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Antimicrobial use regulations are associated with increased susceptibility among bovine *Salmonella* isolates from a U.S. surveillance system

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ABSTRACT

Health authorities around the world have called to limit antimicrobial use in food-producing animals. In the United States, two recent regulatory actions have changed the use of antimicrobials in livestock, banning production uses in 2017 and restricting extra-label use of cephalosporins in 2012. This study aimed to assess the impact of the 2012 and 2017 regulations on antimicrobial use in cattle in the United States by analyzing 18,627 bovine *Salmonella* AMR susceptibility patterns using data from the National Antimicrobial Resistance Monitoring System (NARMS). Logistic regression was used to model the odds of being a susceptible isolate. Additionally, interval-censored accelerated failure time (AFT) models were used to analyze changes in minimum inhibitory concentrations (MICs) over time and by serotype. The most common serotypes were Montevideo ($n = 3003$), Anatum ($n = 1394$), Cerro ($n = 1373$), and Typhimurium ($n = 1213$). Susceptibility was highest for azithromycin (99 %), ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole (all 98 %), and lowest for tetracycline (76 %), chloramphenicol (86 %), and ampicillin (85 %). Serotypes Typhimurium, Newport, and Dublin exhibited lower susceptibility compared to other serotypes. Susceptibility to all antimicrobials increased during the periods 2013–2017 and 2018–2022 compared to isolates before 2012, with a greater increase in 2018–2022. MICs decreased for most antimicrobials except for chloramphenicol and gentamicin, which showed increased median MIC for the periods 2013–2017 and 2018–2022, respectively. In conclusion, antimicrobial use restrictions appear correlated with a reduction in *Salmonella* AMR, although this effect cannot be untangled from the effect of time in this dataset.

1. Introduction

Salmonella enterica is a significant global contributor to diarrheal diseases [1], with an estimated 1.2 million infections occurring annually in the United States alone [2]. Non-typhoidal *Salmonella* can be hosted or carried by many animal species and may further contaminate the environment, water, and food [3]. While symptoms of salmonellosis typically manifest as self-limiting enteritis, certain demographics, such as children and the elderly, are susceptible to severe dehydration and invasive infections, which can be life-threatening [1].

Antimicrobial resistance (AMR) in *Salmonella* poses a significant global public health concern, given the emergence of resistant serotypes [1]. Infections caused by multidrug-resistant (MDR) *Salmonella* strains exhibit a two-fold higher mortality rate compared to susceptible strains [4]. AMR and MDR *Salmonella* have been associated with antimicrobial use (AMU) in food-producing animals and subsequent transmission to humans (Bennani et al., 2020). For instance, a study found that 70 % of MDR *Salmonella* outbreaks in the United States were linked to land animal-derived foods [5]. In California, one study reported a 50 % MDR prevalence among *Salmonella* isolates from cattle fecal samples, with higher MDR probabilities observed in serotypes Newport, Typhimurium, and Dublin [6]. The same study reported decreasing resistance to most antimicrobial drug classes from 2002 to 2016, except for cephalosporins and quinolones [6].

Many countries have implemented legislation to reduce AMU in agriculture. The restrictions range from elimination of some

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Table 1
Number and proportion [n (%)] of minimum inhibitory concentration (μg/mL) for each antimicrobial. Color indicates the interpretations as susceptible (green), intermediate (orange), and resistant (red), according to NARMS breakpoints [15].

Antimicrobial (n tested)	Minimum inhibitory concentration (mg/L) [n (%)]										
Amoxicillin-clavulanic acid (n=16,141)	≤ 1	=1	= 2	= 4	= 8	= 16	= 32	> 32			
	9,962 (62)	3,328 (20)	547 (3)	93 (1)	423 (3)	288 (2)	249 (2)	1,341 (8)			
Ampicillin (n=16,253)	≤ 1	≤2	= 2	= 4	= 8	= 16	= 32	> 32	= 64	> 64	
	9,439 (58)	3,369 (21)	834 (5)	224 (1)	13 (<1)	4 (<1)	7 (<1)	2,334 (14)	1 (<1)	28 (<1)	
Ceftriaxone (n=16,253)	≤ 0.25	= 0.5	= 1	= 2	= 4	= 8	= 16	> 16	= 32	= 64	> 64
	14,620 (90)	18 (<1)	8 (<1)	16 (<1)	57 (<1)	311 (2)	742 (5)	1 (<1)	381 (2)	77 (1)	22 (<1)
Cefoxitin (n=14,332)	≤ 1	= 1	= 2	≤ 4	= 4	= 8	= 16	> 16	= 32	> 32	
	62 (<1)	471 (13)	5,262 (37)	1,888 (13)	4,415 (31)	656 (5)	171 (1)	279 (2)	447 (3)	680 (5)	
Azithromycin (n=7,808)	≤ 1	= 2	= 4	= 8	= 16	> 16	= 32	> 32	= 64	> 64	
	9 (<1)	1,066 (14)	5,524 (71)	1,147 (15)	39 (1)	2 (<1)	3 (<1)	5 (<1)	7 (<1)	6 (1)	
Chloramphenicol (n=16,253)	≤ 2	= 4	<= 4	= 8	= 16	= 32	> 32				
	147 (1)	4,681 (29)	3,292 (20)	5,921 (36)	134 (1)	160 (1)	1,918 (12)				
Ciprofloxacin (n=16,253	≤ 0.015	= 0.03	= 0.06	= 0.125	= 0.25	= 0.5	= 1	> 4			
	14,410 (89)	1,531 (9)	57 (<1)	56 (<1)	102 (1)	90 (1)	6 (<1)	1 (<1)			
Gentamicin (n=16,252)	≤ 0.25	= 0.5	= 1	= 2	= 4	= 8	= 16	> 16			
	6,562 (40)	7,906 (49)	1,376 (9)	101 (1)	20 (<1)	59 (<1)	97 (1)	131 (1)			
Tetracycline (n=16,252)	≤ 4	= 8	<= 8	= 16	> 16	= 32	> 32	= 64	> 64		
	11,783 (73)	46 (<1)	658 [a] (4)	210 (1)	216 (1)	598 (4)	2,696 (17)	33 (<1)	12 (<1)		
Trimethoprim-sulfamethoxazole (n=16,252)	≤ 0.125	= 0.125	≤ 0.25	= 0.25	= 0.5	= 1	= 2	= 4	> 4		
	13,347 (82)	442 (3)	1 (<1)	1,822 (11)	278 (2)	26 (<1)	14 (<1)	22 (<1)	300 (2)		

[a]Not interpretable concentration.

antimicrobial uses or individual antimicrobials to complete restriction of AMU in livestock [7]. The impact of these restrictions on AMR in animals and humans is challenging to measure and has been primarily studied in Europe and North America [7,8]. A meta-analysis of AMU restrictions around the world prior to 2016 found that they reduced AMR in some host-pathogen-antimicrobial combinations but not in others [8]. The association was particularly strong for humans with direct exposure to food animals [8]. Broader restrictions had a larger effect on AMR than narrow restrictions (e.g., single antibiotic restrictions) [7].

In the United States, the Food and Drug Administration (FDA) restricted extra-label uses of cephalosporins in 2012, prohibiting the use of cephalosporin drugs at unapproved dose, frequency, duration, and route of administration, as well as prohibiting using cephalosporins for disease prevention. The use of cephalosporins to treat or control extra-label disease indications is still allowed [9]. Subsequently, in 2017, the FDA transitioned in-feed and in-water medically important antimicrobials used in food-producing animals from over-the-counter status to prescription status, requiring veterinary oversight, and eliminated production uses [10]. Antimicrobial sales for use in food producing animals decreased by 36 % from 2015 to 2023 [11]. This study aimed to assess the impact of these regulatory actions on AMR by analyzing bovine *Salmonella* susceptibility patterns using data from the National Antimicrobial Resistance Monitoring System (NARMS), with a focus on the years coinciding with regulatory changes.

2. Methods

The NARMS dataset was downloaded from the official website on December 3, 2024, available at https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/integrated-reportssummaries. NARMS is a national public health surveillance system established in 1996 to monitor trends in AMR among foodborne enteric bacteria from humans, retail meats, and food animals. The program focuses on meat and poultry products available at retail and on food animals at slaughter with intestinal (cecal) content samples and routine raw product verification (HACCP and ground beef) [12]. Cecal samples are randomly selected from the lots of animals in the slaughterhouses [13]. Prior to 2006, HACCP samples were collected from both random sampling of eligible establishments and non-random sampling from establishments that did not meet HACCP performance standards. After 2006, sampling was risk-based, focused on establishments with higher prevalence of *Salmonella* [12]. Starting in 2014, *Salmonella* surveillance was paired with Shiga toxin-producing *Escherichia coli* surveillance, which uses a random and systematic sampling approach [14].

Salmonella isolates from cattle were selected from the "HACCP 1997–2005", "HACCP 2006-present", and "Cecal 2013-present" databases, resulting in 18,627 observations. Amoxicillin-clavulanic acid, ampicillin, ceftriaxone, cefoxitin, tetracycline, gentamicin, azithromycin, chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole were selected for analysis. Isolates were classified as susceptible using the NARMS breakpoints (Table 1) [15]. The proportion

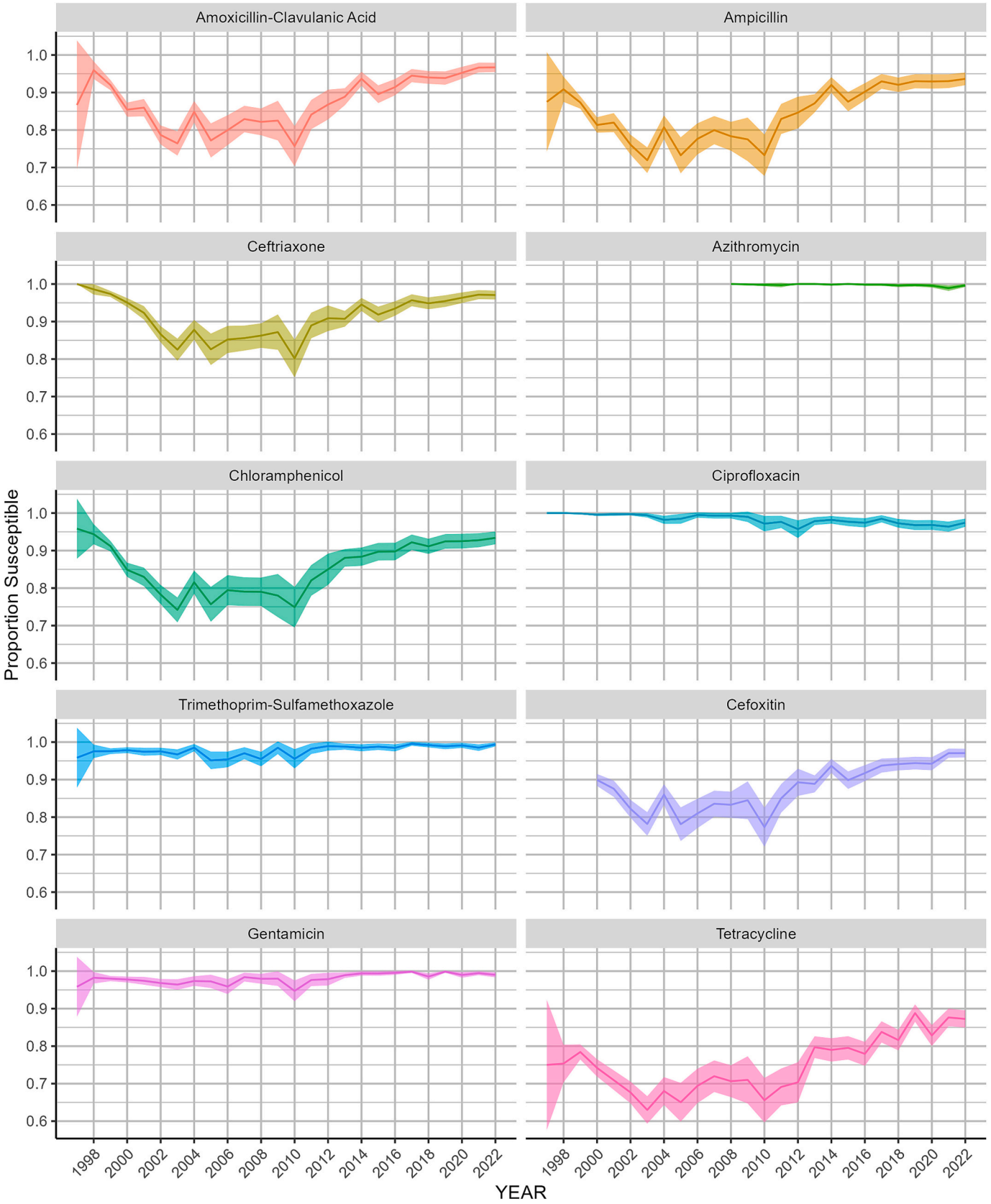

Fig. 1. Proportion (line) and 95 % confidence interval (shaded area) of *Salmonella* isolates susceptible to each antimicrobial per year.

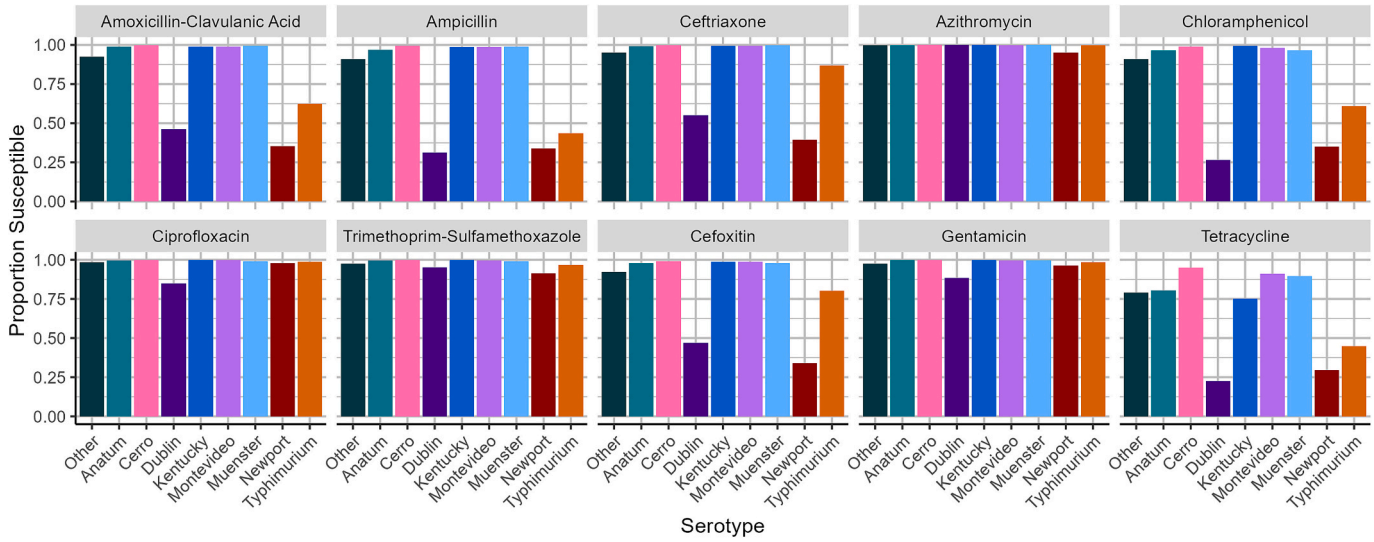

Fig. 2. Proportion of susceptible isolates per serotype to each antimicrobial. Bars are colored by serotype.

of susceptible isolates and a 95 % confidence interval (standard error = $\sqrt{\frac{p(1-p)}{n}}$, where p is the proportion and n is the number of isolates) were compared across the two data sources.

Descriptive analyses of AMR and serotype prevalence were conducted using frequency tables and bar plots. Susceptibility was modeled using logistic regression, with periods when regulations changed (i.e., before 2012, 2013–2017, and 2018–2022) and serotype group as predictors. Serotypes Dublin, Newport, and Typhimurium were grouped in a single category to compare them with other serotypes, based on previous reports on higher AMR prevalence [16] and the exploratory analysis.

Significant interaction terms were retained. Azithromycin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole were excluded from modeling due to over 95 % susceptibility (Table 1). In addition, changes in minimum inhibitory concentrations (MICs) over time were analyzed using interval-censored accelerated failure time (AFT) models with the loglogistic survival distribution, as described in previous studies [17]. The MIC intervals were as follows. For MICs that were reported as "=" a value, the upper limit of the interval was the reported MIC value and the lower limit was half of the reported value (e.g., an MIC of =2 μg/dL had an interval of 1,2). For MICs that were reported as "≤" a value, to prevent predicted MICs of zero, the lower limit of the interval was selected randomly using a uniform distribution between 0 and the reported value and the upper limit was the reported value (e.g., an MIC of ≤1 μg/dL had an interval of r, 1, where r is a random number from 0 to 1, exclusive). For MICs that were reported as ">" a value, the reported value was the lower limit, and the interval was considered right-censored (e.g., upper limit of infinity). MICs for ceftriaxone, ciprofloxacin, and trimethoprim-sulfamethoxazole were not modeled because over 85 % of the MIC values were a single value (Table 1). AFT models were built with the *ic_par* function from the *icenReg* R package, which allows for right-censored intervals [18].

R code and data to reproduce the analysis are publicly available (doi: https://doi.org/10.5281/zenodo.14628972).

3. Results

The majority of the observations came from HACCP sampling (n = 11,850; 66 %). Cecal sampling encompassed 34 % of isolates, which were from beef (n = 3578) and dairy (n = 3199) cattle. The most common serotype was Montevideo (n = 3003), followed by Anatum (n = 1394), Cerro (n = 1373), and Typhimurium (n = 1213) (Appendix A Fig. A.1). The proportion of each serotype over time is shown in the Fig. A.2.

Table 2
Odds ratio (95 % confidence interval) from the logistic regression for the odds of an isolate being susceptible to the antimicrobials included in the National Antimicrobial Resistance Monitoring System (NARMS) dataset. Reference isolates were from pre-2012 and all other serotypes.

Antimicrobial	Intercept	Year 2013–2017	Year 2018–2022	Serotype: Dublin, Newport, or Typhimurium
Amoxicillin-clavulanic acid	15.67	2.99	7.64	0.06
	(14.23–17.31)	(2.30–3.96)	(5.31–11.49)	(0.05–0.07)
Interaction: year and serotype		0.35	0.20	
		(0.24–0.48)	(0.12–0.31)	
Ampicillin	14.45	1.83	2.33	0.03
	(13.27–15.77)	(1.58–2.13)	(1.99–2.74)	(0.03–0.04)
Ceftriaxone	27.47	2.31	3.58	0.07
	(24.26–31.26)	(1.70–3.23)	(2.53–5.22)	(0.06–0.08)
Interaction: year and serotype		0.36	0.37	
		(0.23–0.57)	(0.22–0.61)	
Cefoxitin	13.02	3.00	6.11	0.10
	(11.78–14.43)	(2.34–3.89)	(4.49–8.54)	(0.08–0.11)
Interaction: year and serotype		0.29	0.23	
		(0.20–0.40)	(0.15–0.34)	
Chloramphenicol	15.12	1.41	1.75	0.05
	(13.87–16.51)	(1.22–1.63)	(1.50–2.03)	(0.04–0.05)
Tetracycline	4.05	1.52	2.12	0.11
	(3.83–4.29)	(1.37–1.69)	(1.90–2.37)	(0.10–0.12)

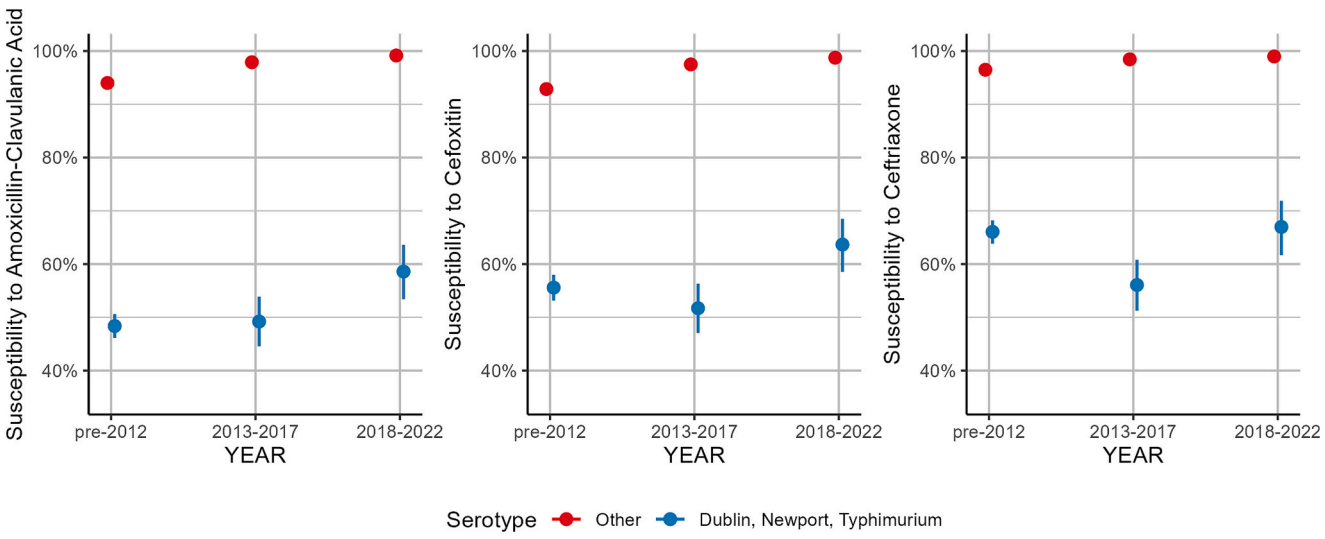

Fig. 3. Predicted prevalence and 95 % confidence intervals of susceptibility to amoxicillin-clavulanic acid, cefoxitin, and ceftriaxone, showing the interaction between year and serotype, using logistic regression models.

3.1. Susceptibility

Overall antimicrobial susceptibility was highest for azithromycin (99 %), followed by ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole (98 %) (Table 1; Fig. 1). Conversely, susceptibility was lowest for tetracycline (76 %), followed by chloramphenicol (86 %) and ampicillin (85 %). Fig. 1 illustrates *Salmonella* susceptibility per year to all the antimicrobials included in this analysis. Serotypes Dublin, Newport, and Typhimurium consistently showed lower susceptibility to most analyzed antimicrobials compared to other serotypes (Fig. 2). There was one percentage point or less difference in the average proportion of susceptible isolates between the cecal and HACCP datasets after 2013 for azithromycin, trimethoprim-sulfamethoxazole, and gentamicin (Appendix A Fig. A.3). There were larger but still modest differences for the β-lactam antimicrobials (amoxicillin-clavulanic acid, 7 percentage points; ampicillin, 8; ceftriaxone, 5; cefoxitin, 6), chloramphenicol (9 percentage points), and ciprofloxacin (5 percentage points). Given that these were relatively small differences compared to the longitudinal trends, the cecal and HACCP datasets were combined for the analysis. There was a larger difference in tetracycline susceptibility (14 percentage points), which was explored further in model sensitivity analyses. Overall, the cecal dataset showed higher susceptibility than the HACCP dataset.

3.2. Logistic regression model for susceptibility

A logistic regression model was applied to six out of ten antimicrobials analyzed. The results revealed that susceptibility to most of the antimicrobials increased during the periods of 2013–2017 and 2018–2022 compared to the baseline (pre–2012) (Table 2), with the 2018–2017 period always the larger odds ratio. The exception was ceftriaxone, where the significant interaction between the serotype group (Dublin, Newport, and Typhimurium) and the year category revealed a reduction in susceptibility for the Dublin group in the 2013–2017 period (Fig. 3). There were also significant interactions for amoxicillin-clavulanic acid and cefoxitin; there was a larger increase in susceptibility over time for these serotypes compared to other serotypes (Fig. 3). The 2018–2022 period generally had a higher odds ratio than the 2013–2017 period (Table 2, Fig. 3). The logistic model was not fit for azithromycin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole due to more than 95 % of the isolates being susceptible (Table 1). Inclusion of the data source (i.e., HACCP vs cecal isolates) in the tetracycline model reduced the magnitude of the year period odds ratios but did not change the direction of the associations; the 2013–2017 period was no longer statistically significant (Appendix A Table A.1). As expected, HACCP isolates had lower odds of susceptibility to tetracycline than cecal isolates.

Table 3
Minimum inhibitory concentration ratio (95 % confidence interval) from the accelerated failure time models. The reference group was *Salmonella* spp. isolated before 2012 and all other serotypes.

Antimicrobial	Intercept [a]	Year 2013–2017 [b]	Year 2018–2022 [b]	Serotype: Dublin, Newport, or Typhimurium [b]
Amoxicillin-clavulanic acid	0.97 (0.06–0.99)	0.81 (0.79–0.84)	0.78 (0.76–0.81)	11.36 (10.76–11.99)
Ampicillin	1.26 1.24–1.29	0.72 (0.69–0.75)	0.70 (0.68–0.73)	33.73 (31.80–35.78)
Cefoxitin	2.42 (2.38–2.46)	0.96 (0.92–0.99)	0.92 (0.89–0.95)	2.65 (2.51–2.79)
Azithromycin	2.87 (2.84–2.90)	0.89 (0.85–0.94)	0.97 (0.92–1.03)	1.02 (0.99–1.05)
Chloramphenicol	4.91 (4.85–4.98)	1.25 (1.21–1.29)	1.18 (1.14–1.21)	4.70 (4.49–4.93)
Gentamicin	0.28 (0.28–0.28)	1.47 (1.44–1.50)	1.43 (1.40–1.46)	1.01 (0.99–1.04)
Tetracycline	3.99 (3.92–4.06)	0.89 (0.86–0.93)	0.81 (0.78–0.84)	6.79 (6.43–7.18)

[a] The intercept (alpha) represents the median minimum inhibitory concentration (MIC_{50}) of the baseline group.
[b] Minimum inhibitory concentration ratio represents the expected change in the median minimum inhibitory concentration (MIC_{50}) assuming significance.

3.3. Accelerated failure time models

Consistent with the logistic regression models, the AFT models showed that MICs for β-lactams and tetracycline decreased in the years 2013–2017 and 2018–2022 compared to the reference category (before 2012) (Table 3). However, MICs for gentamicin and chloramphenicol increased in the same time periods. Serotypes Dublin, Newport, or Typhimurium exhibited MICs much higher than other serotypes. The serotype group was not a significant predictor for azithromycin and gentamicin MICs. Ceftriaxone and trimethoprim-sulfamethoxazole were not modeled because over 90 % of the MICs were a single value.

4. Discussion

This study analyzed *Salmonella* isolates from the NARMS dataset, with a focus on associations with AMU regulatory changes in the United States. The main findings indicate that *Salmonella* susceptibility has increased over the years (Table 2, Fig. 3) and the MICs decreased between 10 % to 20 % for most antimicrobials (Table 3). There tended to be a larger increase in susceptibility or decrease in MICs for 2018–2022 compared to 2013–2017, suggesting that the broader veterinary feed directive AMU restrictions had a larger impact on AMR than the narrow cephalosporin restrictions, consistent with other studies on broad vs. narrow AMU restrictions [7,8]. A study that analyzed cecal *Salmonella* isolates from NARMS in 2013–2016 vs 2017–2019 found that the odds of tetracycline resistance in cattle isolates was lower in the later time period, consistent with our findings (Table 2, Table 3) [19]. Another study examined combined cecal, HACCP, and retail meat *Salmonella* isolates from NARMS in 2012–2015 vs 2016–2019. The analysis also revealed that AMR trends varied by serotype (e.g., very few statistically significant differences in resistance for Dublin vs increasing susceptibility to several antimicrobials for Montevideo) [20].

The overall susceptibility was high (above 80 %) for all antimicrobials except for tetracycline. Susceptibility to ceftriaxone was high, including increasing susceptibility in Dublin, Typhimurium, and Newport isolates (Fig. 3), suggesting that ceftriaxone remains an effective empiric treatment for human salmonellosis [2]. In contrast, other studies have identified a higher prevalence of *Salmonella* resistance to most antimicrobials [21,22]. The differences in susceptibility could be related to the sampling frame, breakpoint application, and laboratory methodologies. The data used in the present study come from national surveillance, in which selection of samples is expected to be independent of likelihood of resistance when sampling is done randomly. Samples from diagnostic laboratories may be biased toward resistant isolates because samples are from animals with clinical disease and it is more common to submit samples after treatment failure [23]. This study used the NARMS breakpoints, which are based on MIC breakpoints for human pathogens from the Clinical and Laboratory Standards Institute [24]. The application of veterinary-specific breakpoints, which are considerably lower, would result in a higher proportion of reported resistance. For example, the breakpoint for amoxicillin-clavulanic acid is 0.5 μg/mL for Enterobacterales in the veterinary guidelines (CLSI, 2024), whereas the NARMS breakpoint was 4 μg/mL. Some studies may not report MIC values at all, which impedes future comparisons with different breakpoint applications. These differences in breakpoints make it challenging to directly compare susceptibility results from this study with other veterinary studies, but overall susceptibility proportions are similar to those reported for humans [25].

A combination of methods to analyze susceptibility and MICs as quantitative data enabled the identification of changes in the MICs that could not be reflected in the susceptibility analysis [17]. In this analysis, the odds of being susceptible to chloramphenicol were higher after 2012 (Table 2). However, the AFT model for chloramphenicol indicated an increase in the MIC for the periods of 2013–2017 and 2018–2022, respectively, compared to before 2012 (Table 3). This could be related to bacterial changes in its tolerance to chloramphenicol (e.g., a shift in MIC values within either the susceptible or resistant categories) or an artifact due to changes in the MIC panels used to determine antimicrobial susceptibility (i.e., some isolates were tested down to 2 μg/dL of chloramphenicol and others were tested only to 4 μg/dL). Previous studies have also indicated an increase in chloramphenicol resistance in *Salmonella* isolated from humans in Iran (1995 to 2019) [26] and China (2014 to 2021) [27]. *Salmonella* isolates resistant to chloramphenicol have been associated with resistance to other antimicrobials such as ampicillin, streptomycin, and tetracycline [28]. Florfenicol, related to chloramphenicol, has several labeled indications for cattle [29], which could lead to selection pressure on gastrointestinal flora, including *Salmonella*. The increasing MICs underscores the need for ongoing monitoring and research to address potential AMR development.

The combination of analytic approaches also enables identification of trends for antimicrobials with very low prevalences of resistance. Gentamicin had very high susceptibility and therefore was not suitable for logistic regression models. However, gentamicin MICs increased, although the relevance of this finding to human health is unclear since gentamicin is not used to treat human salmonellosis. There are no approved uses for aminoglycosides in cattle. However, a study of *Salmonella* isolated from humans and chickens found that aminoglycoside resistance genes sometimes resided on plasmids with β-lactam, tetracycline, and phenicol resistance genes, thus co-selection for aminoglycoside resistance could occur following the use of other antimicrobials [30]. It is important to consider that, according to CLSI, *Salmonella* susceptibility should not be reported for aminoglycosides and first- and second-generation cephalosporins and cephamycins, even though they may appear active *in vitro*, due to lack of clinical efficacy in humans [24]. However, testing these antimicrobials is important for surveillance purposes to identify resistance patterns and mechanisms, to guide policy and regulatory changes, and for public health preparedness [31].

These results are in agreement with previous research demonstrating that antimicrobial resistance in *Salmonella* is highly dependent on the serotype [22], with lower susceptibility and significantly higher MICs for the serotypes Dublin, Newport, and Typhimurium. These serotypes are often characterized by the presence of the plasmid-mediated AmpC gene (bla_{CMY}) and class I integrons, which are associated with resistance to ampicillin, cephalosporins, chloramphenicol, streptomycin, sulfonamide, and tetracycline [32]. Serotype Dublin isolates have little genetic diversity, frequently carry multiple AMR genes, and often carry the IncA/C2 plasmid, which confers MDR [21,33]. A recent genomic analysis of NARMS *Salmonella* isolates identified that all serotype Newport isolates analyzed carried the MDR IncC plasmid [34]. In the same study, Typhimurium was one of the serotypes with more diversity of AMR-inserted plasmids, which confer resistance to many antimicrobial types and higher likelihood of MDR [34]. Similarly, one study associated resistance to chloramphenicol, ampicillin, streptomycin, and tetracycline with *Salmonella Typhimurium* [28], which is one of the most common serotypes worldwide [35]. Over the last few decades, *Salmonella Typhimurium* strain DT104 has evolved and rapidly disseminated across the globe [35]. This strain has the ability to develop MDR due to a genomic island that conferred improved adaptability [28]. *Salmonella Typhimurium* has been used as a sentinel serotype to monitor AMR trends in *Salmonella* [28].

Consistent with these results, previous studies have reported that *Salmonella* Newport and Typhimurium represent approximately 50 % of all *Salmonella* isolates from cattle [16]. *Salmonella* serotype Cerro and Dublin were the most frequently isolated in clinical samples from the northeastern United States between 2007 and 2021 [22]. The emergence of *Salmonella* Dublin is a current concern for both veterinary and human medicine. Although *Salmonella* Dublin is a bovine-adapted serotype, human infections with this serotype are associated with invasive disease and septicemia. The case fatality rate for *Salmonella* Dublin has been reported as the highest compared to other *Salmonella* serotypes, and approximately six times greater than *Salmonella Typhimurium* [36]. The prevalence of *Salmonella* Dublin is reported to be increasing over time in cattle [22] and humans [37]. In the present analysis, there was a peak in the *Salmonella* Dublin prevalence in 2010 followed by a decreasing trend in the prevalence in the following years (Fig. A.2). These differences may be related to the surveillance nature of the data used in this study, compared to clinical isolates in other studies [22], which could overrepresent isolates that cause clinical disease in cattle. It is important to continue monitoring *Salmonella* Dublin and to further research the mechanisms behind its emergence.

This study has some limitations. First, it would have been ideal to include information about AMU, but this was not possible as biomass-adjusted cephalosporin sales for cattle are only available post-2016 from the U.S. Food and Drug Administration. The impact of the

cephalosporin restrictions in cattle may have been minor, since use for extra-label indications was still permitted. Studies that compare AMU and resistance are necessary to quantify the effect of bans and other measures aimed at reducing antimicrobial use on AMR. Finally, the effect of AMU restrictions cannot be separated from other effects of time in this dataset. It is possible that *Salmonella* AMR would have trended downward over this time period regardless of the AMU regulations. For example, changes in serotype or sequence type prevalence can drive temporal changes in resistance. Similarly, there is not an available control group where the regulations were not applied because they were applied uniformly across the United States.

Second, there is limited information about the host or geographic origin of the isolates, which could impact AMR patterns [21]. Although the NARMS database includes a variable that identifies the type of animal from which the isolate was obtained (e.g., calf, dairy cow, beef), this information is only available post-2013. Consequently, these data could not be integrated into the models comparing isolates before 2012, when the first regulations were implemented. This information should be included in future studies, especially if AMU data are available, since antimicrobials are used differently in beef vs. dairy cattle. For example, the Veterinary Feed Directive regulation in 2017 likely impacted beef cattle AMU more than dairy cattle [38] since there were no in-feed antimicrobials approved for use in adult dairy cattle [39].

Moreover, the sampling was not entirely random. Over half of the isolates came from HACCP samples, selected based on the risk of not meeting HACCP standards. While these standards are unrelated to AMR, the HACCP isolates were more resistant than the cecal isolates (Fig. A.3). The sensitivity analysis comparing HACCP and cecal samples found minimal differences in resistance between the two groups for most antimicrobials (Fig. A.3).

In conclusion, although AMU restrictions appear correlated with reduced *Salmonella* AMR, as evidenced by increased susceptibility and decreasing MICs for most antimicrobials over the past decade (except for chloramphenicol), it is impossible to attribute this change to the AMU restrictions alone vs. effects of time and serotype. Some serotypes have shown slower changes in AMR, with Dublin, Newport, and Typhimurium responsible for most resistant isolates. Continuous monitoring of *Salmonella* prevalence and AMR is essential, ideally with comprehensive data on hosts, origins, and unbiased sampling methodologies.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT, an AI language model developed by OpenAI, to proof-read the initial manuscript draft for grammar and spelling errors. Microsoft Co-pilot, an AI language model developed by OpenAI, was used to assist with R code revisions. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Claudia Cobo-Angel: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Maya Craig:** Writing – review & editing, Methodology. **Marwan Osman:** Writing – review & editing, Methodology, Data curation. **Kevin J. Cummings:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Casey L. Cazer:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors do not have conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2025.100983.

Data availability

All data is publicly available at https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/integrated-reportssummaries.

The code to reproduce the analysis presented in this study is available at https://doi.org/10.5281/zenodo.14628972.

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