soil samples (Fig. 3b, d, f). PMQR genes are the second most prevalent, and they account for 19.2, 12.6, and

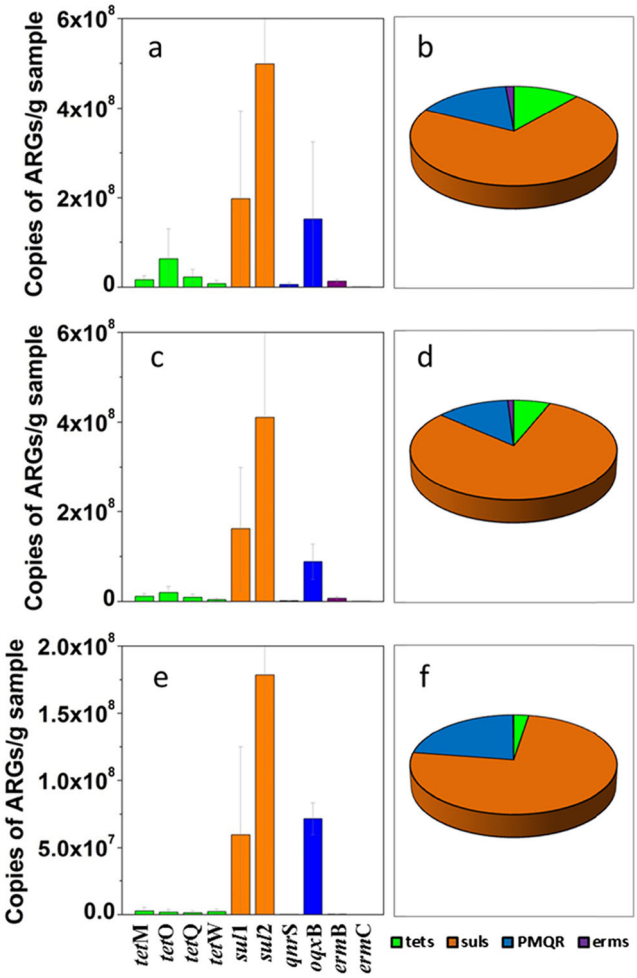

Fig. 3 Concentrations of ARGs in soil adjacent to chicken (**a**), swine (**c**), and cattle (**e**) feedlots, and percentages of the *tet*, *sul*, PMQR, and *erm* ARGs accounting for the total amount of ARGs in soil adjacent to chicken (**b**), swine (**d**), and cattle animal feedlots (**f**)

22.5 % of the total ARGs in soils from chicken, swine, and cattle feedlots, respectively. Compared to PMQR and *sul* ARGs, *tet* ARGs account for lower percentages of the total ARGs in soil samples. The percentage of *erm* ARGs was the lowest and accounted for only approximately 1.0 %, which is smaller than the percentages in feces.

Specifically, the most abundant ARGs in soil were sulfonamide resistance genes *sul*1 *and sul*2. In general, the average concentration of *sul* ARGs were 7–343 times higher than other ARGs detected in soil. The average concentration of *sul*1 was $(2.21\pm1.34)\times10^9$ copies/g dry weight in soil (data can be found in Supplementary Information, Tables S4and S5) and $(1.01\pm0.56)\times10^9$ copies/g dry weight in soil for *sul*2. Since the background levels of *sul*1 ($(8.21\pm0.75)\times10^4$ copies/g dry weight) and *sul*2 ($(5.49\pm0.44)\times10^4$ copies/g dry weight) were low in the reference soil of this area, it is likely that high concentrations of *sul* genes in soil came from manure application. Though the concentration of *oqx*B was the highest in feces, its concentration was significantly

($p<0.05$) lower than *sul*1 and *sul*2 genes (still higher than *tet* and *erm* genes) in soil samples (Fig. 3a, c, e). The *oqx*B gene is located on larger plasmids, which have a narrow host range. In addition, larger plasmids are less prone to horizontal transfer among different species or genera of bacteria. This might be related to the lower abundance of *oqx*B gene in soil samples. The average concentrations of *tet* genes were ($1.21 \pm 0.87) \times 10^8$ copies/g dry weight in soil, lower than those of PMQR and *sul* genes, among which the most abundant gene type was *tet*M. The abundance of *tet* genes in soil was similar to previous studies (Table 3). For example, in research by Wu et al. (2010), *tet*M, *tet*O, *tet*Q, and *tet*W ranged from 1.46×10^6 to 2.62×10^9 copies/g sample in soils adjacent to swine feedlots in China. *erm* genes were less abundant than other ARGs in soil.

In general, the concentrations of ARGs in soil were higher than that in the reference (Tables S4 and S5), but were significantly lower than that in feces, possibly owing to the dilution effect of the soil matrix during the fertilization process. $R_{s/f}$ value was used to evaluate the ARG attenuation from feces to the adjacent soil, and it is defined as the average concentration of each subtype of ARG in feces divided by the concentration of the same gene in the corresponding soil. Significant dependence on different subtypes of ARG was observed for $R_{s/f}$ values. $R_{s/f}$ is 23 for *sul*1 and 31 for *sul*2, whereas $R_{s/f}$ is larger than 50 for most of the *tet* and quinolone resistance genes (for instance, $R_{s/f}$ for *tet*M is 68, and for *oqx*B is 77). The significantly lower $R_{s/f}$ values of *sul* genes suggest that *sul* genes are more persistent than other ARGs during the long-term transport from feces to soils. In previous studies, *sul* genes (*sul*1 and *sul*2) have also been reported to be more recalcitrant than tetracycline resistance genes in wastewater treatment processes (McKinney et al. 2010). The different fates of various ARGs during the transport from feces to soil might be related to their molecular carrier, including the plasmid, integrons, and transposons located in the microbes. For example, s*ul*1 has been previously proved to be associated with class 1 integrons, which are widely distributed in indigenous bacteria and facilitate the horizontal transfer of the antibiotic resistance genes, therefore facilitating the proliferation and dissemination of ARGs in soil environment (Luo et al. 2010, 2014).

Conclusion

Overall, this study demonstrates that livestock farming is a reservoir for various ARGs in the environment. The high abundance of PMQR genes and sulfonamide resistance genes demonstrates high occurrence of antibiotic resistance genes in livestock farming in the studied area, which showed the necessity of strengthening management of antibiotic usage in animal feedlots of northern China. This study helps to have a better scope of the pollution profile of ARGs and thus to mitigate their risks to public health.

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