A comprehensive assessment of environmental emissions, fate and risks of veterinary antibiotics in China: an environmental fate modelling approach

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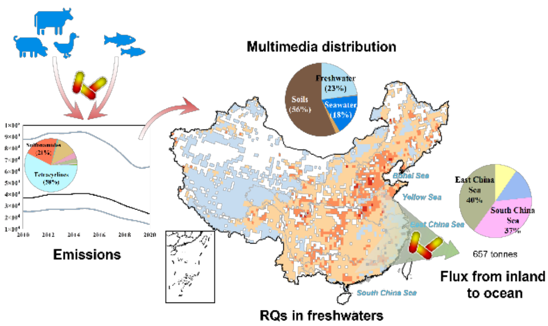
ABSTRACT

China is one of the major global consumers of veterinary antibiotics. Insufficient recognition of emissions and environmental contamination hampers global efforts to prevent antibiotic resistance development. This pioneering study combined empirical data and modelling approaches to predict total 2010–2020 emissions of 80 veterinary antibiotics ranging from 23,110 to 40,850 tonnes/year, after 36–50% antibiotic removal by manure treatment. Following an initial increase of 10% from 2010 to 2015, emissions declined thereafter by 43%. While 85% of emissions discharged into soils, approximately 56%, 23% and 18% of environmental residue were ultimately distributed in soils, freshwaters and seawaters under steady state conditions. In 2020, 657 (319–1470) tonnes entered ocean from inland freshwaters. Median Σantibiotics concentrations were estimated at 4.7×10^3 ng/L in freshwaters and 2.9 ng/g in soils, with tetracyclines and sulfonamides the predominant components. We identified 44 veterinary antibiotics potentially posing high risks of resistance development in freshwaters, with seven exhibiting high risks in >10% of Chinese freshwater areas. Tetracyclines were the category with the most antibiotics exhibiting elevated risks, however sulfamethylthiazole demonstrated the highest individual compound risk. The Haihe River Basin displayed the highest susceptibility overall. The findings offer valuable support for control of veterinary antibiotic contamination in China.



Keywords: veterinary antibiotics, antibiotic emission, antibiotic multimedia distribution, antibiotic discharge to ocean, risk of resistance development

Synopsis: Veterinary antibiotic emission and contamination probably decreased after 2015, with 44 antibiotics exhibiting potential high risks of resistance development in

Chinese freshwaters in 2020. Soil is the major sink of most antibiotics.

INTRODUCTION

Antibiotic resistance is now one of the major threats to human health worldwide. It is estimated to have caused 4.95 million deaths globally in 2019.[1,2] Demand for animal protein drives meat production, which consumes ~75% of antibiotics sold globally for growth promotion and disease prevention of livestock.[3,4] The subsequent environmental releases and contamination of antibiotics could prompt the development and spread of antibiotic resistance.[5,6] This increases the likelihood of human infections by pathogens containing resistant genes and compromises the effectiveness of human medicines.[3,5-7] China is one of the top consumers of veterinary antibiotics, and is one of the largest hotspots of resistance.[3,4,8,9] Rising incomes in China have resulted in an unparalleled expansion in the demand for animal protein, which may elevate veterinary antibiotic use.[10] Meanwhile, various regulations have been issued in China since 2016 to reduce the use of antibiotics in food animals and to control environmental contamination of antibiotics as high-priority novel contaminants.[11-15] It is therefore essential to know the status of emission and contamination of veterinary antibiotics in different media across China, to combat development of resistance both in China and worldwide.

Recent studies have reported widely different emissions sourced from food animals in China. One estimated that 45,360 tonnes of 36 widely used antibiotics were emitted by food animals into the Chinese environment in 2013; in contrast, a much lower release from food animals was recently reported - only 4,131 tonnes in China in 2014.[16,17] Existing studies on antibiotic emission, as well as environmental transport and distribution in China have limitations, such as: on spatial resolution and substance coverage, or on considerations of antibiotic removal during livestock excretion treatment, inter-media chemical transport processes and ionizable features of antibiotics in the modelling approach.[17-22] Global studies including China have mainly investigated the use of veterinary antibiotics, but rarely assessed environmental release and contamination.[3,23,24] Furthermore, previous estimates normally assumed identical application of antibiotics to individual animals within the same livestock species in a country. However, notable geographical disparities were found in residues of some veterinary antibiotics in manures, implying potential differences in regional antibiotic application.[25] Such limitations have introduced large uncertainties in our knowledge of contamination and fate of antibiotics sourced from food animals in China, which limits our understanding of antibiotic environmental exposure.

This study - for the first time - comprehensively assesses environmental emissions, spatial and multimedia concentrations, and the risks of a relatively complete range of veterinary antibiotics used in livestock farming and aquaculture in China. Temporal patterns of antibiotic emissions and concentrations are analyzed for 2010–2020, during which veterinary antibiotic use policies have been considered. We conducted a literature survey of measurements in livestock faeces and aquaculture water to compile the emission inventories of veterinary antibiotics, taking account of spatially varied application preferences. A previously well-constructed and validated national scale multimedia model - the SESAMe v3.4 model (with a spatial resolution at 0.5°) - was utilized to predict multimedia concentrations of antibiotics across China, with a full consideration of ionizable features of substances (e.g. triclosan and triclocarban with different pKa values, and thus showing distinct degrees of ionization and behaviors in the environment) and relatively complete inter-media transport processes, compared with many other multimedia environmental fate models.[19] The study identifies: i. the antibiotics with higher risks of resistance development; ii. highly-contaminated regions and media requiring priorities in risk control, and iii. the predominant emission pathways and sinks of antibiotics, with discharges to ocean being estimated. This wide-ranging assessment can support contamination prevention and control of veterinary antibiotics in China. The same framework is applicable worldwide, especially for countries with serious antibiotic emission and contamination issues.

MATERIALS AND METHODS

Data search and selection strategy for collecting antibiotic residual measurements

To acquire a relatively complete list of veterinary antibiotics being widely used in China, a literature survey was conducted to review measurements of antibiotics in livestock faeces (including swine, cattle, and poultry) and aquaculture water. Papers related to livestock farming and aquaculture were searched from Web of Science and the Chinese Academic Journal Network Database by December 1, 2023. Key search terms including ((livestock OR pig OR swine OR cattle OR cow OR yak OR sheep OR goat OR chicken OR poultry OR feedlots OR farms) AND (antibiotic* OR veterinary antibiotic*) AND (faeces OR urine OR manure OR animal wastes OR animal dung OR residues) AND (China)) and ((antibiotic* OR antimicrobiotic OR veterinary) and (aquaculture farm OR mariculture OR cultured freshwater OR aquafarm OR fishpond) and (China OR Chinese)) were searched via Web of Science to find English articles for livestock and aquaculture, respectively. ((farms OR livestock manure OR "pig manure" OR "cattle manure" OR "poultry manure" OR "sheep manure") AND (antibiotic* OR veterinary antibiotic*) AND ("antibiotic residue")) and ((antibiotic) AND (fishery OR aquaculture OR marine aquaculture OR freshwater aquaculture OR pond)) were searched via the Chinese Academic Journal Network Database to find Chinese articles for livestock and aquaculture, respectively.

A total of 3773 publications were found (see Figure S1 in the Supporting Information (SI). A total of 780 review articles and non-peer-reviewed publications including thesis and conference papers were then excluded. Another 2888 articles with irrelevant contents were excluded after examining the title and abstract of remaining articles. A careful full-text reading was then conducted to exclude 18 studies without specific location information of sampling sites and available concentrations for individual antibiotics. Then 55 (38 English articles and 17 Chinese articles) and 32 (18 English articles and 14 Chinese articles) relevant papers for measured antibiotics in livestock faeces and aquaculture water, respectively, were finally included in this study. Antibiotics widely used in food animals were targeted and their measurements were collected from these papers for estimating emissions. Relevant information is presented in Table S1–S2 in SI and Appendix A.

Emissions

Emissions from both livestock farming and aquaculture were estimated during 2010–2020. Livestock farms were categorized as either intensive farms or family farms in this study. Family farms are operated on a small to moderate scale, and use land contracted by a family for production activities, with family members as primary labour forces.[26] Intensive farms are industrial agriculture practices. Three livestock types were considered, namely swine, cattle, and poultry. Veterinary antibiotics can be released to soils and freshwaters with livestock excrement after being (partially) treated.[27] All excreta of poultry, consisting of a mixture of faeces and urine, was assumed to be released into soils.[28] Regarding swine and cattle, since family farms in China typically involve both crop cultivation and animal husbandry, all excretion from livestock will commonly be used as organic fertilizer through land application with or without treatment.[29] Therefore, excreted antibiotics in this sector were assumed to be all released to soils. Untreated urine in intensive farms was assumed to be directly discharged into freshwaters, while the treated urine and all treated and untreated faeces were assumed to be release to soils.[30] The calculation of the emission for individual antibiotics from livestock farming is shown as equations (Eqs.) 1-4.

$$Ex_i = \sum_i [Exu_i + Exf_i] = \sum_i [(F_i \times C_i \div f_i \times u_i) + (F_i \times C_i)] \quad (1)$$

$$F_i = \sum_i N_i \times p_i \quad (2)$$

$$Ems_i = \sum_{i,j,x} [r_{a,i} \times (Exf_i \times P_{a,i,j} \times R_j + Exu_i \times P_{a,i,x} \times R_x) + r_{b,i} \times Ex_i \times P_{b,i,j} \times R_j] \quad (3)$$

$$Emw_i = \sum_i r_{a,i} \times Exu_i \times P_{a,i,l} \times R_l \quad (4)$$

Where i represents the type of livestock; Ex_i, Exu_i and Exf_i represent the antibiotics excreted with all livestock excrement, and those excreted with urine and faeces, respectively, for livestock i (tonnes/year). F_i and C_i refer to the annual amount of faeces produced by livestock i (kg/year) and residual concentrations of antibiotics therein (μg/kg). f_i and u_i represent excretion rates of antibiotics (%) through faeces and urine, respectively, from livestock i (Table S3). N_i stands for the number of livestock i. The p_i represents the faeces production volume per unit of livestock i per year (kg/unit/year) (Table S4), which was calculated by multiplying the faeces production (kg/unit/day) by growth period (days) of livestock i.[31] Because limited literature has reported urinary concentrations for livestock, urinary excretion has been estimated from an adjustment by fecal excretion rates, i.e. (1) a backward calculation of antibiotic usage using fecal excretion rates and fecal excretion amount of antibiotics, and then (2) forward estimation of urinary excretion using usage and urinary excretion rates.

The emission through different pathways was calculated as Eqs. 3-4. Ems_i and

Emw_i represent the amount of antibiotics (tonnes/year) discharged into soils and freshwaters, respectively, by livestock i. Where j represents the faecal treatment process, including agricultural utilization, composting, and anaerobic digestion; and x represents the urinary treatment process: agricultural utilization and anaerobic digestion. $r_{a,i}$ and $r_{b,i}$ represent the proportion (%) of intensive livestock farms (a) and family livestock farms (b) respectively, for livestock i, which are provincially and temporally varied. Their detailed calculation method is given in section S1 in SI. $P_{a,i,j}$ and $P_{a,i,x}$ represent the proportion (%) of the faecal and urinary treatment process in intensive livestock farms for livestock i; $P_{a,i,l}$ represents the proportion of direct discharges from intensive farms for livestock i (%). The three parameters vary regionally, with values given in Appendix A.[30] $P_{b,i,j}$ represents the proportion of the faecal treatment process in family livestock farms for livestock i. The multi-year (2015-2019) average value was taken for $P_{b,i,j}$ at a provincial level (Appendix A).[32-36] R_j and R_x represents the antibiotic residue rate (%) in the faecal and urinary treatment process, respectively (Table S5). R_l represents the antibiotic residue rate of the direct discharge (R_l=100%).

Both freshwater aquaculture and coastal marine aquaculture were considered in this study. According to different aquaculture practices, freshwater aquaculture comprises pen culture, cage culture, and industrialized culture, while marine aquaculture includes pond culture, cage culture, deep-sea cage culture, raft culture, hanging culture, industrialized culture, and bottom-sowing culture.[37] Bottom-sowing culture allows organisms to grow naturally without using antibiotics (GB/T 20014.18-2013), so it was not considered in this study, while all other practices were considered. All antibiotic use in aquaculture is released into freshwaters or seawaters directly without treatment.[38,39] The emission was calculated as Eqs. 5-7.[16]

$$Emw_{a,f} = \sum_{f=1}^{3} C_f \times A_f \times D_f \times T_f \tag{5}$$

$$Emw_{a,m} = \sum_{m=1}^{6} C_m \times A_m \times D_m \times T_m \tag{6}$$

$$Emw_{aqu} = Emw_{a,f} + Emw_{a,m} \tag{7}$$

$Emw_{a,f}$ and $Emw_{a,m}$ represent the amount of antibiotics (tonnes/year) discharged into freshwaters and seawaters, respectively, in aquaculture. C_f and C_m represent the concentrations of antibiotics (ng/L) in freshwater aquaculture and marine aquaculture, respectively (Appendix A). A_f and A_m represent the areas of freshwater and marine aquaculture, respectively (m^2) (Appendix A). D_f and D_m represent the water depth in freshwater and marine aquaculture area (m) (Appendix A). T_f and T_m represent the water exchange times between aquaculture area and surrounding waters in freshwater and marine aquaculture per year. Instantaneous homogenization was assumed for each water exchange event. Ten times water exchange was assumed for pond culture per year, whilst everyday water exchange (365 times/year) was assumed for other aquaculture practice types.[40]

Livestock production data of swine, cattle, and poultry (N_i) on a county level, as well as freshwater and marine aquaculture area on a provincial level from 2010 to 2020 were collected from the China Statistical Yearbook or local statistical bureaus.[41-43] For counties lacking livestock data in the literature, information was collected by contacting individual local survey teams of the National Bureau of Statistics. Based on empirical data collected in the last section, an average antibiotic concentration was taken for samples collected within the same province to acquire the provincial-level residue in faeces and aquaculture water. For provinces lacking measurements, a national average was taken. The data on livestock production, aquaculture area and measured antibiotic residue in faeces and aquaculture water on a provincial level is given in Appendix A.

To thoroughly consider the effect of use policy on veterinary antibiotics in China and uncertainties of adopted values for parameters, a best-guess (BG) emission scenario and two extreme emission scenarios, i.e. low-emission (LE) and high-emission (HE) scenarios, were constructed to demonstrate the most appropriate and reliable total emissions with a potential range in China. The BG scenario generally followed the prohibition of veterinary antibiotic use in China, unless any banned veterinary antibiotics were still detected in livestock faeces or aquaculture water after the prohibition.[44,45] Meanwhile, average values of the faeces production volume per unit of livestock i per year (p_i), water depth in aquaculture (D_f and D_m) collected from the literature, and national or industrial standards were taken in the BG scenario. Within contrast to the BG scenario, the minimum values were used for p_i, D_f and D_m in the LE scenario. In the HE scenario, it was assumed that all identified veterinary antibiotics were used throughout the 11 years (2010–2020) without prohibition. The maximum values were used for p_i, D_f and D_m, and all excrement was assumed to be directly discharged without treatment (R_j and R_x=100%). The investigation was mainly under the BG scenario.

Environmental fate modelling

The SESAMe v3.4 (Sino Evaluative Simplebox-MAMI model) model was utilized to predict antibiotic concentrations in environments.[46] The model is able to predict concentrations in multiple environmental compartments (i.e., air, different types of soils, freshwaters and sediments, seawaters and sediments, and vegetations) in the Chinese mainland with a high resolution of 0.5°. It has been applied and well validated on chemicals with varying characteristics, such as semi-volatile organic compounds and ionizable chemicals (e.g. active ingredients of personal care products).[19,46,47] As indicated in the previous study, due to introduction of the activity approach, the model has advantages in predicting multimedia concentrations of ionizable chemicals, such as antibiotics, as it considers varying partitioning and transport behaviors of ionic and neutral forms of organic chemicals and has incorporated spatially varying pH in freshwaters, sediments and soils, such that the model has the potential to consider a spatially varied degree of ionization and the consequent varying behavior of a specific substance in different media and areas.[19] The model principle and basic physical processes are introduced in section S2 in SI. The elimination half-life of various targeted antibiotics in the Chinese environment was calculated using the model. The results indicated that different antibiotics reached the steady-state concentrations (after ~5 elimination half-lives) within a maximum of 96 days, which is less than one year.[48] This justifies the utilization of the steady-state model for investigating the annual pattern of concentrations.

Estimated emissions and physicochemical properties (molecular weight (g/mol), vapor pressure at 25°C (Pa), water solubility at 25°C (mg/L), log-transformed octanol-water partition coefficients (log K_{ow}) (-), pKa acid and pKa basic (-), and half-life (hr) in the air, water, soil and sediment) of the identified target veterinary antibiotics (SI Table S1) were input to the model for predictions. A complete literature review was conducted to collect measurements of animal-use-only (AUO) antibiotics in freshwaters and soils across China for model validation. Information on locations of sampling sites and sampling years collected from 74 peer-reviewed papers are given in Table S6. It was found that only nine AUO antibiotics have sufficient measured data for a comparison with predictions, and these were therefore used for model validation. Monte Carlo simulation was performed to conduct the uncertainty analysis by running the model 10,000 times. Parameter values were randomly taken from the environmental parameter databases of the model and emission databases developed in this study for the simulation.

Environmental risk assessment

As the risk of antibiotic resistance selection is currently one of the major global concerns on public health, this study mainly assessed this type of risk induced by antibiotic contamination in freshwaters. However, to implement more comprehensive assessment and offer a broader perspective, risk for aquatic toxicity has been additionally evaluated. Values of predicted no effect concentrations for resistance development ($PNEC_{res}$) and aquatic toxicity ($PNEC_{tox}$) were collected from the literature (Table S7). The $PNEC_{res}$ value serves as a guide to the maximum residue level of antibiotics in the environment, below which resistance is less likely to develop.[49] They were derived from experiments on resistance to clinically relevant bacteria. The values of $PNEC_{res}$ for most antibiotics were collected from studies by Bengtsson-Palme et al. and Zhang et al.,[50,51] and 100 ng/L from a study by Le Page et al. was taken for the remaining antibiotics without a chemical-specific value, for a conservative assessment.[52] In addition, aquatic toxicity data (LC_{50} or EC_{50}) were available for 57 antibiotics, mostly obtained from toxicity assays on algae (Table S7). $PNEC_{tox}$ was calculated as LC_{50} or EC_{50} / AF (Assessment factor = 1000).[53] The risk quotient (RQ), obtained by dividing predicted environmental concentrations (PECs) by PNECs (i.e., RQ = PECs / PNECs) was employed to predict the environmental risks in freshwaters for individual chemicals across China. A nominal RQ classification of RQ < 0.1, 0.1 <

RQ < 1 and RQ > 1 predicts low (or insignificant), medium and high risks in the environment, respectively.[54,55]

RESULTS AND DISCUSSION

Emissions

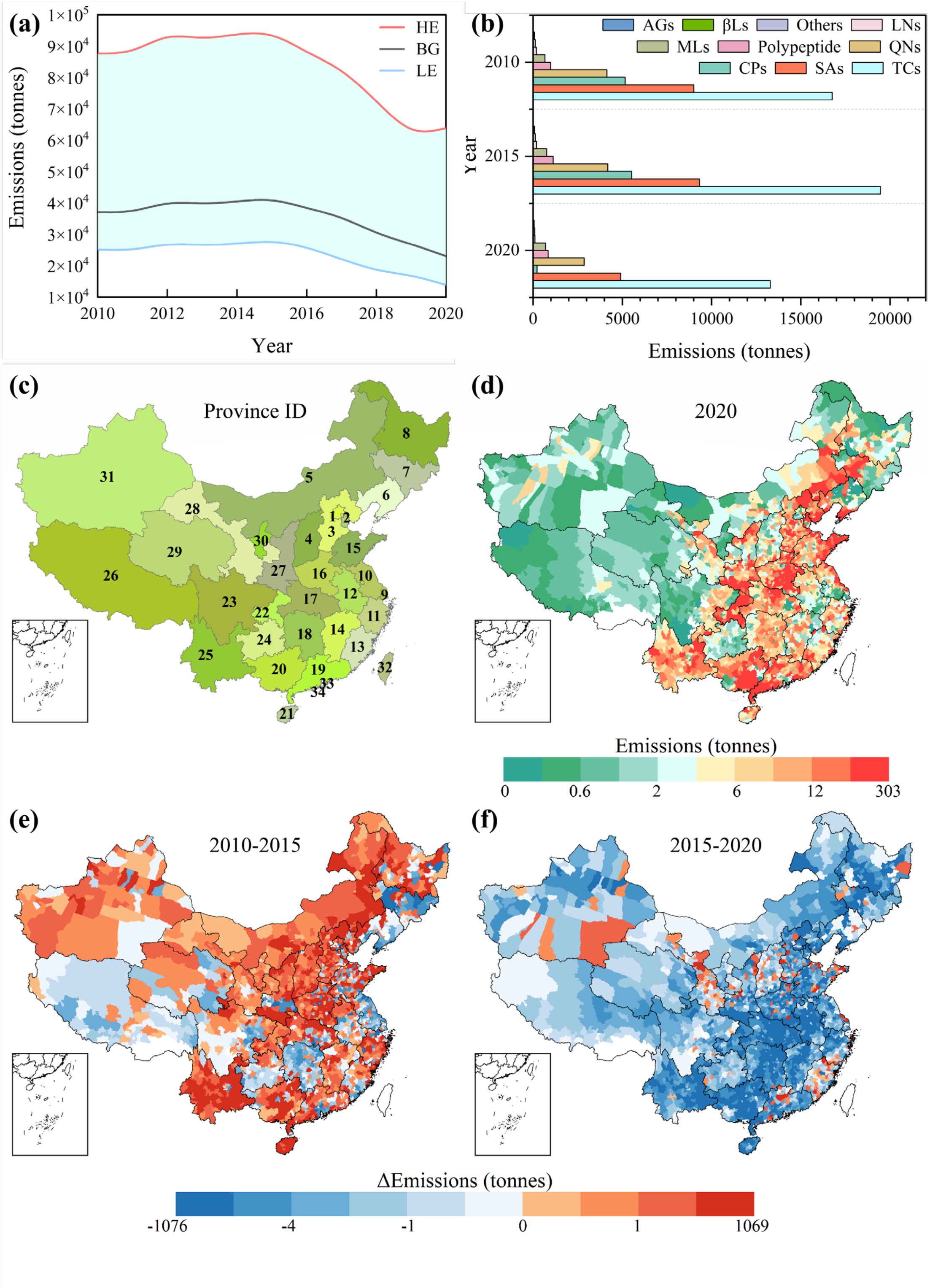


Figure 1. (a) Temporal trends of total antibiotic emissions in low-emission (LE), best-

guess (BG), and high-emission (HE) scenarios from 2010 to 2020; (b) emissions of various antibiotic categories in 2010, 2015, and 2020; (c) Province IDs of China (1. Beijing; 2. Tianjin; 3. Hebei; 4. Shanxi; 5. Inner Mongolia; 6. Liaoning; 7. Jilin; 8. Heilongjiang; 9. Shanghai; 10. Jiangsu; 11. Zhejiang; 12. Anhui; 13. Fujian; 14. Jiangxi; 15. Shandong; 16. Henan; 17. Hubei; 18. Hunan; 19. Guangdong; 20. Guangxi; 21. Hainan; 22. Chongqing; 23. Sichuan; 24. Guizhou; 25. Yunnan; 26. Tibet; 27. Shaanxi; 28. Gansu; 29. Qinghai; 30. Ningxia; 31. Xinjiang; 32. Taiwan; 33. Hong Kong; 34. Macau); (d) spatial distribution of total antibiotic emissions in 2020; (e-f) difference in emissions at the county level between 2010 and 2015, as well as between 2015 and 2020.

A total of 84 veterinary antibiotics were measured in livestock excrement and aquaculture water based on the comprehensive literature review, of which 80 were detected above the limits of detection (LOD) and investigated in this study. A total of 20 are AUO antibiotics (7 quinolones, 5 macrolides, 4 sulfonamides, florfenicol, ceftiofur, monensin, and carbadox (Table S1)), and the other 60 are also used on humans. The 80 antibiotics mainly belong to 9 classes: (1) 23 sulfonamides (SAs); (2) 6 tetracyclines (TCs); (3) 17 quinolones (QNs); (4) 12 macrolides (MLs); (5) 5 β-lactams (βLs); (6) 2 lincomycins (LNs); (7) 3 chloramphenicols (CPs); (8) 2 Aminoglycosides (AGs); (9) 3 Polypeptide, and other 7 antibiotics (carbadox, furazolidone, monensin, nalidixic acid, novobiocin, rifampicin, and salinomycin). Information of the antibiotics is summarized in Table S1.

The annual emission of Σantibiotics (the sum of the 80 antibiotics) used in livestock and aquaculture under the BG scenario ranged from 23,110 to 40,850 tonnes during 2010–2020, peaking in 2015 (Figure 1a). Annual emissions ranged from 13,870 to 27,560 tonnes in the LE scenario, whereas it reached 63,620 to 93,780 tonnes in the HE scenario. According to the HE scenario, there would be a rebound in emissions after 2019, if there was not any restriction on use, mainly due to a 30% increase in the quantity of livestock. The LE and HE scenarios only provide insight to potential emission ranges, considering uncertainties in parameters. The following results and discussion are based on the BG scenario. The emission increased by 10% from 2010 to

2015 and decreased by 43% from 2015 to 2020. The total emission was around 23,110 tonnes in 2020. The increase in the numbers of swine and poultry resulted in the increasing emissions between 2010 and 2015. The decrease in emissions after 2015 was mainly driven by fluctuations in total livestock numbers, freshwater aquaculture area and proportions of livestock farming types. The populations of swine, cattle and poultry declined by 24%, 21% and 5% from 2015 to 2020, while the freshwater aquaculture area reduced by 96%. The reduction in farming volumes contributed 84% of the emission decline after 2015. Meanwhile, a 12% increase in the proportion of intensive farms versus a corresponding decrease in the proportion of family farms contributed 12% of the emission decline after 2015. The more dramatic decline after 2018 was probably attributed to (1) the outbreak of African swine fever (ASF) in China with a mortality rate of nearly 100%, (2) the prohibition of veterinary use of chloramphenicol, carbadox, and vancomycin in 2020, leading to 14% emission reduction compared to 2019, and (3) a 74% reduction in pen culture area from 2017 to 2018.[45,56]

On average 47% of the excreted antibiotics by livestock were removed in manure treatment processes in the BG scenario every year. Due to the temporal variation in proportions of intensive farms and family farms, the percentage varied from 36 to 50% during 2010–2020. Therefore, manure treatment has been effective at removing antibiotics before being discharged to environments. Livestock farming accounted for the majority of veterinary antibiotic emissions (80–88%), while aquaculture only accounted for 12–20% of total emissions. Excretion from swine accounted for >60% of the total livestock emission in the 11 years. This is because China is one of the world's largest producers and consumers of pork, and farms more swine than cattle and poultry.[56] Cattle and poultry contributed, on average, 22% and 14% to total livestock emissions, respectively. Freshwater and marine aquaculture contributed 44% and 56% of the total aquaculture emission, respectively.

Emissions projected under the BG scenario in this study are considered reliable, after being compared with estimates from other studies. Our estimated livestock emission is higher than the antibiotic consumption by livestock in China estimated by Van Boeckel et al. for 2010 (29,720 versus 14,525 tonnes), but is generally comparable with the emission derived from their newly estimated consumption for corresponding years using the updated data, if excretion rates at 30–90% by livestock are applied.[3,23,24] Furthermore, our estimated emission (39,900 tonnes for the 80 antibiotics) is lower than that estimated from food animals by Zhang et al., which was 45,360 tonnes from 36 antibiotics in 2013.[17] This is because removal of antibiotics during manure treatment processes before release to the environment was considered in this study, whereas Zhang et al.'s study assumed that all antibiotics excreted by livestock were directly released into the environment. The estimated excretion of 80 antibiotics in 2013 reached 92,720 tonnes in this study, which would be directly discharged if no removal before release was assumed. The aquaculture emission estimated by our study is also comparable with the antibiotic consumption in aquaculture in China estimated by Schar et al. for 2017 (5,143 versus 5,940 tonnes) and Yang et al. for 2014 (3,226 versus 2052 tonnes).[16,57]

The average annual emissions during 2010–2020 varied from 0.04 to 7,048 tonnes/year for individual antibiotics (Table S8). There were 31 veterinary antibiotics with an annual average emission >100 tonnes/year. Tetracyclines, sulfonamides, chloramphenicols, and quinolones were four predominant antibiotic categories throughout the 11 years (except 2020 due to the ban of chloramphenicol), accounting for 94% on average of the total emissions (Figure 1b). The proportion of individual antibiotics in emissions remained generally steady over the 11 years, without considering the prohibition of use. Although only six substances were in the tetracyclines category, they were the predominant component constituting 58% (13,290 tonnes) of the total emission in 2020. Tetracyclines accounted for 66%, 50%, 67%, and 18% of the emissions from swine, cattle, poultry, and aquaculture, respectively, in 2020. Oxytetracycline, tetracycline, and chlortetracycline were the top three tetracyclines, with an emission of 5,539, 4,242 and 2,237 tonnes, respectively, in 2020. Tetracyclines are extensively used as feed additives to promote animal growth, primarily due to their low cost, wide availability, and broad-spectrum antibacterial activity.[58] The average residual concentration of individual tetracyclines in swine faeces reached 9,890 μg/kg, much higher than that for other categories (126–1,230 μg/kg) (SI Table S2). Before chloramphenicol was banned, chloramphenicols comprised the primary component (53%) of emissions from cattle, although only constituting 14% of the total emission in 2019. Chloramphenicol exhibited the highest emission within this category at 3,762 tonnes in 2019. In contrast to livestock, sulfonamides were the main antibiotic categories, which accounted for 63% and 29% of the emissions from freshwater and marine aquaculture, respectively, in 2020. The AUO antibiotics contributed only 8.0% of the total emission in 2020 (SI Table S8). Enrofloxacin is the single most emitted AUO antibiotic, with an emission of 1,135 tonnes in 2020, 1.6 times higher than the summed emission of all other AUO antibiotics.

The spatial pattern of emissions was mainly ascribed to distribution of livestock farming volumes and aquaculture areas, while geographically varied residues in faeces and aquaculture water (reflecting use variations), as well as proportions of two major livestock farm types with distinct manure treatment rates are also influential factors. Seven provinces, namely Hebei, Henan, Shandong, Guangxi, Guangdong, Yunnan, and Shaanxi, exhibited elevated emissions compared to the other regions, in all contributing 49–61% of the total emissions during 2010–2020 (Figure 1d). In the livestock sector, Henan province generally exhibited the highest antibiotic emissions at 2,410–3,490 tonnes/year due to the consistently highest swine and poultry farming volumes throughout 2010–2020, contributing to 10–12% of the emission from livestock farming. Sichuan was also among the provinces with a high swine farming volume, almost comparable with Henan. However, it had substantially lower emissions (490–870 tonnes/year), probably due to the lower antibiotic use (lower residual concentrations in faeces). In contrast to livestock farming, Anhui and Shandong showed the highest antibiotic emissions at 48–2,180 tonnes/year and 954–1,282 tonnes/year, respectively, from aquaculture during 2010–2020, which was driven by the high aquaculture area in the two provinces.

As the majority of counties experienced extremely limited temporal changes (<10 tonnes) in emissions, with ca. 95% of counties from 2010 to 2015 and ca. 80% of counties from 2015 to 2020, a relatively constant spatial pattern was found for emissions during 2010–2020 (Figure S2). However, exceptionally large variations occurred in certain regions (Figure 1e-f). For instance, in contrast to most regions, emissions in Gongzhuling city (Jilin province) dramatically increased by ca. 67 times (120 tonnes) from 2010 to 2015, meanwhile Tibet and Jilin provinces showed a significant decrease in emissions by 50% (Figure 1e). From 2015 to 2020, Susong county in Anhui province exhibited a quicker decline by 96% (188 tonnes), while counties in Hebei and Shaanxi provinces showed the largest increase at 30% in emissions across China (Figure 1f). In comparison to 2010, emissions of veterinary antibiotics were reduced in 82% of China's counties, but primarily increased in Shanxi, Gansu and Fujian provinces in 2020, with a summed increase of 143 tonnes.

Antibiotic budget

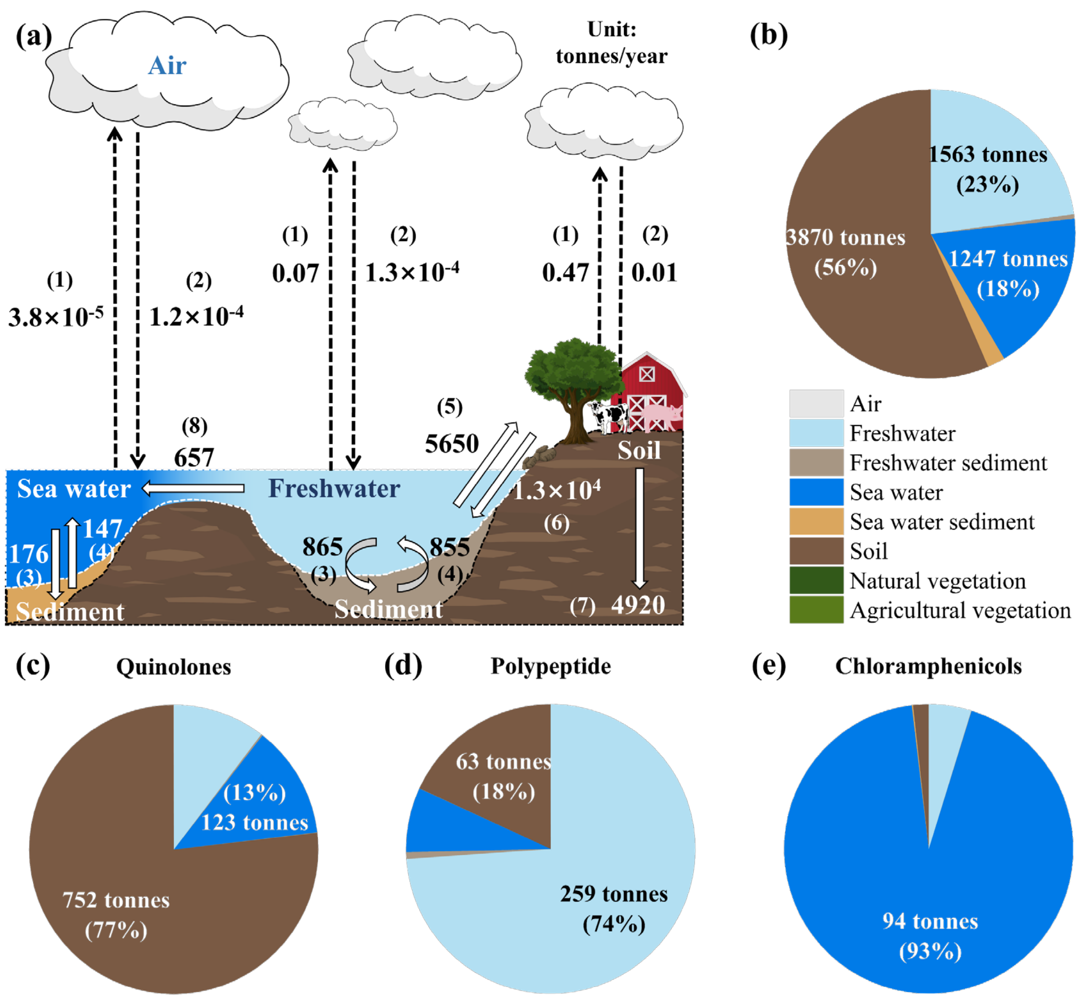


Figure 2. (a) Antibiotic budget in the multimedia environment at the steady state in 2020 (Unit, tonnes/year), and (b-e) multi-media distribution of all antibiotics, quinolones, polypeptide and chloramphenicols at the steady state in 2020. (The inter-

media processes in Figure 2a are explained as follows: (1) volatilization; (2) gas absorption and deposition (dry deposition and wet deposition); (3) absorption and sedimentation; (4) desorption and resuspension; (5) irrigation; (6) runoff and erosion; (7) leaching; (8) discharges from inland freshwater to the ocean)

In 2020, we estimated that around 85% (19,650 tonnes) of the total emissions in China were released to soils, with the remainder discharged into freshwaters and seawaters. When reaching the steady state (i.e. when all mass transfer and degradation fluxes became constant), there were only 56% (3,870 tonnes) of residual antibiotics in the environment distributed in soils, and another 23% (1,563 tonnes) and 18% (1,247 tonnes) were in freshwaters and seawaters, respectively (Figure 2b). Therefore, on average, soil was generally the sink of veterinary antibiotics at the national level in China. Surface runoff and soil water erosion were the major processes transporting 1.3×10^4 tonnes veterinary antibiotics from soils to freshwaters per annum (Figure 2a). This far surpassed the direct source emission to freshwaters, and became the major route of antibiotics into freshwater systems. Irrigation transported 5,650 tonnes/year veterinary antibiotics back to soils from freshwaters. Meanwhile, soil leaching transported 4,920 tonnes/year of antibiotics into deeper soil layers, posing a potential risk of groundwater contamination. Approximately 1.1×10^4 tonnes/year of veterinary antibiotics were degraded, with freshwater and soil experiencing the most significant degradation at 5,290 and 3,850 tonnes/year, respectively. The exchange between freshwaters and sediments was both around 850 tonnes/year, with slightly more transport from freshwaters to sediments. The antibiotic exchange between air and land (soils and freshwaters) was minimal compared to the fluxes at the interface between the other compartments, ranging 10^{-5}–10^{-1} tonnes/year. Antibiotics are hardly volatilized to air. Furazolidone and flumequine were the most volatile antibiotics, contributing almost the 79% of the annual flux from land to air.

However, different antibiotics demonstrated different multimedia distribution patterns, due to their distinct physico-chemical properties (Figure 2 and Table S9). For instance, quinolones and lincomycins were mainly distributed in soils (77–92%), while polypeptide (74%) was primarily in freshwaters, aminoglycosides, chloramphenicols, nalidixic acid, novobiocin, and monensin were mainly distributed in seawaters (93–99%) (Figure 2c-e and S3). Macrolides have a higher percentage in freshwater sediments (0–13%) than the other categories (0–1.0%). Macrolides have lower water solubility (7.8×10^{-3}–107 mg/L) and higher log K_{ow} (0.6–4.75) than other antibiotic categories, so are more likely to remain in soils after being released. In contrast, aminoglycosides and chloramphenicols have relatively high water solubility (2.5×10^{3}–4.1×10^{5} mg/L) and lower log K_{ow} (-1.48–1.14), while the log K_{ow} of polypeptide are extremely low at only -3.10 to -6.83 (Table S1). Some quinolones are different to above antibiotics. For example, ciprofloxacin, difloxacin, enrofloxacin, ofloxacin and norfloxacin also have high water solubility (850–1.8×10^{5} mg/L) and low log K_{ow} (-1.03–2.32), but have a low proportion in freshwaters, ranging from 0.37–13%. This is because their half-lives in freshwaters are much shorter than those in soils. Many antibiotics in other categories have comparable proportions in both freshwaters and soils, with slightly higher percentages typically found in soil (Figure S3).

Predicted environmental concentrations

Although only ca. 15% of veterinary antibiotics was released to freshwaters, the concentration therein was not low. The median PECs (5^{th}–95^{th} percentile range in brackets) of Σantibiotics in freshwaters were 1.2×10^{4} (137–1.2×10^{5}), 1.3×10^{4} (126–1.4×10^{5}) and 4.7×10^{3} (48–6.6×10^{4}) ng/L in China in 2010, 2015 and 2020, respectively. The median PECs of Σantibiotics in soils were 5.3 (0.03–91), 6.0 (0.02–100) and 2.9 (0.02–61) ng/g in China in 2010, 2015 and 2020, respectively. Statistical characteristics of PECs for individual antibiotics are shown in Table S10–S11. Elevated PECs of Σantibiotics in freshwaters in 2020 were found in the Haihe River Basin (Hebei province), Huaihe River Basin (Henan province) and the section of Yangtze River Basin in Henan province (2,044–1.3×10^{6}, 600–3.0×10^{5} and 1.8×10^{4}–2.6×10^{5} ng/L) (Figure 3a-b). Meanwhile, Northwest Inland River Basin, Songhua and Liaohe River Basin, and Southwest River Basin demonstrated lower PECs (0.30–8.4×10^{5}, 0.01–2.4×10^{5}, and 0–1.0×10^{5} ng/L) compared to other basins. The highest PECs in freshwaters were found in the Haihe River Basin in Hebei province, while the lowest PECs were predicted in Southern Tibet Rivers (Southwest River Basin) in Tibet. This is consistent with the findings of Zhang et al.[17] The geographical pattern is also generally consistent with that of emissions, but with regional contrast driven by environmental parameters, such as dilution of freshwater discharge flows (SI Figure S2). In addition, Hebei, Guangxi, and Henan provinces exhibited higher PECs in agricultural soils (11–582, 0.74–360, and 4.5–194 ng/g) than other areas. The highest PECs of antibiotics in soils were found in Hebei province and the lowest PECs were found in Liaoning province (Figure 3c). Resembling emissions, the provinces with elevated soil concentrations were typically the seven provinces with the higher emission levels than other regions. In line with the temporal pattern of emissions, most regions showed an increasing PECs of Σantibiotics from 2010 to 2015 followed by a decline after 2015. The PECs increased 0.02–8,150% in 46% of freshwater area and 0.001–4,130% in 75% of soil area (Figure 3d and 3g). However, variations of PECs in around 70% of regions was actually <30%. Contrarily, a high percentage of regions (84% for freshwaters and 96% for soils) exhibited a decline PECs of Σantibiotics over 80% from 2015 to 2020 (Figure 3e and 3h).

Generally consistent with emissions, tetracyclines, chloramphenicols (except for 2020) and sulfonamides were major components in both freshwaters and soils, with tetracyclines contributing the most (Figure 3f and 3i). Tetracyclines are a group of more stable antibiotics in environments among the three highly emitted antibiotic categories identified in this study.[59] The average half-life of tetracyclines is 1.2-6.0 times that of chloramphenicols and sulfonamides in freshwaters and sediments, while it is comparable with or 1.2 times that of chloramphenicols and sulfonamides in soils (Table S1). Contrary to emissions, the proportion of chloramphenicols in freshwaters reached 35–36% (2015 and 2010), compared to around 14% in emissions, resulting from their higher water solubility and lower log K_{ow} than the other categories - as indicated above (Table S1). While chloramphenicol was banned for veterinary use in 2020, tetracyclines became the major components, contributing 59% in freshwaters. Meanwhile in soils, besides the above three major components, macrolides and quinolones were extra essential components with proportions ranging from 11–14% and from 18–22%, respectively. This is much higher compared to <1% and around 2% of macrolides, as well as 6–8% and around 11% of quinolones in freshwaters and emissions, respectively. The higher proportion of macrolides in soils than in freshwaters was attributed to its hydrophobicity as stated above. The rising proportion of quinolones in soils was mainly driven by the high soil PECs of enrofloxacin, due to its relatively higher log K_{ow} (2.32) compared to other quinolones.[60]

Before the ban, chloramphenicol (median, 2,959 ng/L, and the 5^{th}–95^{th} percentile range, 34–3.6×10^4 ng/L), should be the antibiotic with the highest PECs in freshwaters in 2019, which is consistent with the findings of Zhang et al. (2015). In contrast to Zhang et al.'s study, certain tetracyclines, specifically chlortetracycline (577 ng/L, 9.0–1.4×10^4 ng/L) and oxytetracycline (310 ng/L, 3.1–1.4×10^4 ng/L) have the second highest PECs in freshwaters.[17] Chloramphenicol (0.60 ng/g, 3.8×10^{-3}–10 ng/g) also showed the highest concentration in soils in 2019. However, after it was banned, oxytetracycline (0.23 ng/g, 2.4×10^{-3}–14 ng/g) and enrofloxacin (0.23 ng/g, 2.2×10^{-3}–12 ng/g) became the antibiotics with the highest concentrations in soils in 2020 (Table S11).

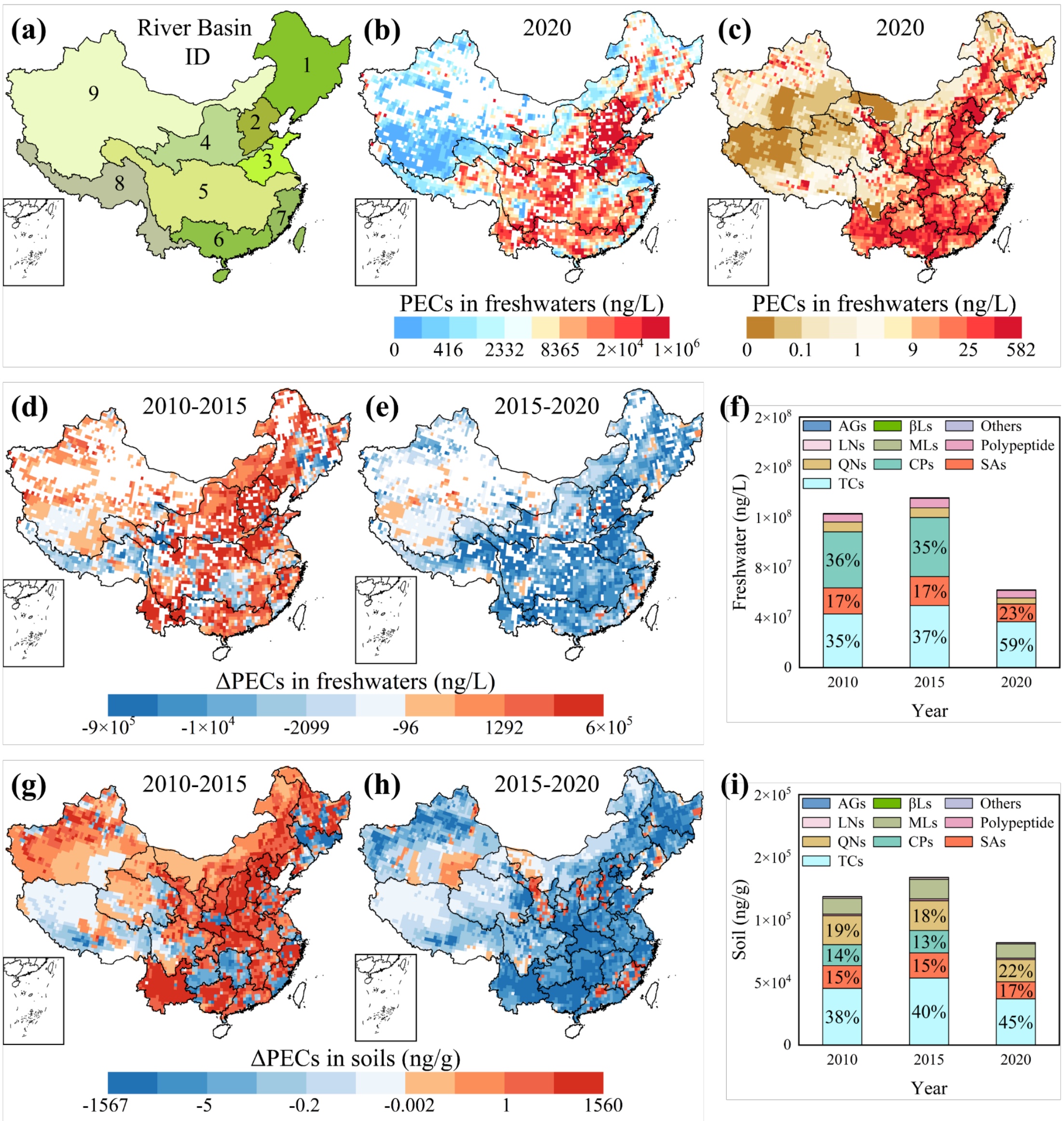


Figure 3. (a) River Basin IDs: 1. Songhua and Liaohe River Basin; 2. Haihe River Basin; 3. Huaihe River Basin; 4. Yellow River Basin; 5. Yangtze River Basin; 6. Pearl River Basin; 7. Southeast River Basin; 8. Southwest River Basin; 9. Northwest Inland River Basin. Predicted environmental concentrations (PECs) of antibiotics in freshwaters (b) and soils (c) across China in 2020; variations in PECs of antibiotics in freshwaters (d-e) and soils (g-h) from 2010 to 2015 and 2015 to 2020; composition of antibiotics in freshwaters (f) and soils (i) in 2010, 2015 and 2020.

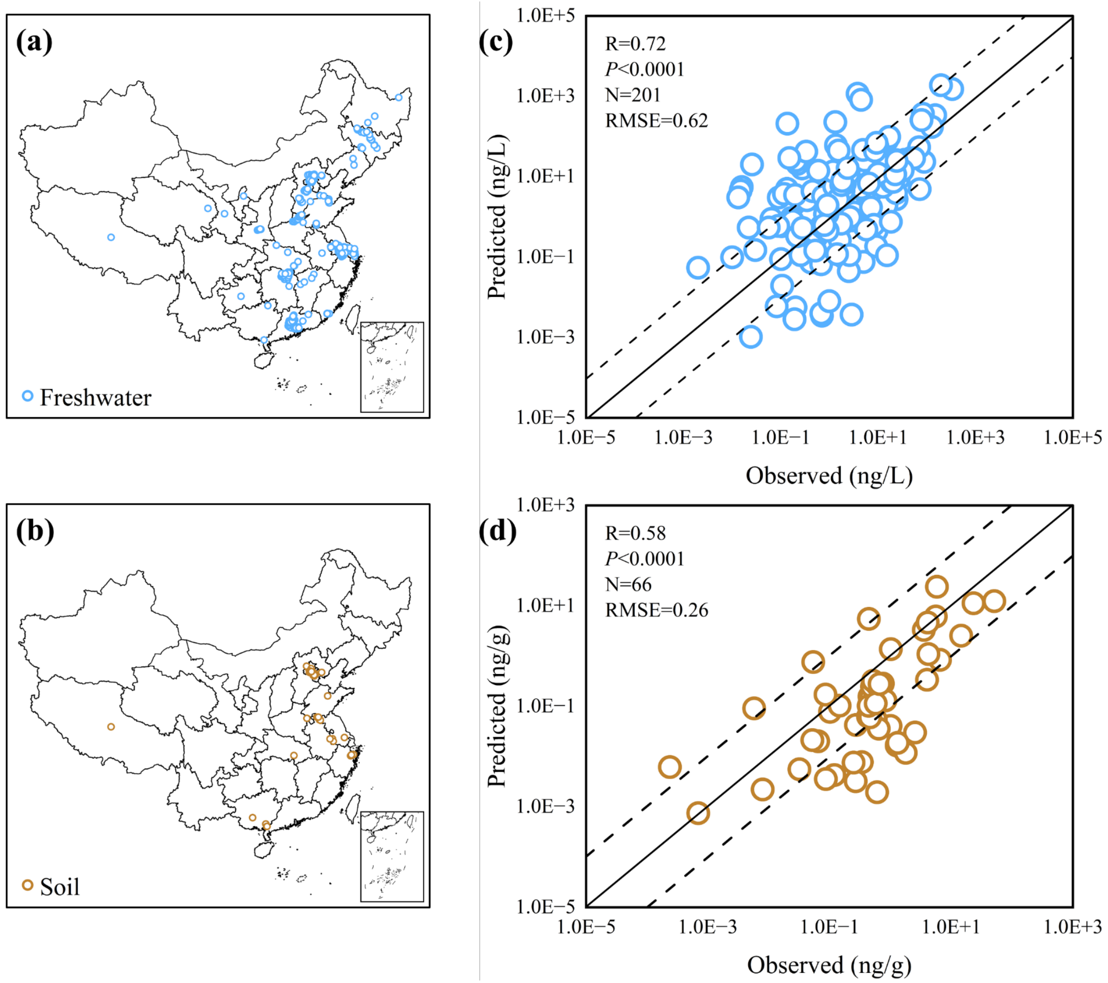


Figure 4. (a-b) Sampling sites of observed data in freshwaters and soils; (c-d) point-to-point comparison of predictions and observations in freshwaters and soils for the nine animal-use-only (AUO) antibiotics. The solid line is 1:1 line. The dashed lines are 10:1 and 1:10 lines. The root mean square error (RMSE) is logarithmic scaled.

Model performance was evaluated by comparing predictions with measurements of the nine AUO antibiotics with relatively sufficient monitoring data in freshwaters and soils. As our predictions are merely derived from veterinary sources, it is not reasonable to validate the model using field measurements of the veterinary antibiotics having human excretion as additional emission sources, even though some of them have abundant measured data. Sampling sites for all validated antibiotics in each compartment are shown in Figure 4a and 4b. Most major catchments in China are covered, whilst soil samples are mainly located in Beijing and Jiangsu province. A generally good agreement was found between predictions and measurements for both freshwaters and soils with a root mean square error (RMSE) at 0.62 and 0.26 log units, respectively (Figure 4c-d). Significant and strong correlation (Pearson correlation coefficient (R) > 0.5, $P < 0.0001$) was found between predictions and measurements. The model exhibits better performance on soils than freshwaters, demonstrated by the lower RMSE for soils. Because soil is less mobile and exhibits more stable characteristics compared to freshwaters, and the residue in soils can better reflect a long-term situation. This better aligns with the nature of modelling results, which are annual average concentrations. The underestimation in soils is mostly for the regions with sampling sites close to livestock farms but having limited spatial coverage within the grid cell to reflect an average concentration. Freshwater samples reflect momentary concentrations, which can be easily affected by various factors, such as river discharge flow rates and instantaneous release of pollutants. They usually cannot reflect a long-term average contamination level. Therefore, although measurements may exhibit generally consistent spatial patterns and contamination levels with predictions in freshwaters, large local uncertainties may exist. This caused an under- or over-estimation over one order of magnitude at several sites, with the corresponding points falling out of the 1:10 lines. Therefore, the uncertainty falls within the acceptable range. Considering the previous external validation of the model on other substances as indicated in the method section, it is feasible and reliable to extrapolate the model on other antibiotics having human sources or without sufficient measurements for model validation. This is a common practice in modelling approaches especially for large scale. The result of the uncertainty analysis conducted by Monte Carlo simulation is shown in Figure S4–S5.

Discharges to the ocean

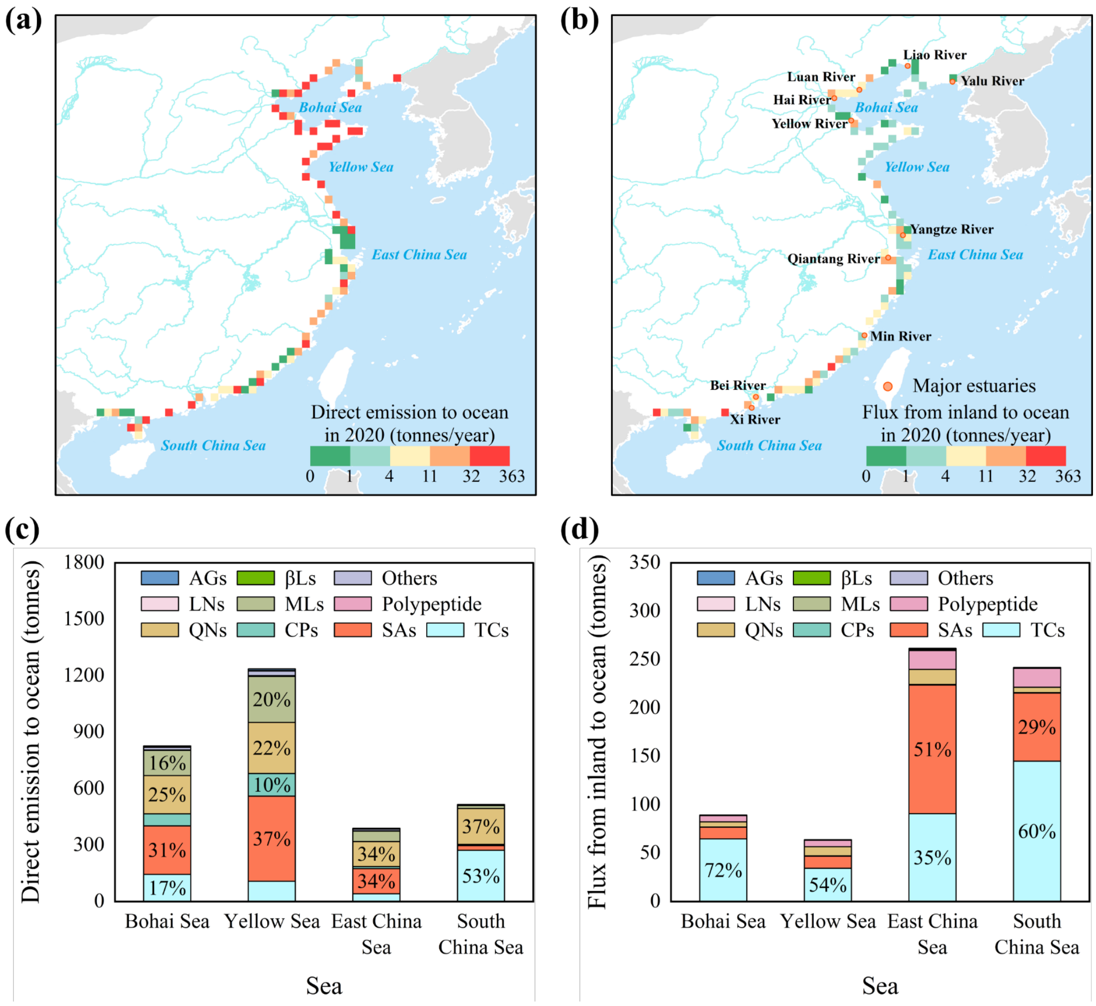


Figure 5. Antibiotic direct emission to ocean from marine aquaculture (a) and fluxes from inland freshwaters to ocean (b) in 2020; composition of antibiotics in (c) marine aquaculture and (d) fluxes from inland freshwaters entering the Bohai Sea, Yellow Sea, East China Sea, and South China Sea, respectively.

The total discharges to the seawater along coastal regions of the Chinese Mainland were estimated, which comprised of source emissions from marine aquaculture and discharges from ten major inland rivers. In 2020, the total discharge to the ocean was 3,626 (2,457–4,961) tonnes, resulting in a median PEC of Σantibiotics reaching 1,094 ng/L in seawaters. Direct emissions from marine aquaculture accounted for 82% of total discharges to the ocean. Around 42% and 28% of marine aquaculture emissions entered the Yellow Sea (1,237 tonnes) and Bohai Sea (827 tonnes), respectively (Figure 5a), due to the high marine aquaculture area in Shandong and Liaoning provinces. This resulted in a higher median PECs of Σantibiotics in Yellow Sea (8,310 ng/L) and Bohai

Sea (4,119 ng/L) than in East China Sea (581 ng/L) and South China Sea (653 ng/L). In contrast, the 657 (319–1470) tonnes of veterinary antibiotics delivered from inland rivers primarily entered the East China Sea (262 tonnes) and South China Sea (242 tonnes), due to high discharge flow rates at the river estuaries here (Figure 5b). For example, the Yangtze River, with an annual freshwater discharge flow of 8.5×10^{11} m^3/year, delivered at least 22 tonnes of veterinary antibiotics to the East China Sea in 2020. Sulfonamides, tetracyclines, quinolones, and macrolides were the primary components in the source emissions from marine aquaculture into all the four seas (Figure 5c), which accounted for 87–98% of the source emissions to individual seas in 2020. However, tetracyclines and sulfonamides were the primary components in discharges from inland freshwaters into all the four seas (Figure 5d), which accounted for 35–72% and 14–51% of discharges to individual seas in 2020. Sulfonamides were the predominant components in the freshwater discharge to the East China Sea, while tetracyclines were the predominant component for the other three seas. The distinct use pattern of veterinary antibiotics in varying regions may influence the composition in the discharge to different seas.

Risks

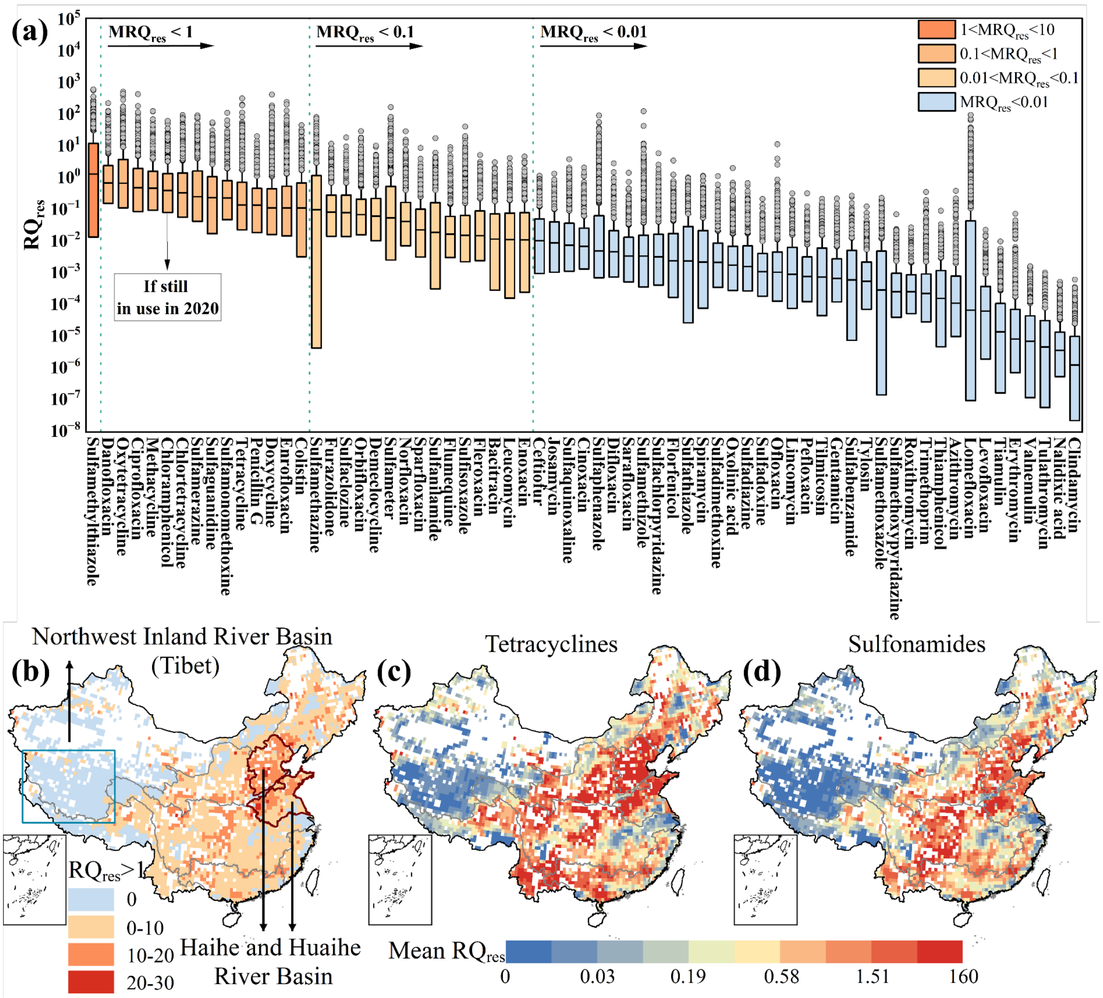


Figure 6. (a) Boxplot of predicted RQ_{res} in freshwater for 67 antibiotics in China; the horizontal solid line in the box is the median RQ_{res} (MRQ_{res} as shown, the MRQ_{res} of all antibiotics are greater than 0); the top and bottom of the box are the 75th (Q3) and 25th (Q1) percentiles, respectively; the top and bottom of the whisker are the highest and lowest values within 1.5 times of the interquartile range (IQ, i.e., [Q1−1.5IQ, Q3 + 1.5IQ]). The circles are outliers with RQ_{res} out of the range of the whiskers. (b) Spatial distribution of the number of antibiotics in freshwaters at high risk ($RQ_{res} > 1$) of antibiotic resistance development; and spatial distribution of mean RQ_{res} for tetracyclines (c) and sulfonamides (d). The other 13 antibiotics only occur in a few cells due to the only application in aquaculture, so the MRQ_{res} across China is zero.

The PECs in freshwaters in 2020 under the BG scenario were applied to be compared with $PNEC_{res}$, to assess the risk of antibiotic resistance development (SI Table S7). A total of 44 antibiotics sourced from veterinary use had a high risk of antibiotic resistance development ($RQ_{res} > 1$) in freshwaters in at least one grid cell (Figure 6a). There were seven of them exhibiting the high risk in >10% of the total freshwater area (ranging from 1.6×10^4–5.0×10^4 km^2), namely sulfamethylthiazole, danofloxacin, oxytetracycline, chlortetracycline, methacycline, sulfamerazine, ciprofloxacin, and sulfamethazine. Sulfamethylthiazole was the antibiotic demonstrating the highest risk with a median RQ_{res} (MRQ_{res}) > 1. Over 20 antibiotics simultaneously exhibited a high risk in ca. 610 km^2 freshwater areas across China, with most areas distributed in the Haihe River Basin (130 km^2) as well as the Huaihe River Basin (310 km^2), accounting for 4.2% and 3.0% of the freshwater area in the respective river basins. The highest risk appeared in the Haihe River Basin in the Beijing-Tianjin-Hebei region, with 30 antibiotics exhibiting a high risk ($RQ_{res} > 1$) in at least one grid cell within the basin (Figure 6b). Notably, the prediction was an average level within a 0.5° grid cell. An identified high risk implied the existence of near sources or local areas having hotspots of potentially extremely high risk. A total of 13 antibiotics demonstrated $0.1 < MRQ_{res} < 1$, while 63 antibiotics had a $MRQ_{res} < 0.1$. There were 38 out of the 63 antibiotics possessing a low risk ($RQ_{res} < 0.1$) in more than 99% of the total freshwater area in China. The risk associated with these antibiotics was deemed negligible. A generally lower risk was observed in the Northwest Inland River Basin within the Tibet (Figure 6b).

Tetracyclines had a generally higher risk, with 5 out of 6 antibiotics having a $MRQ_{res} > 0.1$, followed by sulfonamides (4 antibiotics having a $MRQ_{res} > 0.1$) (Figure 6c-d). All other categories of antibiotics, apart from danofloxacin, ciprofloxacin, penicillin G, enrofloxacin, and colistin, had a $MRQ_{res} < 0.1$. This was consistent with the wide distribution of sulfonamide (antibiotic resistance genes (ARGs) and tetracycline ARGs in the aquatic environment.[61,62] Concentrations of tetracyclines and sulfonamides exhibited concordance with levels of the *tet* and *sul* genes.[63] Therefore, high concentrations of tetracyclines and sulfonamides emitted from veterinary sources may allow high levels of relevant ARGs to be transmitted in the environment, posing a risk to human health. A risk screening based on aquatic toxicity is presented in Figure S6, illustrating that 26 antibiotics may pose potential high risks to freshwater ecosystems.

Uncertainties and limitations

Limitations exist due to the lack of data, which may introduce uncertainties in the estimation and assessment. (1) Livestock farming volumes were lacking in a few regions for certain years, which were estimated by temporal extrapolation based on data present for other years. This may introduce uncertainties to emission estimation. However, the uncertainty should be extremely limited, as the data were collected on a county-level basis, which has ensured the highest accuracy. The farming yield should not change dramatically in a short time. (2) Spatially varied measurements of antibiotics in faeces and aquaculture water were applied regionally to reflect the potential regional difference in use. However, for regions without sampling sites, a national average data was applied, which would possibly result in uncertainties regionally. (3) Manure treatment might not be fully implemented in reality and follow the treatment rate recorded in the literature. The application of literature data may lead to slight underestimation. (4) The input of physico-chemical parameters largely relied on predictions, which may have uncertainties especially for half-lives. More measurements of these parameters are required to refine the prediction of environmental concentrations. (5) $PNEC_{res}$ for more antibiotics are needed for a better assessment of risks for resistance development in environments. Future improvement can be achieved with the acquisition of more precise data, particularly antibiotic use data accounting for spatial variations, removal rates in different manure treatment processes and $PNEC_{res}$. The acquisition of such data is pivotal in enhancing the accuracy and robustness of the research. Despite all the above limitations, the method and relevant data we adopted are mature and the optimal choice based on current available data, so far as we know.

Implications

This is the first study to conduct a comprehensive assessment on emissions and environmental fate for a relatively complete range of veterinary antibiotics with a high spatial resolution over the past 11 years in China. The result indicates that multiple measures should be taken to further control antibiotic emissions and reduce potential risks of resistance development in environments, sourced from animal use. Firstly, the prohibition of veterinary use of certain antibiotics, such as pefloxacin, ofloxacin and norfloxacin, as well as combination therapy should be better supervised in China by relevant governmental agencies.[64] Because (a) pefloxacin, ofloxacin and norfloxacin can still be detected in fresh faeces samples taken even after being banned in 2016, and (b) multiple antibiotics are often detected in faeces of certain livestock within one farm during a single monitoring campaign.[65,66] Techniques to efficiently remove antibiotics from manure should be investigated and adopted in manure treatment processes. Meanwhile, it is easier to improve and implement advanced manure treatments at intensive livestock farms which can improve the removal efficiency of antibiotics before release. Supervision of manure treatment could be more easily implemented in intensive farms. On the other hand, considering that the risk of resistance development in freshwaters is mainly caused by the transport of antibiotics from soils rather than the direct source emission to freshwaters, the current manure discharge measure may not be appropriate. Efforts should be made to explore optimized discharge measures aimed at retaining more antibiotics in the ground, rather than allowing them to be easily transported to freshwater, which is a mobile compartment more capable of spreading the contamination. Finally, colistin, as the last-resort antibiotic for multidrug resistant gram-negative infections in humans was detected in livestock faeces. There have also been reports indicating its extensive use in livestock in China and Vietnam.[67] Veterinary use of such high-end antibiotics should be heavily controlled under strict supervision by the government. This is a concern because once resistance to them appears, it will spread rapidly among different types of bacteria, leaving the human population at risk of being exposed to a range of untreatable infections. This comprehensive assessment is therefore an important reference source for policy makers.

Supporting Information

The Supporting Information is available at https://pubs.acs.org/

Supplemental methods, tables, and figures (PDF)

The raw data of livestock numbers and aquaculture areas; proportion of treatment process in livestock farms; concentration of antibiotic residues in aquaculture and faeces; proportion of intensive farms (Data S1-S17) (Appendix A)

ACKNOWLEDGMENTS

This study is supported by the National Natural Science Foundation of China (41977359, 72088101) and the National Key R&D Program of China (No. 2022YFC3105800). We also acknowledge the National Tibetan Plateau Data Center for providing data related to the livestock farming in Qinghai Province.

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