12-2023

Effects of the U.S. Veterinary Feed Directive Final Rule on the Prevalence of Violative Antibiotic Residues and Antibiotic-Resistant Bacteria in Animal Products

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I am submitting herewith a dissertation written by Md Shamim Sarkar entitled "Effects of the U.S. Veterinary Feed Directive Final Rule on the Prevalence of Violative Antibiotic Residues and Antibiotic-Resistant Bacteria in Animal Products." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

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A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Md Shamim Sarkar
December 2023

DEDICATION

I would like to dedicate this dissertation to my parents, Md Shahjahan Sarker and Rowshonara Begum, as well as my wife and daughters. Their sacrifices, constant love, and unwavering support have been instrumental in my academic achievements.

ACKNOWLEDGEMENTS

I am eternally thankful to Allah (God), who made this possible, and will be forever grateful. I would like to express my gratitude and recognize the invaluable assistance of several people who contributed to the completion of this dissertation. I am sincerely grateful for the opportunity to learn and work under the supervision of Dr. Chika C. Okafor. His expertise, guidance, and dedication allowed me to overcome challenges and succeed. Thank you, Dr. Chika Okafor, for your patience and understanding in this journey. I would like to extend my appreciation to the members of my committee: Dr. Stephen Kania, Dr. Brian Whitlock, and Dr. Russell Zaretzki. I am grateful for the academic and professional support each of you has given me. To my wife, Fatema Hasan, my daughters, Shadha Walijah and Suhaira Farishta, my sisters, Tania and Sadia; and all of my family and friends, thank you for your constant encouragement, prayers, and support. Finally, I thank Allah (God) for his mercy and blessings on me.

ABSTRACT

Antibiotic-resistant is a public health concern. The dissertation objective was to assess the effect of the implementation of the Veterinary Feed Directive (VFD) rule changes on the prevalence of violative antibiotic residues and antibiotic-resistant bacteria in food-animal tissues, retail meats, and cecal samples from food animals compared to the pre-VFD rule change period in the U.S. To understand the effect of implemented VFD rule changes on violative antibiotic residues in food-animal tissues, inspector-generated sampling (IGS) data from the U.S. National Residue Surveillance Program (NRP) was analyzed. An important observation was that implementing VFD rule changes was associated with the decreased prevalence of violative sulfonamide and penicillin residues in food-animal tissues. However, implementing VFD rule changes did not significantly affect the prevalence of violative tetracycline residues in tissues. To further understand the effect of the VFD rule changes, retail meat surveillance data from the National Antimicrobial Resistance Monitoring System (NARMS) was analyzed. The results indicated that implementing VFD rule changes significantly reduced the prevalence of tetracycline-resistant *Campylobacter* and *Escherichia* in chicken and turkey meats. However, the study did not observe a significant effect on tetracycline-resistant *Salmonella* and *Escherichia* prevalence in beef and pork. To expand understandings of the effects of the VFD rule changes, cecal samples collected from food animals' surveillance data in NARMS were analyzed. The results indicated that implementing VFD rule changes significantly reduced the prevalence of tetracycline-resistant *Escherichia* in cecal samples of chickens and turkey, and erythromycin-resistant *Campylobacter* in cecal samples of chickens. However, the study revealed that implementing VFD rule changes significantly increased tetracycline-resistant *Escherichia* in cecal samples of swine and erythromycin-resistant *Campylobacter* in cecal samples of cattle. In conclusion,

implementing VFD rule changes significantly related to reducing the prevalence of tetracycline-resistant bacteria in meats and cecal samples of chickens and turkeys. Conversely, the implementation of VFD rule changes did not impact the prevalence of tetracycline-resistant bacteria in meats and cecal samples of cattle and swine, suggesting a potential surge in usage of injectable tetracycline, which is evident from the lack of reduction in the violative tetracycline residues in food animal tissues that should be further investigated.

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LIST OF ABBREVIATIONS AND ACRONYMS

FDA: U.S. Food and Drug Administration

FSIS: Food Safety and Inspection Service

USDA: United States Department of Agriculture

AVMA: American Veterinary Medical Association

EPA: Environmental Protection Agency

ARS: Agricultural Research Service

CDC: Centers for Disease Control and Prevention

WHO: World Health Organization

WOAH: World Organization for Animal Health

FAO: Food and Agriculture Organization of the United Nations

AMR: Antimicrobial Resistance

AMS: Antimicrobial Stewardship

CLSI: Clinical Laboratory Standards Institute

AMU: Antimicrobial Use

MIC: Minimum Inhibitory Concentration

VFD: Veterinary Feed Directive

MRL: Maximum Residue Limits

NRP: National Residue Program

IGS: Inspector-Generated Sampling

PHV: Public Health Veterinarian

KIS: Kidney Inhibition Swab Test

NARMS: National Antimicrobial Resistance Monitoring System

HACCP: Hazard Analysis and Critical Control Point

CI: Confidence Interval

OR: Odds Ratio

PR: Pathogen Reduction

TN: Tennessee

UTCVM: University of Tennessee College of Veterinary Medicine

INTRODUCTION

The emergence of antimicrobial resistance (AMR) bacteria is a major global public health threat that causes millions of deaths annually. In the United States (U.S.) alone, over 2.8 million people suffer from antimicrobial-resistant infections each year, leading to more than 35,000 fatalities [1]. Antimicrobial usage in livestock production is perceived as a critical driver of AMR [2, 3]. Antimicrobials are widely used in food-producing animals for disease treatment, control, and prevention in the U.S. [4]. The overuse and misuse of antimicrobials plays an important factor associated with the occurrence of antimicrobial residues and the emergence of antimicrobial-resistant bacteria in food-producing animals and their products [5-12]. This phenomenon arises from the utilization of antibiotics for therapeutic, prophylactic, or growth promotion purposes. Each of these applications exert selection pressure, resulting in the development of antimicrobial-resistant bacteria [13, 14]. Restricting or reducing the usage of antimicrobials has been proven to have direct and positive impact on the prevalence of antimicrobial-resistant bacteria in food-producing animals. Evidence indicates that abolishment of the use of antimicrobial agents for growth promotion in food-producing animals has decreased the occurrence of antimicrobial resistant enterococci in fecal samples of food-producing animals in Denmark [15]. Another piece of evidence indicates changing levels of ceftiofur use in chicken hatcheries, resulted in changes in ceftiofur-resistant *Salmonella* and *Escherichia* isolates from chickens in Canada [16]. Evidence shows a reduction in vancomycin-resistant enterococci in poultry and their meat following the restriction of avoparcin usage in food-producing animals. This has been observed in Denmark [17], Germany [18], and Italy [19].

The Veterinary Feed Directive (VFD) final rule changes were implemented by the U.S. Food and Drug Administration (FDA) in 2017 to restrict the use of medically important antimicrobial drugs in the feed and water of food-producing animals, except for treating illnesses. To ensure proper usage, a licensed veterinarian must oversee the usage of these drugs under this rule. I hypothesize that the implementation of VFD rule changes in 2017 has an impact on the occurrence of violative antimicrobial residues in the tissue of food-producing animals, as well as the occurrence of antimicrobial-resistant bacteria isolates in retail meat and cecal samples of food-producing animals in the U.S. The effect of the 2017 VFD rule changes on the occurrence of medically-important antimicrobial violative residues in tissue of food animals and bacteria resistant to medically-important antimicrobials in retail meats, and cecal samples of food-producing animals is yet to be investigated in the U.S.

The National Antimicrobial Resistance Monitoring System (NARMS) surveillance programs monitors the antimicrobial susceptibility of enteric bacteria in food-producing animals (USDA), retail meats (FDA), and ill people (CDC) in the U.S. [20]. This study examines whether implementation of the VFD rule changes is associated with a decrease or increase in the risk of a violative antimicrobial residue and antimicrobial-resistant bacteria in food animals' products in the United States. To test this hypothesis, three nationwide surveillance datasets for information on antibiotic residues in the tissue of food animals and antibiotic-resistant bacteria in retail meat and cecal samples of food-producing animals in the U.S were analyzed. These datasets encompassed the National Residues Program (NRP), specifically focusing on inspector-generated sampling for violative antibiotic residues. Additionally, we analyzed NARMS data, mainly the retail meat surveillance component, which monitors antibiotic-resistant bacteria in retail meats, and NARMS's food animal surveillance component, which monitors for antibiotic-resistant bacteria in cecal samples of food animals in the U.S.

The overall goal of this dissertation is to provide quantitative evidence of whether implementation of the VFD rule changes in 2017 was associated with a decrease or increase in the occurrence of violative antimicrobial residues and bacteria resistant to medically-important antimicrobials in food animals' products in the U.S. The studies reported in this dissertation contribute firsthand evidence on the effect of the implementation of the VFD rule changes measures in 2017 by providing quantitative evidence into: (1) association of the implementation of the VFD rule changes in 2017 with the detection of violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, tilmicosin, florfenicol residues in the tissue of food animals from inspector-generated samples at slaughterhouses in the U.S., (2) association of the implementation of the VFD rule changes in 2017 with the occurrence of tetracycline and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats (chicken breast, ground turkey, ground beef, and pork chop) in the United States; (3) association of the implementation of the VFD rule changes in 2017 with the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples of food-producing animals (cattle, chicken, turkey, and swine) at slaughterhouses in the U.S.

Overview of this dissertation

This dissertation is organized in a manuscript format and is composed of three individual studies that collectively address the effect of the implementation of the VFD rule changes on the occurrence of violative antibiotic residues and antibiotic-resistant bacteria in food animals' products in the U.S. Chapters 1, 2, and 3 are complete individual studies with distinct sections (abstract, introduction, materials and methods, results, discussion, and conclusion).

The overall aims of the studies reported in this dissertation are to:

1. Evaluate the effect of the implementation of the VFD rule changes on the violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, tilmicosin and florfenicol residues in the tissue of food animals in the U.S. (Chapter 1)
2. Evaluate the effect of the implementation of the VFD rule changes on the occurrence of tetracycline- and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats in the U.S. (Chapter 2)
3. Evaluate the effect of the implementation of the VFD rule changes on the occurrence of tetracycline- and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples of food-producing animals in the U.S. (Chapter 3)

Finally, the dissertation concludes with general conclusions, recommendations, future research directions, and my VITA.

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CHAPTER 1

Effect of changes in veterinary feed directive regulations on violative antibiotic residues in the tissue of food animals from the inspector-generated sampling in the United States

A version of this chapter was originally published by Shamim Sarkar, Chika C Okafor (2022). Effect of Changes in Veterinary Feed Directive Regulations on Violative Antibiotic Residues in the Tissue of Food Animals from Inspector-Generated Sampling in the United States. Microorganisms 10(10), 2031. DOI: https://doi.org/10.3390/microorganisms10102031

My contribution to this paper included gathering and review of literature, conceptualization, data analysis, interpretation of results, and drafting and editing of the manuscript.

Abstract

The presence of antibiotic residues in the tissue of food animals is a growing concern due to the adverse health effects that they can cause in humans, such as antibiotic resistance bacteria. An inspector-generated sampling (IGS) dataset from the United States National Residue Surveillance Program, collected between 2014 and 2019, was analyzed to investigate the association of changes in the veterinary feed directive (VFD) regulations on the detection of violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, tilmicosin, and florfenicol, residues in the tissue of food animals. Multivariable logistic regression models were used for analysis. While the animal production class was significantly associated with residue violations for tetracycline, having a sample collection date after the implementation of change in VFD regulations was not. However, the odds of detecting violative sulfonamide and penicillin residues in the tissue of food animals following the implementation of the change in VFD regulations were 36% and 24% lower than those collected before the implementation of the change in VFD regulations period, respectively, irrespective of animal production class. Violative desfuroylceftiofur, tilmicosin, and florfenicol residues in the tissue of food animals were not significantly associated with the implementation of changes in the VFD regulations. Further investigation of the factors that influence the presence of violative antibiotic residues in the tissue of food animals following the change in VFD regulations would lend clarity to this critical issue.

Key words: Antibiotic; residues; violative; penicillin; tetracycline; florfenicol; VFD; sulfonamides.

Introduction

Antibiotics have been widely used for the treatment, control, and prevention of livestock diseases in the United States (U.S.) [1-3]. Inappropriate use of antibiotics in food animals is one factor associated with the presence of violative antibiotic residues (ARs) in food animal products [4]. A prior study found violative tetracycline, gentamicin, oxytetracycline, and penicillin residues in bob veal calves in the U.S. [5]. Likewise, penicillin was the most frequently identified antibiotic with violative residue levels in culled cows in the U.S. [5]. Foods of animal origin containing ARs have adverse health effects among consumers. For example, ingestion of antibiotic-containing meat products can induce resistance in the normal flora of the human gastrointestinal tract [1].

The Veterinary Feed Directive (VFD) regulations were updated by the U.S. Food and Drug Administration (FDA) on 1 October 2015 and fully implemented on 1 January 2017 in accordance with FDA's Guidance for Industry #213[6]. This VFD rule change guideline discusses FDA's concerns regarding the development of antimicrobial resistance in human and animal bacterial pathogens when medically important antimicrobials drugs are used in food-producing animals in an injudicious manner. So, the modified VFD rule aims to promote the judicious use of medically important antimicrobials in food-producing animals in the U.S. [7]. The VFD rule changes restrict the use of medically important antimicrobials administered in feed and water for therapeutic purposes only and require the supervision of a licensed veterinarian [7]. How-ever, a recent qualitative study reported that the VFD could create more black-market access to in-feed antimicrobials [8]. Previous studies reported increased use of antimicrobials for therapeutic purposes in food-producing animals after a rule restricting antimicrobials use (AMU) for growth promotion in food animals was implemented in Denmark and Sweden [9,10]. On the other hand, implementing a rule restricting AMU in Taiwan in 2000 was associated with decreased resistance to vancomycin among enterococci in chickens [11].

In 1976, the U.S. established the U.S. National Residue Program (NRP), a national residue surveillance system to monitor chemical residues, including antibiotic residues, in meat, poultry, and egg products. This surveillance program was aimed at protecting the health and welfare of consumers. The NRP is an interagency program conducted by the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) [12]. The NRP has three sampling schemes: surveillance sampling, inspector-generated sampling (IGS), and unique project sampling [12]. The inspector-generated sampling targets individual suspect animals, suspect animal populations, and animals retained or condemned for specific pathologies. The following steps are involved in the inspector-generated sampling: a Public Health Veterinarian (PHV) selects a carcass for sampling based on the criteria (i.e., an animal with disease signs and symptoms, producer history of violative levels of residues, or as a follow-up to result from random scheduled sampling). Then, the PHV performs a Kidney Inhibition Swab (KIS™) test (in-plant screening test) for the presence of antibiotic drug residues. If the KIS™ test result is positive, the sample is submitted to FSIS field laboratories for confirmation.

With this background information, we hypothesize that the use of injectable anti-microbial drugs may have increased in food animals in the U.S. after the implementation of change in VFD regulations, which could increase the detection of violative antibiotic residues in the tissue of food animals in the U.S. Violative antibiotic tissue residues may pose a risk of adverse health effects in humans, such as an increase in resistant bacteria [13,14], allergic reaction [14,15], altering gut microbiota [16] and obesity [16,17] from consuming such residues. To our knowledge, no study has quantified the association of the VFD rule changes on the presence of violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, florfenicol, and tilmicosin residues in the tissue of food animals in the US. These antibiotics are commonly used in food-producing animals in the U.S. Therefore, this study aimed to investigate the association of the implementations of revised VFD regulations on the detection of violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, tilmicosin, and florfenicol residues in the tis-sue of food animals from IGS samples in slaughterhouses in the U.S. Our study results could provide a baseline understanding of the relationship changes in VFD regulations to detection rates of violative residues of penicillin, tetracyclines, sulfonamides, desfuroylceftiofur, florfenicol, and tilmicosin in the tissue of food animals in the U.S.

Materials and Methods

Data source

The inspector-generated sampling (IGS) data used for this study were retrieved from the U.S. NRP for meat, poultry, and egg products [18]. These data covered the period between 2014 and 2019. Penicillin, tetracyclines, sulfonamides, desfuroylceftiofur, florfenicol, and tilmicosin were selected as target antibiotics for analysis because they are commonly used in food animals in the U.S. and are important antibiotics in human health.

Data preparation and variables

The IGS dataset contains the following variables: antibiotic residues (penicillin, tetracyclines, sulfonamides, desfuroylceftiofur, florfenicol, and tilmicosin), testing results (violative and non-violative), date of collection (month and year), animal species (cattle, goat, sheep, swine, and turkey), tissue name (kidney, liver, and muscle), and analyte name (drug name). The dataset was transferred from Microsoft Excel (version 2019, Microsoft Corporation, Redmond, WA, USA) to

STAT 16.1 software (Stata Corporation, College Station, TX, USA). Then, all variables in the dataset were assessed for completeness and accuracy. Next, the 'year' variable was collapsed into a dichotomous variable, “VFD rule changes”: ‘after VFD rule changes (2017 to 2019) and 'before VFD rule changes’ (2014 to 2016) for analysis. This VFD rule changes variable was the primary exposure of interest in this analysis. Others included the type of animal and type of tissue sampled. The animal species' variable was considered the animal production class’ variable and was categorized based on production class such as bob veal, beef cow, dairy cow, bull, heifer, steer, goat, sheep, swine, and turkey. Besides, the 'tissue name' variable was collapsed into a dichotomous variable as 'type of tissue sampled' (kidney vs. others (liver/muscle)). Chlortetracycline, oxytetracycline, tetracycline, and doxycycline were aggregated as the antibiotic group 'tetracyclines'. Similarly, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine, and sulfamethoxazole were aggregated as the antibiotic group 'sulfonamides'. The outcome of interest for each antibiotic or antibiotic group was whether violative residue was present compared to absence in the tissue of food animals from the IGS.

Statistical analyses

All statistical analyses were conducted using Stata 16.1 (Stata Corporation, College Station, TX, USA). Categorical predictor variables were summarized using frequencies and percentages. Chi-square or Fisher's exact test (if the expected cell count was <5) was used to investigate the distribution of the outcome variables with respect to categorical predictor variables. The differences were then assessed for significance by p-values, with $p < 0.05$ considered significant. Separate logistic regression models were built for the six antibiotic residues: penicillin, tetracyclines, sulfonamides, desfuroylceftiofur, tilmicosin, and florfenicol.

Each model-building process involved two steps. The first step involved fitting univariable logistic regression models to assess crude associations between potential predictor variables and detection of violative residues in tissue samples. A relaxed p value of 0.2 was used to identify potentially significant predictors, and variables with a $p \leq 0.2$ in the univariable analysis were considered for further investigation in multivariable models in step two. Pair-wise collinearity of these variables was examined in order to prevent the inclusion of collinear variables in the multivariable models. When two variables were highly correlated (absolute value of rho > 0.70; $p < 0.05$), only one was selected for consideration in the multivariable models. The decision regarding which of a pair of highly correlated variables to include in step two was based on biological and statistical considerations.

The multivariable logistic regression model was initially built by fitting a full model that included all non-correlated variables with univariable $p \leq 0.20$. In addition, the variable after VFD regulation rule changes was included in each full model regardless of the p-value obtained from univariable regression. Non-significant predictor variables were removed using manual backward elimination, with a critical p-value of ≤ 0.05. However, non-significant variables were considered potential confounders if their removal from the model resulted in a large (greater than 20%) change in the coefficients of any of the remaining variables in the model and were considered for retention in the final model. Two-way interaction terms between VFD rule changes, animal production class, and type of tissue sampled were assessed for statistical significance. The fitness of the final model was assessed using Hosmer-Lemeshow goodness-of-fit statistics [19]. When the Hosmer-Lemeshow goodness-of-fit test was not appropriate, the area under the curve (AUC) value was used to evaluate the final model. Results of the final model were reported as odds ratio (OR) with a 95% confidence interval (CI).

Results

The original IGS dataset contained 7762 records of testing results for drug residues in the tissues of food animals. A total of 4391 records contained results of testing for residues of the antibiotics of interest in this study (penicillin: 1310; tetracyclines: 983; sulfonamides: 901; desfuroylceftiofur: 809; florfenicol: 181; and tilmicosin: 207) and were included in the analysis.

Univariable logistic regression results

Type of tissue samples was significantly associated with the detection of violative penicillin residue in the tissue of food animals from the IGS (Table 1.1). Similarly, animal production class and type of tissue sampled were significantly associated with the detection of violative tetracycline residues in the tissue of food animals from the IGS samples (Table 1.2). In addition, sample collection following the implementation of changes in VFD regulations was significantly associated with detecting violative sulfonamide residues in the tissue of food animals from the IGS (Table 1.3). Furthermore, the type of tissue sample was significantly associated with detecting violative desfuroylceftiofur residues in the tissue of food animals from the IGS (Table S1.4). There was no statistically significant association between animal production classes, type of tis-sue sample, and VFD rule changes with the detection of violative tilmicosin (Table S1.5) and florfenicol (Table S1.6) residues in the tissue of food animals from the IGS.

Multivariable logistic regression results

In the final multivariable logistic regression model for penicillin, which included 1310 observations, significant predictors associated with detecting violative residues in the tissue of food animals included the type of tissue sampled **(Table 1.7)**.

Table 1. 1: Univariable association between predictors and detection of violative residues of penicillin in the tissue of food animals (n=1,310) from the IGS, 2014-2019.

Predictor	**Categories**	**Violation N (%)**	**Non-violation N (%)**	**OR**	**95% CI**	***p*-value**
VFD rule change						0.116
	Before VFD rule change (2014-2016)	460 (72)	182 (28)	Referent		
	After VFD rule change (2017-2019)	452 (68)	216 (32)	0.82	0.65, 1.04	0.117
Animal production class						0.501
	Bob veal	58 (65)	31 (35)	0.91	0.57, 1.45	0.704
	Beef cow	74 (73)	27 (27)	1.33	0.83, 2.14	0.225
	Dairy cow	430 (67)	210 (33)	Referent		
	Bull	117 (71)	47 (29)	1.21	0.83, 1.77	0.309
	Heifer	131 (64)	75 (36)	0.85	0.61, 1.18	0.343
	Steer	17 (74)	6 (26)	1.38	0.53, 3.56	0.501
	Goat	2 (50)	2 (50)	0.48	0.06, 3.49	0.475
	Sheep	4 (100)	0 (0)	1	NA	NA
	Swine	36 (100)	0 (0)	1	NA	NA
	Turkey	43 (100)	0 (0)	1	NA	NA
Type of tissue sampled						<0.001
	Muscle	34 (32)	73 (68)	Referent		
	Kidney	878 (73)	325 (27)	5.8	3.78, 8.88	<0.001

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

Table 1. 2: Univariable association between predictors and detection of violative residues of tetracycline in the tissue of food animals (n=983) from the IGS, 2014-2019.

Predictor	**Categories**	**Violation N (%)**	**Non-violation N (%)**	**OR**	**95% CI**	***p*-value**
VFD rule change						0.244
	Before VFD rule change (2014-2016)	40 (8)	465 (92)	Referent		
	After VFD rule change (2017-2019)	48 (10)	430 (90)	1.29	0.83, 2.01	0.245
Animal production class						<0.001
	Bob veal	13 (5)	267 (95)	0.45	0.22, 0.90	0.024
	Beef cow	17 (10)	150 (90)	1.05	0.55, 2.00	0.863
	Dairy cow	27 (10)	252 (90)	Referent		
	Bull	10 (12)	75 (88)	1.24	0.57, 2.68	0.578
	Heifer	7 (7)	96 (93)	0.68	0.28, 1.61	0.383
	Steer	1 (5)	19 (95)	0.49	0.06, 3.81	0.497
	Goat	8 (40)	12 (60)	6.22	2.33, 16.55	<0.001
	Sheep	5 (83)	1 (17)	46.66	5.25, 414.23	0.001
	Swine	0 (0)	12 (100)	1	NA	NA
	Turkey	0 (0)	11 (100)	1	NA	NA
Type of tissue sampled						0.002
	Kidney	80 (8)	882 (92)	Referent		
	Others (muscle)	8 (38)	13 (62)	6.78	2.73, 16.85	<0.001

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

Table 1. 3: Univariable association between predictors and detection of violative residues of sulfonamides in the tissue of food animals (n=901) from the IGS, 2014-2019.

Predictor	Categories	Violation N (%)	Non-violation N (%)	OR	95% CI	*p*-value
VFD rule change						0.014
	Before VFD rule change (2014-2016)	417 (87)	64 (13)	Referent		
	After VFD rule change (2017-2019)	339 (81)	81 (19)	0.64	0.44, 0.91	0.015
Animal production class						0.082
	Bob veal	188 (91)	19 (9)	2.01	1.16, 3.48	0.012
	Beef cow	42 (88)	6 (12)	1.42	0.57, 3.50	0.441
	Dairy cow	290 (83)	59 (17)	Referent		
	Bull	70 (79)	19 (21)	0.74	0.42, 1.33	0.329
	Heifer	100 (78)	28 (22)	0.72	0.43, 1.20	0.214
	Steer	33 (83)	7 (17)	0.95	0.40, 2.27	0.924
	Goat	7 (78)	2 (22)	0.71	0.14, 3.51	0.677
	Sheep	1 (100)	0 (0)	1		
	Swine	20 (83)	4 (17)	1.01	0.33, 3.08	0.976
	Turkey	5 (83)	1 (17)	1.01	0.11, 8.86	0.988
Type of tissue sampled						NA
	Others (muscle/liver)	642 (82)	145 (18)	Referent		
	Kidney	114 (100)	0 (0)	1	NA	NA

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

Table 1. 4: Univariable association between predictors and detection of violative residues of Desfuroylceftiofur in the tissue of food animals (n=809) from the IGS, 2014-2019.

Predictor	Categories	Violation N (%)	Non-violation N (%)	OR	95% CI	*P*-value
VFD rule change						0.765
	Before VFD rule change (2014-2016)	522 (87)	81 (13)	Referent		
	After VFD rule change (2017-2019)	180 (87)	26 (13)	1.07	0.66, 1.72	0.767
Animal production class						0.963
	Bob veal	37 (88)	5 (12)	1.14	0.43, 3.03	0.782
	Beef cow	42 (88)	6 (12)	1.08	0.44, 2.66	0.858
	Dairy cow	387 (87)	60 (13)	Referent		
	Bull	6 (100)	0 (0)	1	NA	NA
	Heifer	203 (88)	28 (12)	1.12	0.69, 1.81	0.633
	Steer	18 (82)	4 (18)	0.69	0.22, 2.13	0.528
	Goat	9 (82)	2 (18)	0.69	0.14, 3.30	0.650
	Sheep	0 (0)	1 (100)	1	NA	NA
	Swine	0 (0)	1 (100)	1	NA	NA
	Turkey	NA	NA	NA	NA	NA
Type of tissue sampled						0.001
	Others (muscle/liver)	4 (36)	7 (64)	Referent		
	Kidney	698 (87)	100 (13)	12.21	3.51, 42.47	<0.001

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

Table 1. 5. Univariable association between predictors and detection of violative residues of tilmicosin in the tissue of food animals (n=207) from the IGS, 2014-2019.

Predictor	**Categories**	**Violation N (%)**	**Non-violation N (%)**	**OR**	**95% CI**	***P*-value**
VFD rule change						0.739
	Before VFD rule change (2014-2016)	74 (69)	33 (31)	Referent		
	After VFD rule change (2017-2019)	67 (67)	33 (33)	0.90	0.50, 1.62	0.739
Animal production class						0.855
	Bob veal	43 (81)	10 (19)	1.43	0.49, 4.11	0.504
	Beef cow	28 (78)	8 (22)	1.16	0.38, 3.58	0.788
	Dairy cow	24 (75)	8 (25)	Referent		
	Bull	12 (75)	4 (25)	1	0.25, 3.99	1.000
	Heifer	20 (77)	6 (23)	1.11	0.33, 3.73	0.865
	Steer	11 (65)	6 (35)	0.611	0.17, 2.19	0.450
	Goat	3 (60)	2 (40)	0.49	0.07, 3.54	0.488
	Sheep	NA	NA	NA	NA	NA
	Swine	0 (0)	12 (100)	1	NA	NA
	Turkey	0 (0)	10 (100)	1	NA	NA
Type of tissue sampled						
	Others (muscle/liver)	141 (76)	44 (24)	Referent		
	Kidney	0 (0)	22 (100)	1	NA	NA

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

Table 1. 6: Univariable association between predictors and detection of violative residues of florfenicol in the tissue of food animals (n=181) from the IGS, 2014-2019.

Predictor	Categories	Violation N (%)	Non-violation N (%)	OR	95% CI	*P*-value
VFD rule change						0.3306
	Before VFD rule change (2014-2016)	70 (76)	22 (24)	Referent		
	After VFD rule change (2017-2019)	62 (70)	27 (30)	0.72	0.37, 1.39	0.332
Animal production class						0.1737
	Bob veal	42 (78)	12 (22)	2.39	0.92, 6.21	0.073
	Beef cow	22 (71)	9 (29)	1.67	0.58, 4.77	0.336
	Dairy cow	19 (59)	13 (41)	Referent		
	Bull	14 (93)	1 (7)	9.57	1.11, 82.06	0.039
	Heifer	13 (62)	8 (38)	1.11	0.35, 3.43	0.854
	Steer	17 (77)	5 (23)	2.32	0.68, 7.89	0.175
	Goat	3 (75)	1 (25)	2.05	0.19, 21.97	0.552
	Sheep	2 (100)	0 (25)	1	NA	NA
	Swine	NA	NA	NA	NA	NA
	Turkey	NA	NA	NA	NA	NA
Type of tissue sampled						
	Others (muscle/liver)	124 (72)	49 (28)	Referent		
	Kidney	8 (100)	0 (0)	1	NA	NA

95% confidence interval (CI); odds ratio (OR); NA (not applicable).

The Hosmer-Lemeshow test was not used as a summary goodness-of-fit measure for the final penicillin model because there were only two covariate patterns (at least 6 covariate patterns should be present when using the Hosmer-Lemeshow test) [20]. Hence, the final penicillin model was assessed using the area under the curve (AUC), indicating the proportion of outcomes correctly classified by the model (AUC value = 0.5914).

The implementation of changes in VFD regulations was significantly associated with detecting violative penicillin residues in the tissue of food animals from the IGS. The odds of detecting penicillin residue violations decreased by 24% after the implementation of VFD regulations rule changes compared to before the VFD rule change implementation, and this finding was statistically significant **(Table 1.7)**.

The interaction term (VFD rule changes*type of tissue sampled) was statistically significant in the final model. Hence, we reported the relationship between types of tissue samples and detecting violative penicillin residues in the tissue of food animals by VFD rule change categories (before VFD rule change and after VFD rule change). The odds of detecting penicillin residue violations was about 4 times higher in the kidney than in other tissue (muscle) before implementing the VFD rule change **(Table 1.8)**. However, the odds of detecting penicillin residue violations was about 13 times higher in kidneys than in other tissue after implementing the VFD rule change **(Table 1.8)**. The final multivariable logistic regression model for tetracycline had 960 observations. The type of animal and type of tissue sampled were significant predictors of tetracycline residue violations in food animal tissues from the IGS **(Table 1.9)**. The p-value for the Hosmer-Lemeshow test was 0.0833, indicating that the final tetracycline model fit the data well.

Table 1. 7: Results of multivariable logistic regression for predictors of detection of violative residues of penicillin in the tissue of food animals (n=1,310) from the IGS, 2014-2019.

Predictor	**Categories**	**OR**	**95% CI**	***p*-value**
VFD rule change				0.030
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	0.76	0.59, 0.97	0.031
Type of tissue sampled				<0.001
	Others (muscle)	Referent		
	Kidney	6.01	3.91, 9.23	<0.001
VFD rule change*type of tissue sampled		0.3009283	0.11, 0.80	0.017

95% confidence interval (CI); odds ratio (OR); interaction (*) between VFD rule change and type of tissue sampled

Table 1. 8: Results of association between type of tissue sampled and penicillin residues in the tissue of food animals by VFD rule change categories

Predictor	Categories	OR	95% CI	*p*-value
Before the VFD rule change (2014-2016), *n*=642				
Type of tissue sampled	Others (muscle)	Referent		
	Kidney	3.95	2.32, 6.73	<0.001
After the VFD rule change (2017-2019), *n*=668				
Type of tissue sampled	Others (muscle)	Referent		
	Kidney	13.14	5.75, 30.02	<0.001

95% confidence interval (CI); odds ratio (OR).

Animal production class was significantly associated with detecting violative tetracycline residues in the tissue of food animals. The magnitude of association varied according to animal production class. For example, the odds of detecting violative tetracycline residues in the tissue of bob veal was 74% decreased compared to the tissue of dairy cows **(Table 1.9)**. On the other hand, the odds of detecting violative tetracycline residues in the tissue of sheep was 40 times higher than in the tissue of dairy cows **(Table 1.9)**. The odds of detecting violative tetracycline residues were about 8 times high in other tissue (muscle) samples compared to kidney samples **(Table 1.9)**.

Although the odds of detecting violative tetracycline residues were 54% higher for samples collected following the implementation of the VFD rule change compared to those collected prior to the VFD rule change, this finding was not statistically significant **(Table 1.9)**. Again, none of the interaction terms assessed (VFD rule changes*type of animal and VFD rule changes*type of tissue sampled) were statistically significant in the final tetracycline model.

The final multivariable logistic regression model for sulfonamides had 901 observations **(Table 1.10).** The Hosmer-Lemeshow test was not used as a summary goodness-of-fit measure for the final sulfonamide model because there were only two co-variate patterns (at least 6 covariate patterns should be present when using the Hosmer-Lemeshow test) [20]. Hence, the final sulfonamide model was assessed using the area under the curve (AUC), indicating the proportion of outcomes correctly classified by the model (AUC value = 0.56). The implementation of changes in VFD regulations was significantly associated with detecting violative sulfonamide residues in the tis-sues of food animals.

Table 1. 9. Results of multivariable logistic regression for predictors of detection of violative residues of tetracyclines in the tissue of food animals (n=960) from the IGS, 2014-2019.

Predictor	Categories	OR	95% CI	*p*-value
VFD rule change				0.092
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	1.54	0.93, 2.55	0.092
Animal production class				0.001
	Dairy cow	Referent		
	Bob veal	0.36	0.17, 0.76	0.007
	Beef-cow	0.97	0.50, 1.88	0.942
	Bull	0.98	0.43, 2.20	0.962
	Heifer	0.56	0.22, 1.39	0.218
	Steer	0.54	0.06, 4.24	0.562
	Goat	6.11	2.27, 16.47	<0.001
	Sheep	40.24	4.45, 363.69	0.001
	Swine	1		
	Turkey	1		
Type of tissue sampled				<0.001
	Kidney	Referent		
	Others (muscle)	7.71	3.02, 19.70	<0.001

The odds of detecting sulfonamide residue violations decreased by 36% after the implementation of changes in VFD regulations compared to before the VFD rule change period, and this finding was statistically significant **(Table 1.10).**

Regarding desfuroylceftiofur, the final multivariable logistic regression model had 809 observations **(Table 1.11).** The final model was assessed using the area under the curve (AUC), indicating the proportion of outcomes correctly classified by the model (AUC value = 0.5308). Although the odds of detecting violative desfuroylceftiofur residues were 2% decreased for samples collected following the implementation of the VFD rule change compared to those collected before the VFD rule change, this finding was not statistically significant **(Table 1.11).** The odds of detecting desfuroylceftiofur residue violations was 12 times higher in the kidney than in other tissue (muscle) **(Table 1.11).**

The final multivariable logistic regression model for tilmicosin had 207 observations **(Table 1.12).** The final model was assessed using the area under the curve (AUC), indicating the proportion of outcomes correctly classified by the model (AUC value = 0.5124). The odds of detecting violative tilmicosin residues were 10% decreased for samples collected following the implementation of the VFD rule change compared to those collected before the VFD rule change. However, this finding was not statistically significant **(Table 1.12).**

Regarding florfenicol, the final multivariable logistic regression model had 181 observations **(Table 1.13).** The final model was assessed using the area under the curve (AUC), indicating the proportion of outcomes correctly classified by the model (AUC value = 0.5407). The odds of detecting violative florfenicol residues were 28% decreased for samples collected following the implementation of the VFD rule change compared to those collected before the VFD rule change. However, this finding was not statistically significant **(Table 1.13).**

Discussion

To the best of our knowledge, this is the first report describing the association of changes in VFD regulations on the detection rates of violative penicillin, tetracycline, sulfonamide, desfuroylceftiofur, tilmicosin, and florfenicol residues in the tissues of food animals in slaughterhouses in the U.S. Our study highlights three critical findings. Firstly, compared to the period before changes in VFD regulations, the odds of detecting violative sulfonamide and penicillin residues in the tissues of food animals sampled (from the IGS) following VFD implementation decreased by 36% and 24%, respectively, irrespective of the animal production class. Secondly, animal production class was significantly associated with the detection of violative tetracycline residues. However, the implementation of change in the VFD rule was not significantly associated with the tetracycline residue violation rates in the tissue of food animals from the IGS. Finally, the type of tissue sampled was significantly associated with tetracycline and desfuroylceftiofur residues violation. However, the implementation of the change in VFD rule was not associated with the desfuroylceftiofur residues violation rates in the tissue of food animals from the IGS.

Table 1. 10. Results of multivariable logistic regression for predictors of detection of violative residues of sulfonamides in the tissue of food animals (n=901) from the IGS, 2014-2019.

Predictor	**Categories**	**OR**	**95% CI**	***p*-value**
VFD rule change				0.014
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	0.64	0.44, 0.91	0.015

95% confidence interval (CI); odds ratio (OR).

Table 1. 11. Results of multivariable logistic regression for predictors of detection of violative residues of Desfuroylceftiofur in the tissue of food animals (n=809) from the IGS, 2014-2019.

Predictor	Categories	OR	95% CI	*p*-value
VFD rule change				0.963
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	0.98	0.61, 1.59	0.964
Type of tissue sampled				0.001
	Others (muscle)	Referent		
	Kidney	12.24	3.50, 42.85	<0.001

95% confidence interval (CI); odds ratio (OR).

Table 1. 12. Results of multivariable logistic regression for predictors of detection of violative residues of tilmicosin in the tissue of food animals (n=207) from the IGS, 2014-2019.

Predictor	**Categories**	**OR**	**95% CI**	***p*-value**
VFD rule change				0.739
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	0.90	0.50, 1.62	0.739

95% confidence interval (CI); odds ratio (OR).

Table 1. 13. Results of multivariable logistic regression for predictors of detection of violative residues of florfenicol in the tissue of food animals (n=181) from the IGS, 2014-2019.

Predictor	**Categories**	**OR**	**95% CI**	***p*-value**
VFD rule change				0.330
	Before VFD rule change (2014-2016)	Referent		
	After VFD rule change (2017-2019)	0.72	0.37, 1.39	0.332

95% confidence interval (CI); odds ratio (OR).

Before this study, cattle producers perceived that changes in VFD regulations would lead to increased use of injectable antibiotics by producers [8] and an overall increase in residue violations. Results of the current study showed that after the implementation of change in the VFD rule, the detection of violative sulfonamide and penicillin residues decreased significantly in the tissue of food animals from the IGS. There are several potential explanations for these findings. For instance, revised VFD regulations may not have impacted the use of sulfonamide and penicillin injectables. Alternatively, the use of injectable sulfonamides and penicillin may have increased following the implementation of VFD regulations; however, the relatively short withdrawal period (Sulfonamides:5 days; penicillin G: range from 4 to 10 days, as label withdrawal time) [21] may have increased the likelihood of farmers' compliance, leading to non-violative residues in our study. Other potential factors could be associated with this finding depending on dose/route/duration and animal production class. Payne MA et al., [22] reported that extra-label use of penicillin in food-producing animals under the direction of a veterinarian as the labeled dose of penicillin is not effective, and the extra-labeled requires an extended withdrawal period, typically at least 21-30 days depending on dose/route/duration [22]. Also, clinical illness can impact the withdrawal time (as the withdrawal time is established in healthy animals), and may also play role in the risk of antibiotic residue violation in tissues of food animals.

In contrast, the odds of detecting violative tetracycline residues among samples collected following the implementation of change in the VFD rule were not decreased significantly compared to before the implementation of change in the VFD rule. Multiple factors could explain these findings. Farmers have expressed displeasure with rule changes in VFD regulations because non-therapeutic use of medically important antimicrobials in medicated feed for growth promotion and feed efficiency, which was, permitted prior to implementation of VFD rule changes, may have prevented or reduced clinical diseases later in animals' life [8]. If cases of clinical disease among food animals were more frequent following changes in VFD regulations, injectable (including extra-label) use of tetracyclines might have increased to treat these animals. Furthermore, injectable tetracycline has relatively lengthy withdrawal periods of 28 days [21]. Adhering to these withdrawal periods could be more challenging than sulfonamides, leading farmers to send treated animals to slaughter with violative tissue levels of antibiotic residues. In addition, farmers with limited experience using injectable antibiotics may be unaware of proper dosing. Hence, imprudent use of tetracycline, including incorrect dosage and route of administration [23-25], may have contributed to the residue violations observed in this study. Previous studies have reported that failure to follow meat withdrawal periods and extra-label use of injectable tetracycline may be associated with antibiotic residues in the tissues of food animals [23,26-28]. Future studies are warranted to investigate practices of injectable antibiotic administration, including extra-label use, treatment documentation, and knowledge of anti-biotic withdrawal periods in food animals with clinical illnesses, to elucidate the spectrum of these issues (after the implementation of changes in VFD regulations) at the farm level in the U.S.

This study revealed significant differences in the odds of detecting violative tetracycline and penicillin residues between kidney and other tissue samples (muscle/liver). Kidney tissue samples had higher odds of penicillin residue violations than samples from other tissues (muscle/liver). This magnitude of association varied before and after VFD rule changes; for instance, higher odds (OR=13.14) of detecting violative penicillin residues after VFD rule change than before the implementation of VFD rule changes (OR=3.95). This is an expected finding because most (60-90%) of parenterally administered penicillin is eliminated in the urine, and kidneys represented the majority (92%) of the sampled tissues in the dataset. Hence, this finding is consistent with the results of Paturkar et al. [29], regardless of any regulatory change.

On the other hand, muscle tissue samples had higher odds of tetracycline residue violations compared to kidney samples. Several factors may have contributed to this finding, including the route of administration and extra-label use of tetracycline in food animals. For example, tetracycline residues have been found at the injection site as many as 35 days after intramuscular administration [30]. In addition, previous studies have reported that tetracycline residue levels were higher in muscles than in kidneys [31-34], regardless of regulations. Future experimental and epidemiologic field studies could generate knowledge on host- and farm-level factors associated with tissue levels of tetracycline residues in food animals in the U.S.

The results of multivariable logistic regression models showed that bob veal samples had lower odds of residue violations for tetracycline compared to dairy cows. On the other hand, compared to dairy cows, sheep and goats had higher odds of detecting tetracycline residue violations. This finding indicates that withdrawal times set for antibiotic use in goats and sheep are not always followed or are inaccurate because the use of antibiotics in goats and sheep is predominantly extra-label [35,36]. Practices of extra-label antibiotics could be more common in sheep and goats [30] because there are limited FDA-approved labeled antibiotic products in the U.S. [37] A study reported that extra-label antibiotic use is more common in small ruminants than in cattle [37]. This inappropriate or extra-label antibiotic use in these animal classes [38] may play a role in the risk of tetracycline residue violations. However, extra-label use of medicated feed is not prohibited in these animal classes [37]. It requires a written recommendation by a licensed veterinarian within the confines of a valid veterinarian-client-patient relationship in the U.S [37]. A previous study reported that goats had a higher frequency of antibiotic residues at slaughter in Missouri [38]. A study from Alberta indicates that tetracycline was one of the most common injectable antibiotics used in sheep [39]. Our findings warranted further research and highlighted that increased labeled antibiotic options for these animal classes would provide producers with appropriate withdrawal times to follow. Also, there is a need to improve working relationships between veterinarians and goat/sheep farmers to promote appropriate antibiotic use [38] to prevent the occurrence of antibiotic residues in the tissue of these animal classes at slaughter.

The result of this study would not be generalizable because the tissue samples were collected using the targeted sampling of food-producing animals under the IGS. The tissue samples from the IGS were chosen based on clinical signs or pathologic lesions on food-producing animals during antemortem and post-mortem examination by a veterinarian authorized to collect the tissue samples. So current study results may over-represent the violation of antibiotic residues in tissues of food-producing animals from the IGS than all other food-producing animals brought to the slaughterhouse. Our study findings only apply to the samples collected under the IGS, not the entire food-producing animals brought into the slaughterhouse.

Besides, our study used at least 181 observations for each class of antibiotic of interest, and a larger sample size could be more helpful. However, given the number of covariates used in our model, we consider this sample size adequate for the study. In addition, there were limited variables in the dataset, so we suggest including animal-level information such as age, sex, breed, pathologic lesions or signs, and location (state-level) of sampled animals under the IGS scheme. Besides, results of the animal production class should be generalized cautiously because dairy cows are used as the reference population (as there is no VFD use of antimicrobials in dairy cattle) in the animal production class variable for all analyses in the study. Cull dairy cows are one of the most likely production categories to have violative residues identified, although this varies based on antibiotic class. For example, penicillin was the most frequently identified antibiotic with violative residue levels in culled cows in the U.S. [5].

Conclusion

In summary, the implementation of the VFD rule changes in 2017 did not increase the detection of violative residues of injectable antibiotics in the tissues of food animals from the IGS. Actually, the VFD rule changes had a positive impact on violative residues of a few injectable antibiotics. Violative residues of sulfonamides and penicillin were reduced, but violative residues of tetracyclines, desfuroylceftiofur, tilmicosin, and florfenicol did not change. In addition to the practical benefits of the VFD rule changes, multi-sectoral coordinated educational interventions to food animal producers and farmers concerning withdrawal periods, record-keeping, and compliance with label instructions of antibiotics is critical. Such wholistic approach would further reduce violative antibiotic residues in the tissues of food animals in the U.S.

Author Contributions: Writing-original draft preparation: S.S.; conceptualization, and writing: S.S.; Data analysis: S.S.; Review and Editing: SS, C.C.O.; Supervision: C.C.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable. Our study did not directly involve humans or animals.

Data Availability Statement: All the datasets used in this study are secondary datasets, and the links to access the datasets are given in this article. These data sets are publicly available, and no specific rights are required to access these datasets.

Acknowledgments: We would like to thank the Comparative and Experimental Medicine Program of University of Tennessee, Knoxville, to support this study. We acknowledged that funding for open access to this research was provided by University of Tennessee's Open Publishing Sup-port Fund. Also, we would like to thanks Jennifer Lord for checking the English language expression in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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CHAPTER 2

Effect of veterinary feed directive rule changes on tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats in the United States

A version of this chapter was originally published by Shamim Sarkar, Chika C Okafor (2023). Effect of veterinary feed directive rule changes on tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats in the United States. PLoS ONE 18(8): e0289208. https://doi.org/10.1371/journal.pone.0289208

My contribution to this paper included gathering and review of literature, conceptualization, data analysis, interpretation of results, and drafting and editing of the manuscript.

Abstract

Antimicrobial-resistant bacteria are a growing public health threat. In 2017 the U.S. Food and Drug Administration implemented Veterinary Feed Directive (VFD) rules changes to limit medically important antimicrobial use in food-producing animals, combating antimicrobial-resistant bacteria. The effect of the VFD rule changes on the occurrence of bacteria resistant to medically-important antimicrobials in retail meats is yet to be investigated in the U.S. This study investigates whether the VFD rule changes affected the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats in the U.S. Multivariable mixed effect logistic regression models were used to analyze 2002-2019 retail meats surveillance data from the National Antimicrobial Resistance Monitoring System (NARMS) in the U.S. Variables included VFD rule changes, meat type, quarter of year, and raising claims. A potential association between these variables and the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in retail meats was estimated. Analysis included data regarding tetracycline-resistant *Salmonella* (n=8,501), *Escherichia* (n=20,283), *Campylobacter* (n=9,682), and erythromycin-resistant *Campylobacter* (n=10,446) in retail meats. The odds of detecting tetracycline-resistant *Escherichia* (OR=0.60), *Campylobacter* (OR=0.89), and erythromycin-resistant *Campylobacter* (OR=0.43) in chicken breast significantly decreased after the VFD rule changes, compared to the pre-VFD rule change period. The odds of detecting tetracycline-resistant *Salmonella* (0.66), *Escherichia* (OR=0.56), and *Campylobacter* (OR=0.33) in ground turkey also significantly decreased. However, the odds of detecting tetracycline-resistant *Salmonella* (OR=1.49) in chicken breast and erythromycin-resistant *Campylobacter* (OR=4.63) in ground turkey significantly increased. There was no significant change in the odds of detecting tetracycline-resistant *Salmonella* and *Escherichia* in ground beef or pork chops. The implementation of VFD rule changes had a beneficial effect by reducing the occurrence of tetracycline-resistant and erythromycin-resistant bacteria in chicken and ground turkey. Ongoing surveillance of antimicrobial resistance and antimicrobial use could complement the implementation of stewardship, such as the VFD rule in food-producing animals in the U.S.

Keywords: Antimicrobial resistance, veterinary feed directives, tetracycline, erythromycin, Salmonella, Escherichia, and Campylobacter, retail meats, chicken breast, ground turkey, ground beef, pork chops, NARMS.

Introduction

Animal-sourced protein consumption has increased globally over the last decade [1]. The animal-sourced protein consumed in the United States (U.S.) mostly comes from the industrial food-animal production system [2], which is characterized by large-scale, densely stocked conditions [3], and the widespread usage of antimicrobials to treat sick animals and control disease [4, 5]. Before the implementation of the Veterinary Feed Directive (VFD) rule changes in the U.S., antimicrobials were commonly used in food-producing animals as preventive measures. However, since the VFD rule changes were implemented, antimicrobials can no longer be used in a prophylactic manner [4].

Antimicrobial resistance is a global health concern [6, 7]. Multiple factors are associated with the emergence of antimicrobial resistance bacteria [8]. Overuse and misuse of antimicrobials are important factors associated with resistance to antimicrobial drugs [9, 10]. The use of antimicrobials in food-producing animals in the U.S. varies across different classes of antimicrobials. In the U.S., specifically in 2020, tetracycline was the most frequently used antimicrobial, representing 66% (3,948,745 kg) of total use and 7% (433,394 kilograms) of macrolides in food-producing animals [11]. Antimicrobial resistance has emerged due to the selective pressure exerted by antimicrobial use in food-animal production [12-14]. The improper use of antimicrobial drugs in food-producing animal farming has increased antimicrobial resistance bacteria in animal food products [15, 16]. The dose, route, duration, and class of antimicrobials (selective pressure) are important variables that may affect the development of antimicrobial resistance [17, 18]. Also, antibiotic usage, whether for therapeutic, prophylactic, or growth promotion purposes, all contribute to the selection pressure leading to antibiotic-resistant development [19, 20]. Reducing the misuse of antibiotics, whether for therapeutic, growth promotion, or prophylactic purposes, may strengthen the prevention and control of the development of antimicrobial resistance [18]. Biologically, reducing antimicrobial use can decrease the selection pressure for developing resistance [21]. For example, the resistance level in hospital-acquired pathogens may change rapidly within weeks or months after reforms in antimicrobial use [22]. Multifaceted interventions are needed to reduce the improper use of antimicrobials [23] and promote the judicious use of medically important antimicrobials in food-producing animals to control the development of antimicrobial resistance. Resistance to tetracycline and erythromycin were selected for the current analysis because these are medically important antibiotics [24] approved for use in food-producing animals in the U.S. [25].

The VFD rule changes are an important strategy to ensure the judicious use of medically important antimicrobials in food-producing animals in the U.S. On October 1, 2015, the U.S. Food and Drug Administration (FDA) changed the rules for the VFD. The rules were fully enforced on January 1, 2017, following the FDA's Guidance for Industry # 2013 [4]. This VFD rule change highlighted the FDA's concerns about developing antimicrobial resistance (AMR) pathogenic bacteria in humans and animals when medically important antimicrobial drugs are imprudently used in food-producing animal production. The changes made to the VFD rules limit the use of medically important antimicrobial drugs given to animals via feed and water, only allowing usage for treating illness. The VFD has impacted a number of medically important antimicrobial classes, such as tetracyclines, macrolides, and penicillins. However, it is important to note that these antimicrobials can still usage for therapeutic purposes through the administration via drinking water under the supervision of a licensed veterinarian [26, 27]. The VFD rule changes have reduced the presence of violative sulfonamide and penicillin residues in the tissue of food animals at U.S. slaughterhouses, compared to before the VFD rule changes [28]. The effect of the VFD rule changes on the occurrence of bacteria resistant to medically important antimicrobials in retail meats is yet to be investigated in the U.S.

The National Antimicrobial Resistance Monitoring System (NARMS) was set up in 1996 as a joint initiative involving state and local public health departments, universities, the FDA, the Center for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture's (USDA) [29]. This national surveillance system has routinely collected data on the antimicrobial susceptibility of enteric bacteria from meats sold in stores since 2002 [29]. This program aims to monitor changes in the antimicrobial susceptibility of enteric bacteria in the US., with a specific component of the surveillance being devoted to retail meats.

Meat and meat products can be an important source of exposure to antimicrobial-resistant bacteria in humans [30-33]. Numerous studies have indicated the existence of antimicrobial-resistant bacteria found in meats sold at retail stores globally [31, 34-39]. Antimicrobial-resistant bacteria originating from meats can be transmitted to humans through direct contact with meats or the consumption of meat products [40, 41]. In cases where humans contract antimicrobial-resistant bacteria, the resulting infection may be challenging to manage or lead to unfavorable clinical consequences [42, 43]. Foodborne illness associated with enteric bacteria is a significant public health issue in the U.S. Approximately 1 in 6 Americans suffer annually from a foodborne illness, resulting in almost 48 million cases, 128,000 hospitalizations, and 3000 deaths in the U.S. [44]. Three food-associated enteric bacteria, namely *Salmonella*, *Escherichia*, and *Campylobacter*, are responsible for approximately 60% of these illnesses and hospitalizations in the U.S. [44, 45]. These bacteria also infect food-producing animals, making them a suitable focus for the current study.

Several studies have been conducted on the prevalence of antimicrobial-resistant bacteria in retail meats in the U.S. Most of these studies aimed to characterize and assess the profiles of antimicrobial-resistant bacteria isolates in retail meats, using cross-sectional surveys conducted in the U.S. between 1998 and 2018. [32, 46-48]. A recent study utilized a subset of NARMS data covering the period from 2008 to 2017 and compared *Salmonella* prevalence and antibiotic susceptibility profiles in retail poultry meats with and without antibiotic-related claims [49]. Another study explored the associations between meat production methods and AMR and bacterial contamination of retail meats, using NARMS data covering 2012 to 2017 [50].

However, recent studies utilizing NARMS data did not evaluate the potential impact of VFD rule changes on the risk of antimicrobial-resistant bacteria in retail meats in the U.S. Therefore, our study's objective was to investigate the effect of the 2017 VFD rule changes on the occurrence of tetracycline and erythromycin-resistant bacteria (*Salmonella*, *Escherichia*, and *Campylobacter*) in the U.S. using the NARMS dataset. The findings of this study will provide evidence of the magnitude of the impact that VFD rule changes have had on the risk of AMR bacteria isolates in retail meats in the U.S.

Materials and Methods

Data source and retrieval

On March 1, 2023, the retail meats surveillance dataset from 2002 to 2019 was downloaded from the NARMS, which is publicly available data [51]. Under this surveillance system, chicken breast, ground beef, ground turkey, and pork chops are collected from grocery stores and tested for bacterial contamination and their resistance to antimicrobial drugs [51]. Subsequently, the obtained data was transferred from Microsoft Excel to STATA 17.1 software (Stata Corporation, College Station, TX, USA) for data validation and analysis.

Data preparation and variables

The primary variable of interest utilized in selecting the final dataset for analysis was the presence of the minimum inhibitory concentration (MIC) values of tetracycline and erythromycin. The other selected variables for analysis in each dataset included month, year, state, meat type, raising claims (regarding how the animals were raised and whether they were given antibiotics), and type genera of bacteria found (*Salmonella*, *Escherichia*, and *Campylobacter*). The bacteria categories analyzed in this study include *Salmonella*, *Escherichia*, and *Campylobacter*, which encompass the aggregation of their respective serotypes. The "raising claims" variable has numerous text-form responses, which was further categorized into three groups (conventional, without any antibiotics, and not specified). Responses that included words such as "organic", or "no antibiotic" or "antibiotic-free" (without any antibiotics) were classified together as “without any antibiotics” categories [52]. Likewise, responses without specific antibiotic-free claims such as “all-natural”, “use antibiotic responsibly”, “grass-fed” were grouped together as “conventional”. Similarly, responses containing the words “none” or “not specified” were grouped together as "not specified". After analyzing the data, we found that there was no difference between the conventional and not specified categories. As a result, we merged the not specified category with the conventional category. The final raising claims were either ‘without any antibiotic’ or ‘conventional’. The “year” variable was treated as an individual year variable. Also, we collapsed “year” variable into a categorical variable called “years of sampling”. This categorization was based on three years: ‘2002-2004’, ‘2005-2007’, ‘2008-2010’, ‘2011-2013’, ‘2014-2016’, and ‘2017-2019’. Additionally, we also created a dichotomous variable called “VFD rule change,” which collapse the “year” variable into two categories: “before VFD rule change (2002-2016)” and “after VFD rule change (2017-2019).” The “VFD rule changes” is a two-level categorical variable, with ‘after VFD rule changes’ meaning the retail meats were sampled and tested between 2017 and 2019, and ‘before VFD rule changes’ meaning the retail meats were sampled and tested between 2002 and 2016.

The "month" variable was categorized into four quarters: Quarter 1 (January, February, and March), Quarter 2 (April, May, and June), Quarter 3 (July, August, and September), and Quarter 4 (October, November, and December).

The outcome variables were the presence of tetracycline-resistant *Salmonella*, *Escherichia*, and *Campylobacter* isolates in retail meats and the presence of erythromycin- resistant *Campylobacter* isolates in retail meats. Tetracycline-resistant *Salmonella* and *Escherichia* isolates in retail meat were categorized based on the MIC breakpoint values of tetracycline (≥16 µg/ml). Similarly, the tetracycline-resistant *Campylobacter* in retail meats was categorized based on the MIC values of tetracycline (≥4 µg/ml). Erythromycin-resistant *Campylobacter* in retail meats was defined based on the MIC breakpoint values of erythromycin (≥8 µg/ml). The breakpoints were based on the 2021 NARMS Interpretive Criteria for Susceptibility Testing [53].

Statistical analysis

Frequencies and percentages were used to summarize categorical predictor variables. Four mixed-effect logistic regression models were built, using a random intercept to control for state-level clustering for the tetracycline-resistant *Salmonella*, *Escherichia*, and *Campylobacter* isolates and erythromycin-resistant *Campylobacter* isolates in retail meats in this study.

For each model-building process, two steps were involved. In the first step, a univariable mixed effect logistic regression model using the state as a random intercept was fitted to assess the unadjusted associations between potential predictor variables and the outcome variable. We conducted the univariable analysis to investigate the association of the "year", "years of sampling", "VFD rule change", meat type, quarter of year, and raising claims with the resistant outcome.

A relaxed p-value ≤ 0.2 [54-57] was used to identify predictors that were chosen for further examination in the multivariable mixed-effect logistic regression models in step two. To prevent the inclusion of collinear variables in the multivariable models, the pairwise collinearity of these variables was examined using Spearman's rank correlation coefficient. If the correlation coefficient between two variables is ≥0.6 [58], only the variable with the highest odds ratio, the fewest missing observations in the initial screening, and biological plausibility would be included in the multivariable model. Categorical variables with more than two levels of categories were analyzed to evaluate pairwise differences using the Tukey-Kramer adjustment for multiple comparisons. Temporal graphs were generated in excel to visualize the temporal patterns of resistant outcomes by years of sampling. However, for the variables measuring similar characteristics (e.g., year, year of sampling, and VFD rule change), only one of the variables was used in model building, determined by the results of the univariable analysis.

In the second step, a multivariable mixed-effect logistic regression model (PROC GLIMMIX, SAS version 9.4) was built using a manual backward elimination method. A full model was first constructed by including all the screened variables and non-correlated as fixed factors, and subsequently, the state was fitted as a random effect and state as a random effect. We included the VFD rule change in every full multivariable model, regardless of the p-value obtained from the univariable analysis, as it was our primary exposure of interest for the analysis. Non-significant variables were removed through a manual backward elimination process. However, if the removal of a non-significant variable resulted in a substantial change (more than 20%) in the coefficients of any remaining variables in the model, it was considered a potential confounder and was retained in the final model [59]. Each final multivariable model was checked for possible multicollinearity using variance inflation factor (VIF) to avoid modeling issues associated with multicollinearity. If the VIF value exceeded 10, it indicated the of presence of multicollinearity [60]. The relevant pairwise significant interaction term was assessed in the final multivariable models [61]. For instance, the two-way interaction between VFD rule changes and the meat type was evaluated. For all final multivariable model results were presented as an odds ratio (OR) with a corresponding 95% confidence interval (CI), and p-values. A p-value ≤ 0.05 was considered statistically significant [62]. The overall assessment of the final multivariable model was done using the Akaike's Information Criterion (AIC) [63]. The model with the lowest AIC values was considered as the best-fitting model.

Results

The original dataset contained 342,041 records from 2002 to 2019. However, only 67,731 (19.8%) and 39,720 (11.6 %) of these records had MIC values for the target antibiotics, tetracycline and erythromycin, respectively. Data regarding tetracycline-resistant *Salmonella* (N=8,501), *Escherichia* (N=20,283), *Campylobacter* (N=9,682), and erythromycin-resistant *Campylobacter* (N=10,446) from records of retail meats were included in the statistical analysis.

Univariable mixed effect logistic regression results

Meat type, quarter of year, raising claims, and year were significantly associated with the occurrence of tetracycline-resistant *Salmonella* in retail meats **(Table 2.1).** Additionally, the estimated odds ratios from the years of sampling did not demonstrate a distinct linear pattern across all comparisons for tetracycline-resistant *Salmonella* in retail meats **(Table 2.2).** Furthermore, no linear trend was observed in the proportion of tetracycline-resistant *Salmonella* in retail meats based on our graphical analysis **(Figure 2.1).** Similarly, meat type, quarter of year, raising claims, year, and the VFD rule changes were significantly associated with tetracycline-resistant *Escherichia* in retail meats **(Table 2.3).** Additionally, the estimated odds ratios from the years of sampling did not demonstrate a distinct linear pattern across all comparison's tetracycline-resistant *Escherichia* in retail meats **(Table 2.4).** Furthermore, no linear trend was observed the proportion of tetracycline-resistant *Escherichia* in retail meats based on our graphical analysis **(Figure 2.2).** Additionally, meat type, quarter, year, and VFD rule changes were significantly associated with tetracycline-resistant *Campylobacter* in retail meats **(Table 2.5).** However, there was no statistically significant association between raising claims and quarter year for tetracycline-resistant *Campylobacter* in retail meats **(Table 2.5).** In addition, the estimated odds ratios from the years of sampling did not demonstrate a distinct linear pattern across all comparison's tetracycline-resistant *Campylobacter* in retail meats **(Table 2.6).** Furthermore, no linear trend was observed in the proportion of tetracycline-resistant *Campylobacter* in retail meats based on our graphical analysis **(Figure 2.3).** Lastly, meat type, year, and VFD rule changes were significantly associated with erythromycin-resistant *Campylobacter* in retail meats **(Table 2.7).** In addition, the estimated odds ratios from the years of sampling did not demonstrate a distinct linear pattern across all comparison's erythromycin-resistant *Campylobacter* in retail meats **(Table 2.8).** Furthermore, no linear trend was observed in the proportion of erythromycin-resistant *Campylobacter* in retail meats based on our graphical analysis **(Figure 2.4).**

Multivariable mixed effect logistic regression model results

The final model was fitted for tetracycline-resistant *Salmonella*, which included 8,501 records of retail meats **(Table 2.9).** Variables significantly associated with detecting tetracycline-resistant *Salmonella*--controlling for other variables--was meat type. No multicollinearity issue was found in the final model. However, there was a significant interaction between the VFD rule changes and meat type after controlling for all other variables in the model. The significant interaction between the VFD rule changes and meat type implies that the effect of the VFD rule changes on the odds of detecting tetracycline-resistant *Salmonella* was not the same across the meat types. For example, the odds of detecting tetracycline-resistant *Salmonella* were decreased by 44% in ground turkey following implementation of the VFD rule changes compared to ground turkey in the period prior to implementation (OR= 0.66, 95% CI: 0.56, 0.77; $p<0.0001$) **(Table 2.9).**

Table 2. 1: Results of the univariable mixed-effect logistic regression model, using a random intercept to control for state-level clustering for tetracycline-resistant *Salmonella* in retail meats in the United States, 2002-2019

Variable	**Categories**	**Tetracycline-resistant**		**OR**	**95% CI**	**p-value**
		Yes n (%)	**No n (%)**			
Year (n=8,501)						<0.001
	2002	70 (45.75)	83 (54.25)	0.89	0.61, 1.29	0.554
	2003	76 (35.85)	136 (64.15)	0.60	0.43, 0.83	0.003
	2004	161 (49.69)	163 (50.31)	1.10	0.82, 1.46	0.502
	2005	146 (41.36)	207 (58.64)	0.83	0.63, 1.10	0.216
	2006	166 (49.11)	172 (50.89)	1.14	0.87, 1.51	0.327
	2007	178 (55.63)	142 (44.38)	1.55	1.17, 2.06	0.002
	2008	268 (54.58)	223 (45.42)	1.42	1.11, 1.83	0.005
	2009	298 (61.19)	189 (38.81)	1.77	1.37, 2.28	<0.001
	2010	218 (54.50)	182 (45.50)	1.37	1.05, 1.79	0.018
	2011	213 (59.66)	144 (40.34)	1.73	1.31, 2.28	<0.001
	2012	164 (46.20)	191 (53.80)	0.97	0.73, 1.27	0.828
	2013	183 (51.84)	170 (48.16)	1.26	0.96, 1.65	0.090
	2014	137 (52.29)	125 (47.71)	1.32	0.98, 1.78	0.063
	2015	185 (45.45)	222 (54.55)	0.95	0.73, 1.23	0.722
	2016	184 (43.91)	235 (56.09)	0.91	0.70, 1.18	0.509
	2017	257 (44.93)	315 (55.07)	Referent		
	2018	311 (44.05)	395 (55.95)	0.97	0.77, 1.21	0.795
	2019	1119 (56.17)	873 (43.83)	1.54	1.28, 1.87	<0.001
VFD rule changes (n=8,501)						0.084
	Before VFD rule changes (2002-2016)	2647 (50.60)	2584 (49.40)	Referent		
	After VFD rule changes (2017-2019)	1687 (51.59)	1583(48.41)	1.09	0.98, 1.20	0.085

OR = odds ratio; CI = confidence interval

Table 2. 1. continued

Variable	Categories	Tetracycline-resistant		OR	95% CI	p-value
		Yes n (%)	**No n (%)**			
Meat type (n=8,501)						<0.001
	Chicken breast	2382 (52.11)	2189(47.89)	1.00	0.92, 1.10	0.838
	Ground beef	61 (27.35)	162 (72.65)	0.36	0.26, 0.49	<0.001
	Ground turkey	1738 (51.79)	1618(48.21)	Referent		
	Pork chops	153 (43.59)	198 (56.41)	0.72	0.58, 0.91	0.006
Quarter of year (n=8,501)						0.0187
	Quarter 1	1107 (51.54)	1041 (48.46)	1.14	1.00, 1.28	0.035
	Quarter 2	1139 (53.20)	1002 (46.80)	1.21	1.07, 1.36	0.002
	Quarter 3	1001 (48.06)	1082 (51.94)	Referent		
	Quarter 4	1087 (51.06)	1042 (48.94)	1.14	1.00, 1.28	0.035
Raising claims (n=3,300)						0.0001
	Conventional	672 (47.93)	730 (52.07)	Referent		
	Without any antibiotics	1,027(54.11)	871(45.89)	1.33	1.15, 1.54	<0.001

OR = odds ratio; CI = confidence interval

Table 2. 2: Univariable mixed-effect logistic regression of association between years of sampling and tetracycline-resistant *Salmonella* in retail meats in the United States

Variable	Categories	OR	95% CI	p-value
Years of sampling (n= 8501)				<0.0001
	2002-2004 vs. 2017-2019	0.68	0.52, 0.88	0.0002
	2005-2007 vs. 2017-2019	0.87	0.69, 1.08	0.4621
	2008-2010 vs. 2017-2019	1.17	0.96, 1.43	0.205
	2011-2013 vs. 2017-2019	0.99	0.80, 1.23	1
	2014-2016 vs. 2017-2019	0.79	0.64, 0.97	0.014
	2002-2004 vs. 2005-2007	0.78	0.58, 1.04	0.1237
	2002-2004 vs. 2008-2010	0.58	0.44, 0.76	<0.0001
	2002-2004 vs. 2011-2013	0.68	0.51, 0.91	0.0021
	2002-2004 vs. 2014-2016	0.86	0.64, 1.15	0.6604
	2005-2007 vs. 2008-2010	0.74	0.58, 0.95	0.0062
	2005-2007 vs. 2011-2013	0.88	0.68, 1.13	0.6955
	2005-2007 vs. 2014-2016	1.11	0.86, 1.43	0.8701
	2008-2010 vs. 2011-2013	1.18	0.93, 1.50	0.3355
	2008-2010 vs. 2014-2016	1.49	1.18, 1.89	<0.0001
	2011-2013 vs. 2014-2016	1.26	0.98, 1.62	0.088

OR = odds ratio; CI = confidence interval

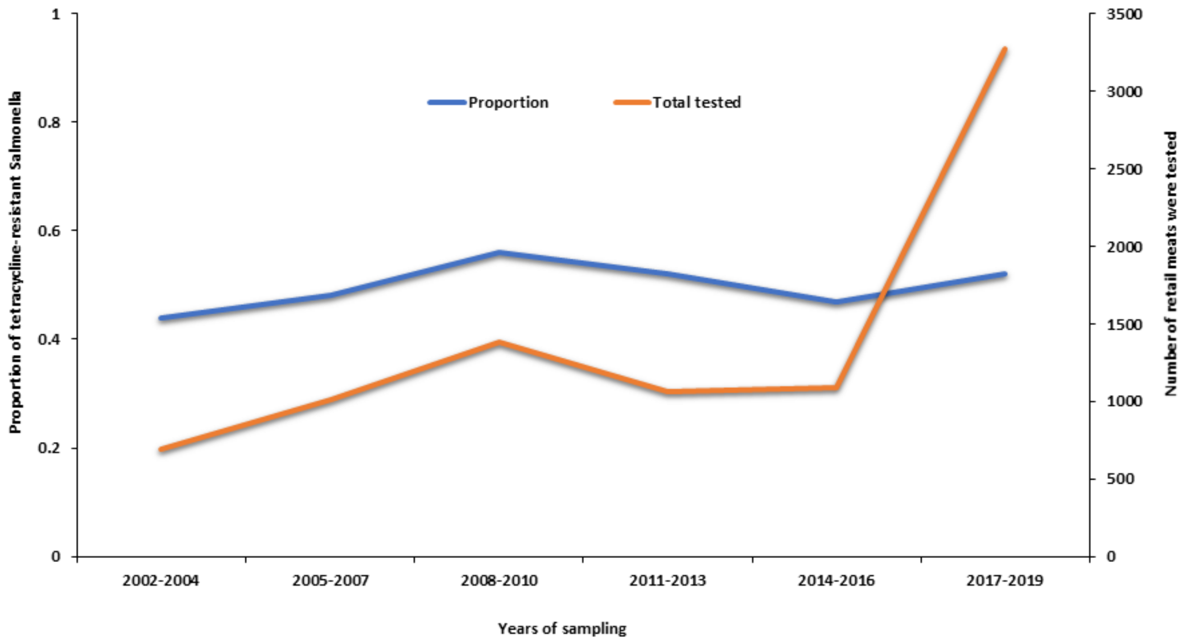

Figure 2. 1: Temporal patterns of the proportion of tetracycline-resistant *Salmonella* in retail meats by years of sampling in the United States

Table 2. 3: Results of the univariable logistic regression models, using a random intercept to control for state-level clustering for tetracycline-resistant *Escherichia* in retail meats in the United States, 2002-2019

Variable	Categories	Tetracycline-resistant		OR	95% CI	p-value
		Yes n (%)	No n (%)			
Year (n=20,283)						<0.001
	2002	552 (51.83)	513 (48.17)	1.46	1.22, 1.74	<0.001
	2003	608 (48.33)	650 (51.67)	1.22	1.03, 1.45	0.016
	2004	678 (50.37)	668 (49.63)	1.31	1.11, 1.55	0.001
	2005	638 (48.70)	672 (51.30)	1.24	1.04, 1.46	0.012
	2006	679 (52.92)	604 (47.08)	1.45	1.22, 1.72	<0.001
	2007	505 (49.41)	517 (50.59)	1.23	1.03, 1.47	0.019
	2008	531 (52.99)	471 (47.01)	1.43	1.20, 1.71	<0.001
	2009	497 (48.97)	518 (51.03)	1.22	1.02, 1.46	0.024
	2010	537 (45.59)	641 (54.41)	1.07	0.90, 1.27	0.390
	2011	539 (50.37)	531 (49.63)	1.30	1.09, 1.55	0.003
	2012	577 (47.73)	632 (52.27)	1.17	0.99, 1.39	0.062
	2013	592 (50.64)	577 (49.36)	1.33	1.12, 1.58	0.001
	2014	572 (50.89)	552 (49.11)	1.35	1.13, 1.60	0.001
	2015	541 (50.66)	527 (49.34)	1.33	1.12, 1.59	0.001
	2016	445 (52.17)	408 (47.83)	1.42	1.18, 1.71	<0.001
	2017	585 (43.98)	745 (56.02)	Referent		
	2018	303 (45.50)	363 (54.50)	1.04	0.86, 1.25	0.680
	2019	604 (45.93)	711 (54.07)	1.06	0.90, 1.23	0.457
VFD rule changes (n=20,283)						<0.001
	Before VFD rule changes (2002-2016)	8491 (50.03)	8481(49.97)	Referent		
	After VFD rule changes (2017-2019)	1492 (45.06)	1819 (54.94)	0.79	0.71, 0.87	<0.001

OR = odds ratio; CI = confidence interval

Table 2. 3 continued

Variable	Categories	Tetracycline-resistant		OR	95% CI	p-value
		Yes n (%)	No n (%)			
Meat type (n=20,283)						<0.001
	Chicken breast	2489 (42.24)	3403(57.76)	0.23	0.22, 0.25	<0.001
	Ground beef	1014 (22.12)	3571(77.88)	0.09	0.08, 0.10	<0.001
	Ground turkey	4914(74.95)	1642 (25.05)	Referent		
	Pork chops	1566 (48.18)	1684 (51.82)	0.31	0.28, 0.33	<0.001
Quarter of year (n=20,283)						<0.001
	Quarter 1	2720 (51.44)	2568 (48.56)	1.19	1.10, 1.29	<0.001
	Quarter 2	2588 (49.95)	2593(50.05)	1.12	1.03, 1.21	0.004
	Quarter 3	2315 (46.88)	2623(53.12)	Referent		
	Quarter 4	2360 (48.40)	2516 (51.60)	1.05	0.97, 1.14	0.200
Raising claims (n=2,945)						<0.001
	Conventional	637 (49.57)	648 (50.43)	Referent		
	Without any antibiotics	687 (41.39)	973 (58.61)	0.71	0.61, 0.83	<0.001

OR = odds ratio; CI = confidence interval

Table 2. 4: Univariable mixed-effect logistic regression of association between years of sampling and tetracycline-resistant *Escherichia* in retail meats in the United States

Variable	**Categories**	**OR**	**95% CI**	**p-value**
Years of sampling (n=20283)				0.0001
	2002-2004 vs. 2017-2019	1.29	1.09, 1.53	0.0004
	2005-2007 vs. 2017-2019	1.27	1.07, 1.51	0.0008
	2008-2010 vs. 2017-2019	1.19	1.00, 1.42	0.0473
	2011-2013 vs. 2017-2019	1.23	1.04, 1.46	0.0078
	2014-2016 vs. 2017-2019	1.33	1.11, 1.58	<0.0001
	2002-2004 vs. 2005-2007	1.01	0.88, 1.16	0.9999
	2002-2004 vs. 2008-2010	1.08	0.94, 1.24	0.6314
	2002-2004 vs. 2011-2013	1.05	0.91, 1.20	0.9343
	2002-2004 vs. 2014-2016	0.97	0.84, 1.12	0.9904
	2005-2007 vs. 2008-2010	1.07	0.93, 1.23	0.7672
	2005-2007 vs. 2011-2013	1.03	0.90 1.19	0.9812
	2005-2007 vs. 2014-2016	0.96	0.83, 1.10	0.9602
	2008-2010 vs. 2011-2013	0.97	0.84, 1.12	0.9887
	2008-2010 vs. 2014-2016	0.90	0.78, 1.04	0.2970
	2011-2013 vs. 2014-2016	0.93	0.81, 1.07	0.6596

OR = odds ratio; CI = confidence interval

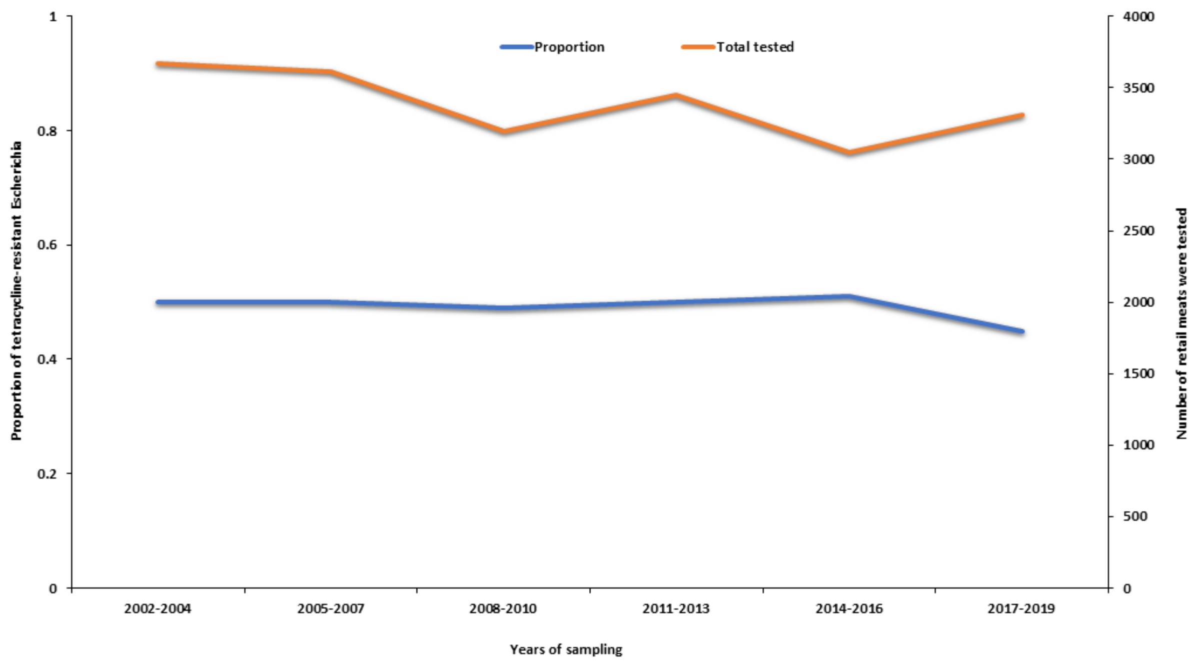

Figure 2. 2: Temporal patterns of the proportion of tetracycline-resistant *Escherichia* in retail meats by years of sampling in the United States

Table 2. 5: Results of the univariable logistic regression models, using a random intercept to control for state-level clustering for tetracycline-resistant *Campylobacter* in retail meats in the United States, 2004-2019

Variable	Categories	Tetracycline-resistant		OR	95% CI	p-value
		Yes n (%)	No n (%)			
Year (n= 9,698)						<0.001
	2004	354 (49.10)	367 (50.90)	1.17	0.94, 1.45	0.149
	2005	269 (46.86)	305 (53.14)	1.10	0.87, 1.39	0.386
	2006	289 (48.49)	307 (51.51)	1.11	0.88, 1.39	0.346
	2007	251 (48.46)	267 (51.54)	1.08	0.86, 1.37	0.480
	2008	279 (51.76)	260 (48.24)	1.34	1.06, 1.69	0.012
	2009	274 (45.29)	331 (54.71)	1.01	0.80, 1.26	0.916
	2010	200 (38.83)	315 (61.17)	0.77	0.61, 0.98	0.039
	2011	328 (51.74)	306 (48.26)	1.35	1.08, 1.68	0.007
	2012	330 (48.82)	346 (51.18)	1.23	0.99, 1.53	0.055
	2013	305 (47.81)	333 (52.19)	1.19	0.95, 1.48	0.112
	2014	243 (46.91)	275 (53.09)	1.20	0.94, 1.50	0.130
	2015	262 (45.02)	320 (54.98)	1.13	0.90, 1.41	0.264
	2016	270 (48.74)	284 (51.26)	1.21	0.97, 1.52	0.088
	2017	332 (41.97)	459 (58.03)	Referent		
	2018	205 (36.61)	355 (63.39)	0.75	0.60, 0.95	0.017
	2019	333 (49.19)	344 (50.81)	1.20	0.97, 1.48	0.091
VFD rule changes (n= 9,698)						0.008
	Before VFD rule changes (2004-2016)	3654 (47.64)	4016(52.36)	Referent		
	After VFD rule changes (2017-2019)	870 (42.90)	1158(57.10)	0.85	0.76, 0.96	0.008
Meat type (n= 9,698)						<0.001
	Chicken breast	4322 (45.90)	5095(54.10)	0.29	0.22, 0.38	<0.001
	Ground beef	1 (20.00)	4 (80.00)	0.10	0.01, 0.92	0.043
	Ground turkey	194 (73.21)	71 (26.79)	Referent		
	Pork chops	7 (63.64)	4 (36.36)	0.54	0.15, 1.95	0.355

OR = odds ratio; CI = confidence interval

Table 2. 5 continued

Variable	Categories	Tetracycline-resistant		OR	95% CI	p-value
		Yes n (%)	**No n (%)**			
Quarter of year (n=9,698)						0.105
	Quarter 1	1060(44.31)	1332(55.69)	Referent		
	Quarter 2	1023(46.35)	1184(53.65)	1.07	0.95, 1.20	0.256
	Quarter 3	1178(47.56)	1299(52.44)	1.11	0.99, 1.25	0.062
	Quarter 4	1263(48.17)	1359(51.83)	1.14	1.02, 1.28	0.020
Raising claims (n=2,276)						0.312
	Conventional	245 (43.91)	313 (56.09)	Referent		
	Without any antibiotics	808 (47.03)	910 (52.97)	1.11	0.90, 1.36	0.312

OR = odds ratio; CI = confidence interval

Table 2. 6: Univariable mixed-effect logistic regression of association between years of sampling and tetracycline-resistant *Campylobacter* in retail meats in the United States

Variable	Categories	OR	95% CI	*P*-value
Years of sampling (n= 9698)				0.0049
	2002-2004 vs. 2017-2019	1.18	0.90, 1.55	0.4947
	2005-2007 vs. 2017-2019	1.11	0.90, 1.38	0.7184
	2008-2010 vs. 2017-2019	1.03	0.84, 1.28	0.9976
	2011-2013 vs. 2017-2019	1.27	1.04, 1.55	0.0085
	2014-2016 vs. 2017-2019	1.20	0.97, 1.47	0.1339
	2002-2004 vs. 2005-2007	1.06	0.82, 1.38	0.9863
	2002-2004 vs. 2008-2010	1.14	0.88, 1.48	0.6983
	2002-2004 vs. 2011-2013	0.93	0.72, 1.20	0.9661
	2002-2004 vs. 2014-2016	0.99	0.76, 1.29	1.0000
	2005-2007 vs. 2008-2010	1.08	0.88, 1.32	0.9106
	2005-2007 vs. 2011-2013	0.88	0.72, 1.07	0.3881
	2005-2007 vs. 2014-2016	0.93	0.76, 1.15	0.9260
	2008-2010 vs. 2011-2013	0.82	0.67, 0.99	0.0329
	2008-2010 vs. 2014-2016	0.87	0.70, 1.07	0.3618
	2011-2013 vs. 2014-2016	1.06	0.87, 1.29	0.9510

OR = odds ratio; CI = confidence interval

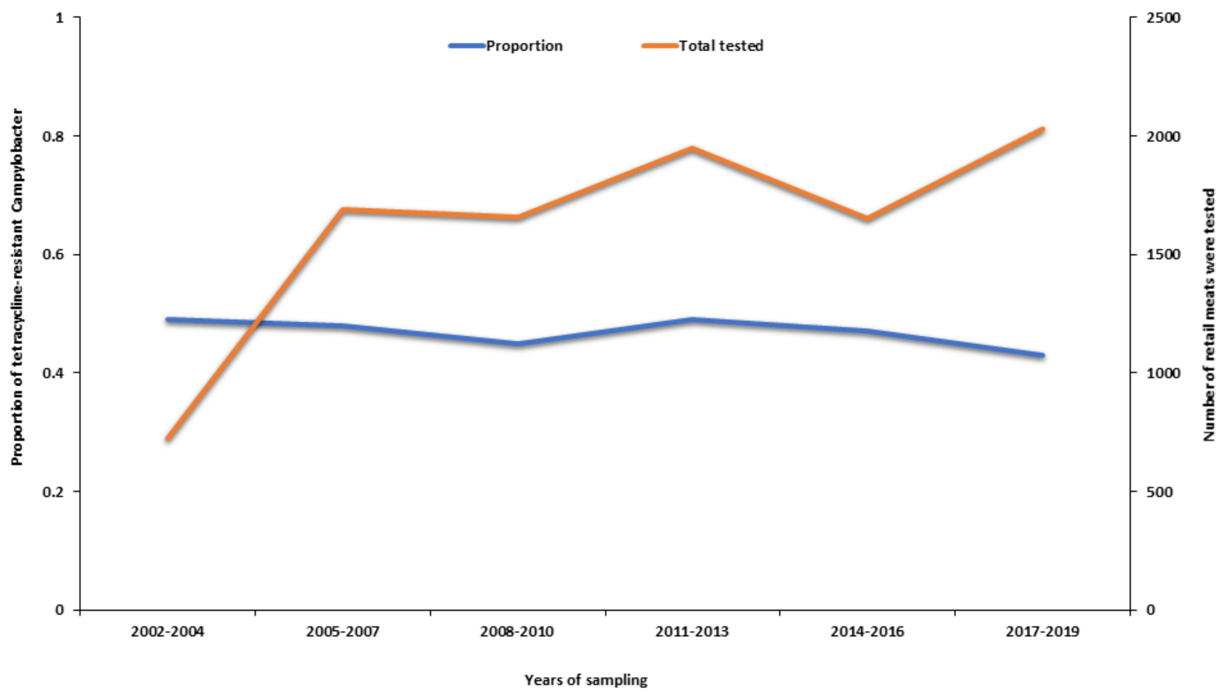

Figure 2. 3: Temporal patterns of the proportion of tetracycline-resistant *Campylobacter* in retail meats by years of sampling in the United States

Table 2. 7: Results of the univariable logistic regression models, using a random intercept to control for state-level clustering for erythromycin-resistant *Campylobacter* in retail meats in the United States, 2004-2019

Variable	Categories	Erythromycin-resistant		OR	95% CI	p-value
		Yes n (%)	No n (%)			
Year (n=10,472)						<0.001
	2002	18 (6.06)	279 (93.94)	3.01	1.48, 6.11	0.002
	2003	16 (3.35)	461 (96.65)	2.00	0.97, 4.11	0.059
	2004	24 (3.33)	697 (96.67)	1.99	1.03, 3.84	0.040
	2005	19 (3.31)	555 (96.69)	1.93	0.97, 3.85	0.061
	2006	13 (2.18)	583 (97.82)	1.28	0.60, 2.73	0.511
	2007	14 (2.70)	504 (97.30)	1.59	0.76, 3.34	0.214
	2008	25 (4.64)	514 (95.36)	2.67	1.39, 5.12	0.003
	2009	12 (1.98)	593 (98.02)	0.85	0.39, 1.85	0.699
	2010	9 (1.75)	506 (98.25)	0.89	0.39, 2.06	0.803
	2011	14 (2.21)	620 (97.79)	1.04	0.50, 2.17	0.910
	2012	28 (4.14)	648 (95.86)	1.88	1.00, 3.53	0.049
	2013	27 (4.23)	611 (95.77)	2.31	1.23, 4.34	0.009
	2014	18 (3.47)	500 (96.53)	1.73	0.87, 3.44	0.115
	2015	34 (5.84)	548 (94.16)	2.96	1.61, 5.44	<0.001
	2016	16 (2.89)	538 (97.11)	1.48	0.73, 2.99	0.275
	2017	17 (2.15)	774 (97.85)	Referent		
	2018	5 (0.89)	555 (99.11)	0.38	0.13, 1.04	0.062
	2019	13 (1.92)	664 (98.08)	0.81	0.38, 1.72	0.602
VFD rule changes (n=10,472)						<0.001
	Before VFD rule changes (2004-2016)	287 (3.40)	8,157(96.60)	Referent		
	After VFD rule changes (2017-2019)	35 (1.73)	1,993(98.27)	0.43	0.29, 0.63	<0.001

OR = odds ratio; CI = confidence interval

Table 2. 7 continued

Variable	Categories	Erythromycin-resistant		OR	95% CI	p-value
		Yes n (%)	**No n (%)**			
Meat type (n=10,472)						<0.001
	Chicken breast	301 (2.96)	9,871(97.04)	0.64	0.37, 1.13	0.127
	Ground turkey	14 (5.11)	260 (94.89)	Referent		
	Ground beef & Pork	7 (26.92)	19 (73.08)	8.72	3.07, 24.75	<0.0001
Quarter of year (n=10,472)						0.816
	Quarter 1	82 (3.20)	2,481(96.80)	1.14	0.83, 1.56	0.397
	Quarter 2	77 (3.22)	2,318(96.78)	1.14	0.83, 1.57	0.409
	Quarter 3	83 (3.09)	2,602(96.91)	1.10	0.80, 1.50	0.546
	Quarter 4	80 (2.83)	2,749(97.17)	Referent		
Raising claims (n=22,76)						0.404
	Conventional	11 (1.97)	547 (98.03)	Referent		
	Without any antibiotics	24 (1.40)	1,694(98.60)	0.72	0.34, 1.53	0.405

OR = odds ratio; CI = confidence interval

Table 2. 8: Univariable mixed-effect logistic regression of association between years of sampling and erythromycin-resistant *Campylobacter* in retail meats in the United States

Variable	Categories	OR	95% CI	p-value
Years of sampling (n= 10,472)				0.0004
	2002-2004 vs. 2017-2019	2.29	1.23, 4.27	0.0021
	2005-2007 vs. 2017-2019	1.59	0.83, 3.05	0.3213
	2008-2010 vs. 2017-2019	1.62	0.85, 3.11	0.2761
	2011-2013 vs. 2017-2019	2.09	1.14, 3.81	0.0068
	2014-2016 vs. 2017-2019	2.43	1.33, 4.46	0.0004
	2002-2004 vs. 2005-2007	1.44	0.81, 2.56	0.4630
	2002-2004 vs. 2008-2010	1.41	0.79, 2.52	0.5262
	2002-2004 vs. 2011-2013	1.09	0.65, 1.85	0.9957
	2002-2004 vs. 2014-2016	0.94	0.56, 1.59	0.9995
	2005-2007 vs. 2008-2010	0.98	0.53, 1.80	1.0000
	2005-2007 vs. 2011-2013	0.76	0.44, 1.33	0.7319
	2005-2007 vs. 2014-2016	0.65	0.37, 1.14	0.2511
	2008-2010 vs. 2011-2013	0.78	0.45, 1.36	0.7893
	2008-2010 vs. 2014-2016	0.67	0.38, 1.17	0.3016
	2011-2013 vs. 2014-2016	0.86	0.52, 1.42	0.9519

OR = odds ratio; CI = confidence interval

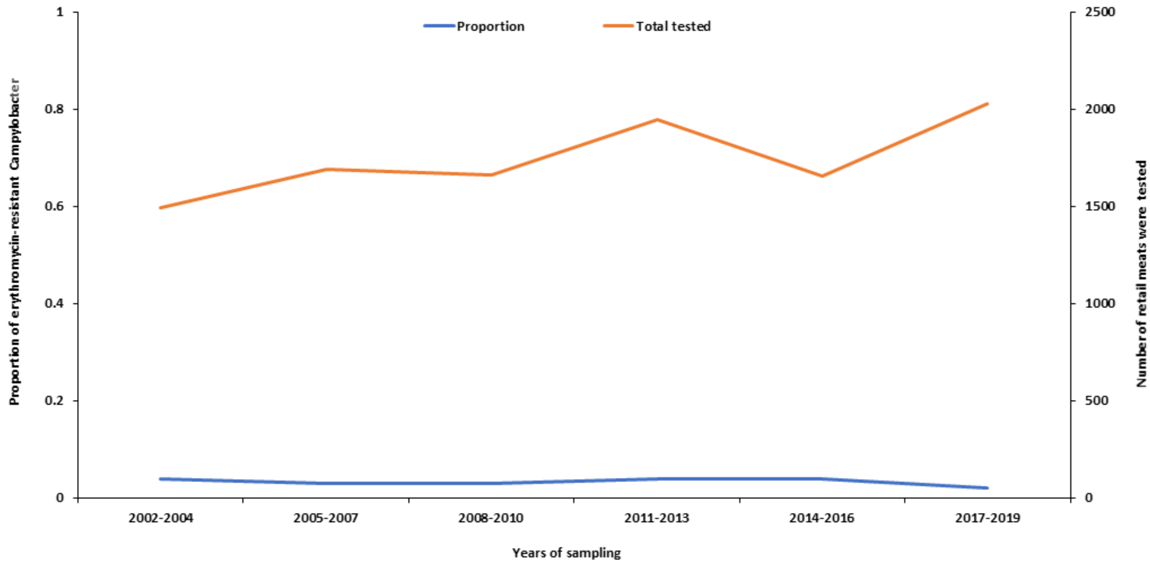

Figure 2. 4: Temporal patterns of the proportion of erythromycin-resistant *Campylobacter* in retail meats by years of sampling in the United States

In contrast, the odds of detecting tetracycline-resistant *Salmonella* were increased by 49% in the chicken breast following implementation of the VFD rule changes, compared to chicken breast in the period prior to implementation (OR= 1.49; 95% CI: 1.31, 1.69; p<0.0001) **(Table 2.9).**

The final model was fitted for tetracycline-resistant *Escherichia*, which included 20,283 records of retail meat **(Table 2.10).** Variables significantly associated with detecting tetracycline-resistant *Escherichia* --controlling for other variables--were the VFD rule changes, meat type, and sampling quarter. No multicollinearity issue was found in the final model. However, there was a significant interaction between the VFD rule changes and meat type after controlling for all other variables in the model. The significant interaction between the VFD rule changes and meat type implies that the effect of the VFD rule changes on the odds of detecting tetracycline-resistant *Escherichia* was not the same across the meat types. For example, the odds of detecting tetracycline-resistant *Escherichia* were decreased by 40 % in the chicken breast following the implementation of the VFD rule changes compared to chicken breast in the period prior to implementation (OR= 0.60, 95% CI: 0.50, 0.72; p<0.0001) **(Table 2.10).** Likewise, the odds of detecting tetracycline-resistant *Escherichia* were decreased by 44% in ground turkey following implementation of the VFD rule changes compared to ground turkey in the period prior to implementation (OR= 0.56, 95% CI: 0.48, 0.65; p<0.0001) **(Table 2.10).** After the VFD rule changes, there was no significant difference in the odds of detecting tetracycline-resistant *Escherichia* in ground beef and pork chop compared to before the VFD rule changes **(Table 2.10).** Considering quarter of year, the odds of detecting tetracycline-resistant *Escherichia* in Quarter 1 and Quarter 2 were 22% (OR=1.22, 95% CI: 1.09, 1.36); p<0.0001) and 12% (OR=1.12, 95% CI: 1.00, 1.26, p= 0.008) higher, compared to Quarter 3 **(Table 2.10).**

The final model was fitted for tetracycline-resistant *Campylobacter*, which included 9,682 records of retail meats **(Table 2.11).** Variables significantly associated with detecting tetracycline-resistant *Campylobacter* --controlling for other variables--were the VFD rule changes and meat type. No multicollinearity issue was found in the final model. However, there was a significant interaction between the VFD rule changes and meat type after controlling for all other variables in the model. The significant interaction between the VFD rule changes and meat type implies that the effect of the VFD rule changes on the odds of detecting tetracycline-resistant *Campylobacter* was not the same across the meat types. For example, the odds of detecting tetracycline-resistant *Campylobacter* were decreased by 11 % in the chicken breast following implementation of the VFD rule changes, compared to chicken breast in the period prior to implementation (OR= 0.89, 95% CI: 0.79, 0.99; p= 0.0370) **(Table 2.11).** Likewise, the odds of detecting tetracycline-resistant *Campylobacter* were decreased by 67 % in ground turkey following implementation of the VFD rule changes, compared to ground turkey in the period prior to implementation (OR= 0.33, 95% CI: 0.17, 0.66; p=0.0017) **(Table 2.11).**

The final model was fitted for erythromycin-resistant *Campylobacter*, which included 10,446 records of retail meats (**Table 2.12**). Variables significantly associated with detecting erythromycin-resistant *Campylobacter* --controlling for other variables--was meat type. No multicollinearity issue was found in the final model. However, there was a significant interaction between the VFD rule changes and meat type after controlling for all other variables in the model. The significant interaction between the VFD rule changes and meat type implies that the effect of the VFD rule changes on the odds of detecting erythromycin-resistant *Campylobacter* were not the same across the meat types. For example, the odds of detecting erythromycin-resistant *Campylobacter* were decreased by 57 % in the chicken breast following implementation of the VFD rule changes, compared to chicken breast in the period prior to implementation (OR= 0.43, 95% CI: 0.29, 0.64; p<0.0001). In contrast, the odds of detecting erythromycin-resistant *Campylobacter* were 4.63 times higher in ground turkey following implementation of the VFD rule changes compared to ground turkey in the period prior to implementation (OR= 4.63; 95% CI: 1.48, 14.44; p = 0.0084) (**Table 2.12**).

Discussion

The VFD rules changes were implemented in 2017 to reduce the use of medically important antibiotics in food-producing animals. These rule changes are crucial in decreasing the amount of medically important antibiotics used and, consequently, reducing the development of antibiotic-resistant bacteria in food-producing animals and their products in the U.S. This current investigation sought to determine the association between VFD rule changes and the occurrence of tetracycline and erythromycin-resistant bacteria isolated from retail meats obtained under the NARMS in the U.S., after controlling for other variables. After controlling for other variables in the multivariable models, there was a significant interaction between the VFD rule changes and meat type on the occurrence of tetracycline-resistant *Salmonella* in retail meats **(Table 2.9).** Additionally, after controlling for other variables in the multivariable models, there was a significant interaction between the VFD rule changes and meat type on the occurrence of tetracycline-resistant *Escherichia* in retail meats **(Table 2.10).** Likewise, after controlling for other variables in the multivariable models, there was a significant interaction between the VFD rule changes and meat type on the occurrence of tetracycline-resistant *Campylobacter* in retail meats **(Table 2.11).**

Table 2. 9: Final multivariable mixed-effect logistic regression model using a random intercept to control for state-level clustering for tetracycline-resistant *Salmonella* in retail meats (n= 8,501) in the United States, 2002-2019

Variable	Categories	OR	95% CI	p-value
VFD rule changes				0.4146
	After the VFD rule changes (2017-2019) vs. Before the VFD rule changes (2002-2016))	0.91	0.72, 1.15	0.4146
Meat type				<0.0001
	Ground beef vs. Ground turkey	0.36	0.22, 0.63	<0.0001
	Chicken breast vs. Ground turkey	1.11	0.98 1.26	0.1462
	Pork chop vs. Ground turkey	0.75	0.55, 1.02	0.0804
VFD rule changes* Meat type				<0.0001
Ground beef	After VFD rule changes vs. before VFD rule changes	0.89	0.41 1.96	0.7741
Chicken breast	After VFD rule changes vs. before VFD rule changes	1.49	1.31, 1.69	<0.0001
Pork chop	After VFD rule changes vs. before VFD rule changes	0.77	0.49, 1.22	0.2708
Ground turkey	After VFD rule changes vs. before VFD rule changes	0.66	0.56, 0.77	<0.0001

OR — Odds ratio; CI — Confidence interval

Table 2. 9 continued

Variable	**Categories**	**OR**	**95% CI**	**p-value**
Before VFD rule changes				
	Ground beef vs. Ground turkey	0.32	0.21, 0.50	<0.0001
	Chicken breast vs. Ground turkey	0.74	0.63, 0.86	<0.0001
	Pork chop vs. Ground turkey	0.69	0.48, 0.99	0.0385
	Chicken breast vs. Ground beef	2.29	1.47, 3.55	<0.0001
	Chicken breast vs. Pork chop	1.07	0.75, 1.52	0.9665
	Ground beef vs. Pork chop	0.47	0.27, 0.81	0.0019
After the VFD rule changes				
	Ground beef vs. Ground turkey	0.44	0.17, 1.13	0.1133
	Chicken breast vs. Ground turkey	1.67	1.37, 2.03	<0.0001
	Pork chop vs. Ground turkey	0.81	0.49, 1.36	0.7242
	Chicken breast vs. Ground beef	3.83	1.49, 9.85	0.0015
	Chicken breast vs. Pork chop	2.06	1.24, 3.41	0.0013
	Ground beef vs. Pork chop	0.54	0.19, 1.55	0.4327

OR — Odds ratio; CI — Confidence interval

Table 2. 10: Final multivariable mixed-effect logistic regression model using a random intercept to control for state-level clustering for tetracycline-resistant *Escherichia* in retail meats (n= 20,283) in the United States, 2002-2019

Variable	Categories	OR	95% CI	p-value
VFD rule changes				<0.0001
	After the VFD rule changes (2017-2019) vs. Before the VFD rule changes (2002-2016))	0.72	0.64, 0.81	<0.0001
Meat type				<0.0001
	Ground beef vs. Ground turkey	0.11	0.09, 0.13	<0.0001
	Chicken breast vs. Ground turkey	0.24	0.21, 0.27	<0.0001
	Pork chop vs. Ground turkey	0.36	0.31, 0.42	<0.0001
Sampling quarter				<0.0001
	Quarter 1vs. Quarter 3	1.22	1.09, 1.36	<0.0001
	Quarter 2 vs. Quarter 3	1.12	1.00, 1.26	0.0399
	Quarter 4 vs. Quarter 3	1.04	0.93, 1.16	0.8419
VFD rule changes* Meat type				<0.0001
Ground beef	After VFD rule changes vs. before VFD rule changes	0.89	0.72, 1.10	0.2742
Chicken breast	After VFD rule changes vs. before VFD rule changes	0.60	0.50, 0.72	<0.0001

OR — Odds ratio; CI — Confidence interval

Table 2. 10 continued

Variable	**Categories**	**OR**	**95% CI**	**p-value**
Pork chop	After VFD rule changes vs. before VFD rule changes	0.90	0.74, 1.10	0.3091
Ground turkey	After VFD rule changes vs. before VFD rule changes	0.56	0.48, 0.65	<0.0001
Before VFD rule changes				
	Ground beef vs. Ground turkey	0.09	0.07, 0.10	<0.0001
	Chicken breast vs. Ground turkey	0.23	0.20, 0.25	<0.0001
	Pork chop vs. Ground turkey	0.28	0.25, 0.32	<0.0001
	Chicken breast vs. Ground beef	2.68	2.37, 3.03	<0.0001
	Chicken breast vs. Pork chop	0.80	0.71, 0.91	<0.0001
	Ground beef vs. Pork chop	0.30	0.26, 0.35	<0.0001
After the VFD rule changes				
	Ground beef vs. Ground turkey	0.14	0.10 0.18	<0.0001
	Chicken breast vs. Ground turkey	0.25	0.19, 0.32	<0.0001
	Pork chop vs. Ground turkey	0.46	0.35, 0.60	<0.0001
	Chicken breast vs. Ground beef	1.81	1.33, 2.47	<0.0001
	Chicken breast vs. Pork chop	0.53	0.40, 0.71	<0.0001
	Ground beef vs. Pork chop	0.29	0.21, 0.41	<0.0001

OR — Odds ratio; CI — Confidence interval

Table 2. 11: Final multivariable mixed-effect logistic regression model using a random intercept to control for state-level clustering for tetracycline-resistant *Campylobacter* in retail meats (n= 9,682) in the United States, 2002-2019

Variable	Categories	OR	95% CI	p-value
VFD rule changes				0.0007
	After the VFD rule changes (2017-2019) vs. Before the VFD rule changes (2002-2016))	0.54	0.38, 0.77	0.0007
Meat type				<0.0001
	Chicken breast vs. Ground turkey	0.40	0.28, 0.56	<0.0001
VFD rule changes* Meat type				0.0058
Chicken breast	After VFD rule changes vs. before VFD rule changes	0.89	0.79, 0.99	0.0370
Ground turkey	After VFD rule changes vs. before VFD rule changes	0.33	0.17, 0.66	0.0017
Before VFD rule changes				
	Chicken breast vs. Ground turkey	0.24	0.18, 0.34	<0.0001
After the VFD rule changes				
	Chicken breast vs. Ground turkey	0.65	0.35, 1.20	0.1685

OR — Odds ratio; CI — Confidence interval

Table 2. 12: Final multivariable mixed-effect logistic regression model using a random intercept to control for state-level clustering for erythromycin-resistant *Campylobacter* in retail meats (n= 10,446) in the United States, 2002-2019

Variable	**Categories**	**OR**	**95% CI**	***P*-value**
VFD rule changes				0.2584
	After the VFD rule changes (2017-2019) vs. Before the VFD rule changes (2002-2016))	1.41	0.78, 2.58	0.2584
Meat type				<0.0001
	Chicken breast vs. Ground turkey	0.30	0.16, 0.54	<0.0001
VFD rule changes* Meat type				0.0001
Chicken breast	After VFD rule changes vs. before VFD rule changes	0.43	0.29, 0.64	<0.0001
Ground turkey	After VFD rule changes vs. before VFD rule changes	4.63	1.48, 14.44	0.0084
Before VFD rule changes				
	Chicken breast vs. Ground turkey	0.97	0.47, 1.99	0.9286
After the VFD rule changes				
	Chicken breast vs. Ground turkey	0.09	0.04, 0.24	<0.0001

OR — Odds ratio; CI — Confidence interval

Moreover, after controlling for other variables in the multivariable models, there was a significant interaction between the VFD rule changes and meat type on the occurrence of erythromycin-resistant *Campylobacter* in retail meats **(Table 2.12).** The significant interaction between the VFD rule changes and meat type implies that the effect of the VFD rule changes on the odds of detecting antibiotic-resistant bacteria were not the same across the meat types.

The findings of this study reveal that the implementation of VFD rule changes in the U.S. resulted in a significant reduction in the odds of detecting tetracycline-resistant *Escherichia*, *Campylobacter*, and erythromycin-resistant *Campylobacter* isolated from chicken breast. Also, our data revealed significant reduction in the odds of detecting tetracycline-resistant *Salmonella*, *Escherichia*, and *Campylobacter* isolated from ground turkey. To our knowledge, this report is the first to provide quantitative evidence of the association between changes in VFD rules changes and the odds of detecting tetracycline-resistant *Escherichia*, *Campylobacter*, and erythromycin-resistant *Campylobacter* isolated from chicken breast, as well as tetracycline-resistant *Salmonella*, *Escherichia*, and *Campylobacter* in ground turkey in the U.S.

Multiple factors could explain the decreased odds of detecting tetracycline-resistant *Escherichia* (40 %) and *Campylobacter* (11%) isolated from chicken breast compared to the period before implementation. One is the VFD rule changes potentially decreased the use of tetracycline in chicken production, leading to a decrease in the occurrence of tetracycline-resistant bacteria in chicken breast in the U.S. Reduced use of tetracycline may have reduced the selective pressure for the emergence of tetracycline-resistant bacteria in food animals, including chickens [13, 15]. The U.S. FDA's recent report indicates that tetracycline sales decreased in chicken production after the VFD rule changes were implemented [11]. Recent data on on-farm antimicrobial use in the U.S. broiler chicken industry shows a substantial decline in the utilization of medically important in-feed and water-soluble tetracycline since 2017 [64].

Similarly, data on on-farm antimicrobial use in U.S. turkey production demonstrates a substantial reduction in the usage of medically important in-feed and water-soluble tetracycline in 2017 [65]. The reduction of antimicrobial use in food-producing animals is associated with a reduction in the occurrence of antibiotic-resistant bacteria originating from food animals [15]. An observational study conducted by Stuart B. Levy et al. [66] demonstrated that six months after the removal of tetracycline-supplemented feed from a chicken farm, there was a lower frequency of tetracycline-resistant *Escherichia* isolates compared to before the removal of the tetracycline-supplemented feed in the farm. There is also evidence that a reduction in antimicrobial use is associated with reduced occurrence of antimicrobial-resistant bacteria isolates from humans [21, 56]. In addition, chicken production type and management practices could be associated with decreased odds of detecting tetracycline-resistant *Escherichia* and *Campylobacter*. For instance, extensive and comprehensive cleaning and hygiene practices in chicken farms could reduce the reservoirs of resistant bacteria [67] that may have occurred on dirty floors and fomites within the chicken houses [68]. Also, improved feeding systems, such as providing safe and antibiotic-free feed and water in chicken production, could reduce the risk of the occurrence of resistant bacteria [67] in chicken meats. Furthermore, the organic food industry is rapidly growing in the U.S., with organic meat and poultry sales increasing by 2.5% and 4.7%, respectively, in 2021 [69]. Transitioning from conventional chicken production to organic chicken production, which involves the elimination of antibiotic use, can significantly reduce the occurrence of antibiotic-resistant bacteria in chicken meat in the U.S. There is evidence that the prevalence of antibiotic-resistant *Enterococcus* significantly decreased in poultry farms in the U.S. which transitioned from conventional farms to organic farming practices [70].

Another explanation could be that the VFD rule changes may have improved veterinary-client-patient relationships, resulting in better oversight of antimicrobial usage in chicken production by licensed veterinarians. For instance, following the implementation of VFD rule changes in the U.S., administering medically important antimicrobials to food animals via feed or drinking water has been brought under licensed veterinarian supervision [71]. Additionally, it is possible that the VFD rule changes led to increased awareness and adoption of responsible antimicrobial use practices among both chicken producers and veterinarians, resulting in more judicious use of antimicrobials to help mitigate the development of resistance to antimicrobials [72, 73]. Also, it is possible that the reduction in antimicrobial-resistant bacteria from retail chicken meats observed after the implementation of VFD rule changes may be due to factors not directly related to the rule changes-- such as natural variations in resistance patterns in meat-associated bacterial population over time or improved hygiene practices during meat processing at slaughterhouses, in storing, or handling. Overall, this study demonstrates that the VFD rule changes were beneficial in reducing the occurrence of tetracycline-resistant *Escherichia* and *Campylobacter* in chicken breasts in the U.S.

However, the multivariable logistic regression analysis showed that the odds of detecting tetracycline-resistant Salmonella were significantly increased in the chicken breast following the VFD rule changes compared to the pre-VFD rule changes period. The findings of our study is consistent with previous research that has reported a higher level of tetracycline-resistant *Salmonella* in chicken breast compared to ground turkey in California in 2018 [57]. The exact cause of this outcome remains unknown. However, it is possible that the composition of

Salmonella serotypes within chicken production may vary over time [74], or there could be other unidentified factors that explain our study's findings. Control of tetracycline-resistant *Salmonella* in chicken meat is difficult as chickens can serve as perpetual vectors and reservoirs without exhibiting symptoms [57]. Tetracycline-resistant *Salmonella* can contaminate the chicken farm environment and potentially spread the bacteria by infected chicken and contaminated eggs [74]. It is important to conduct further investigations to explore potential risk factors associated with an increased likelihood of tetracycline-resistant *Salmonella* in chicken meat.

Our research shows that there was no significant change in the likelihood of detecting tetracycline-resistant *Salmonella* and *Escherichia* in ground beef and pork chops after the VFD rule changes were implemented in 2017, compared to before the changes. Our study's results align with recent research that found no significant change in the prevalence of tetracycline-resistant *Escherichia* in cecal samples of swine at slaughter in the U.S. between 2013 and 2019 [75]. There is no systematic collection of medically important antibiotic consumption data at the farm-level in the U.S. [71]. Such data could provide important information to explain the observed findings. However, the FDA's medically important antibiotic drug and species-specific sales data between 2016 and 2019 could serve as a proxy for antibiotic consumption data at the farm-level in the U.S. [11]. The data shows that tetracycline sales were highest in 2016 for both cattle (2,840,519 kilograms) and swine (2,520,680 kilograms) production. Tetracycline sales sharply decreased for cattle (1,560,542 kilograms) and swine (1,579,145 kilograms) production in 2017 when the VFD rule changes were implemented. However, tetracycline sales increased in both cattle and swine production in 2018 and 2019 in the U.S. [11]. This pattern of tetracycline sales suggests that there was no consistent reduction in tetracycline consumption in cattle and swine production following the implementation of the VFD rule changes measures in the U.S.

Furthermore, the increased usage of this drug for therapeutic purposes could also contribute to the rising sales of tetracycline following the implementation of the VFD rule changes. It is important to conduct further investigations to explore potential factors associated with the increased usage of tetracycline in cattle and swine production just one year after implementing the VFD rule changes.

The current study also shows that there were lower odds of detecting erythromycin-resistant *Campylobacter* in chicken breast (57%), following the VFD rule changes implementation period, compared to the period prior to implementation. These observed lower odds of detecting erythromycin-resistant *Campylobacter* in chicken breast may be due to declines in the frequency of usage of erythromycin following the implementation of VFD rule changes. This decrease in usage could result in lower selective pressure [72, 73]. The U.S. FDA antimicrobials sold data indicates that the sale of macrolides, including erythromycin, sharply decreased in chicken production following the 2017 VFD rule changes [11]. Alternative explanations may include improved farming management and biosecurity practices, resulting in a decreased occurrence of bacterial disease (respiratory disease, infectious sinusitis, air sacculitis), and, thus, a reduction of the need for macrolide (erythromycin, tylosin) usage in chicken production. There is evidence from other studies that reducing or restricting antimicrobial use in food-producing animals reduces the occurrence of antimicrobial-resistant bacteria by decreasing selective pressure [72, 73] on the bacteria originating from the food animals [15, 76]. Likewise, a low prevalence of ciprofloxacin-resistant *Campylobacter* in Louisiana retail chickens was observed after the enrofloxacin ban [77]. In human studies, evidence of low use of azithromycin (macrolide) during summer months was found to be associated with decreased prevalence of resistant pneumococci among children [18, 78]. The findings of our work show that implementing the VFD rule changes has had a significant effect on the occurrence of erythromycin-resistant *Campylobacter* in chicken breast in the U.S.

However, the odds of detecting erythromycin-resistant *Campylobacter* were significantly increased in ground turkey following the implementation of the VFD rule changes compared to the pre-VFD rule changes period. There was no systematic collection of medically important antibiotic consumption data at the turkey farm level in the U.S. [71]. Such data could provide important information to explain this finding. However, the FDA's medically important antibiotic drug and species-specific sales data between 2016 and 2019 could serve as a proxy for antibiotic consumption data at the turkey farm-level in the U.S. The data shows a sharp increase in sales of macrolide, including erythromycin, in 2017 (1307 kilograms) when the VFD rule changes were implemented [11]. An increasing trend in macrolide sales was observed in 2018 (1653 kilograms) and 2019 (1944 kilograms) in the U.S. [11]. This sales pattern suggests an increasing consumption of macrolide, including erythromycin/tylosin, in turkey production following the implementation of the VFD rule changes in the U.S., which can create selective pressure in developing erythromycin-resistant bacteria. Previous research identified erythromycin-resistant *Campylobacter* in commercial turkey farms and at slaughterhouses in Ohio, USA [79]. There is evidence of an association between antimicrobial usage and antimicrobial-resistant bacteria isolated from fecal samples in turkey farms in European countries [80]. Other possible reasons may be the presence of poor hygiene, inadequate flock health management, and intensive farming conditions at turkey farms. These factors can contribute to a higher prevalence of infectious diseases such as respiratory disease/infectious sinusitis/air sacculitis in turkeys. These conditions often require treatment with antimicrobial drugs from the macrolide group. The usage of antibiotics can increase the selection pressure for erythromycin-resistant bacteria among turkeys [81]. As a result, there is an increased likelihood of erythromycin-resistant bacteria-infected turkeys entering slaughterhouse establishments. Additionally, suppose turkeys are exposed to feed or water contaminated with erythromycin-resistant bacteria or genes. In that case, they can become carriers of these resistant bacteria. Furthermore, improper sanitary and cleanliness of slaughterhouses and fecal contamination during slaughtering processing [82, 83] can result in resistant-bacteria cross-contamination in turkey meat. Evidence of improper scalding or scalding processes without scalding water temperature control was significantly associated with bacteria contamination of chicken meat in slaughterhouses [84]. The U.S. is the world's largest producer of turkey and turkey meat. In 2019, approximately 229 million turkeys were raised in the U.S., resulting in a total live weight production of 7.4 billion pounds. It is important to conduct further investigations at the turkey farm-level to explore potential factors associated with the emergence of erythromycin-resistant *Campylobacter* within U.S. turkey production following the implementation of the VFD rule changes.

While this study provides valuable insights into the reduction of tetracycline-resistant *Escherichia*, *Campylobacter*, and erythromycin-resistant *Campylobacter* in chicken breast, as well as tetracycline-resistant *Salmonella*, *Escherichia*, and *Campylobacter* in ground turkey, it is important to note that these findings may not apply to all chicken and turkey retail meats sold in the U.S. This limitation arises from the sampling strategy employed during the study period (2002-2019), which involved convenience sampling [85]. The utilization of convenience sampling could potentially introduce sample selection bias.

To analyze the occurrence of antibiotic-resistant bacteria in retail meats, we initially examined both the individual year and three-year trend using a univariable mixed effect logistic regression model. Additionally, we visually assessed the linear trend of the proportion of antibiotic-resistant bacteria in retail meats at three-year intervals. However, the estimated odds ratios from both the individual year and three-year wise analyses did not demonstrate a consistent linear pattern across all comparisons **(Table 2.2, Table 2.4, Table 2.6, and Table 2.8).** Furthermore, no linear trend of the proportion of antibiotic-resistant bacteria in retail meats was observed based on our graphical analysis **(Figure 2.1, Figure 2.2, Figure 2.3, and Figure 2.4).** These non-linear relationships suggest that the impact of individual years or three-year intervals on antibiotic-resistant bacteria in retail meats vary across different points of comparison. Considering these observations, we chose to analyze the variable "VFD rule change" as a reasonable approach. We categorized data into two groups: "before VFD rule change (2002-2016)" and "after VFD rule change (2017-2019)". Our research question aimed to investigate significant differences in antibiotic-resistant bacteria in retail meats, and the "VFD rule change" variable allowed us to examine the overall effect of the period after the implementation of VFD rule changes compared to the reference period (pre-VFD period: 2002-2016). Furthermore, collapsing these years into binary variable increased the sample size of the category, thereby improving the statistical power of our analysis. Additionally, we employed a mixed-effect logistic regression model to address the clustering effect of state-level during the analysis to avoid biased estimates [86].

The findings of this study suggest that the implementation of VFD rule changes had a positive impact on reducing the presence of tetracycline and erythromycin-resistant bacteria (*Escherichia* and *Campylobacter*) in retail meats, specifically chicken breast and ground turkey. The implementation of VFD rule changes has likely contributed to a decrease in antibiotic-resistant bacteria isolates found in retail meats by reducing the usage of antibiotics in food animals. These findings highlight the importance of continued efforts to promote the judicious use of medically important antimicrobials in food-producing animals to combat the emergence and spreading of antimicrobial-resistant bacteria. Further research is necessary covering a more extended period after the implementation of VFD rule changes could more comprehensively evaluate the effectiveness of the VFD rule changes on the occurrence of bacteria resistant to medically-important antimicrobials in retail meats in the U.S. Additionally, it is crucial to assess the impact of the implementation of the VFD rule changes on human health in the U.S.

Acknowledgments

The authors are grateful to the National Antimicrobial Resistance Monitoring System (NARMS) as the data source. We thank the Comparative and Experimental Medicine Program of University of Tennessee, Knoxville, for supporting this study. Also, we would like to thank Jessica Vidlund for checking the English language expression in the manuscript.

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CHAPTER 3

Effect of veterinary feed directive rule changes on tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) isolated from cecal samples of food-producing animals in the United States

A version of this chapter has submitted to Peer J on July 31, 2023 by Shamim Sarkar, Chika C. Okafor. Effect of veterinary feed directive rule changes on tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) isolated from cecal samples of food-producing animals in the United States

My contribution to this paper included gathering and review of literature, conceptualization, data analysis, interpretation of results, and drafting and editing of the manuscript.

Abstract

Antimicrobial resistance is a growing public health concern. This study investigates whether the 2017 Veterinary Feed Directive (VFD) rule changes affected the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples of food-producing animals at slaughter establishments in the United States (U.S.). Multivariable logistic regression was used to analyze 2013-2019 cecal samples of food-producing animals surveillance data from the National Antimicrobial Resistance Monitoring System (NARMS) in the U.S. Variables included year (used to evaluate VFD rule changes), host, and quarter of year. The potential association between these variables and the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples of cattle, swine, chickens, and turkeys) were estimated.

Regarding tetracycline-resistant *Salmonella*, controlling for all other variables in the final model, there were significant interactions between VFD rule changes and host, which indicates the effect of VFD rule changes on the odds of detecting tetracycline-resistant *Salmonella* were not the same across the host levels. For example, the odds of detecting tetracycline-resistant *Salmonella* decreased by 41% in cattle following implementation of the VFD rule changes compared to cattle prior to implementation (OR=0.59, 95% CI: 0.47, 0.74; $p<0.0001$). In contrast, the odds of detecting tetracycline-resistant *Salmonella* were 1.71 times higher in chickens following implementation of the VFD rule changes compared to chickens in the period prior to implementation (OR= 1.71, 95% CI: 1.36, 2.15; $p<0.0001$). Regarding tetracycline-resistant *Escherichia*, controlling for all other variables in the final model, the quarter and interaction term between VFD rule changes and host were significant. For instance, the odds of detecting tetracycline-resistant *Escherichia* decreased by 30% in chickens following implementation of the VFD rule changes compared to chickens prior to implementation (OR= 0.70, 95% CI: 0.56, 0.87; p=0.0017). In contrast, the odds of detecting tetracycline-resistant *Escherichia* were 1.22 times higher in swine following implementation of the VFD rule changes compared to swine in the period prior to implementation (OR= 1.22, 95% CI: 1.05, 1.41; p=0.0090). Furthermore, controlling for all other variables in the final model for erythromycin-resistant *Campylobacter,* there were significant interactions between VFD rule changes and the host. For example, the odds of detecting erythromycin-resistant *Campylobacter* were 2.68 times higher in cattle following implementation of the VFD rule changes compared to cattle in the period prior to implementation (OR= 2.68, 95% CI: 1.83, 3.93; p<0.0001). In contrast, the odds of detecting erythromycin-resistant *Campylobacter* decreased by 62% in chickens following implementation of the VFD rule changes compared to chickens prior to implementation (OR= 0.38, 95% CI: 0.22, 0.66; p=0.0005). The implementation of VFD rule changes has been beneficial in reducing the odds of detecting tetracycline-resistant *Escherichia* and erythromycin-resistant *Campylobacter* in chickens, as well as tetracycline-resistant *Salmonella* in cattle. However, there was an increase in the odds of detecting tetracycline-resistant *Salmonella* in chicken, tetracycline-resistant *Escherichia* in swine, and erythromycin-resistant *Campylobacter* in cattle. Continued monitoring of antimicrobial resistance and antimicrobials usage can aid in supporting stewardship efforts, such as the VFD rule for food-producing animals in the U.S.

Keywords: Antimicrobial-resistant, veterinary feed directives, tetracycline, erythromycin, Salmonella, Escherichia, and Campylobacter, cecal sample, food-producing animal, NARMS.

Introduction

Medically important antimicrobial-resistant bacteria are recognized as an important threat to global health [1, 2]. Inappropriate or unnecessary use of medically important antimicrobial drugs is an important factor that increases the risk of developing antimicrobial-resistant bacteria in food-producing animals [3, 4]. The amount, length of time, and type of antimicrobial drugs used all play a role in developing antimicrobial-resistant bacteria [3, 5, 6]. Bacteria exposure to antimicrobials is associated with an increased selective proliferation of resistant bacteria [7]. Reducing inappropriate antimicrobial use in food-producing animals may improve antimicrobial-resistant prevention and control [5]. The judicious use of antimicrobials can reduce the selection pressure for developing resistant bacteria [8] in food-producing animals. An observational study demonstrated that six months after the removal of tetracycline-supplemented feed from a chicken farm, there was a lower frequency of tetracycline-resistant *Escherichia* isolates compared to before the removal of the tetracycline-supplemented feed in the farm [9].

In 2017, the United States (U.S.) Food and Drug Administration (FDA) implemented the Veterinary Feed Directive (VFD) rule changes to limit medically important antimicrobial drugs administered to food-producing animals through feed and water, allowing usage for treating illness [10]. A licensed veterinarian must supervise the use of these antimicrobial drugs under this rule. The changes to the VFD rules are an important strategy for ensuring the judicious use of medically important antimicrobials in food-producing animals in the U.S. [10]. Antimicrobial drugs were frequently administered as a preventative measure in food-producing animals before the VFD rule changes were introduced in the U.S. However, with the implementation of the VFD rule changes, the prophylactic use of antimicrobials is no longer allowed in the U.S. [10].

Violative sulfonamide and penicillin residues in the tissues of food animals have decreased at U.S. slaughter establishments following the implementation of the VFD rule changes compared to the period prior to implementation [11]. The use of antimicrobials in food-producing animals varies by antimicrobial class in the U.S. In 2020, tetracycline was the most commonly sold antimicrobial, comprising 66% (3,948,745 kg) of the total usage and 7% (433,394 kilograms) of macrolides in the U.S. food-producing animals [12], providing an adequate focus of analysis for the current study.

To support animal health authorities in implementing evidence-based targeted interventions, monitoring the antimicrobial-resistant bacteria isolates from food-producing animals is essential to identify emerging antimicrobial-resistant bacteria. In 1997, the animal component of the National Antimicrobial Resistance Monitoring System (NARMS) was started by the Agricultural Research Services (ARS) of the U.S. Department of Agriculture (USDA) [13]. They tested *Salmonella* isolates obtained through the Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) program of the USDA Food Safety Inspection Services (FSIS) [13]. Later in 2013, the FSIS and FDA began the cecal sampling program to monitor the antimicrobial susceptibility of bacteria in food-producing animals [13]. Samples of the cecal contents from cattle, swine, chickens, and turkeys were taken from slaughter establishments that are regulated by the FSIS. The establishments were randomly sampled in a tiered manner, depending on their slaughter volume, which resulted in a representative sample of national food animals' production. These cecal samples were then tested for enteric bacteria, and their antimicrobial susceptibility was obtained at the FSIS laboratory [13, 14].

In the U.S., foodborne illness caused by enteric bacteria poses a public health threat. Around 1 in 6 Americans are affected annually by a foodborne illness, leading to about 48 million cases, 128,000 hospitalizations, and 3000 deaths [15]. Contact with infected food-producing animals acts as a risk factor for *Salmonella* infections in humans in the U.S. [16, 17]. Three types of enteric bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) commonly found in food-producing animals are responsible for almost 60% of these illnesses and hospitalizations in the U.S. [18] making them the primary focus of analysis for the present study.

Several studies have been conducted on the prevalence and trends of antimicrobial-resistant bacteria isolates from food-producing animals in the U.S. Most of the studies aimed to isolate, characterize, and antimicrobial susceptibility profiles of bacteria in food producing-animals, using cross-sectional and retrospective study design in the U.S.[19-22]. Another study evaluated antimicrobial susceptibility patterns in *Campylobacter* isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern U.S. [23]. A recent study utilized NARMS data to assess antimicrobial-resistant patterns and temporal trends in commensal *Escherichia* isolated from cecal samples of swine in the U.S. [24]. The effect of the 2017 VFD final rule changes on the occurrence of bacteria resistant to medically-important antimicrobials in cecal samples of food-producing animals at slaughter establishment is yet to be investigated in the U.S. Therefore, our study's objective was to investigate whether the 2017 VFD final rule changes affected the occurrence of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples obtained from food-producing animals at slaughter establishments in the U.S. The findings of this study will provide evidence of the magnitude of impact VFD rule changes have had on the risk of tetracycline-resistant and erythromycin-resistant bacteria (*Salmonella*, *Campylobacter*, and *Escherichia*) in cecal samples obtained from food-producing animals at slaughterhouses facilities in the U.S.

Materials and Methods

Data sources and retrieval

On March 21, 2023, cecal samples of food-producing animal surveillance dataset from 2013 to 2019 was downloaded from the NARMS, which is publicly available data [25]. Under this surveillance system cattle, swine, chicken, and turkey cecal are collected at slaughter facilities throughout the U.S. by the FDA and FSIS. The cecal samples are tested for enteric bacteria and their antimicrobial susceptibility [13]. The obtained data was transferred from Microsoft Excel to STATA 17.1 software for data validation.

Data validation and variables used in the analysis

The primary variable of interest utilized in selecting the final dataset for analysis was the presence of the minimum inhibitory concentration (MIC) values of tetracycline and erythromycin. The other selected variables for analysis in each dataset included year, month, host, and type genera of bacteria found (*Salmonella*, *Campylobacter,* and *Escherichia*). The bacteria categories analyzed in this study include *Salmonella*, *Escherichia*, and *Campylobacter*, which comprise their respective serotypes' aggregation. The “year” variable was first analyzed for differences between years. Additionally, we collapsed the “year” variable into a categorical variable called “years of sampling.” This categorization was done as follows: “2013-2014”, “2015-2016”, and “2017-2019”. We also created a dichotomous variable called “VFD rule change,” which was collapsed the “year” variable into two categories: “before VFD rule change (2013-2016)” and “after VFD rule change (2017-2019)”. The “VFD rule changes” was a binary categorical variable. Cecal samples collected and tested between 2017 and 2019 were considered to be “after VFD rule changes,” whereas cecal samples collected and tested between 2013 and

2016 were considered as “before VFD rule changes” period. The "month" variable was categorized into four quarters: Quarter 1 (January, February, and March), Quarter 2 (April, May, and June), Quarter 3 (July, August, and September), and Quarter 4 (October, November, and December). The outcome variables were the presence of tetracycline-resistant *Salmonella*, *Campylobacter*, and *Escherichia*, as well as erythromycin-resistant *Campylobacter* isolates in cecal samples from cattle, swine, chicken, and turkey.

Tetracycline-resistant *Salmonella and Escherichia* isolates in cecal samples were categorized based on the MIC breakpoint values of ≥16 µg/ml. Similarly, tetracycline-resistant *Campylobacter* in cecal sample was categorized based on the MIC breakpoint values of ≥4 µg/ml. Additionally, erythromycin-resistant *Campylobacter* in cecal sample was defined based on the MIC breakpoint values of erythromycin (≥8 µg/ml). The breakpoints were based on the 2021 NARMS Interpretive Criteria for Susceptibility Testing [26].

Statistical analysis

Frequencies and percentages were used to summarize categorical predictor variables. Four multivariable logistic regression models were built for the tetracycline-resistant *Salmonella*, *Campylobacter*, and *Escherichia*, as well as erythromycin-resistant *Campylobacter* isolates in cecal samples of food-producing animals (cattle, swine, chicken, and turkey) in this study. The proportion of antibiotic-resistant bacteria isolates for tetracycline and erythromycin was determined by dividing the number of resistant isolates by the total number of bacteria isolates tested for each antibiotic. The Cochran-Armitage trend tests were used to evaluate temporal trends in the proportion of cecal samples resistant to individual antibiotics, categorized by hosts, from 2013 to 2019.

For each model-building process, two steps were involved. In the first step, a univariable logistic regression model was fitted to assess the unadjusted associations between potential independent variables and the outcome variable. We conducted the univariable logistic regression analysis to investigate the association of the “year”, “years of sampling”, “VFD rule change”, host, and quarter of year with the resistant outcomes. A relaxed p-value ≤ 0.2 [27-30] was used to identify the predictors that were chosen for further examination in the multivariable logistic regression models in step two.

To prevent the inclusion of collinear variables in the multivariable models, the pairwise collinearity of these variables was examined using Spearman's rank correlation coefficient. If the correlation coefficient between the two variables was ≥ 0.6 [31], only the variable with the highest odds ratio, the fewest missing observations in the initial screening, and biological plausibility would be included in the multivariable model. Categorical variables with more than two levels of categories were analyzed to evaluate pairwise differences using the Tukey-Kramer adjustment for multiple comparisons. Temporal graphs were generated in Excel to visualize the temporal patterns of resistant outcomes by years of sampling. If variables had similar characteristics, such as year, year of sampling, and VFD rule change, only one was included in the multivariable model based on the results of the univariable analysis. In the second step, a multivariable logistic regression model was built using a manual backward elimination method. A full model was first constructed by including all the screened variables. We included the VFD rule change in every full multivariable model, regardless of the p-value obtained from the univariable analysis, as it was our primary exposure of interest for the analysis. Non-significant variables were removed through a manual backward elimination process. However, if the removal of a non-significant variable resulted in a substantial change (more than 20%) in the coefficient of any remaining variables in the model, it was considered a potential confounder and was retained in the final model [32]. Each final multivariable model was checked for possible multicollinearity using variance inflation factor (VIF) to avoid modeling issues associated with multicollinearity. If the VIF value exceeded 10, it indicated the presence of multicollinearity [33]. The relevant pairwise significant interaction was assessed in the final model [32]. For instance, the two-way interaction between VFD rule changes and the host categories was assessed. All final multivariable model results were presented as an odds ratio (OR) with a corresponding 95% confidence interval (CI), and p-value. A p-value ≤ 0.05 was considered statistically significant. The overall assessment of the final multivariable model was done using Akaike's Information Criterion (AIC) [34]. The model with the lowest AIC values was considered as the best-fitting model. Statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The original dataset contained 54,115 records from 2013 to 2019. However, only 47,016 (86.88%) and 25,430 (47 %) of these records had MIC values for the target tetracycline and erythromycin, respectively.

Temporal trends in the proportion of antibiotic-resistant bacteria

There was a decreasing trend in the proportion of tetracycline-resistant *Salmonella* in cattle ($p < .0001$), with a distinct downward trend observed after 2018 **(Figure 3.1).** Conversely, there was an increasing trend in the proportion of tetracycline-resistant *Salmonella* in chickens ($p < .0001$) **(Figure 3.1).** No significant trend was observed in the proportion of tetracycline-resistant *Salmonella* in swine ($p = 0.758$) and turkeys ($p = 0.259$) **(Figure 3.1).** No significant trend was observed in the proportion of tetracycline-resistant *Campylobacter* in cattle ($p= 0.334$), chickens ($p= 0.097$), swine ($p = 0.872$), and turkeys ($p = 0.930$) **(Figure 3.1).** Likewise, there was a decreasing trend in the proportion of tetracycline-resistant *Escherichia* in chickens ($p= 0.014$). Similarly, there was a decreasing trend in the proportion of tetracycline-resistant *Escherichia* in turkeys ($p= 0.006$), with a distinct downward trend after 2018 **(Figure 3.2).** No significant trend was observed in the proportion of tetracycline-resistant *Escherichia* in cattle ($p= 0.462$) and swine ($p= 0.133$) **(Figure 3.3).** Additionally, there was decreasing trend in the proportion of erythromycin-resistant *Campylobacter* in cattle ($p= 0.002$) and chickens ($p < .0001$) with a distinct downward trend after 2018 **(Figure 3.4).** No significant trend was observed in the proportion of erythromycin-resistant *Campylobacter* in swine ($p= 0.072$) and turkeys ($p= 0.719$) **(Figure 3.4).**

Univariable logistic regression results

Year, years of sampling, VFD rule changes, host, and quarter of the year were significantly associated with the odds of tetracycline-resistant *Salmonella* isolated in cecal samples of food-producing animals **(Table 3.1, Table 3.2).** Additionally, no distinct linear pattern was observed across different categories of sampling years regarding the odds of detecting tetracycline-resistant *Salmonella* in cecal samples obtained from food-producing animals **(Table 3.2).**

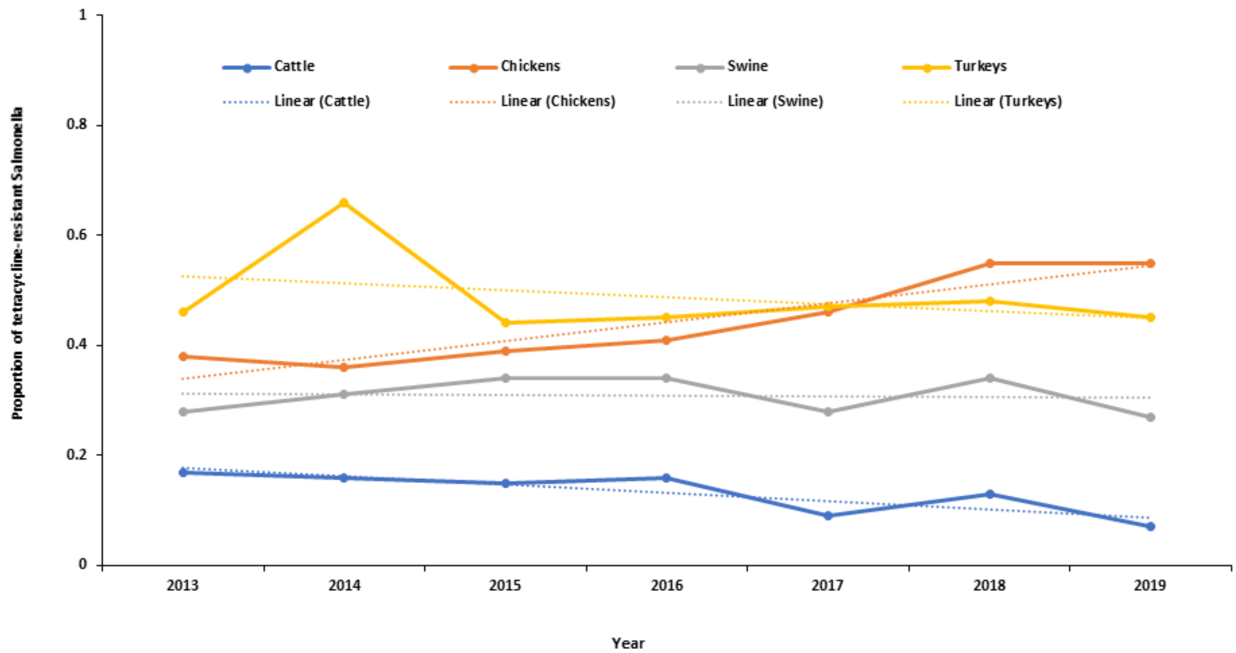

Figure 3. 1: Temporal trends in the proportion of tetracycline-resistant *Salmonella* isolated from cecal samples of food animals in the United States, 2013-2019.

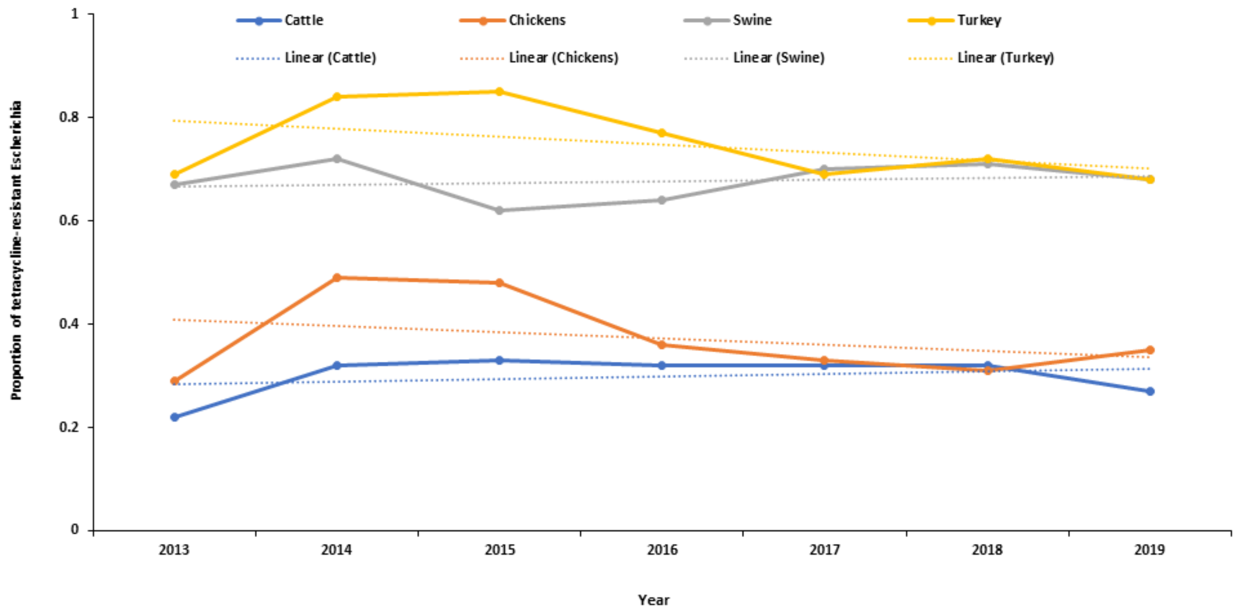

Figure 3. 2: Temporal trends in the proportion of tetracycline-resistant *Campylobacter* isolated from cecal samples of food animals in the United States, 2013-2019.

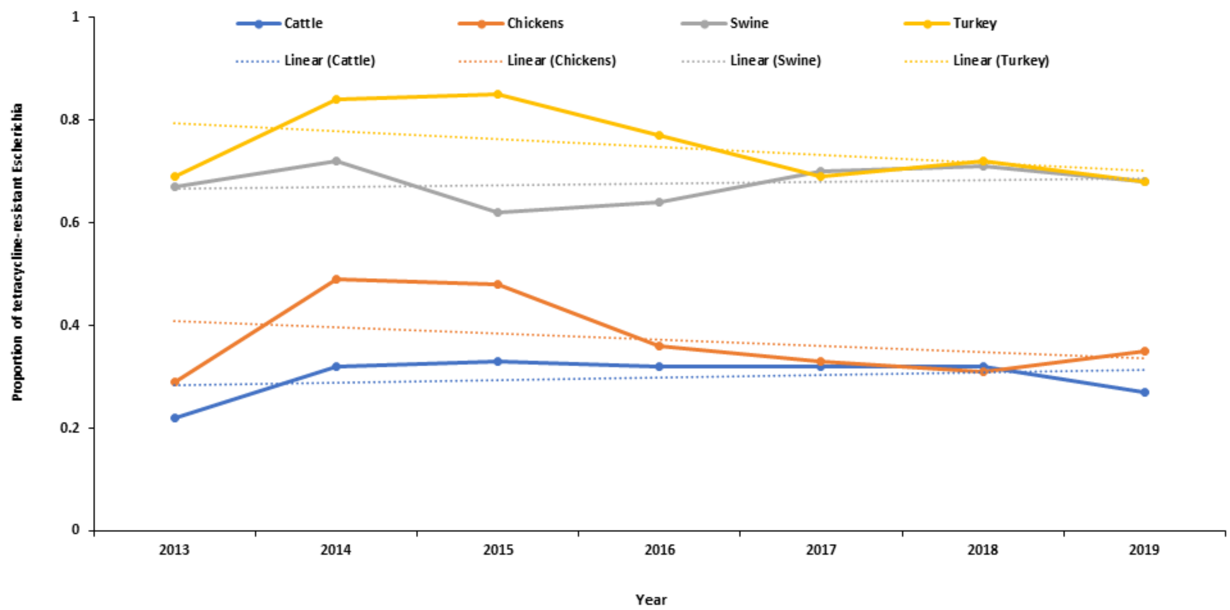

Figure 3. 3: Temporal trends in the proportion of tetracycline-resistant *Escherichia* isolated from cecal samples of food animals in the United States, 2013-2019.

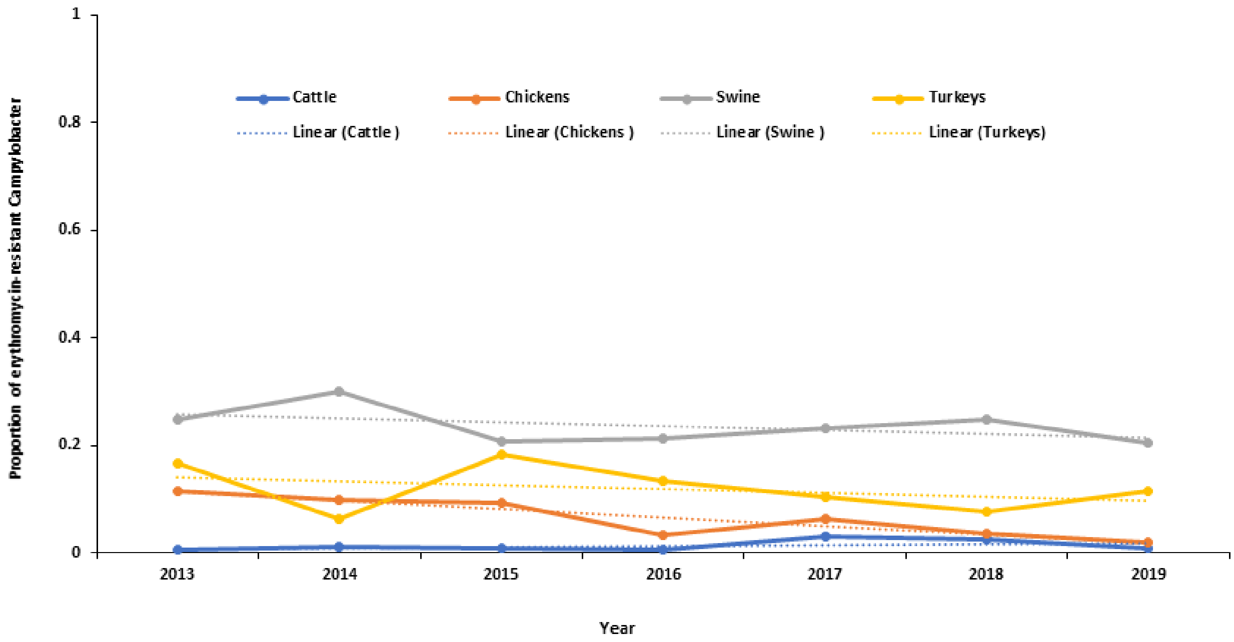

Figure 3. 4: Temporal trends in the proportion of erythromycin-resistant *Campylobacter* isolated from cecal samples of food animals in the United States, 2013-2019.

Moreover, the graphical analysis **(Figure 3.5)** revealed no discernible linear trend in the overall proportion of tetracycline-resistant *Salmonella* in the cecal samples. Similarly, year, years of sampling, VFD rule changes, and host were significantly associated with the odds of tetracycline-resistant *Campylobacter* isolated from cecal samples of food-producing animals **(Table 3.3, Table 3.4).** Additionally, no distinct linear pattern was observed across different categories of sampling years regarding the odds of detecting tetracycline-resistant *Campylobacter* in cecal samples obtained from food-producing animals **(Table 3.4).** Moreover, the graphical analysis **(Figure 3.6)** revealed that there was no distinct linear trend in the overall proportion of tetracycline-resistant *Campylobacter* in the cecal samples.

Additionally, year, host, and quarter of the year were significantly associated with the odds of tetracycline-resistant *Escherichia* isolated from ceca samples of food-producing animals **(Table 3.5, Table 3.6).** Additionally, no distinct linear pattern was observed across different categories of sampling years regarding the odds of detecting tetracycline-resistant *Escherichia* in cecal samples obtained from food-producing animals **(Table 3.6).** Moreover, the graphical analysis **(Figure 3.7)** revealed no discernible linear trend in the overall proportion of tetracycline-resistant *Escherichia* in the cecal samples. Lastly, year, years of sampling, host, and quarter of the year were significantly associated with the odds of erythromycin-resistant *Campylobacter* isolated from cecal samples of food-producing animals **(Table 3.7, Table 3.8).** Additionally, no distinct linear pattern was observed across different categories of sampling years regarding the odds of detecting erythromycin-resistant *Campylobacter* in cecal samples obtained from food-producing animals **(Table 3.8).** Moreover, the graphical analysis **(Figure 3.8)** revealed no discernible linear trend in the overall proportion of erythromycin-resistant *Campylobacter* in the cecal samples.

Table 3. 1: Results of univariable logistic regression analysis for tetracycline-resistant *Salmonella* isolated from cecal samples of food-producing animals in the United States, 2013-2019

Variable	Category	Tetracycline		OR	95% CI	p-values
		Resistant n (%)	No resistant n (%)			
Year (n=8,968)						<0.001
	2013	261 (24.42)	808 (75.58)	0.87	0.73, 1.05	0.162
	2014	306 (28.71)	760 (71.29)	1.09	0.91, 1.30	0.311
	2015	283 (28.08)	725 (71.92)	1.06	0.88, 1.27	0.513
	2016	339 (29.22)	821 (70.78)	1.12	0.94, 1.33	0.184
	2017	393 (26.88)	1,069(73.12)	Referent		
	2018	549 (33.74)	1,078(66.26)	1.38	1.18, 1.61	<0.001
	2019	453 (28.74)	1,123(71.26)	1.09	0.93, 1.28	0.253
VFD rule changes (n=8,968)						0.0176
	Before VFD rule changes (2013-2016)	1,189(27.63)	3,114(72.37)	Referent		
	After VFD rule changes (2017-2019)	1,395(29.90)	3,270(70.10)	1.11	1.01, 1.22	0.018
Host (n=8,968)						<0.001
	Cattle	376 (13.11)	2,491(86.89)	0.15	0.12, 0.20	<0.001
	Chickens	749 (48.64)	791 (51.36)	0.98	0.77, 1.25	0.891
	Swine	1,303(30.71)	2,940(69.29)	0.46	0.36, 0.57	<0.001
	Turkeys	156 (49.06)	162 (50.94)	Referent		

OR — Odds ratio; CI — Confidence interval.

Table 3. 1 continued

Variable	Category	Tetracycline		OR	95% CI	p-values
		Resistant n (%)	No resistant n (%)			
Quarter of year (n=8,965)						0.0008
	Quarter 1	653 (31.61)	1,413(68.39)	1.25	1.10, 1.42	<0.001
	Quarter 2	665 (29.04)	1,625(70.96)	1.11	0.97, 1.26	0.101
	Quarter 3	679 (26.91)	1,844(73.09)	Referent		
	Quarter 4	586 (28.09)	1,500(71.91)	1.06	0.93, 1.20	0.372

OR — Odds ratio; CI — Confidence interval.

Table 3. 2: Univariable logistic regression of association between years of sampling and tetracycline-resistant *Salmonella* isolates in cecal samples of food-producing animals in the United States

Variable	Categories	OR	95% CI	*P*-value
Years of sampling (n= 8,968)				0.0183
	2013-2014 vs. 2017-2019	0.85	0.74, 0.97	0.0132
	2015-2016 vs. 2017-2019	0.94	0.83, 1.08	0.5620
	2013-2014 vs. 2015-2016	0.90	0.77, 1.06	0.2615

OR — Odds ratio; CI — Confidence interval.

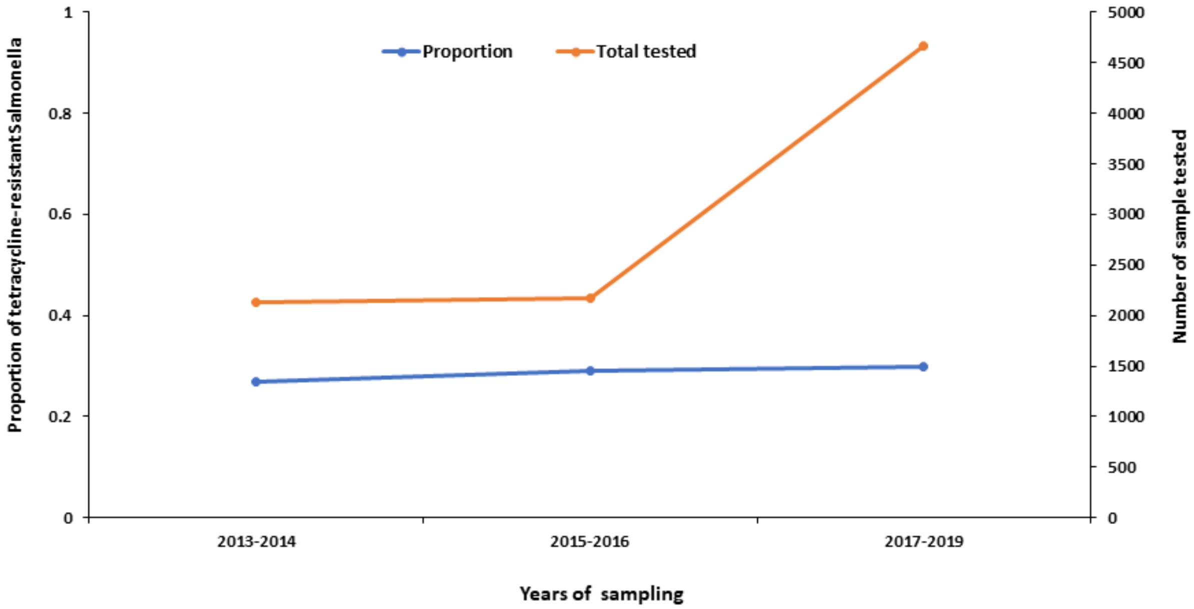

Figure 3. 5: Temporal trends in the proportion of tetracycline-resistant *Salmonella* by years of sampling

Table 3. 3: Results of univariable logistic regression analysis for tetracycline-resistant *Campylobacter* isolated from cecal samples of food-producing animals in the United States, 2013-2019

Variable	**Category**	**Tetracycline**		**OR**	**95% CI**	**p-value**
		Resistant n (%)	**No resistant n (%)**			
Year (n=13,160)						<0.001
	2013	1,195(68.52)	549 (31.48)	1.05	0.92, 1.21	0.440
	2014	1,184(72.37)	452 (27.63)	1.27	1.10, 1.46	0.001
	2015	1,021(70.17)	434 (29.83)	1.14	0.98, 1.31	0.077
	2016	1,023(70.45)	429 (29.55)	1.15	0.99, 1.33	0.051
	2017	1,361(67.34)	660 (32.66)	Referent		
	2018	1,465(64.17)	818 (35.83)	0.86	0.76, 0.98	0.029
	2019	1,657(64.50)	912 (35.50)	0.88	0.77, 0.99	0.044
VFD rule changes (n=13,160)						<0.001
	Before VFD rule changes (2013-2016)	4,423(70.35)	1,864(29.65)	Referent		
	After VFD rule changes (2017-2019)	4,483(65.23)	2,390(34.77)	0.79	0.73, 0.85	<0.001
Host (n=13,160)						<0.001
	Cattle	6,131(68.38)	2,835(31.62)	0.93	0.77, 1.13	0.515
	Chickens	630 (44.30)	792 (55.70)	0.34	0.27, 0.42	<0.001
	Swine	1,792(79.08)	474 (20.92)	1.63	1.32, 2.03	<0.001
	Turkeys	353 (69.76)	153 (30.24)	Referent		

OR: odds ratio; CI: confidence intervals.

Table 3. 3 continued

Variable	Category	Tetracycline		OR	95% CI	p-value
		Resistant n (%)	**No resistant n (%)**			
Quarter of year (n=13, 157)						0.162
	Quarter 1	2,411(68.07)	1,131(31.93)	1.06	0.95, 1.17	0.290
	Quarter 2	2,393(67.93)	1,130(32.07)	1.05	0.94, 1.16	0.351
	Quarter 3	1,983(66.84)	984 (33.16)	Referent		
	Quarter 4	2,117(67.74)	1,008(32.26)	1.04	0.93, 1.15	0.450

OR: odds ratio; CI: confidence intervals.

Table 3. 4: Univariable logistic regression of association between years of sampling and tetracycline-resistant *Campylobacter* isolates in cecal samples of food-producing animals in the United States

Variable	Categories	OR	95% CI	p-value
Years of sampling (n= 13,160)				<0.0001
	2013-2014 vs. 2017-2019	1.27	1.14, 1.41	<0.0001
	2015-2016 vs. 2017-2019	1.26	1.13, 1.41	<0.0001
	2013-2014 vs. 2015-2016	1.00	0.88, 1.14	0.9979

OR — Odds ratio; CI — Confidence interval.

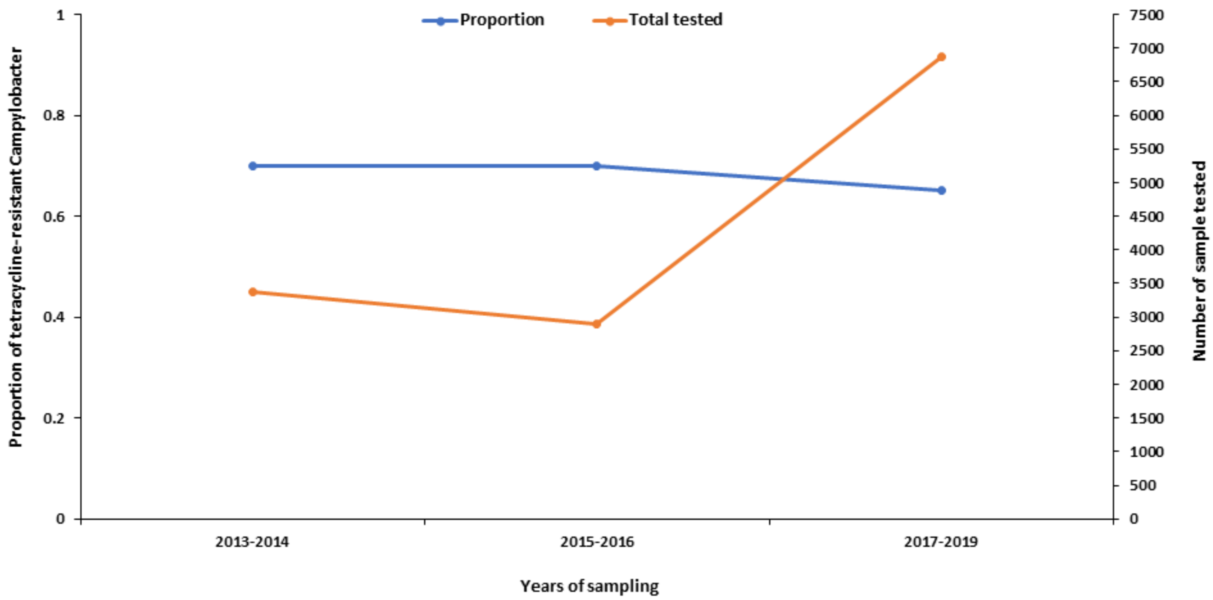

Figure 3. 6: Temporal trends in the proportion of tetracycline-resistant *Campylobacter* by years of sampling

Table 3. 5: Results of univariable logistic regression analysis for tetracycline-resistant *Escherichia* isolated in cecal samples of food-producing animals in the United States, 2013-2019

Variable	Category	Tetracycline		OR	95% CI	p-value
		Resistant n (%)	No resistant n (%)			
Year (n=12,618)						<0.001
	2013	313 (36.23)	551 (63.77)	0.73	0.62, 0.86	<0.001
	2014	432 (47.95)	469 (52.05)	1.19	1.02, 1.39	0.025
	2015	712 (45.41)	856 (54.59)	1.07	0.94, 1.22	0.256
	2016	935 (44.15)	1,183(55.85)	1.02	0.90, 1.15	0.700
	2017	1,034(43.57)	1,339(56.43)	Referent		
	2018	1,105(43.42)	1,440(56.58)	0.99	0.88, 1.11	0.913
	2019	952 (42.33)	1,297(57.67)	0.95	0.84, 1.06	0.393
VFD rule changes (n=12,618)						0.397
	Before VFD rule changes (2013-2016)	2,392(43.88)	3,059(56.12)	Referent		
	After VFD rule changes (2017-2019)	3,091(43.13)	4,076(56.87)	0.96	0.90, 1.04	0.398
Host (n=12,618)						<0.001
	Cattle	2,188(30.65)	4,950(69.35)	0.16	0.13, 0.18	<0.001
	Chickens	513 (35.77)	921 (64.23)	0.20	0.16, 0.24	<0.001
	Swine	2,189(67.62)	1,048(32.38)	0.76	0.64, 0.90	0.002
	Turkeys	593 (73.30)	216 (26.70)	Referent		
Quarter of year (n=12,617)						0.0005
	Quarter 1	1,433(46.33)	1,660(53.67)	1.20	1.09, 1.33	<0.001
	Quarter 2	1,410(42.22)	1,930(57.78)	1.02	0.92, 1.12	0.668
	Quarter 3	1,358(41.69)	1,899(58.31)			
	Quarter 4	1,282(43.80)	1,645(56.20)	1.08	0.98, 1.20	0.095

OR: odds ratio; CI: confidence intervals.

Table 3. 6: Univariable logistic regression of association between years of sampling and tetracycline-resistant *Escherichia* isolates in cecal samples of food-producing animals in the United States

Variable	Categories	OR	95% CI	p-value
Years of sampling (n= 12,618)				0.1584
	2013-2014 vs. 2017-2019	0.96	0.85, 1.09	0.7645
	2015-2016 vs. 2017-2019	1.07	0.97, 1.17	0.2694
	2013-2014 vs. 2015-2016	0.904	0.79, 1.04	0.1972

OR — Odds ratio; CI — Confidence interval.

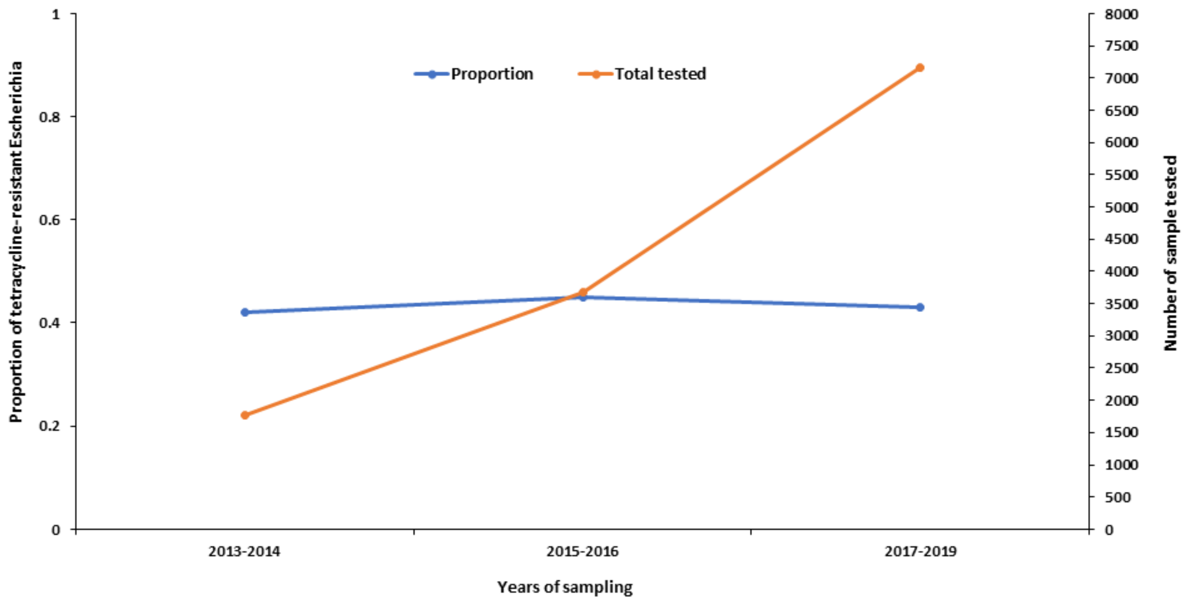

Figure 3. 7: Temporal trends in the proportion of tetracycline-resistant *Escherichia* by years of sampling

Table 3. 7: Results of univariable logistic regression analysis for erythromycin-resistant *Campylobacter* isolated in cecal samples of food-producing animals in the United States, 2013-2019

Variable	Category	Erythromycin		OR	95% CI	p-value
		Resistant n (%)	No resistant n (%)			
Year (n=13,160)						0.0011
	2013	109 (6.25)	1,635(93.75)	0.92	0.71, 1.19	0.552
	2014	125 (7.64)	1,511(92.36)	1.14	0.89, 1.47	0.287
	2015	69 (4.74)	1,386(95.26)	0.69	0.51, 0.92	0.015
	2016	72 (4.96)	1,380(95.04)	0.72	0.53, 0.97	0.031
	2017	136 (6.73)	1,885(93.27)	Referent		
	2018	144 (6.31)	2,139(93.69)	0.93	0.73, 1.18	0.576
	2019	127 (4.94)	2,442(95.06)	0.72	0.56, 0.92	0.010
VFD rule changes (n=13,160)						0.917
	Before VFD rule changes (2013-2016)	375 (5.96)	5,912(94.04)	Referent		
	After VFD rule changes (2017-2019)	407 (5.92)	6,466(94.08)	0.99	0.85, 1.14	0.917
Host (n=13,160)						<0.001
	Cattle	128 (1.43)	8,838(98.57)	0.12	0.08, 0.17	<0.001
	Chickens	62 (4.36)	1,360(95.64)	0.38	0.26, 0.57	<0.001
	Swine	539(23.79)	1,727(76.21)	2.66	1.97, 3.60	<0.001
	Turkeys	53 (10.47)	453 (89.53)	Referent		
Quarter of year (n=13,160)						0.0007
	Quarter 1	224 (6.32)	3,319(93.68)	1.36	1.09, 1.69	0.005
	Quarter 2	197 (5.59)	3,327(94.41)	1.19	0.95, 1.49	0.115
	Quarter 3	140 (4.72)	2,828(95.28)	Referent		
	Quarter 4	221 (7.07)	2,904(92.93)	1.53	1.23, 1.91	<0.001

OR — Odds ratio; CI — Confidence interval.

Table 3. 8: Univariable logistic regression of association between years of sampling and erythromycin-resistant *Campylobacter* isolates in cecal samples of food-producing animals in the United States

Variable	Categories	OR	95% CI	p-value
Years of sampling (n= 13,160)				0.0026
	2013-2014 vs. 2017-2019	1.18	0.97, 1.44	0.1205
	2015-2016 vs. 2017-2019	0.81	0.64, 1.03	0.0894
	2013-2014 vs. 2015-2016	1.46	1.13, 1.89	0.0017

OR — Odds ratio; CI — Confidence interval.

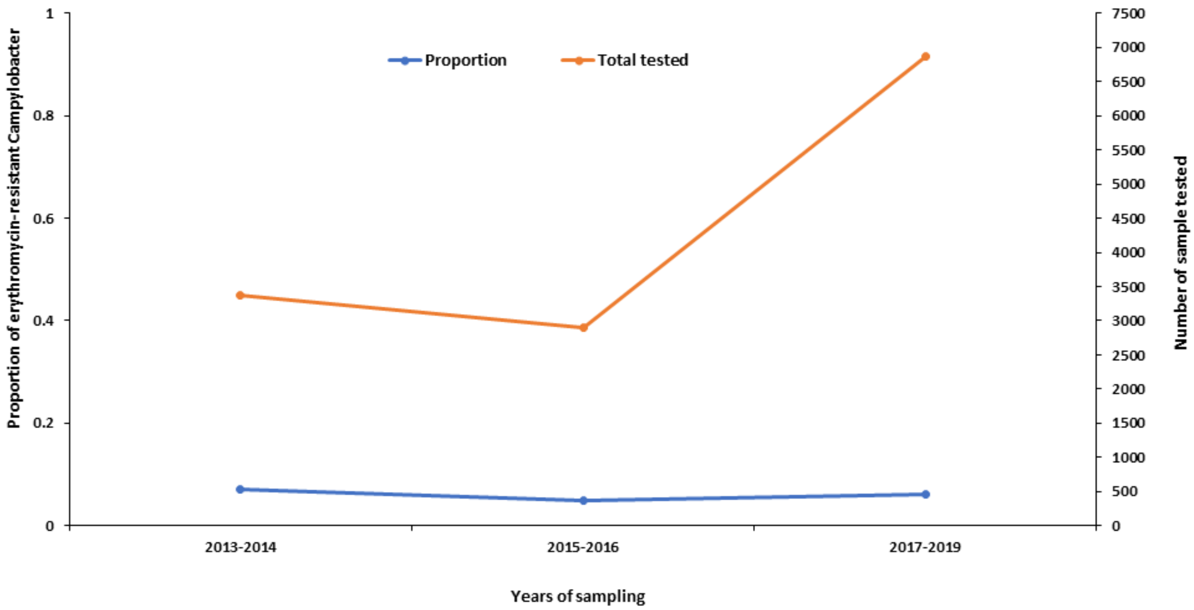

Figure 3. 8: Temporal trends in the proportion of erythromycin-resistant *Campylobacter* by years of sampling

Multivariable logistic regression results

The final model was fitted for tetracycline-resistant *Salmonella,* which included 8968 observations **(Table 3.9).** No multicollinearity issue was found in the final model. There were significant interactions between VFD rule changes and the host after controlling for all other variables in the model. The significant interaction between VFD rule changes and host implies that the effect of VFD rule changes on the odds of detecting tetracycline-resistant *Salmonella* were not the same across the host levels. For example, the odds of detecting tetracycline-resistant *Salmonella* were decreased by 41% in cattle following implementation of the VFD rule changes compared to cattle in the period prior to implementation (OR= 0.59, $p<0.0001$) **(Table 3.9).** In contrast, the odds of detecting tetracycline-resistant *Salmonella* were 1.71 times higher in chickens following implementation of the VFD rule changes compared to chickens in the period prior to implementation (OR= 1.71, $p<0.0001$) **(Table 3.9).** Additionally, specific to the period following implementation of the VFD rule changes, the odds of detecting tetracycline-resistant *Salmonella* were decreased by 90% in cattle compared to chickens (OR= 0.10, $p<0.0001$), 73% in cattle compared to swine (OR= 0.27, $p<0.0001$), 87% in cattle compared to turkeys (OR= 0.13, $p<.0001$), and 53% in swine compared to turkeys (OR= 0.47, $p<0.0001$) (Table 5). In contrast, the odds of detecting tetracycline-resistant *Salmonella* were 2.59 times higher in chickens compared to swine (OR= 2.59, $p<0.0001$) for the same period as above **(Table 3.9).**

The final model was fitted for tetracycline-resistant *Campylobacter*, which included 13,160 observations **(Table 3.10).** No multicollinearity issue was found in the final model. Variables significantly associated with the odds of detection of tetracycline-resistant *Campylobacter* – controlling for other variables--was the host **(Table 3.10).** There was borderline association between the VFD rule change and the odds of detecting tetracycline-resistant *Campylobacter* isolated from cecal samples from food-producing animals (OR=0.93, p=0.0598) **(Table 3.10).**

The final model was fitted for tetracycline-resistant *Escherichia*, which included 12,617 observations **(Table 3.11).** No multicollinearity issue was found in the final model. Variables significantly associated with the odds of detecting tetracycline-resistant *Escherichia* – controlling for other variables--were VFD rule changes, host, and quarter of the year. However, there were significant interactions between VFD rule changes and the host after controlling for all other variables in the model. The significant interaction between VFD rule changes and the host implies that the effect of VFD rule changes on the odds of detecting tetracycline-resistant *Escherichia* were not the same across the host levels. For example, the odds of detecting tetracycline-resistant *Escherichia* decreased by 30% in chickens following the VFD rule changes compared to chickens prior to implementation (OR= 0.70, p=0.0017) **(Table 3.11).** In contrast, the odds of detecting tetracycline-resistant *Escherichia* were 1.22 times higher in swine following implementation of the VFD rule changes compared to swine in the period prior to implementation (OR= 1.22, p= 0.0090). In addition, specific to the period following the implementation of the VFD rule changes, the odds of detecting tetracycline-resistant *Escherichia* were decreased by 81 % in cattle compared to swine (OR= 0.19, p<0.0001), 79% in chickens compared to swine (OR=0.21, p<0.0001), and 79% in chickens compared to turkeys (OR= 0.21, p<0.0001) for the same period as above **(Table 3.11).**

The final model was fitted for erythromycin-resistant *Campylobacter*, which included 13,160 observations **(Table 3.12).** No multicollinearity issue was found in the final model. Variables significantly associated with detecting erythromycin-resistant *Campylobacter* – controlling for other variables-was hosts.

Table 3. 9: Final multivariable model of factors associated with tetracycline-resistant *Salmonella* isolated in cecal samples of food-producing animals (n= 8,968) in the United States, 20013-2019

Variable	**Category**	**OR**	**95% CI**	**p-value**
VFD rule changes				0.3736
	After VFD rule changes (2017-2019) vs. Before VFD rule changes (2013-2016)	0.94	0.82 1.08	0.3736
Host				<0.0001
	Cattle vs. Turkey	0.15	0.11, 0.21	<0.0001
	Chickens vs. Turkey	0.86	0.62, 1.20	0.6518
	Swine vs. Turkey	0.46	0.34, 0.62	<0.0001
VFD rule changes* Host				<0.0001
Cattle	After VFD rule changes vs. before VFD rule changes	0.59	0.47, 0.74	<0.0001
Chickens	After VFD rule changes vs. before VFD rule changes	1.71	1.36, 2.15	<0.0001
Swine	After VFD rule changes vs. before VFD rule changes	0.91	0.8, 1.04	0.1699
Turkey	After VFD rule changes vs. before VFD rule changes	0.84	0.54, 1.31	0.4473

OR: odds ratio; CI: confidence intervals.

Table 3. 9 continued

Variable	Category	OR	95% CI	p-value
Before VFD rule changes				
	Cattle vs. Turkeys	0.18	0.11, 0.29	<0.0001
	Chickens vs. Turkeys	0.61	0.37, 0.99	0.0479
	Swine vs. Turkeys	0.44	0.28, 0.68	<0.0001
	Cattle vs. Chickens	0.30	0.22, 0.41	<0.0001
	Cattle vs. Swine	0.41	0.33, 0.51	<0.0001
	Chicken vs. Swine	1.39	1.05, 1.84	0.0159
After VFD rule changes				
	Cattle vs. Turkeys	0.13	0.08, 0.20	<0.0001
	Chicken vs. Turkeys	1.23	0.81, 1.87	0.5922
	Swine vs. Turkeys	0.47	0.31, 0.72	<0.0001
	Cattle vs. Chickens	0.10	0.08, 0.14	<0.0001
	Cattle vs. Swine	0.27	0.21, 0.35	<0.0001
	Chicken vs. Swine	2.59	2.12, 3.16	<0.0001

OR: odds ratio; CI: confidence intervals.

Table 3. 10: Final multivariable model of factors associated with tetracycline-resistant *Campylobacter* isolated in cecal samples of food animals (n=13,160) in the United States, 20013-2019

Variable	**Categories**	**OR**	**95% CI**	**p-value**
VFD rule changes				0.0598
	After the VFD rule changes (2017-2019) vs. Before VFD rule changes (2013-2016)	0.93	0.75, 1.00	0.0598
Host				<0.0001
	Cattle vs. Turkeys	0.91	0.70, 1.18	0.7811
	Chickens vs. Turkeys	0.34	0.26, 0.46	<0.0001
	Swine vs. Turkeys	1.59	1.19, 2.11	0.0002
	Cattle vs. chicken	2.65	2.27, 3.09	<0.0001
	Cattle vs. Swine	0.57	0.50, 0.66	<0.0001
	Chicken vs. Swine	0.22	0.18, 0.26	<0.0001

OR: odds ratio; CI: confidence intervals.

Table 3. 11: Final multivariable model of factors associated with tetracycline-resistant *Escherichia* isolated in cecal samples of food-producing animals (n=12,617) in the United States, 20013-2019

Variable	**Categories**	**OR**	**95% CI**	***P*-value**
VFD rule changes				0.0019
	After the VFD rule changes (2017-2019) vs. Before VFD rule changes (2013-2016)	0.83	0.74, 0.94	0.0019
Host				<0.0001
	Cattle vs. Turkeys	0.14	0.11, 0.18	<0.0001
	Chickens vs. Turkeys	0.19	0.14, 0.25	<0.0001
	Swine vs. Turkeys	0.68	0.53, 0.87	0.0004
Quarter of year				0.0015
	Quarter 1 vs. Quarter 3	1.23	1.07, 1.42	0.0008
	Quarter 2 vs. Quarter 3	1.06	0.92, 1.22	0.7011
	Quarter 4 vs. Quarter 3	1.08	0.94, 1.25	0.4762
	Quarter 1 vs. Quarter 2	1.16	1.01, 1.33	0.0279
	Quarter 1 vs. Quarter 4	1.14	0.98, 1.31	0.1012
	Quarter 2 vs. Quarter 4	0.98	0.85, 1.13	0.9794
VFD rule changes* Host				<0.0001
Cattle	After VFD rule changes vs. before VFD rule changes	0.99	0.89, 1.09	0.7760
Chickens	After VFD rule changes vs. before VFD rule changes	0.70	0.56, 0.87	0.0017

OR: odds ratio; CI: confidence intervals.

Table 3. 11 continued

Variable	Categories	OR	95% CI	p-value
Swine	After VFD rule changes vs. before VFD rule changes	1.22	1.05, 1.41	0.0090
Turkeys	After VFD rule changes vs. before VFD rule changes	0.58	0.41, 0.82	0.0025
Before VFD rule changes				
	Cattle vs. Turkeys	0.11	0.07, 0.17	<0.0001
	Chickens vs. Turkeys	0.17	0.11, 0.28	<0.0001
	Swine vs. Turkeys	0.47	0.31, 0.71	<0.0001
	Cattle vs. Chickens	0.64	0.49, 0.82	<0.0001
	Cattle vs. Swine	0.24	0.20, 0.28	<0.0001
	Chickens vs. Swine	0.37	0.28, 0.49	<0.0001
After VFD rule changes				
	Cattle vs. Turkeys	0.19	0.15, 0.24	<0.0001
	Chickens vs. Turkeys	0.21	0.16, 0.28	<0.0001
	Swine vs. Turkeys	0.98	0.75, 1.30	0.9989
	Cattle vs. Chickens	0.90	0.74, 1.10	0.5258
	Cattle vs. Swine	0.19	0.16, 0.22	<0.0001
	Chickens vs. Swine	0.21	0.17, 0.27	<0.0001

OR: odds ratio; CI: confidence intervals.

However, there were significant interactions between VFD rule changes and the host after controlling for all other variables in the model **(Table 3.12).** The significant interaction between VFD rule changes and host implies that the effect of VFD rule changes on the odds of detecting tetracycline-resistant *Campylobacter* were not the same across the host levels. For example, the odds of detecting erythromycin-resistant *Campylobacter* were 2.68 times higher in cattle following implementation of the VFD rule changes compared to cattle in the period prior to implementation (OR= 2.68, $p<0.0001$) **(Table 3.12).** In contrast, the odds of detecting erythromycin-resistant *Campylobacter* decreased by 42% in chickens following implementation of the VFD rule changes compared to cattle prior to implementation (OR= 0.38, $p=0.0005$) **(Table 3.12).** Additionally, specific to the period following the implementation of the VFD rule changes, the odds of detecting erythromycin-resistant *Campylobacter* were decreased by 40% in cattle compared to chickens (OR= 0.60, $p=0.0406$), 93% in cattle compared to swine (OR= 0.07, $p<0.0001$), 88% in chickens compared to swine (OR= 0.12, $p<0.0001$), and 68% in chickens compared to turkeys (OR= 0.32, $p<0.0001$) for the same period as above **(Table 3.12).**

Discussion

When studying antibiotic-resistant bacteria in food-producing animals, the factors associated with them are usually assessed independently. However, examining how the primary exposure variable interacts with other factors to the outcome variable is crucial. The present study investigated the effects of the interactions between the VFD rule changes and host categories on the odds of detecting tetracycline-resistant *Salmonella*, *Campylobacter*, and *Escherichia*, as well as erythromycin-resistant *Campylobacter* isolated from cecal samples of food-producing animals.

Table 3. 12: Final multivariable model of factors associated with erythromycin-resistant *Campylobacter* isolated in cecal samples of food animals (n= 13,160) in the United States, 20013-2019

Variable	Categories	OR	95% CI	p-value
VFD rule changes				0.4690
	After the VFD rule changes (2017-2019) vs. Before VFD rule changes (2013-2016)	0.91	0.70, 1.18	0.4690
Host				<0.0001
	Cattle vs. Turkeys	0.10	0.06, 0.18	<0.0001
	Chickens vs. Turkeys	0.44	0.24, 0.84	0.0060
	Swine vs. Turkeys	2.36	1.37, 4.07	0.0003
VFD rule changes* Host				<0.0001
Cattle	After VFD rule changes vs. before VFD rule changes	2.68	1.83, 3.93	<0.0001
Chickens	After VFD rule changes vs. before VFD rule changes	0.38	0.22, 0.66	0.0005
Swine	After VFD rule changes vs. before VFD rule changes	0.91	0.75, 1.10	0.3232
Turkeys	After VFD rule changes vs. before VFD rule changes	0.73	0.33, 1.63	0.4428

OR: odds ratio; CI: confidence intervals.

Table 3. 12 continued

Variable	Categories	OR	95% CI	p-value
Before VFD rule changes				
	Cattle vs. Turkeys	0.05	0.02, 0.15	<0.0001
	Chickens vs. Turkeys	0.62	0.20, 1.92	0.6910
	Swine vs. Turkeys	2.12	0.79, 5.70	0.2082
	Cattle vs. Chickens	0.09	0.04, 0.18	<0.0001
	Cattle vs. Swine	0.03	0.02, 0.04	<0.0001
	Chickens vs. Swine	0.29	0.16, 0.53	<0.0001
After VFD rule changes				
	Cattle vs. Turkeys	0.19	0.12, 0.31	<0.0001
	Chickens vs. Turkeys	0.32	0.18, 0.57	<0.0001
	Swine vs. Turkeys	2.63	1.68, 4.12	<0.0001
	Cattle vs. Chickens	0.60	0.37, 0.99	0.0406
	Cattle vs. Swine	0.07	0.05, 0.10	<0.0001
	Chickens vs. Swine	0.12	0.08, 0.19	<0.0001

OR: odds ratio; CI: confidence intervals.

The present study identified the significant interactions between the VFD rule changes and host levels that imply the effect of VFD rule changes on the odds of detecting the outcome of interest were not the same across the host levels. The odds of detecting tetracycline-resistant *Salmonella* were significantly decreased in cattle following implementation of the VFD rule changes compared to cattle in the period before implementation. On the other hand, there has been a significant uptick in the odds of detecting tetracycline-resistant Salmonella in chickens, following the changes to the VFD regulations, compared to the period before their implementation. Additionally, the odds of detecting tetracycline-resistant *Escherichia* decreased significantly in chickens following the VFD rule changes in 2017 compared to the period prior to their implementation. In contrast, the odds of detecting tetracycline-resistant *Escherichia* were increased significantly in swine, following the changes to the VFD regulations, compared to the prior implementation period. Moreover, the odds of detecting erythromycin-resistant *Campylobacter* were significantly increased in cattle, following the changes to the VFD regulations, compared to the period before their implementation. In contrast, the odds of detecting erythromycin-resistant *Campylobacter* were significantly decreased in chickens, following the VFD rule changes, compared to the period before their implementation. The results of this study can assist in directing focused research and implementing measures to mitigate the risk of the emergence of antibiotic-resistant bacteria in food-producing animals that have a higher likelihood of the emergence of antibiotic-resistant bacteria.

Implementing the VFD rule changes has significantly decreased the likelihood of detecting tetracycline-resistant *Salmonella* in cattle. This can be attributed to various factors. For instance, the 2017 VFD rule changes have led to a potential decrease in tetracycline use in cattle production. A recent U.S. FDA's antibiotics sales report indicates that tetracycline sales decreased in cattle production following the 2017 VFD rule changes [12]. The VFD rule changes restrict using medically important antibiotics, including tetracycline, for growth promotion in cattle production. It requires veterinary supervision to use tetracycline for disease prevention and control in cattle production. As a result, reduced use of tetracycline may have reduced the selective pressure of the emergence of tetracycline-resistant *Salmonella* in cattle production in the U.S. [35-37]. A review study also reported that the reduction of antibiotic use in food-producing animals is associated with a reduction in the occurrence of antibiotic-resistant bacteria in food animals [38]. Furthermore, the observed favorable outcomes may be attributed to adopting improved biosecurity protocols, improved water, hygiene, and sanitation practices, and the utilization of vaccinations to manage infections in cattle production [39, 40]. Also, after the VFD rule changes, beef and dairy operators in Tennessee (USA) reported increased interactions with licensed veterinarians [41]. Similarly, Ohio (USA) cattle farmers reported a decrease in the use of feed antibiotics, more veterinarian-farmers interactions, and maintained record-keeping following the VFD rule changes [42]. This evidence suggests a positive link between implementing the VFD rule changes and reducing the likelihood of detecting tetracycline-resistant *Salmonella* in U.S. cattle production. Additionally, we have observed a clear downward trend in the occurrence of tetracycline-resistant *Salmonella* in cattle after 2018 (Figure 3.1). This trend implies that the effects of the 2017 VFD rule changes have positively impacted the occurrence of tetracycline-resistant *Salmonella* in cattle production within one year of implementing the rule changes. A study led by Stuart B. Levy et al. observed that after six months of stopping the use of tetracycline-supplemented feed in a chicken farm, the frequency of tetracycline-resistant *Escherichia* decreased compared to before the feed was removed [9]. Another study reported that avoparcin restriction regulations in Italy have decreased vancomycin-resistant enterococci found in poultry products [43]. Similarly, in the Netherlands, from 1997 to 1999, a reduction was observed in humans, broilers, and pigs following the restriction of avoparcin use [44]. The practical benefits of the VFD rule changes are evident in cattle production in the U.S. A recent review study pointed out that the European Union, notably Denmark and the Netherlands, have successfully implemented government regulations that have reduced antibiotic consumption in food animals. As a result, there has been a notable reduction in antibiotic-resistant bacteria among food animals [45].

The findings of our study indicate a higher likelihood of detecting tetracycline-resistant *Salmonella* in the cecal samples of chickens. This suggests that the use of tetracycline may contribute to the development of antibiotic-resistant *Salmonella* in chicken production [46]. The rise of tetracycline-resistant *Salmonella* in chickens may have various factors, including the use of antibiotics in chicken production. Studies have shown a relationship between antibiotic use in livestock, including chickens, and the emergence of antibiotic-resistant bacteria, which is attributed to the selective pressure exerted by antibiotics [47-49]. There is evidence of an association between the consumption of tetracycline and tetracycline-resistant enteric bacteria in Canadian turkey flocks [30], although the direction of association depends on the antibiotic class. It has been observed that the resistance of coliform bacteria to streptomycin in turkeys is linked to the consumption of streptomycin by the turkeys [47]. Our study results are consistent with previous findings that showed increased tetracycline-resistant *Salmonella* isolates in Canadian broiler chickens after implementing the Chicken Farmers of Canada's Antimicrobial Use Reduction Initiative [50]. Additionally, tetracycline-resistant *Salmonella* in chickens can be linked to direct and indirect exposure of tetracycline to chickens. Direct exposure to tetracycline can occur in chickens when treated with tetracycline. Tetracyclines are approved for therapeutic use in poultry production [51], including chickens in the U.S. [52]. In addition, environmental factors could also affect the occurrence of tetracycline-resistant *Salmonella* in chickens by exposure to higher levels of tetracycline in the environment (via drinking water, feed, litter, feces), leading to a higher occurrence of tetracycline-resistant *Salmonella* in chickens. Several studies have reported the presence of tetracycline-resistant bacteria in different farm environments. For instance, studies have reported the presence of tetracycline-resistant *Salmonella* in Florida poultry litter [53] and poultry farms in the southeastern U.S. [54]. Tetracycline-resistant *Escherichia* has been isolated from water, sediment, and biofilms in agricultural watersheds in Canada [55]. Furthermore, tetracycline-resistant *Salmonella* has been detected in poultry litter in Egypt [56]. Another explanation could be genetic factors; for example, *Salmonella* can carry more tetracycline-resistant genes, leading to higher odds of detecting tetracycline-resistant *Salmonella* in chickens' production. Also, epidemiological factors could be associated with the higher odds of detecting tetracycline-resistant *Salmonella* in chickens. For example, changes in the VFD rules have led to restrictions on the preventive use of tetracycline in chickens [10], which may contribute to higher odds of *Salmonella* infections. The higher odds of *Salmonella* infections lead to increased therapeutic use of tetracycline and selection pressure, leading to increased odds of detecting tetracycline-resistant *Salmonella* in chickens. A study found that treating chickens with tetracycline led to an increase in the occurrence of tetracycline-resistant *Salmonella* [46]. Other explanation can be various serovars of *Salmonella* exhibits distinct resistance phenotypes, thereby implying that the distribution of serovars of *Salmonella* can have an impact on this finding [57-59]. Our study did not account for serovar-specific data for *Salmonella* for analysis. Therefore, this limitation can be considered when interpreting overall *Salmonella* data. Other possible explanation can be the co-selection of resistance to tetracycline by exposure to other antimicrobial drugs or to chemicals (e.g., heavy metals, disinfectants) in the chicken's farms environment may explain this finding [60]. Further research is needed to understand why tetracycline-resistant *Salmonella* increased in chicken production compared to other food animal production following the VFD rule changes in the U. S.

Our study shows that the odds of detecting tetracycline-resistant *Escherichia* increased by 22% in the swine population after implementing the 2017 VFD rule changes. This finding can be explained by increased selection pressure due to the increasing use of tetracycline for therapeutic purposes after their restriction (as growth promoters) in swine production in the U.S. For instance, poor farm management, hygiene, and biosecurity practices can increase the chance of infectious diseases occurrence. The subsequent need for the therapeutic use of antibiotic (tetracycline) in swine production in the U.S. Existing studies consistently show a clear link between increased usage of antibiotics in swine and a higher occurrence of antibiotic-resistant *Escherichia* [61-63]. A recent U.S. nationwide monitoring study has demonstrated a high frequency (34 %) of tetracycline-resistant *Escherichia* isolates in swine at slaughter across the U.S. [24]. Future farm-level investigations could explore the factors associated with the tetracycline-resistant *Escherichia* as well as evaluate herd-level interventions, such as improving biosecurity measures and water, sanitation, and hygiene practices [40, 64] to reduce the usage of antibiotics in U.S. swine production.

On the other hand, our study revealed a decrease in tetracycline-resistant *Escherichia* in chickens and turkeys. This finding can be explained by decreased selection pressure due to the decreasing use of tetracycline as their restriction as a growth promoter in poultry production in the U.S. The U.S. FDA 2021 antibiotics sale report shows significant reductions in tetracycline sales in chicken and turkey production [12]. Evidence indicates a decrease in the use of tetracycline and a subsequent reduction in the prevalence of tetracycline-resistant *Escherichia* in broiler chickens following the implementation of the Chicken Farmers of Canada's Antimicrobial Use Reduction Initiative [50]. Another possible explanation could be that genetic mutations in *Escherichia's* DNA could reduce tetracycline-resistant development. The genetic mutation of *Escherichia* could be associated with decreased tetracycline-resistant [65]. There is evidence that genetic mutations of *Escherichia* are beneficial that prevent the induction of resistance mechanisms [65]. Other potential factors could be associated with this phenomenon. Further research is needed to understand this phenomenon fully.

Implementing the VFD rule changes has led to a significantly higher likelihood of detecting erythromycin-resistant *Campylobacter* in the cecal samples of cattle. Several factors could explain this study’s findings. First, to treat campylobacteriosis in cattle production, erythromycin or other macrolides such as tylosin can be administered more frequently, as they are the preferred initial treatment [66]. The higher frequency of therapeutic use of erythromycin or other macrolide increases the selection pressure for erythromycin-resistant *Campylobacter* [67] in cattle. The U.S. FDA’s recent report indicates that erythromycin sales increased in cattle production after the 2017 VFD rule changes. There is evidence relationship between the use of macrolides (such as tylosin and erythromycin) and the emergence of erythromycin-resistant *Campylobacters* in foods of animals origin [68]. Second, increased genetic mutation in *Campylobacter* could be associated with the erythromycin-resistant *Campylobacter* isolated from cecal samples of cattle. There is evidence that macrolide-resistant *Campylobacter* is associated with natural point mutations occurring in the peptidyl-encoding region in domain V of the 23 S rRNA gene, which is the target of macrolides [69, 70]. Further farm-level studies are needed to evaluate the risk factors associated with the higher likelihood of detecting erythromycin-resistant *Campylobacter* in the cecal samples of cattle.

On the contrary, the VFD rule changes were associated with lower odds of detecting erythromycin-resistant *Campylobacter* in chicken cecal samples. The implementation of the VFD now requires a prescription for purchasing erythromycin or any other macrolide, rendering them inaccessible for preventive use in chickens. This change in VFD rules can be attributed to decreased utilization of erythromycin, which helps reduce the selective pressure driving the emergence of erythromycin-resistant *Campylobacter* in chickens. Recent data from the U.S. FDA's antibiotics sales report shows a decline in erythromycin sales for chicken production following the 2017 VFD rule changes [12]. Improved on-farm biosecurity, encompassing measures like sanitation, hygiene practices, and clean water access, may explain the decrease in erythromycin-resistant *Campylobacter* in chickens. Such biosecurity improvements are associated with a decline in infections and a subsequent reduction in antibiotic usage. Consequently, the occurrence of erythromycin-resistant *Campylobacter* in chickens experiences a positive impact. A recent systematic review highlighted that interventions, such as on-farm biosecurity and water, sanitation, and hygiene practices, can directly or indirectly lower infection frequency and minimize antibiotic usage in animal agriculture settings [40].

This study had several strengths, including a large sample size and representative sampling. It also included information about food-producing animal hosts, which helped to understand the impact of VFD rule changes on different food animals production, such as cattle, chickens, swine, and turkeys. To test for effect modification, we analyzed the interactions of main effects with VFD rule changes and host levels. Additionally, we used multiple comparison procedures to reduce the risk of false positive statistical inference (type 1 error). Moreover, since no data was available on specific antibiotic exposure from the food animals from which cecal samples were taken, it was impossible to analyze the association between antibiotic exposure and antibiotic-resistant bacteria. Also, the absence of geographic location data may have a potential confounding effect on the outcome of these study. Also, the absence of demographic (age, sex, and breed) and health status (apparently healthy/sick) of the sampled animals might explain differences in outcomes. Hence, we recommend that future surveillance datasets include antibiotic exposure, geographic location, demographic, and health information for enhanced statistical analysis. Additionally, to analyze the patterns of odds of detecting antibiotic-resistant bacteria in cecal samples of food-producing animals, we initially examined both the year and years of sampling using a univariable logistic regression model. Additionally, we visually assessed the linear trend of the proportion of antibiotic-resistant bacteria in cecal samples by years of sampling. However, the estimated odds ratios by years of sampling categories did not demonstrate a consistent linear pattern across all comparisons (**Table 3.2, Table 3.4, Table 3.6, and Table 3.8**). Furthermore, no linear trend of the overall proportion of antibiotic-resistant bacteria in cecal samples was observed based on our graphical analysis **(Figure 3.5, Figure 3.6, Figure 3.7, and Figure 3.8).** These non-linear relationships suggest that the impact of years of sampling on the odds of detecting antibiotic-resistant bacteria in cecal samples vary across different points of comparison. Considering these observations, we analyzed the variable "VFD rule change" as a reasonable approach. We categorized data into two groups: "before VFD rule change (2013-2016)" and "after VFD rule change (2017-2019)". Our research question aimed to investigate significant differences in antibiotic-resistant bacteria in cecal samples, and the "VFD rule change" variable allowed us to examine the overall effect of the period after the implementation of VFD rule changes compared to the reference period (pre-VFD period: 2013-

2016). Furthermore, collapsing these years into binary variables increased the category's sample size, thereby improving our analysis's statistical power. Furthermore, our study is constrained by a limited timeframe of only two years of post-VFD rule changes data, which limits our ability to comprehensively evaluate the long-term effects of these changes on antibiotic-resistant bacteria isolates in food-producing animals. To overcome this limitation, acquiring a dataset that encompasses a broader range of periods following the implementation of the VFD rule changes in future research endeavors is worthwhile. Despite these limitations, this study provides valuable information on whether the changes in the Veterinary Feed Directive (VFD) rule would lead to a decrease in medically important antibiotic-resistant bacteria in cecal samples obtained from food-producing animals at slaughterhouse facilities in the U.S.

Conclusions

The implementation of VFD rule changes has been beneficial in reducing the occurrence of tetracycline-resistant *Escherichia* and erythromycin-resistant *Campylobacter* in cecal samples obtained from chickens, as well as tetracycline-resistant *Salmonella* in cecal samples obtained from cattle. These findings underscore the significance of ongoing efforts to encourage the responsible and judicious use of medically important antimicrobials in food-producing animals. Additionally, continued monitoring of antimicrobial resistance and antimicrobials usage can aid in supporting stewardship efforts, such as the VFD rule for food-producing animals in the U.S. Such measures are crucial in combating the emergence and dissemination of antimicrobial-resistant bacteria. Nevertheless, it is important to note that there was a notable increase observed in tetracycline-resistant *Salmonella* in cecal samples obtained from chickens, tetracycline-resistant *Escherichia* in cecal samples obtained from swine, and erythromycin-resistant

Campylobacter among in cecal samples obtained from cattle. Further investigation is warranted to understand the underlying factors contributing to the rise of specific antimicrobial-resistant bacteria in particular groups of food-producing animals following the implementation of VFD rule changes in the U.S.

Acknowledgments

The authors thank the National Antimicrobial Resistance Monitoring System (NARMS) as the data source. We want to thank the Comparative and Experimental Medicine Program of the University of Tennessee, Knoxville, for supporting this study.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable. Our study did not directly involve humans or animals.

Data Availability Statement: All the datasets used in this study are secondary datasets, and the reference to access the datasets are given in this article. These data sets are publicly available, and no specific rights are required to access these datasets.

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CONCLUSIONS, RECOMMENDATIONS, AND FUTURE RESEARCH DIRECTIONS

General conclusions

To my knowledge, this is the first study that analyzes three national surveillance datasets to determine quantitatively how the VFD rule changes have impacted the occurrence of violative residues of antibiotics and antibiotic-resistant bacteria in food-animal tissues, retail meats, and cecal samples from food animals compared to the period before implementing the VFD rule changes in the U.S. In this dissertation, many lessons were learned from analyzing the three national surveillance datasets. The implementation of VFD rule changes significantly reduced the prevalence of violative sulfonamide and penicillin residues in food animal tissues, which suggests an increased likelihood of food animal producer's compliance to label withdrawal time of injectable sulfonamide and penicillin, which are relatively short withdrawal period (sulfonamides: 5 days; penicillin G: 4 to 10 days). On the other hand, implementing VFD rule changes did not significantly reduce the prevalence of violative tetracycline residues in food animal tissues, suggesting an increase in the use of injectable tetracycline for therapeutic and control purposes that should be further investigated. Moreover, food animal producers may lack compliance to label withdrawal time of injectable tetracycline, which is a relatively lengthy withdrawal period of 28 days. Besides the implementation of VFD rule changes, other areas that would need improvement include increased veterinary-client-patient relationships, educational training and campaigns about the responsible use of injectable antibiotics (dose, route, duration, withdrawal time, treatment record keeping, proper disposal of unused antibiotics) for food animal producers, and a greater number of food animal veterinarians, to reduce the risk of occurrence of violative antibiotic residues in food animal products.

It was observed that the prevalence of tetracycline-resistant bacteria decreased in retail chicken and turkey meats following the implementation of the VFD. This finding suggests that usage of tetracycline decreased in chicken and turkey production, which is supported by the evidence in this dissertation that the prevalence of tetracycline-resistant bacteria decreased in cecal samples obtained from chickens and turkeys. Additionally, the VFD rule changes likely influenced poultry and turkey producers to develop and implement a plan for preventing disease in their flocks. This led to a greater focus on vaccination and other preventive measures, which helped to reduce the need for antibiotics and subsequently reduced selection pressure. In addition, the use of medically important in-feed antimicrobials could decrease in the poultry industry with the increase in demand for antibiotic-free/raised-without-antibiotics poultry products and improved antibiotic stewardship. The observed reduction of tetracycline-resistant bacteria in retail chicken and turkey meats can help protect public health by reducing the risk of exposure to tetracycline-resistant bacteria through chicken and turkey meats.

Another important finding was that the implementation of VFD rule changes did not significantly impact on the prevalence of tetracycline-resistant *Salmonella* and *Escherichia* in beef and pork. Additionally, the increased prevalence of tetracycline-resistant *Escherichia* was observed in the cecal samples of swine. These findings can be explained that there could be a potential surge in the usage of injectable tetracycline for therapeutic and control purposes in cattle and swine production, which is evident from the lack of reduction in the violative tetracycline residues in food animal tissues that should be further investigated to determine the long-term impact of the VFD rule changes on antibiotic use and antibiotic-resistant bacteria in cattle and swine production. The occurrence of tetracycline-resistant *Salmonella* in cecal samples of cattle and tetracycline-resistant *Escherichia* in the cecal sample of swine pose a potential risk of environmental contamination with tetracycline-resistant bacteria through fecal materials of cattle and swine. In addition to implementing VFD rule changes, multi-sectoral coordinated educational interventions to cattle and swine producers concerning withdrawal periods of injectable antibiotics, record-keeping, compliance with label instructions of antibiotics, vaccination, and other preventive measures are critical. Such a holistic approach would help to reduce violative antibiotic residues and bacteria resistant to medically-important antimicrobials in food animal products in the U.S.

Recommendations

Judicious use of medically important antimicrobials is critical in controlling the emergence of bacteria resistant to medically-important antimicrobials in food-producing animals. In order to tackle the problem of bacteria resistant to medically-important antimicrobials in food-producing animals and their products, below are some recommendations for future consideration.

1. The odds of detecting bacteria resistant to medically-important antimicrobials in food animals and their products did not show the same direction of association with the VFD rule changes in these studies, suggesting variations in antimicrobial use practices across different food animals' production. It is crucial to employ judicious antimicrobial usage at the farm level, guided by culture and susceptibility testing, to minimize selection pressure and slow the development of AMR.
2. Increasing the number of variables in the cecal sample surveillance dataset is recommended to improve the statistical analysis. The current dataset has limited

variables; therefore, additional data should be collected to include antibiotic exposure, geographic location, demographic information (such as age, sex, and breed), and health status information. These additional variables can provide valuable insights and enable a more comprehensive analysis of the surveillance data. It could help better understand the relationship between the prevalence of antibiotic-resistant bacteria and associated factors.

3. The antibiotic residue surveillance dataset had a limited number of variables. It is recommended to expand the dataset by including animal-level information such as age, sex, breed, pathologic lesion or signs, and location (e.g., state/county) of sampled animals. This additional information will facilitate the investigation of factors associated with violative antibiotic residue and any potential interaction effect on the outcome variables.

4. Implement a nationwide campaign to raise awareness among food-producing animal producers about the judicious use of medically important antimicrobials. In addition, effort should be made to improve the veterinary-client-patient relationship throughout the production cycle.

Future research directions

Future research should focus on the following areas:

1. This research is constrained by a limited timeframe of only two years of post-VFD rule changes data, which limits our ability to comprehensively evaluate the long-term effects of these changes on bacteria resistant to medically-important antimicrobials in food-producing animals and violative antibiotic residue in food animal tissue. To overcome

this limitation, acquiring a dataset that encompasses a broader range of periods following the implementation of the VFD rule changes in future research endeavors is worthwhile.

2. Future studies are warranted to investigate the prevalence and practices of injectable antibiotic administration, including extra-label use, treatment documentation/records, and knowledge of antibiotic withdrawal periods in cattle and swine production to characterize the injectable antibiotic usage practices at the farm level following the implementation of the VFD rule changes in the U.S.
3. Future research should evaluate host- and farm-level risk factors associated with the prevalence of violative residues in food animal products and antimicrobial-resistant zoonotic enteric bacteria in food-producing animals to develop an evidence-based farm-level intervention.
4. Future studies should be conducted to identify barriers to practicing the judicious use of antimicrobials at the farm level. This research can help develop targeted interventions and practical guidelines to minimize the barriers and contribute to mitigating the risks of antimicrobial resistance.
5. Future research should examine the effect of implementing the VFD rule changes on the prevalence of bacteria resistant to medically-important antimicrobials in humans and environment in the U.S.

VITA

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Sarkar is interested in furthering his career in research in applied epidemiology and public health. His research and career interests are in emerging and zoonotic infectious diseases epidemiology, epidemiology of transboundary emerging diseases, antimicrobial stewardship program in livestock production, food safety epidemiology, one health approach, and evaluation context and culture-appropriate practical intervention studies to reduce the risk of zoonotic and transboundary emerging diseases and the emergence of antimicrobial resistance in livestock production and human health.