Antimicrobial use and antimicrobial resistance monitoring for pig production in the United States of America

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Summary

Monitoring antimicrobial use (AMU) and antimicrobial resistance (AMR) on farms is recognised as an important component of antimicrobial stewardship yet the process can be resource intensive. This paper describes a subset of findings from the first year of a collaboration across government, academia, and a private sector veterinary practice focused on swine production in the Midwestern United States. The work is supported by participating farmers and the greater swine industry. Twice-annual collection of samples from pigs along with AMU monitoring, occurred on 138 swine farms. Detection

and resistance of *Escherichia coli* from pig tissues was assessed, and associations between AMU and AMR were evaluated. This paper describes the methods utilised and the first-year *E. coli*-related results from this project. Higher minimum inhibitory concentrations (MIC) of enrofloxacin and danofloxacin in *E. coli* from swine tissues were associated with the purchase of fluoroquinolones. There were no other significant associations between MIC and AMU combinations in *E. coli* isolated from pig tissues. This project represents one of the first attempts to monitor AMU as well as AMR in both swine and public health pathogens on a large-scale commercial swine system in the United States of America.

Keywords

AMR – AMU – Antimicrobial resistance – Antimicrobial use – *Enterococcus* – *Escherichia coli* – One Health – *Salmonella* – *Streptococcus suis*.

Introduction

Antimicrobial resistance (AMR) poses a threat to both human and animal health, representing a One Health challenge. Amidst increasing recognition that antimicrobial use (AMU) is one of multiple drivers for AMR [1], there is widespread agreement that monitoring AMU is a critical component of antimicrobial stewardship [2]. Strategies to minimise AMR ideally include biosecurity, vaccination, and hygiene-related components, in addition to monitoring AMU. In the United States of America (USA), recognition of the need for enhanced monitoring of AMR patterns, as well as antimicrobial sales, use, and management practices in production animals, was articulated in the first National Action Plan for Combating Antibiotic-Resistant Bacteria (CARB), published in 2015 [3], and reiterated in the second CARB National Action Plan [4].

Worldwide, 38 active farm-level AMU monitoring systems from 16 countries have been identified. These systems vary in a number of ways, including coverage (full or partial), participation (mandatory by

legislation or quality assurance programme, or voluntary), and funding (private, governmental, or a combination) [5].

In the USA, farm-level monitoring of AMU and AMR in livestock and poultry production is voluntary. Government programmes are costly and generating buy-in from producers is an ongoing challenge. Within this context, designing an AMU and AMR monitoring programme around routine veterinary visits as part of an established veterinarian-client-patient relationship can yield valuable data. This sample survey approach allows us to tailor the programme for long-term sustainability. Approaches to monitoring farm-level AMU vary around the world. The development of such systems is dependent on local needs, goals, and resources [5].

Longitudinal studies on farm can help guide best practices related to AMU and monitor for changes in AMR; however, these kinds of studies can be logistically challenging to maintain. Such longitudinal monitoring programmes are more likely to be sustainable when they account for producer needs, as well as overall animal health and economic concerns. Public–private partnerships are one approach to achieving this goal.

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) coordinates with partners at international, national, and state levels to address AMU and AMR in animal agriculture. The USDA's National Animal Health Monitoring System (NAHMS) has been collecting data about antimicrobial use, stewardship, and resistance for many years with the voluntary support of farmers in a diversity of sectors. This project supports the work APHIS is already doing on AMU and AMR in animal agriculture. Veterinarians and farmers play key roles in antimicrobial stewardship. The farmers participating in this study are clients of Pipestone Veterinary Services (PVS), a veterinary practice working with over 800 pig farmers across the USA. PVS works with farmers that produce more than 8.5 million market pigs per year. For perspective, the USA produced approximately 127.1 million barrows and gilts for federally-inspected slaughter in 2020 [6].

Since 2017, PVS has offered an AMU data collection service to their clients. This service allows participating clients to track their AMU via purchase records (water and injectable) and veterinary feed directives (feed) to optimise use practices on their farms. Purchase records of antimicrobials are used as a proxy for AMU in this system. Recently, efforts expanded to include sampling for AMR in selected pig and food safety-related pathogens. For this project, sample collection, laboratory analysis, and statistical analysis are coordinated among PVS, South Dakota State University's Animal Disease Research and Diagnostic Laboratory (SDSU ADRDL), and NAHMS. Veterinarians are uniquely positioned to understand the animal health context of AMU and AMR, and they are trusted by farmers.

The broad study objectives include monitoring AMU and antimicrobial susceptibility of pathogens of veterinary and food safety importance across a commercial swine production system over time and evaluating associations between AMU and AMR. The goal is to develop a model protocol for AMU and AMR monitoring for the USA swine industry. This paper highlights methodology, key results in *E. coli* from swine tissues from the first year of this longitudinal monitoring programme, and lessons learned. As the study is replicated across multiple years, a version of this approach may be useful for others seeking to design cost-effective, practical AMU and AMR monitoring programmes.

Materials and methods

Participating farms

There were 78 wean-to-finish (WTF) farms and 54 farrow-to-weaning (FTW) farms participating in the study. This represented a near-census of PVS FTW farmers and a convenience sample for PVS WTF farmers. Six farms with both FTW and WTF components to their farms chose to participate as farrow-to-finish farms.

When possible, sampling events from farrow-to-finish farms were assigned to either the FTW site or the WTF site for data analysis; for 12 sampling events, this information was not available, and AMR results from these farms were excluded. This approach was taken because

including these isolates in the inferential analyses resulted in convergence issues due to small numbers of farrow-to-finish farms. Results from farrow-to-finish farms were checked prior to exclusion to be sure they did not carry more indicators of resistance than included isolates. Farms were located in Iowa, Illinois, Minnesota, Missouri, South Dakota, and Wisconsin states.

Biological sampling methods

Participating farms (n = 138) were sampled during routine veterinary visits, generally twice per year. Pig sampling consisted of substandard pigs as well as sick pigs. A substandard pig was defined as not clinically sick or treated with antimicrobials and targeted for euthanasia, having little to no production value. A sick pig was defined as being clinically sick and may have been treated with antimicrobials. For each substandard and sick pool, two pigs were euthanised; lung, liver and intestine were collected for *E. coli* culture. Tissues were pooled on farm within pig type. Two pigs were chosen to optimise bacterial recovery while managing on farm sampling limitations. This was a convenience sample. Power calculations were not performed.

Laboratory methods

Bacterial isolation from tissues

Necropsy tissue surfaces assumed to be contaminated during the collection process were seared before creating incisions with sterile blades to produce internal surfaces for swabbing. Swabs were cultured for generic *E. coli* [7]. Suspect growth was transferred to tryptic soy agar with 5% sheep blood agar plate to verify purity and identity via matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (below). Plates were incubated overnight and read the following day on the Sensititre™ Vizion® System. Organisms were tested for antimicrobial sensitivity using the Sensititre™ Bovine/Porcine Plate [8], with results reported as the minimum inhibitory concentration (MIC) for each isolate and antimicrobial. This panel is standard for clinical swine submissions in the USA.

Antimicrobial use data collection

Annualised antimicrobial purchase data were provided by PVS to NAHMS by antimicrobial class and route (water, drench, feed, injectable) in milligram (mg) active ingredient. Data on dosing regimens were not collected during the first year of data collection.

Farrow-to-weaning farms provide actual head count while WTF farms provide an estimated head count, both annually. Standardised live weights were used for FTW and WTF farms (5.44 kilogram (kg) and 127.01 kg, respectively). The standardised FTW weight was set at the low end of industry guidelines [9]. The standardised WTF weight was based on an average live slaughter weight for participating farms in May 2020. It was slightly lower than the national average slaughter weight of 129.27 kg [10].

Calculation of antimicrobial use

Standardised weights were obtained in order to calculate the population correction unit (PCU), which was calculated as:

> the size of population at risk × estimated live weight (assuming all pigs were at risk over the course of the year).

Then the AMU was expressed as mg/PCU and this was calculated for each antibiotic class as follows:

> mg (the total weight of antibiotic active ingredient used) / PCU.

This was calculated separately for FTW and WTF farms.

Antimicrobial sales for FTW farms include antimicrobials that were used to treat sows. However, live weight information was not available for sows and gilts, therefore, mg/PCU estimates for FTW operations apply to the AMU required to produce weaned piglets.

Statistical analysis

Analysis of AMU and pig sample *E. coli* isolate susceptibility data, reported in MIC, was performed in Statistical Analysis System, version

9.4 using the PROC GLIMMIX procedure [11]. PROC GLIMMIX fits generalized linear mixed models (GLMMs) and can handle non-normal data in the response variable while accounting for clustered or multi-level data. MIC values were treated as ordinal because it is the most direct interpretation from the laboratory testing method. Combinations of MIC (ordinal response) and the associated AMU by route (the continuous predictor of main interest) were assessed via mixed models. Farm identification (ID) (the subject) was a random effect included in all models to account for the clustered nature of the data due to multiple visits being made to the same farms over time.

Model selection was performed by backward stepwise selection by comparing the Akaike information criterion. The fixed effect variables considered for model selection were farm type (FTW or WTF), 'quarter' (each quarter of the calendar year divided into four equal segments, equivalent to season), 'biannual' (a two-level categorical variable: '1' means the farm visit occurred between May to October 2020, and '2' means the farm visit occurred between November 2020 to May 2021), and pig type (sick or substandard). 'Quarter' and 'biannual' were checked in separate model selection processes since they are redundant information. For consistency, the final model selected was the same for all models tested, even though a few models could have kept 'biannual' as an additional fixed effect variable based on their specific model selection process.

If convergence issues occurred, visit frequency was dropped from the model, but further simplification of the model would require dropping farm ID and was not done, and instead the model was reported as not converged in Table I.

Given the sparsity of certain MIC values in Table II (i.e. many of the observed MICs belong to a single category, with the other observed categories having much fewer samples in them), occurrences of complete or quasi-complete separation were checked for each model using the fixed-effects-only version of the model with PROC LOGISTIC since PROC GLIMMIX does not check this [14, 15]. A lack of multicollinearity between AMU and farm type was confirmed by

recoding farm type as a dummy variable and checking correlation between the two variables, not accounting for subject. Extreme outliers were checked by calculating 'dfbeta', which measures the change in the beta coefficient(s) of interest from the model with and without the outlier observation under investigation; no extreme outliers were found [14]. A score test for the proportional odds assumption was performed on the fixed-effects-only version of each model with PROC LOGISTIC since PROC GLIMMIX does not have this feature. A follow-up graphical test on the calculated empirical logits was performed if the score test rejected the proportional odds assumption since the score test is known to be anti-conservative [14, 16].

Using PROC GLIMMIX, mixed-effects proportional odds logistic regression was performed if the proportional odds assumption passed, and mixed-effects multinomial logistic regression was performed if the assumption failed [14]. The highest observed MIC value was chosen as the reference category in multinomial logistic regression, and the proportional odds logistic regressions are modelling the odds of the highest observed MIC value against the cumulative odds of all other observed MIC values.

Some MIC levels were collapsed into a single category if complete or quasi-complete separation occurred (tetracycline in Table III) or if the graphical test for the proportional odds assumption could not be evaluated on the observed MIC levels (ampicillin in Table III). The beta coefficients and resulting *p*-values in proportional odds logistic regression are thought to be unbiased when collapsing levels in the response category [17], although published literature is inconsistent on whether these biases estimates or not [18].

Bias in estimates due to sparse data in the response variable has long been noted in logistic regression [19]. PROC GLIMMIX has several common estimation methods that incorporate small sample bias correction [11]. This study used Laplace's method as recent simulation studies suggest it produces the most accurate estimates compared to other estimation methods [20, 21].

The model type used and *p*-values of type III tests for AMU are reported in Table I. The conventional alpha level of 0.05 was adjusted with a Bonferroni correction to 0.0026, which was calculated from 0.05 divided by the 19 converged models in this paper. We chose to adjust the conventional alpha level of 0.05 because even just 1 of the 19 converged models finding a significant association between MIC and AMU would reject the study-wide null hypothesis, 'AMU is not associated with AMR', derived from the research question common to all 19 converged models, 'is AMU associated with AMR?'.

Results

Farm production

Median annual production for FTW farmers was 103,343 head (25th percentile, 81,184 head; 75th percentile, 164,617 head) and WTF farmers was 29,710 head (25th percentile, 16,467 head; 75th percentile, 61,521 head) during the study period.

Antimicrobial use

Using a mg active ingredient by class and route per PCU live weight production approach, tetracyclines administered via water were the drug class and route most commonly given. Tetracylines administered via feed and beta-lactams administered via water were other more commonly utilised drug classes and routes. Penicillins and lincosamides accounted for the highest use of injectable antimicrobials (Table II).

Sampling on-farm

Farms were visited between one and four times during the period from May 2020 to May 2021 (total: 324 visits). Slightly more than a year was allotted for farm visits to account for COVID-related delays. Nearly all farms (90%; 124/138 visits) were visited twice during this period. Quarterly visits were distributed as follows: smaller percentages of farm visits took place in January to March 2021 (10.8%; 35/324 visits) and in July to September 2020 (19.4%; 63/324 visits) than in June 2020,

April 2021, May 2020 and 2021 (37.7%; 122/324 visits) and in October to December 2020 (32.1%; 104/324 visits).

Detection and resistance of *Escherichia coli* from swine tissue samples

Escherichia coli

For *E. coli*, 97.8% (315/322) sick pig sample pools were culture positive; 97.2% (313/322) of substandard pig sample pools were culture positive. There was no seasonal variation in detection of *E. coli* from pig samples. Two samples from substandard pig pools could not be cultured.

Model selection for antimicrobial use and antimicrobial resistance – *Escherichia coli* from swine tissues

With MIC as the response variable, the final models included the intercepts, AMU via a specific route, and farm type for fixed effects. Random effects were farm ID (the subject) and either 'biannual' or random intercepts.

Looking separately at each AMU and AMR combination, the model selection process found farm type was a significant fixed effect at the alpha level = 0.05; WTF farms display higher MIC values than FTW farms in all AMR classes in Table I except tildipirosin and sulfadimethoxine, which show no significant association. Pig type (sick or substandard) was not a significant fixed effect.

Although 'biannual' as a random effect was not included as part of the model selection process, we preferred to include 'biannual' as a random effect to account for the order of visits in some capacity. However, we found that published literature on modelling repeated measures or longitudinal data is inconsistent in how the repeated time effect is treated and whether effects can be both fixed and random, even for similar study designs [22, 23, 24]. Thus, to be safe, every MIC/AMU model presented in Table I was also modelled with all possible combinations of 'biannual' as fixed, random, both fixed and random, or dropped completely from the model (and in combination with and

without random intercepts, when appropriate); we found no difference in the statistically significant conclusions made in all model combinations, with only minuscule differences in the final *p*-values of the fixed effects, AMU and farm type. For tidiness, we only report one of these combinations for each MIC/AMU model in Table I.

Associations between antimicrobial use and antimicrobial resistance – *Escherichia coli* from swine tissues

The *p*-values for all GLMMs evaluating the association between MIC and AMU are shown in Table I. Models that would not converge are also noted. Nineteen separate regressions were performed and significant associations at the Bonferroni corrected alpha level of 0.0026 were found with use of injectable fluoroquinolones as they were associated with an increase in MIC of both danofloxacin (odds ratio [OR] = 1.65, $p = 0.0002$, 95% confidence interval [CI] [1.27–2.14]) and enrofloxacin (OR = 1.66, $p = 0.0001$, 95% CI [1.28–2.15]) in *E. coli* detected in pigs. The 17 other regressions did not find a statistically significant association between MIC and AMU.

Discussion

Antimicrobial resistance is a One Health challenge, highlighting the interconnectedness of human, animal, and environmental health. In the USA, accessing farms to collect samples for antimicrobial susceptibility testing and gather AMU information has proven to be challenging and resource intensive. By partnering with veterinarians and collecting samples during routine visits, this collaboration of practicing veterinarians, laboratory microbiologists, and federal epidemiologists has been successful. This project provides a practical model for learning more about AMU and AMR on swine farms, including evaluating associations between AMU and AMR.

After reviewing year one, the sampling methods employed resulted in the collection of valuable farm-level data. While multiple associations were evaluated, the only statistically significant finding was that use of injectable fluoroquinolones was associated with a higher MIC of both danofloxacin and enrofloxacin in *E. coli* detected in pigs, which has

been reported previously [25]. However, this is a longitudinal study, so the analysis described in this paper will be updated as additional visits are made to each participating farm. This paper did not investigate trends over time, but trends will be investigated in future publications as more samples are collected for each farm at future time points.

Evaluating associations between AMU and AMR can be difficult, especially given the lack of validated breakpoints (defining the MIC values for susceptible, intermediate, and resistant [SIR] classifications) for many animal pathogens. Because of this, the approach taken in this project and demonstrated using *E. coli* was to evaluate associations between AMU (in mg/PCU by antimicrobial drug class) and increasing MIC for each antimicrobial included on the resistance panels. While evaluating associations between AMU and increasing MIC is challenging, this approach can give more of an early indicator of trend toward resistance than SIR-categorised data.

For pig sampling components, it should be noted that some antimicrobials included on standard veterinary diagnostic laboratory panels are not used in swine production in the USA. Additionally, some antimicrobials utilised in swine production are not available for all classes of pigs or via all routes of administration.

When drugs of a given class are utilised in swine production, in some instances the results show resistance for drugs in the same class that are not utilised in swine production. This information has been provided based on the hypothesis that use of one drug in a given class may be associated with the development of cross-resistance to other drugs in the same class. While this finding has been documented in the literature to some extent, the variable findings are consistent with a complex relationship between AMU and AMR [26, 27, 28].

As previously noted by other researchers [29], route of drug administration may be valuable to consider in AMU and AMR monitoring programmes. There is variable evidence that oral exposure may be associated with increased development of AMR [30, 31].

As noted in the results, WTF farms display higher MIC values than FTW farms in all AMR classes in Table I except tildipirosin and sulfadimethoxine. While many studies demonstrate declining AMR with age in swine, this is not a universal finding. It has been documented that AMR is potentially affected by AMU during the entire rearing period, however, AMU is one of many factors that affect the detection of AMR [1].

There are limitations in the study design that could affect interpretation of these results. Since recruitment to PVS's programme is voluntary and focused on Midwestern states, results should not be interpreted as representative of all USA farms with FTW or WTF pigs. This was a convenience sample, and in general, more progressive WTF farmers may have been more likely to participate. The use of substandard and sick pigs may have introduced bias because they may have been more likely to be treated with antimicrobials. Each farm visit in this study had two pooled samples derived from four pigs, which could introduce bias if this was not a large enough sample size to be representative of the entire farm. Additionally, animal class weights vary slightly from national averages. It is important to highlight that AMU for each antimicrobial class was calculated based on a single one-year total from purchase records of product and not administration at the population or animal level. More granular data may generate different results. Purchase data may overestimate use in cases where drugs are purchased but not utilised in the same year. The mg/PCU measure assumes that all pigs produced during a year are at risk. That is, this measure does not account for differing use as required by variability in animal disease treatment needs throughout the year. However, a version of this approach has been frequently utilised by others [32]. Some AMR patterns have all the samples belonging to one MIC level (e.g. penicillin in Table III), which can't be modelled, although a logical, non-statistical argument can be made that MIC levels being the same level regardless of AMU implies that AMU and AMR are not associated; however, this observation could be a limitation of granularity of the phenotypic analysis or could be due to a lack of sample size.

Use of annualised data reported by antimicrobial class and route for this type of analysis has known limitations [33]. Briefly, not all drugs within a given class are the same potency, so when use in mg is combined, some of the ability to interpret findings is lost.

Model convergence issues are unfortunately common in GLMMs [15]. Besides overall sample size, the sources of convergence issues in this study are likely from one or both of the following reasons:

a) an over-specified model, as most convergence issues in this study are a result of zero variance in the random effects and will converge if all random effects are dropped, and

b) lack of variation in AMU, such as injectable aminoglycosides in Table I.

The first issue will likely be improved over time as each farm receives more visits.

Limitations aside, additional resources are needed for more precise measures of AMU, such as defined daily dose [34]. In the first year of data collection, a balance was sought between convenience for participating farmers and granularity of collected data. Transparency in describing the methods used to create AMU metrics is a recommended best practice.

The USA approach to AMU is focused on prevention and good husbandry to prevent the need for use. National targets are not part of that approach. During this pilot, penicillins and tetracyclines were used in greatest volume. Though reasons for use were not captured, according to a representative national survey, in nursery-age pigs, penicillin G is most commonly given in water to treat meningitis/polyserositis/arthritis, and oxytetracycline is most commonly given in water to treat respiratory disease. Chlortetracycline is most commonly given in feed to treat respiratory disease. Similarly, in grower/finisher pigs, tetracyclines are most commonly given in water and feed to treat respiratory disease [35]. Tetracyclines are also the most commonly reported antimicrobial class used in animals worldwide [32].

In closing, although not perfect, this AMU and AMR monitoring programme provides a practical on-farm example for collecting these data, as well as an approach to evaluating associations between AMU and AMR. This paper highlights the key results from the completion of year one of this public–private partnership. Collaboration between pig farmers, veterinarians, laboratory microbiologists, and federal epidemiologists has enabled the successful collection and analysis of complex data. That said, AMU is only one part of the overall picture of AMR, and animal health and food security are of critical importance.

As this longitudinal monitoring project continues, more data will be collected on AMU and AMR. Further analyses will include evaluating other animal health metrics in association with AMU and AMR as well as assessing trends in AMR patterns. Additionally, beginning in 2022, required sacrifice of pigs will no longer be an element of this programme, rather, samples of enteric *E. coli* and *Salmonella* will be gathered via rectal swab from healthy pigs. Sick pigs will be posted at the request of participating farmers, and pig pathogen testing will be an added benefit of participation in the programme. Options for collection of more granular AMU data are being explored.

Conclusions

The approach to developing an AMU and AMR monitoring programme described here demonstrated that public–private partnerships can be a successful way to collect data on farm. In year one, use of injectable fluoroquinolones was associated with higher MIC values for enrofloxacin and danofloxacin in *E. coli* isolated from pig tissues. There were not any other significant associations between other MIC and AMU combinations in *E. coli* isolated from pig tissues. Continued monitoring will generate more data and allow trends to be monitored over time. Flexibility and collaboration are key when designing sustainable voluntary AMU and AMR monitoring programmes.

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Table I

Models and their associations between antimicrobial use in mg/PCU by antimicrobial drug class and increase in minimum inhibitory concentration in *Escherichia coli* isolated from swine tissues on farrow-to-weaning and wean-to-finish swine farms

Antimicrobial included in resistance panel (response variable)	**Fixed effects**	**Random effects**	**OR of AMU fixed effect (95% CI)**	**Type III *p*-value of association**	**Significance at alpha = 0.05**	**Significance at alpha = 0.0026 [b]**
Gentamicin	Intercept, farm type, **aminoglycoside in water**	Farm ID (subject), biannual	0.98 (0.91–1.05)	0.56	No	No
Gentamicin	Intercept, farm type, **aminoglycoside (drench)**	Farm ID (subject), intercept	[a]	0.20 [a]	No	No
Gentamicin	NA (**injectable aminoglycoside**)	NA	Model would not converge	Model would not converge		
Spectinomycin	Intercept, farm type, **aminoglycoside in water**	Farm ID (subject), intercept	[a]	0.80 [a]	No	No
Spectinomycin	Intercept, farm type, **aminoglycoside (drench)**	Farm ID (subject), intercept	[a]	0.17 [a]	No	No
Spectinomycin	NA (**injectable aminoglycoside**)	NA	Model would not converge	Model would not converge		
Neomycin	Intercept, farm type, **aminoglycoside in water**	Farm ID (subject), biannual	0.99 (0.92–1.07)	0.79	No	No
Neomycin	Intercept, farm type, **aminoglycoside (drench)**	Farm ID (subject), biannual	1.03 (0.88–1.20)	0.75	No	No
Neomycin	NA (**injectable aminoglycoside**)	NA	Model would not converge	Model would not converge		
Danofloxacin	Intercept, farm type**, injectable fluoroquinolone**	Farm ID (subject), biannual	1.65 (1.27–2.14)	0.0002	Yes	Yes

Enrofloxacin	Intercept, farm type, **injectable fluoroquinolone**	Farm ID (subject), biannual	1.66 (1.28–2.15)	0.0001	Yes	Yes
Gamithromycin	NA (**macrolide in water**)	NA	Model would not converge	Model would not converge		
Gamithromycin	NA (**macrolide in feed**)	NA	Model would not converge	Model would not converge		
Gamithromycin	NA (**injectable macrolide**)	NA	Model would not converge	Model would not converge		
Tildipirosin	Intercept, farm type, **macrolide in water**	Farm ID (subject), intercept	1.02 (1.00–1.03)	0.007	Yes	No
Tildipirosin	Intercept, farm type, **macrolide in feed**	Farm ID (subject), intercept	1.00 (0.99–1.01)	0.90	No	No
Tildipirosin	Intercept, farm type, **injectable macrolide**	Farm ID (subject), intercept	1.20 (0.93–1.54)	0.16	No	No
Tulathromycin	Intercept, farm type, **macrolide in water**	Farm ID (subject), intercept	1.02 (1.01–1.04)	0.008	Yes	No
Tulathromycin	Intercept, farm type, **macrolide in feed**	Farm ID (subject), biannual	0.99 (0.98–1.01)	0.29	No	No
Tulathromycin	NA (**injectable macrolide**)	NA	Model would not converge	Model would not converge		
Ampicillin	Intercept, farm type, **beta-lactam in water**	Farm ID (subject), biannual	1.03 (1.00–1.05)	0.08	No	No
Ampicillin	Intercept, farm type, **injectable beta-lactam**	Farm ID (subject), intercept	[a]	0.22 [a]	No	No
Ceftiofur	NA (**injectable cephalosporin**)	NA	Model would not converge	Model would not converge		
Sulfadimethoxine	Intercept, farm type, **sulfa in water**	Farm ID (subject), intercept	0.98 (0.94–1.01) [c]	0.19	No	No
Tetracycline	Intercept, farm type, **tetracycline in water**	Farm ID (subject), biannual	1.01 (1.00–1.02)	0.13	No	No

Tetracycline	Intercept, farm type, **tetracycline in feed**	Farm ID (subject), biannual	1.00 (1.00–1.00)	0.32	No	No
Tetracycline	Intercept, farm type, **injectable tetracycline**	Farm ID (subject), biannual	1.10 (0.97–1.23)	0.13	No	No
Florfenicol	NA (**injectable amphenicol**)	NA	Model would not converge	Model would not converge		

(a) The proportional odds assumption failed for this model, so this *p*-value is from a multinomial logistic regression model with the highest observed MIC value as the reference category, and the ORs are not reported for tidiness because there are multiple ORs for AMU equal to the number of pairwise combinations of MIC. Odds ratios and *p*-values without this footnote were modelled via proportional odds logistic regression

(b) Bonferroni adjusted threshold of alpha = 0.05 divided by the 19 converged tests in this table, which calculates to an adjusted alpha = 0.0026

(c) Binary logistic regression was used because the response variable (sulfadimethoxine) had only two observed MIC levels

AMU: antimicrobial use
CI: confidence interval
FTW: farrow-to-weaning
ID: identification
mg/PCU: milligram/population correction unit
MIC: minimum inhibitory concentration
NA: not applicable
OR: odds ratio
WTF: wean-to-finish

Table II

Antimicrobial purchase amount (mg/PCU; annualised at the farm level) quartiles by production category (FTW, *n* = 54; WTF, *n* = 78; or all farms), antimicrobial class, and route of administration (water, drench, feed, or injectable) for 138 total farms, coded by US Food and Drug Administration (denoting importance to human health) [12] and World Organisation for Animal Health (denoting veterinary importance) [13] class (six farrow-to-finish operations included in totals but not broken out individually)

Antimicrobial class	FDA classification	WOAH classification	Route	FTW farms			WTF farms			All farms		
				25th percentile (mg/PCU)	Median (mg/PCU)	75th percentile (mg/PCU)	25th percentile (mg/PCU)	Median (mg/PCU)	75th percentile (mg/PCU)	25th percentile (mg/PCU)	Median (mg/PCU)	75th percentile (mg/PCU)
Aminoglycosides	Highly important	Critically important	Water	0	0	0.89	0	0.17	1.81	0	0.69	2.32
			Drench	0	0.88	1.74	0	0	0	0	0	0.62
			Injectable	0.01	0.05	0.11	0	0	0	0	0	0.04
Penicillins	Highly important	Critically important	Water	0	0	1.79	4.43	8.53	16.66	0	4.58	11.05
			Injectable	6.49	9.77	15.73	0	0.02	0.12	0	0.18	7.21
Cephalosporins [(a)]			Injectable	0.07	0.48	2.46	0	0.01	0.06	0	0	0.26
Fluoroquinolones	Critically important	Critically important	Injectable	0.24	0.71	1.94	0.08	0.19	0.37	0.12	0.28	0.68
Lincosamides	Highly important	Highly important	Water	0	0	4.47	0	2.22	7.47	0	1.40	5.27
			Feed	0	0	0	0	0	1.19	0	0	0
			Injectable	3.04	5.17	10.41	0	0.08	0.24	0	0.29	4.17
Macrolides	Critically important	Critically important	Water	0	0	5.43	0	0.17	1.80	0	0	2.10
			Feed	0	0	0	0	0	0	0	0	0
			Injectable	0.34	0.66	1.36	0	0	0.01	0	0.01	0.48

Phenicols	Highly important	Critically important	Injectable	0	0	0	0	0	0	0	0	0
Pleuromutilins	Not medically important	Highly important	Water	0	0.21	8.13	0.19	2.16	5.21	0	1.56	5.50
			Feed	0	0	0	0	0	1.23	0	0	0.32
Quinoxalines	Not medically important	Important	Feed	0	0	0	0	0	0.51	0	0	0
Sulfonamides	Critically important	Critically important	Water	0	0	1.69	1.28	3.21	7.26	0	1.73	4.58
Potentiated sulfonamides	Critically important	Critically important	Water	0	0	0.14	0.19	0.51	0.96	0	0.24	0.69
Tetracyclines	Highly important	Critically important	Water [(b)]	0	1.79	22.10	6.50	16.04	29.76	0	13.29	28.50
			Feed	0	0	10.60	0	11.80	31.29	0	1.90	27.95
			Injectable	0	0.90	3.63	0	0	0	0	0	0.62

(a) Cephalosporins are classified by generation, and antimicrobial purchase records only captured the antimicrobial drug class; therefore, categorising by FDA and WOAH classification was not possible

(b) Missing one observation of tetracycline use in water; data in this row includes 137 farms

FDA: Food and Drug Administration
FTW: farrow-to-weaning
mg/PCU: milligram/population correction unit
US: United States
WOAH: World Organisation for Animal Health
WTF: wean-to-finish

Table III

Minimum inhibitory concentration distributions of *Escherichia coli* isolated from farrow-to-weaning and wean-to-finish swine tissues (322 visits to 132 farms)

Antimicrobial class	Antimicrobial	Antimicrobial used in swine [a] (Yes/No)	MIC value (µg/ml)																	Total isolates
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	24	32	48	64	128	256	512	
Aminoglycoside	Gentamicin	Yes							371	19	10	10	212								622
Aminoglycoside	Spectinomycin	Yes										7	298		43		271				619
Aminoglycoside	Neomycin	Yes									424	5	6		184						619
Fluoroquinolone	Danofloxacin	No				364	15	38	205												622
Fluoroquinolone	Enrofloxacin	Yes				364	17	42	30	169											622
Macrolide	Gamithromycin	No								2	56	564									622
Macrolide	Tildipirosin	No								15	295	252	60								622
Macrolide	Tulathromycin	Yes										433	118		13		58				622
Macrolide	Tylosin [d]	Yes													622						622
Macrolide	Tilmicosin [d]	Yes											622								622
Lincosamide	Clindamycin [d]	No											622								622
Pleuromutilin	Tiamulin [d]	Yes													622						622
Penicillin	Penicillin [d]	Yes										622									622
Penicillin	Ampicillin	Yes							7 [b]	118 [b]	105	2 [c]	390 [c]								622
Cephalosporin (3rd generation)	Ceftiofur	Yes					99	272	31	14	12	191									619
Sulfonamide	Sulfadimethoxine	No																	442	174	616

Folate pathway inhibitor	Trimethoprim-sulfamethoxazole [(d)]	No				622			622
Tetracycline	Tetracycline	Yes	1 [(b)]	51 [(b)]	58	2 [(c)]	510 [(c)]		622
Amphenicol	Florfenicol	Yes			41	287	294		622

(a) Please note that not all drugs included in the panels are used in swine production (see National Pork Board, 2021, https://www.porkcdn.com/sites/porkcheckoff/Withdrawal+Period+by+Product+Chart+Horizontal+Approved+January+2022.pdf)
(b) Within each row, MIC values sharing (b) were collapsed together into a single category for regression modelling purposes only
(c) Within each row, MIC values sharing (c) were collapsed together into a single category for regression modelling purposes only
(d) Regression modelling could not be performed on these antimicrobials due to all of the observed data belonging to a single MIC value

FTW: farrow-to-weaning
MIC: minimum inhibitory concentration
µg/ml: microgram/millilitre
WTF: wean-to-finish