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Overview of the Antimicrobial Resistance of *Salmonella* Recovered from US Poultry Processing Plants

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Abstract: The objectives of this study were to analyze the results of antimicrobial susceptibility testing on *Salmonella* isolated from poultry carcass and parts rinsates using a scoring system for antimicrobial resistance (AMR) and to determine whether the resistance of *Salmonella* to selected antimicrobials critically or highly important to human medicine changed from 2017 to 2019. Samples were collected from 26 plants in the United States, analyzed for the presence of *Salmonella*, and tested for susceptibility to 12 antimicrobials ($n = 734$ for 8 antimicrobials; $n = 597$ for 4 antimicrobials). The multidrug resistance (MDR) scores and AMR scores remained the same over time ($P > 0.05$); however, MDR and AMR differed ($P < 0.0001$) by serogroup and serogroup-by-year interactions. Most notably, MDR—and AMR for 7 out of the 12 antimicrobials—was greater ($P < 0.05$) in serogroup C1 than other serogroups and/or lower ($P < 0.05$) in serogroup D1 than other serogroups. The effect year-by-serogroup was also significant for MDR ($P < 0.0001$) and—for 8 out of the 12 antimicrobials—AMR ($P < 0.05$); differences ($P < 0.05$) across years were identified in serogroup C1, B, and C2 but were highly variable. Resistance to ciprofloxacin and ceftriaxone, "highest priority critically important antimicrobials" to human medicine, were not different ($P > 0.05$) across years, but there were significant ($P < 0.05$) serogroup and serogroup-by-year effects for ceftriaxone resistance. Interestingly, gentamicin resistance across years differed ($P < 0.05$) in serogroup B and C. Overall, mean *Salmonella* MDR and AMR scores were stable from year to year, but shifts in AMR in *Salmonella* serogroups across years were identified, emphasizing the need to continue monitoring AMR in *Salmonella* isolated from poultry products in the interest of food safety and human health.

Key words: antimicrobial resistance, broiler, carcass rinsates, parts rinsates, *Salmonella*
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Introduction

One of the major pathogens causing foodborne illness in the United States is *Salmonella* (NARMS, 2019a). It is estimated that over 1.2 million illnesses are caused by nontyphoidal *S. enterica* yearly (Scallan et al., 2011), resulting in 23,000 hospitalizations and 450 deaths (NARMS, 2018). Cases of salmonellosis in humans are often attributed to the consumption of *Salmonella*-contaminated foodstuffs. While *Salmonella* has been isolated from a variety of foodstuffs—including, but not limited to, fruits, vegetables, and animal products—contaminated poultry products are one of the major vehicles of *Salmonella* transmission to humans (Parveen et al., 2007).

Furthermore, there is growing concern over the prevalence of not just antimicrobial-resistant (AMR) but also multidrug-resistant (MDR) strains of *Salmonella* isolated from poultry products and the potential transmission and impact of those strains on human health (Parveen et al., 2007; Berrang et al., 2009; Shah et al., 2017). Severe cases of salmonellosis are more frequently associated with MDR strains of *Salmonella* than susceptible strains (Parveen et al.,

2007) and are often fatal for the young, elderly, and immunocompromised (NARMS, 2018). The Centers for Disease Control and Prevention estimates that drug-resistant nontyphoidal *Salmonella* causes 212,500 infections and 70 deaths annually (CDC, 2019). Two classes of antimicrobials—quinolones/fluoroquinolones (e.g., ciprofloxacin [CIP]) and cephalosporins (e.g., ceftriaxone [CRO])—are commonly used to treat severe *Salmonella* infections in adults and children, respectively (NARMS, 2018). Thus, *Salmonella* isolates resistant to antimicrobials used to treat severe *Salmonella* infections pose a risk to human health and reduce the number of therapeutic options available to treat *Salmonella* infections (Parveen et al., 2007).

Due to the human health risk associated with AMR pathogens, the antimicrobial susceptibility of foodborne pathogens, like *Salmonella*, are monitored in ill persons by the Centers for Disease Control and Prevention, in retail meats by the US Food and Drug Administration, and in food-producing animals by the US Department of Agriculture as part of the National Antimicrobial Resistance Monitoring System (NARMS). Additionally, to protect food safety and human health, Pilgrim's Pride Corporation (hereafter referred to as Pilgrim's) internally monitors the resistance of *Salmonella* isolated from poultry to selected medically important antimicrobials recognized as highest priority critically important, high priority critically important, or highly important to human medicine by the World Health Organization (Table 1). The objectives of this study were to analyze the results of antimicrobial susceptibility testing on *Salmonella* isolated from poultry carcass and parts rinsates using a scoring system for AMR and determine whether the resistance of *Salmonella* to selected antimicrobials critically or highly important to human medicine changed over time.

Materials and Methods

Carcass and parts sampling for Salmonella analysis

Salmonella spp.–positive samples used for antimicrobial susceptibility testing were obtained from routine carcass and parts rinsate sampling conducted to meet the Modernization of Poultry Slaughter Inspection standards at all 26 of Pilgrim's plants in the US and Puerto Rico. Briefly, one prechill and postchill carcass rinse sampling pair was collected per 22,000 birds slaughtered at a plant. Of those, one sampling pair per shift per day was randomly selected and analyzed for *Salmonella* spp. The sampling pair selected for *Salmonella* spp. analysis consisted of a sample taken from one prechill zone and one postchill zone from the same flock. All plants followed the same zone categorization along the production line. One parts rinse sample was collected each processing day and analyzed for *Salmonella*. Rinsate samples were collected following the US Department of Agriculture carcass and parts rinsing procedures (FSIS, 2013). Briefly, samples were collected aseptically by rinsing a whole carcass or approximately 4 lb of parts with 400 mL of buffered peptone water for 1 min. Then, 100 mL of rinsate was poured into a sterile specimen cup. Samples were packaged in a cooler with ice packs and shipped to Pilgrim's laboratories for arrival and testing within 2 d of collection.

Salmonella analysis

Salmonella spp. analysis of carcass and parts rinsates was conducted by Pilgrim's laboratories in Athens, Georgia; Broadway, Virginia; and Pittsburg, Texas. Carcass and parts rinsates (30 mL) were tested

Table 1. Antimicrobials used to test for *Salmonella* antimicrobial susceptibility ranked according to the World Health Organization's categorization of medically important antimicrobials

Categorization of Importance to Human Medicine	Antimicrobial Class	Antimicrobial Name
Highest Priority Critically Important	Cephalosporins (3rd, 4th, and 5th generation)	Ceftriaxone
Highest Priority Critically Important	Quinolones and fluoroquinolones	Ciprofloxacin
High Priority Critically Important	Aminoglycosides	Gentamicin
High Priority Critically Important	Aminoglycosides	Streptomycin
High Priority Critically Important	Carbapenems and other penems	Meropenem
High Priority Critically Important	Penicillins (aminopenicillins)	Ampicillin
High Priority Critically Important	Penicillins (aminopenicillin with β-lactamase inhibitor)	Amoxicillin-clavulanic acid
Highly Important	Amphenicols	Chloramphenicol
Highly Important	Cephalosporins (1st and 2nd generation) and cephamycins	Cefoxitin
Highly Important	Sulfonamides (dihydrofolate reductase inhibitors)	Sulfisoxazole
Highly Important	Sulfonamides (dihydrofolate reductase inhibitors)	Trimethoprim
Highly Important	Tetracyclines	Tetracycline

for *Salmonella* spp. using a Romer RapidCheck *Salmonella* test kit (Romer Labs Inc., Newark, DE), and the manufacturer-recommended procedures were followed. Postchill carcass and parts samples that were presumptive positive for *Salmonella* spp. were selected (approximately every 10th sample) for cultural confirmation and antimicrobial susceptibility testing. Cultural confirmation was completed using methods as described in the Microbiological Laboratory Guidebook (FSIS, 2019). Confirmed *Salmonella* isolates were then serogrouped and used for antimicrobial susceptibility testing. Samples were serogrouped by serological testing with somatic (O) antigen agglutination tests using the following antiserums: poly Ai-Vi, group B, group C1, group C2, group D1, and group E.

Salmonella antimicrobial susceptibility testing

The antimicrobial susceptibility of *Salmonella* spp. isolates was tested from July 12, 2017, through December 5, 2019. From July 2017 to mid-September 2017, the panel of antimicrobials used for antimicrobial susceptibility testing (Panel 1) included trimethoprim (TMP), gentamicin (GEN), amoxicillin-clavulanic acid (AMC), streptomycin (STR), tetracycline (TET), CIP, ampicillin (AMP), chloramphenicol (CHL), erythromycin (ERY), penicillin (PEN), rifampin (RIF), and vancomycin (VAN). From late September 2017 onward, ERY, PEN, RIF, and VAN were replaced with cefoxitin (FOX), CRO, sulfisoxazole (SXZ), and meropenem (MEM) as recommended by the JBS Food Safety Advisory Board. The recommendation was made based on the fact that gram-negative bacteria are intrinsically resistant to those antimicrobials and the replacement of those antimicrobials allowed for the monitoring of more antimicrobials that are highest priority critically important, high priority critically important, and highly important to human medicine. Thus, the antimicrobials TMP, GEN, AMC, STR, TET, CIP, AMP, CHL, FOX, CRO, SXZ, and MEM were included on the latter panel (Panel 2). The susceptibility of *Salmonella* isolates ($n = 137$ analyzed with Panel 1; $n = 597$ analyzed with Panel 2) to selected antimicrobials was determined using the HardyDisk Antimicrobials Sensitivity Test (Hardy Diagnostics, Santa Maria, CA). Standardized methods for agar diffusion testing described by the manufacturer for *Enterobacteriaceae* were followed. The following antimicrobial disks were used:

1. Antimicrobials present on both panels: TMP-5 (TMP), GM-10 (GEN), AmC-30 (AMC), S-10 (STR), Te-30 (TET), CIP-5 (CIP), AM-10 (AMP), and C-30 (CHL)
2. Antimicrobials present only on Panel 1: E-15 (ERY), P-10 (PEN), RA-5 (RIF), and Va-30 (VAN)
3. Antimicrobials present on only Panel 2: FOX-30 (FOX), CRO-30 (CRO), G-0.25 (SXZ), and MEM-10 (MEM)

Table 2. Antimicrobial disk diffusion zone diameter criteria

		Zone Diameter Interpretive Standards (mm)		
Antimicrobial Agent	Antimicrobial Disk	Resistant	Intermediate	Susceptible
Trimethoprim	TMP-5	≤10	11–15	≥16
Gentamicin	GM-10	≤12	13–14	≥15
Amoxicillin-clavulanic acid	AmC-30	≤13	14–17	≥18
Streptomycin	S-10	≤11	12–14	≥15
Tetracycline	Te-30	≤11	12–14	≥15
Ciprofloxacin	CIP5	≤15	16–20	≥21
Ampicillin	AM-10	≤13	14–16	≥17
Chloramphenicol	C-30	≤12	13–17	≥18
Cefoxitin	FOX-30	≤14	15–17	≥18
Ceftriaxone	CRO-30	≤19	20–22	≥23
Sulfisoxazole	G-0.25	≤12	13–16	≥17
Meropenem	MEM-10	≤13	14–15	≥16

The results were categorized as susceptible, intermediate, or resistant using zone diameter (in millimeters) interpretive standards (Table 2) provided by the manufacturer for each antimicrobial, derived from the Clinical and Laboratory Standards Institute (CLSI, 2019).

Statistical methods

Statistical analyses were conducted using JMP version 14.3.0 (SAS Institute Inc., Cary, NC). A significance level of $P < 0.05$ was used for all statistical tests. Assumptions necessary for the results of the statistical analyses to be valid were assessed and found to be met.

Prior to statistical analysis, the antimicrobial sensitivity test results were transformed using a scoring system adapted and developed from Moore et al. (2013) and Ewing et al. (2017). The antimicrobial sensitivity test results of susceptible, intermediate, or resistant were assigned a numerical value of 0, 0.5, or 1, respectively. The values represent *Salmonella* isolate AMR scores for each antimicrobial on a panel ($n = 12$). An MDR score for each *Salmonella* isolate was calculated by adding the 12 AMR scores together for each sample, with a minimum MDR score of 0 and a potential maximum MDR score of 12 (maximum MDR in this

dataset was 8). Intermediate scores were included to investigate changes in susceptibility; classifying all intermediates as susceptible would hinder the identification of shifts in susceptibility. As a result, MDR scores are not always whole numbers. For example, if the *Salmonella* isolate was susceptible to TMP, it would be assigned a 0; if it was susceptible to GEN, it would be assigned a 0; if it was susceptible to AMC, it would be assigned a 0; if it was susceptible to STR, it would be assigned a 0; if it was susceptible to TET, it would be assigned a 0; if it was intermediate to CIP, it would be assigned a 0.5; if it was resistant to AMP, it would be assigned a 1; if it was susceptible to CHL, it would be assigned a 0; if it was resistant to FOX, it would be assigned a 1; if it was susceptible to CRO, it would be assigned a 0; if it was susceptible to SXZ, it would be assigned a 0; and if it was resistant to MEM, it would be assigned a 1. The sum of those AMR scores—$0 + 0 + 0 + 0 + 0 + 0.5 + 1 + 0 + 1 + 0 + 0 + 1 = 3.5$—is the MDR score for that *Salmonella* isolate.

Serogroup Ai-Vi was excluded from the analysis due to a lack of data in 2017. A two-way analysis of variance was conducted to assess the overall effects of year, serogroup, and year by serogroup on the means of *Salmonella* MDR scores (samples analyzed with Panel 2 antimicrobials only, $n = 597$) and AMR scores ($n = 734$, antimicrobials present on both panels; $n = 597$, only Panel 2). A Student *t* test was used to test pairwise comparisons of year, serogroup, and year-by-serogroup combination means.

Table 3. Least-square mean (±SE) of MDR score ($n = 597$) by year, serogroup, and year by serogroup

Effect	MDR Score	Effect *P* Value
Year		
2017	1.78 (0.23)	0.8140
2018	1.95 (0.13)	
2019	1.96 (0.28)	
Serogroup		
B	2.21 (0.10)^{b}	<0.0001
C1	3.27 (0.21)^{a}	
C2	1.77 (0.10)^{c}	
D1	0.63 (0.13)^{d}	
E	1.61 (0.58)^{b,c,d}	
Year × Serogroup		
2017, B	2.57 (0.16)^{b}	<0.0001
2017, C1	1.57 (0.51)^{b,c,d,e}	
2017, C2	2.17 (0.24)^{b,c}	
2017, D1	0.61 (0.22)^{e,f}	
2017, E	2.00 (0.95)^{b,c,d,e,f}	
2018, B	2.20 (0.17)^{b,c}	
2018, C1	4.20 (0.28)^{a}	
2018, C2	1.59 (0.13)^{d}	
2018, D1	0.92 (0.16)^{e,f}	
2018, E	0.83 (0.55)^{d,e,f}	
2019, B	1.85 (0.20)^{c,d}	
2019, C1	4.04 (0.27)^{a}	
2019, C2	1.55 (0.15)^{d}	
2019, D1	0.35 (0.27)^{f}	
2019, E	2.00 (1.35)^{a,b,c,d,e,f}	

[a–f]Least-square means with different superscripts within a column and per effect (year, serogroup, year × serogroup) differ ($P < 0.05$).

MDR = multidrug resistance; SE = standard error.

Results and Discussion

MDR scores

Least-square estimates of the means (with standard errors) for MDR scores are listed in Table 3. Mean MDR scores were not different across years ($P = 0.8140$); however, the effects serogroup and year by serogroup on mean MDR scores were significant (both $P < 0.0001$). Figure 1 illustrates differences in mean MDR scores by serogroup. Most notably, serogroup C1 had the greatest mean MDR score, whereas serogroup D1 had the lowest mean MDR score ($P < 0.05$; 3.27 ± 0.21 vs. 0.63 ± 0.13). Figure 2 illustrates differences in MDR scores by year and serogroup. The mean MDR score for serogroup C1 increased from 2017 to 2018 ($P < 0.05$; 1.57 ± 0.51 vs. 4.20 ± 0.28) but remained the same from 2018 to 2019 ($P > 0.05$; 4.20 ± 0.28 vs. 4.04 ± 0.27). Conversely, the mean

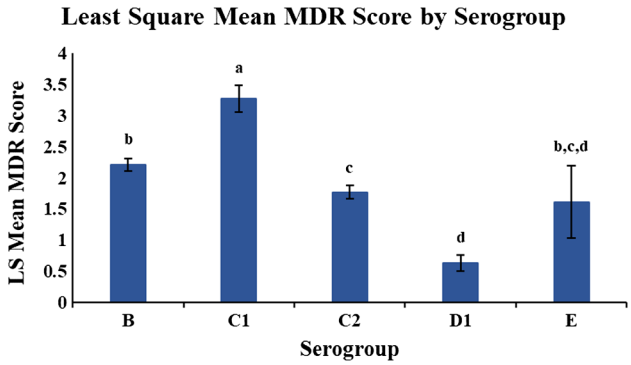

Figure 1. Least-square means (± standard error [SE]) of multidrug resistance (MDR) scores by serogroup.

MDR score for serogroup B decreased over time ($P < 0.05$; 2.57 ± 0.16 [2017] vs. 1.85 ± 0.20 [2019]). Similarly, the mean MDR score for serogroup C2 decreased from 2017 to 2018 ($P < 0.05$; 2.17 ± 0.24 vs. 1.59 ± 0.13) and remained the same from 2018 to

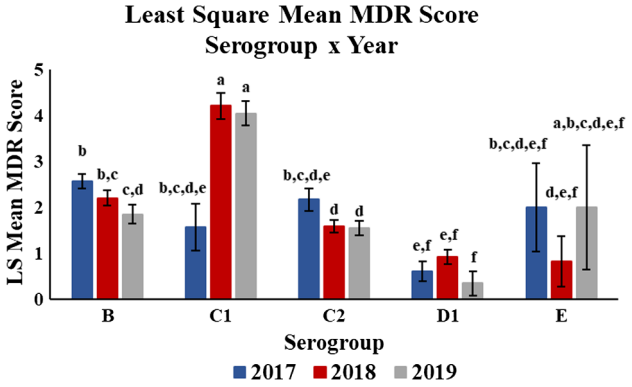

Figure 2. Least-square means (± standard error [SE]) of multidrug resistance (MDR) scores by year and serogroup.

2019 ($P > 0.05$; 1.59 ± 0.13 vs. 1.55 ± 0.15). Furthermore, there were no significant year-by-serogroup interactions in group D1 or group E.

Contrary to these data, the most recent NARMS integrated summary reported a substantial increase (9.5% to 18%) in MDR *Salmonella* recovered from routinely sampled chickens during 2015 to 2017 (NARMS, 2019a). Additionally, the report stated that the increase in the percentage of MDR *Salmonella* over time was attributed to a rise in MDR *Salmonella* Infantis isolates. This is interesting because *Salmonella* Infantis belongs to serogroup C1 (Grimont and Weill, 2007), and in the data presented here, serogroup C1 had the greatest MDR score compared to other serogroups, and MDR nearly doubled from 2017 to 2018. However, MDR *Salmonella* did not change over time because MDR in serogroup B and C2 decreased over time, averaging out the rise in serogroup C1 MDR (Figure 2).

AMR scores: Year

Table 4 lists least-square estimates of the means (with standards errors) for the AMR scores of 12 antimicrobials critical to human medicine (WHO, 2018; Table 1). AMR scores were not significantly different across years for all antimicrobials tested, with the exception of CHL ($P = 0.0037$). CHL mean AMR score increased from 2017 to 2018 ($P < 0.05$; 0.03 ± 0.02 vs. 0.12 ± 0.02) but remained the same from 2018 to 2019 ($P > 0.05$; 0.12 ± 0.02 vs. 0.08 ±.0.03). In agreement with these data, the "NARMS Now: Integrated Data" show an increase in the percentage of carcass rinsate *Salmonella* isolates resistant to CHL from 2016 to 2017 (2.9% vs. 8.2%) (NARMS, 2019b). In general, *Salmonella* resistance to CHL is still low but should continue to be monitored since

Table 4. Least-square means (±SE) of AMR scores ($n = 734$, TMP through CHL; $n = 597$, FOX through MEM) by year

	Year[1]			
AM	17	18	19	*P* Value
TMP	0.01(0.02)	0.07(0.02)	0.06(0.03)	0.0950
GEN	0.08(0.03)	0.13(0.03)	0.09(0.05)	0.4367
AMC	0.06(0.01)	0.02(0.02)	0.02(0.04)	0.2743
STR	0.52(0.05)	0.53(0.04)	0.68(0.08)	0.1856
TET	0.29(0.06)	0.40(0.04)	0.47(0.09)	0.1545
CIP	0.00(0.00)	0.00(0.00)	0.00(0.01)	0.8891
AMP	0.09(0.03)	0.11(0.02)	0.07(0.05)	0.7069
CHL	0.03(0.02)[b]	0.12(0.02)[a]	0.08(0.03)[a,b]	0.0037
FOX	0.06(0.03)	0.01(0.02)	0.04(0.03)	0.2180
CRO	0.09(0.03)	0.0(0.02)	0.07(0.04)	0.7956
SXZ	0.50(0.07)	0.44(0.04)	0.38(0.08)	0.5382
MEM	0.03(0.02)	0.02(0.01)	0.00(0.02)	0.5958

[1]17 = 2017; 18 = 2018; 19 = 2019.

[a–f]Least-square means with different superscripts within a row differ ($P < 0.05$).

AM = antimicrobial; AMP = ampicillin; AMR = antimicrobial resistance; AMC = amoxicillin-clavulanic acid; CHL = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; FOX = cefoxitin; GEN = gentamicin; MEM = meropenem; SE = standard error; STR = streptomycin; SXZ = sulfisoxazole; TET = tetracycline; TMP = trimethoprim.

CHL is a highly important antimicrobial in human medicine (WHO, 2018). It is also worth noting that CHL is not approved for use in food-producing animals in the US (eCFR, 2020).

AMR scores: Serogroup

The effect of serogroup on the means of AMR scores was significant ($P < 0.0001$) for 8 out of the 12 antimicrobials tested: TMP, GEN, STR, TET, AMP, CHL, CRO, and SXZ (Table 5). The majority of antimicrobials with significant ($P < 0.05$) serogroup differences followed 1 of 2 trends (Figure 3): (1) serogroup C1 AMR scores were significantly greater than other serogroups ($P < 0.0001$; TMP, GEN, AMP, CHL, and CRO), or (2) serogroup D1 AMR scores were significantly lower than other serogroups ($P < 0.0001$; STR and TET). SXZ followed a different trend with a significantly ($P < 0.0001$) lower mean AMR score in serogroup C2 compared to serogroup B, C1, and D1.

Of the antimicrobials with greater C1 AMR scores, the increased CRO resistance of serogroup C1 *Salmonella* isolates compared to other serogroups (C1 [0.30 ± 0.03] vs. B [0.03 ± 0.02], C2 [0.02 ± 0.02], D1 [0.01 ± 0.02], E [0.08 ± 0.09]) is concerning because CRO is a cephalosporin and highest priority

Table 5. Least-square means (±SE) of AMR scores ($n = 734$, TMP through CHL; $n = 597$, FOX through MEM) by serogroup

	Serogroup					
AM	B	C1	C2	D1	E	*P* Value
TMP	0.01 (0.01)[b]	0.21 (0.02)[a]	0.01 (0.01)[b]	0.00 (0.01)[b]	0.00 (0.06)[b]	<0.0001
GEN	0.17 (0.02)[b]	0.30 (0.03)[a]	0.03 (0.02)[c]	0.02 (0.02)[c]	0.00 (0.10)[b,c]	<0.0001
AMC	0.05 (0.02)	0.01 (0.02)	0.04 (0.02)	0.02 (0.02)	0.06 (0.07)	0.3300
STR	0.69 (0.03)[b]	0.74 (0.04)[a,b]	0.77 (0.02)[a]	0.07 (0.03)[c]	0.61 (0.15)[a,b]	<0.0001
TET	0.42 (0.03)[a]	0.52 (0.05)[a]	0.50 (0.03)[a]	0.09 (0.04)[b]	0.39 (0.18)[a,b]	<0.0001
CIP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.02)	0.8336
AMP	0.07 (0.02)[b]	0.27 (0.03)[a]	0.06 (0.02)[b,c]	0.01 (0.02)[c]	0.06 (0.10)[b,c]	<0.0001
CHL	0.00 (0.01)[b]	0.35 (0.02)[a]	0.00 (0.01)[b]	0.01 (0.01)[b]	0.00 (0.07)[b]	<0.0001
FOX	0.05 (0.01)	0.02 (0.03)	0.02 (0.01)	0.00 (0.02)	0.08 (0.07)	0.1299
CRO	0.03 (0.02)[b]	0.30 (0.03)[a]	0.02 (0.02)[b]	0.01 (0.02)[b]	0.08 (0.09)[b]	<0.0001
SXZ	0.71 (0.03)[a]	0.58 (0.06)[a,b]	0.28 (0.03)[d]	0.41 (0.04)[c]	0.22 (0.17)[b,c,d]	<0.0001
MEM	0.01 (0.01)	0.05 (0.02)	0.02 (0.01)	0.00 (0.01)	0.00 (0.05)	0.3443

[a–f]Least-square means with different superscripts within a row differ ($P < 0.05$).

AM = antimicrobial; AMP = ampicillin; AMR = antimicrobial resistance; AMC = amoxicillin-clavulanic acid; CHL = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; FOX = cefoxitin; GEN = gentamicin; MEM = meropenem; SE = standard error; STR = streptomycin; SXZ = sulfisoxazole; TET = tetracycline; TMP = trimethoprim.

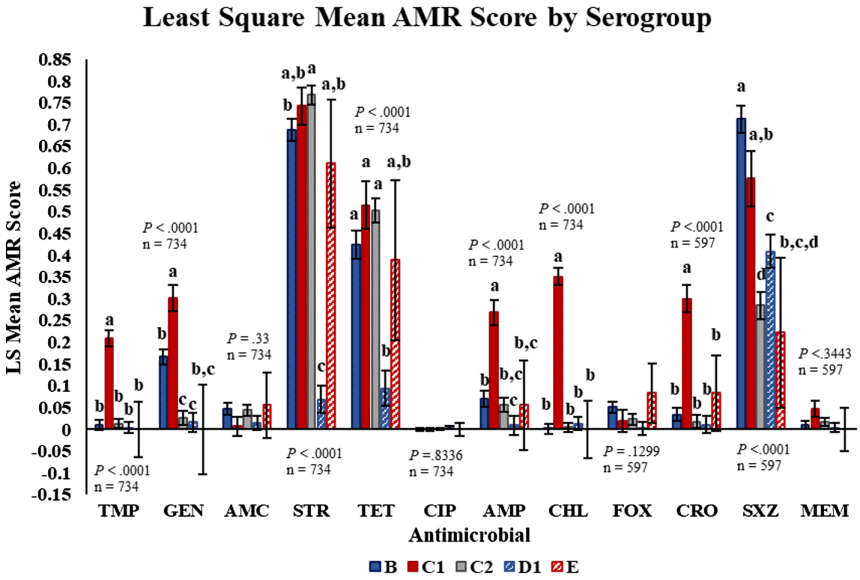

Figure 3. Least-square means (± standard error [SE]) of antimicrobial resistance (AMR) scores for trimethoprim (TMP), gentamicin (GEN), amoxicillin-clavulanic acid (AMC), streptomycin (STR), tetracycline (TET), ciprofloxacin (CIP), ampicillin (AMP), chloramphenicol (CHL), cefoxitin (FOX), ceftriaxone (CRO), sulfisoxazole (SXZ), and meropenem (MEM) by serogroup.

critically important antimicrobial used to treat severe *Salmonella* infections in humans (NARMS, 2018). Serogroup C1 isolates were also more resistant to the critically important antimicrobials GEN (an aminoglycoside) and AMP (a beta-lactam), as well as the highly important antimicrobials TMP (a sulfonamide) and CHL (an amphenicol). Several *Salmonella* isolates belonging to serogroup C1 are commonly isolated from poultry, some of which include *Salmonella* Infantis, *Salmonella* Thompson, *Salmonella* Montevideo, and *Salmonella* Mbandaka. In comparison, previous reports indicate that *Salmonella* Infantis is pan-susceptible, *Salmonella* Thompson is pan-susceptible, *Salmonella* Montevideo has some resistance to sulfonamides, and *Salmonella* Mbandaka has some resistance to sulfonamides and aminoglycosides

(Shah et al., 2017). For the antimicrobials with lower D1 AMR scores, serogroup D1 *Salmonella* isolates were more susceptible to STR (D1 [0.07 ± 0.03] vs. B [0.69 ± 0.03], C1 [0.74 ± 0.04], C2 [0.77 ± 0.02], E [0.61 ± 0.15]) and TET (D1 [0.09 ± 0.04] vs. B [0.42 ± 0.03], C1 [0.52 ± 0.05], C2 [0.50 ± 0.03], E [0.39 ± 0.18]) than other serogroups. Similar results were reported by Liljebjelke et al. (2017) for *Salmonella* Enteritidis, a commonly isolated D1 serogroup in poultry (Shah et al., 2017), in which STR resistance was 3.6% and TET resistance was 0% (Liljebjelke et al., 2017).

AMR scores: Year by serogroup

The effect year by serogroup was significant for TMP ($P < 0.001$), GEN ($P = 0.0046$), STR ($P = 0.0001$), TET ($P < 0.0001$), AMP ($P < 0.0001$), CHL ($P < 0.0001$), CRO ($P = 0.0007$), and SXZ ($P = 0.0006$), which were the same antimicrobials with a significant serogroup effect (Table 6). Significant differences ($P < 0.05$) in mean AMR scores across years and within serogroup were observed in serogroup C1, B, and C2, while no significant differences ($P > 0.05$) in mean AMR scores across years and within serogroup were observed in serogroup D1 or E (Table 6, Figure 4).

The majority of significant differences ($P < 0.05$) in serogroup C1 mean AMR scores across years followed 2 trends: (1) mean AMR scores in serogroup C1 increased from 2017 to 2018 and decreased from 2018 to 2019 (AMP, CRO, and CHL), and (2) mean AMR scores in serogroup C1 increased from 2017 to 2018 and remained the same from 2018 to 2019 (GEN, SXZ, TET, and TMP). STR showed a different trend as mean AMR scores in serogroup C1 increased across years and were significantly ($P = 0.0001$) different between 2017 and 2019.

Of the AMR scores in serogroup C1 that increased from 2017 to 2018 and decreased from 2018 to 2019, the changes in *Salmonella* CRO resistance are of interest because CRO is a highest priority critically important antimicrobial in human medicine, belonging to the cephalosporin class of antimicrobials used to treat severe *Salmonella* infections in humans (NARMS, 2018). While mean CRO resistance in serogroup C1 increased from 2017 to 2018 ($P < 0.05$; 0.14 ± 0.08 vs. 0.48 ± 0.04), mean CRO resistance decreased from 2018 to 2019 ($P < 0.05$; 0.48 ± 0.04 vs. 0.28 ± 0.04). The NARMS integrated summary reported an increase in the percentage of carcass rinsate *Salmonella* isolates resistant to CRO from 2015 to 2017 (6.5% vs. 9.3%), but the magnitude of CRO resistance reported by NARMS was less than that reported here (NARMS, 2019a).

For the AMR scores in serogroup C1 that increased from 2017 to 2018 and remained the same from 2018 to 2019, changes in the mean AMR score of GEN over time (years) are of particular interest since Pilgrim's discontinued the *in ovo* use of GEN in hatcheries on January 1, 2017. The serogroup C1 mean AMR score for GEN increased from 2017 to 2018 ($P < 0.05$; 0.15 ± 0.05 vs. 0.41 ± 0.05) and remained similar from 2018 to 2019 ($P > 0.05$; 0.41 ± 0.05 vs. 0.34 ± 0.05). Although not a direct year-to-year comparison, NARMS (2019b) reported the percent resistance of *Salmonella* Infantis (serogroup C1) to GEN increased from 2015 to 2016 (1.7% vs. 8.9%) and decreased in 2017 (5.3%), while the percent resistance of *Salmonella* Montevideo (serogroup C1) to GEN decreased from 2015 to 2017 (4.8% vs. 0%). Notably, the GEN resistance of serogroup C1 *Salmonella* in 2017, 2018, and 2019 (0.15, 0.41, and 0.34, respectively) reported here is greater than that reported by NARMS in 2015, 2016, and 2017 for *Salmonella* Infantis (1.7%, 8.9%, and 5.3%, respectively) and *Salmonella* Montevideo (4.8%, 0%, and 0%, respectively). Furthermore, despite discontinuing the *in ovo* use of GEN, it appears that resistance to GEN still persists in serotype C1 *Salmonella* isolated from carcass and parts rinsates at Pilgrim's.

There were also significant differences ($P < 0.05$) in serogroup B mean AMR scores across years for the following antimicrobials: GEN, STR, SXZ, and TET (Table 6, Figure 4); however, there were no common trends in serogroup B mean AMR scores across years. GEN serogroup B mean AMR scores were not different from 2017 to 2018 ($P > 0.05$; 0.21 ± 0.02 vs. 0.21 ± 0.03) but decreased from 2018 to 2019 ($P < 0.05$; 0.21 ± 0.03 vs. 0.08 ± 0.04), STR serogroup B mean AMR scores decreased from 2017 to 2018 ($P < 0.05$; 0.73 ± 0.03 vs. 0.55 ± 0.04) and increased from 2018 to 2019 ($P < 0.05$; 0.55 ± 0.04 vs. 0.78 ± 0.16), SXZ serogroup B mean AMR scores decreased from 2017 to 2018 ($P < 0.05$; 0.83 ± 0.05 vs. 0.70 ± 0.05) and remained the same from 2018 to 2019 ($P > 0.05$; 0.70 ± 0.05 vs. 0.59 ± 0.06), and TET serogroup B mean AMR scores increased from 2017 to 2018 ($P < 0.05$; 0.49 ± 0.04 vs. 0.63 ± 0.06) and decreased from 2018 to 2019 ($P < 0.05$; 0.63 ± 0.06 vs. 0.16 ± 0.07).

Interestingly, mean GEN resistance over time differed between *Salmonella* serogroup B and C1 isolates. GEN serogroup B mean AMR scores were not different from 2017 to 2018 ($P > 0.05$;

Table 6. Least-square means (±SE) of AMR scores ($n = 734$, TMP through CHL; $n = 597$, FOX through MEM) by year × serogroup

	Year[1] × Serogroup															
AM	17, B	17, C1	17, C2	17, D1	17, E	18, B	18, C1	18, C2	18, D1	18, E	19, B	19, C1	19, C2	19, D1	19, E	*P* Value
TMP	0.01 (0.01)^{b}	0.04 (0.03)^{b}	0.01 (0.02)^{b}	0.00 (0.02)^{b}	0.00 (0.09)^{b}	0.00 (0.02)^{b}	0.30 (0.03)^{a}	0.01 (0.02)^{b}	0.00 (0.02)^{b}	0.00 (0.06)^{b}	0.02 (0.02)^{b}	0.28 (0.03)^{a}	0.01 (0.02)^{b}	0.00 (0.03)^{b}	0.00 (0.15)^{a,b}	<0.0001
GEN	0.21 (0.02)^{b}	0.15 (0.05)^{b,c,d}	0.05 (0.03)^{d,e}	0.01 (0.03)^{e}	0.00 (0.015)^{b,c,d,e}	0.21 (0.03)^{b,c}	0.41 (0.05)^{a}	0.00 (0.02)^{e}	0.04 (0.03)^{e}	0.00 (0.03)^{c,d,e}	0.08 (0.04)^{d,e}	0.34 (0.05)^{a}	0.03 (0.03)^{e}	0.00 (0.05)^{e}	0.00 (0.25)^{a,b,c,d,e}	0.0046
AMC	0.06 (0.02)	0.02 (0.04)	0.04 (0.02)	0.03 (0.02)	0.17 (0.11)	0.04 (0.02)	0.00 (0.04)	0.04 (0.02)	0.01 (0.02)	0.00 (0.07)	0.05 (0.03)	0.00 (0.04)	0.05 (0.02)	0.00 (0.04)	0.00 (0.18)	0.9776
STR	0.73 (0.03)^{b,c,d}	0.59 (0.07)^{d,e}	0.86 (0.04)^{a}	0.07 (0.05)^{f}	0.33 (0.21)^{d,e,f}	0.55 (0.04)^{e}	0.76 (0.07)^{a,b,c,d}	0.73 (0.03)^{b,c,d}	0.13 (0.04)^{f}	0.50 (0.15)^{c,d,e}	0.78 (0.05)^{a,b,c}	0.88 (0.07)^{a,b}	0.71 (0.04)^{c,d}	0.00 (0.07)^{f}	1.00 (0.36)^{a,b,c,d,e}	0.0001
TET	0.49 (0.04)^{b,e}	0.22 (0.09)^{c,d,f}	0.64 (0.05)^{a}	0.08 (0.06)^{f}	0.00 (0.26)^{a,b,c,d,e}	0.63 (0.06)^{a}	0.61 (0.09)^{a,b}	0.45 (0.04)^{b,e}	0.16 (0.05)^{d,f}	0.17 (0.18)^{c,d,e,f}	0.16 (0.07)^{d,f}	0.72 (0.09)^{a}	0.42 (0.05)^{b,c,e}	0.04 (0.05)^{f}	1.00 (0.45)^{a,b,c,d,e}	<0.0001
CIP	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.01)	0.01 (0.00)	0.00 (0.00)	0.00 (0.02)	0.00 (0.01)	0.00 (0.01)	0.00 (0.00)	0.00 (0.01)	0.00 (0.04)	0.8648
AMP	0.12 (0.02)^{c}	0.09 (0.05)^{c,d}	0.06 (0.03)^{c,d}	0.02 (0.03)^{d}	0.17 (0.14)^{b,c,d}	0.05 (0.03)^{c,d}	0.49 (0.05)^{a}	0.03 (0.02)^{d}	0.01 (0.03)^{d}	0.00 (0.10)^{c,d}	0.05 (0.04)^{c,d}	0.24 (0.05)^{b}	0.08 (0.03)^{c,d}	0.00 (0.05)^{d}	0.00 (0.25)^{a,b,c,d}	<0.0001
CHL	0.00 (0.01)^{d}	0.13 (0.03)^{c}	0.00 (0.02)^{d}	0.00 (0.02)^{d}	0.00 (0.09)^{c,d}	0.00 (0.02)^{d}	0.52 (0.03)^{a}	0.01 (0.02)^{d}	0.04 (0.02)^{d}	0.00 (0.07)^{c,d}	0.00 (0.02)^{d}	0.40 (0.03)^{b}	0.00 (0.02)^{d}	0.00 (0.03)^{d}	0.00 (0.16)^{c,d}	<0.0001
FOX	0.06 (0.02)	0.00 (0.06)	0.00 (0.03)	0.00 (0.03)	0.25 (0.11)	0.02 (0.02)	0.00 (0.03)	0.02 (0.02)	0.01 (0.02)	0.00 (0.07)	0.08 (0.02)	0.06 (0.03)	0.05 (0.02)	0.00 (0.03)	0.00 (0.16)	0.4742
CRO	0.06 (0.02)^{c}	0.14 (0.08)^{b,c}	0.00 (0.04)^{c}	0.00 (0.04)^{c}	0.25 (0.14)^{a,b,c}	0.00 (0.03)^{c}	0.48 (0.04)^{a}	0.01 (0.02)^{c}	0.03 (0.02)^{c}	0.00 (0.08)^{c}	0.05 (0.03)^{c}	0.28 (0.04)^{b}	0.04 (0.02)^{c}	0.00 (0.04)^{c}	0.00 (0.20)^{b,c}	0.0007
SXZ	0.83 (0.05)^{a}	0.21 (0.15)^{e,f}	0.47 (0.07)^{d,e}	0.47 (0.07)^{d,e}	0.50 (0.28)^{a,b,c,d,e,f}	0.70 (0.05)^{b,c}	0.67 (0.08)^{a,b,c,d}	0.22 (0.04)^{f}	0.44 (0.05)^{e}	0.17 (0.16)^{e,f}	0.59 (0.06)^{c,d}	0.84 (0.08)^{a,b}	0.16 (0.05)^{f}	0.31 (0.08)^{e,f}	0.00 (0.40)^{c,d,e,f}	0.0006
MEM	0.01 (0.01)	0.14 (0.04)	0.00 (0.02)	0.00 (0.02)	0.00 (0.08)	0.02 (0.01)	0.00 (0.02)	0.05 (0.01)	0.01 (0.01)	0.00 (0.05)	0.00 (0.02)	0.00 (0.02)	0.00 (0.01)	0.00 (0.02)	0.00 (0.12)	0.0875

[1]17 = 2017; 18 = 2018; 19 = 2019.

[a–f]Least-square means with different superscripts within a row differ ($P < 0.05$).

AM = antimicrobial; AMP = ampicillin; AMR = antimicrobial resistance; AMC = amoxicillin-clavulanic acid; CHL = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; FOX = cefoxitin; GEN = gentamicin; MEM = meropenem; SE = standard error; STR = streptomycin; SXZ = sulfisoxazole; TET = tetracycline; TMP = trimethoprim.

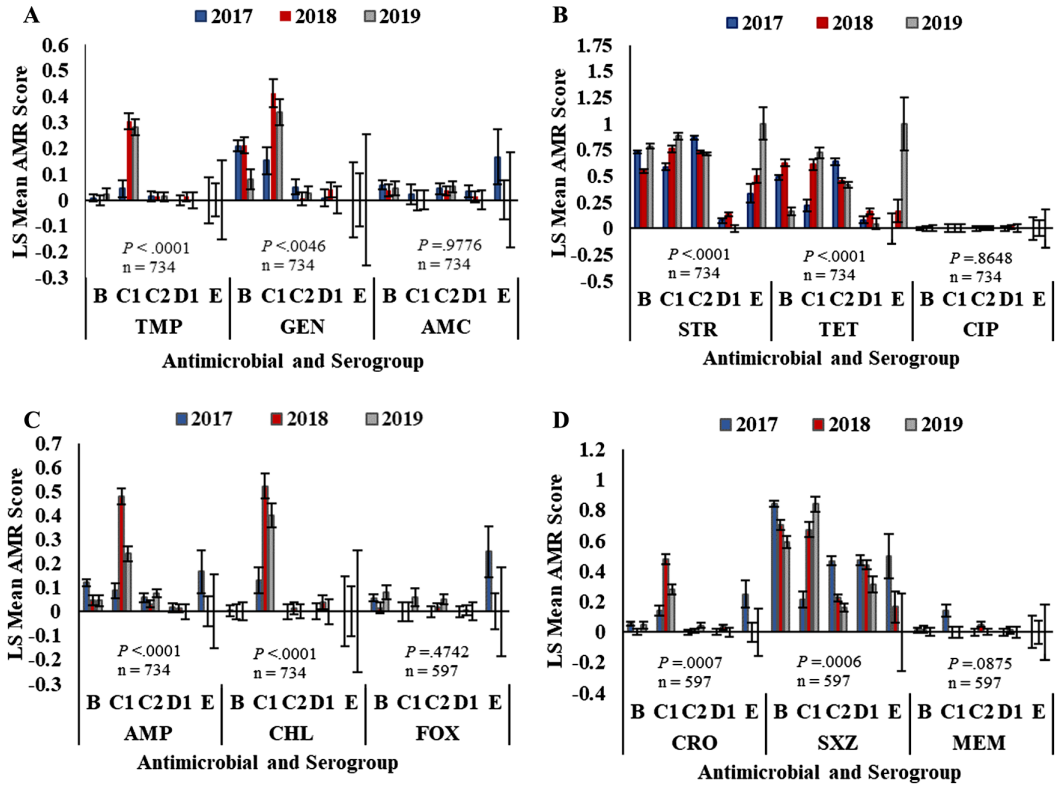

Figure 4. Least-square means (± standard error [SE]) of antimicrobial resistance (AMR) scores for (A) trimethoprim (TMP), gentamicin (GEN), and amoxicillin-clavulanic acid (AMC); (B) streptomycin (STR), tetracycline (TET), and ciprofloxacin (CIP); (C) ampicillin (AMP), chloramphenicol (CHL), and cefoxitin (FOX); and (D) ceftriaxone (CRO), sulfisoxazole (SXZ), and meropenem (MEM) by serogroup and year.

0.21 ± 0.02 vs. 0.21 ± 0.03) but decreased from 2018 to 2019 ($P < 0.05$; 0.21 ± 0.03 vs. 0.08 ± 0.04), while serogroup C1 mean AMR score for GEN increased from 2017 to 2018 ($P < 0.05$; 0.15 ± 0.05 vs. 0.41 ± 0.05) and remained similar from 2018 to 2019 ($P > 0.05$; 0.41 ± 0.05 vs. 0.34 ± 0.05). Improvements in serogroup B GEN susceptibility over time (2018 to 2019) could be related to the removal of GEN use in Pilgrim's hatcheries on January 1, 2017; however, the discrepancy in serogroup C1 and B mean AMR scores across years highlights the complexity of AMR.

Lastly, there were significant ($P < 0.05$) differences in serogroup C2 mean AMR scores across years (Table 6, Figure 4). STR, SXZ, and TET serogroup C2 mean AMR scores decreased from 2017 ($P < 0.05$; 0.86 ± 0.04, 0.47 ± 0.07, and 0.64 ± 0.05, respectively) to 2018 ($P < 0.05$; 0.73 ± 0.03, 0.22 ± 0.04, and 0.45 ± 0.04, respectively) and remained the same in 2019 ($P > 0.05$; 0.71 ± 0.04, 0.16 ± 0.05, and 0.42 ± 0.05, respectively). In contrast, the percent resistance reported by NARMS of *Salmonella* Kentucky (serogroup C2) to STR, SXZ, and TET in 2015 (76.8%, 2.7%, and 45.5%, respectively), 2016 (76.2%, 4.1%, and 47.7%, respectively), and 2017 (78.8%, 3.5%, and 54.0%, respectively) was more variable (NARMS, 2019b).

Conclusions

The results presented in this paper demonstrate how a scoring system for AMR can be used to monitor AMR in *Salmonella* isolated from poultry products and assess how changes in production practices affect AMR. The MDR and AMR scores of *Salmonella* isolated from carcass and parts rinses at Pilgrim's processing plants remained the same from 2017 to 2019; however, MDR and AMR scores differed by serogroup and serogroup-by-year interactions. Most notably, MDR scores—and AMR scores for 7 out of the 12 antimicrobials tested—were greater in serogroup C1 than other serogroups and/or lower in serogroup D1 than other serogroups. The effect of year by serogroup was also significant for MDR scores, and for the AMR scores of 8 out of the 12 antimicrobials tested. Significant differences in both MDR and AMR scores across years were identified in serogroup C1, B, and C2 but were highly variable.

Of particular interest was the AMR of CIP and CRO because of the classification of these

antimicrobials as highest priority critically important antimicrobials to human medicine and the use of these antimicrobials in the treatment of severe *Salmonella* infections in humans. The results indicated that *Salmonella* isolated from carcass and parts rinsates were susceptible to CIP across the years and serogroups evaluated. In contrast, CRO resistance did not differ by year but was greater in serogroup C1 *Salmonella* isolates, and it increased from 2017 to 2018 and decreased from 2018 to 2019. *Salmonella* resistance to GEN was also of interest since the *in ovo* use of GEN was discontinued on January 1, 2017. GEN resistance in serogroup B was not different from 2017 to 2018 but decreased from 2018 to 2019, while GEN resistance in serogroup C1 increased from 2017 to 2018 and remained similar from 2018 to 2019, highlighting the complexity of AMR. Overall, mean *Salmonella* MDR and AMR scores were stable from year to year, but shifts in AMR in *Salmonella* serogroups across years were identified and emphasize the need to continue monitoring AMR in *Salmonella* isolated from poultry products in the interest of food safety and human health. Furthermore, the scoring system used for AMR in *Salmonella* is a useful and effective method for monitoring changes in AMR.

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Literature Cited

Berrang, M. E., J. S. Bailey, S. F. Altekruse, W. K. Shaw Jr., B. L. Patel, R. J. Meinersmann, and P. J. Fedorka-Cray. 2009. Prevalence, serotype, and antimicrobial resistance of Salmonella on broiler carcasses postpick and postchill in 20 U.S. processing plants. J. Food Protect. 72:1610–1615. https://doi.org/10.4315/0362-028X-72.8.1610.

Centers for Disease Control and Prevention (CDC). 2019. Antibiotic resistance threats in the United States, 2019. U.S. Department of Health and Human Services, CDC. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf. (Updated December 2019; Accessed 8 January 2020).

CLSI. 2019. Performance standards for antimicrobial susceptibility testing. Supplement M100. 29th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

eCFR. 2020. CFR530.41: Drugs prohibited for extralabel use in animals. Electronic Code of Federal Regulations. https://www.ecfr.gov/cgi-bin/text-idx?SID=edceb98dee197013888157a7ee885307&mc=true&node=se21.6.530_141&rgn=div8. Updated 20 April 2020. (Accessed 15 April 2020).

Ewing, J., J. McCaughan, J. Moore, D. Fairley, B. Sutherland, and D. Downey. 2017. Relative resistance index (RRI) - a scoring system for antibiotic resistance in Pseudomonas aeruginosa. Brit. J. Biomed. Sci. 74:198–202. https://doi.org/10.1080/09674845.2017.1338500.

FSIS. 2013. FSIS Directive 10, 250.1: *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. 20 September 2013. United States Department of Agriculture (USDA) Food Safety and Inspection Service. https://www.fsis.usda.gov/wps/wcm/connect/ebf83112-4c3b-4650-8396-24cc8d38bf6c/10250.1.pdf?MOD=AJPERES. (Accessed 12 January 2020).

FSIS. 2019. Microbiology Laboratory Guidebook (MLG) method 4.10: Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg, and siluriformes (fish) products and carcass and environmental sponges. 2 January 2019. United States Department of Agriculture (USDA) Food Safety and Inspection Service. https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/mlg-4.pdf?MOD=AJPERES. (Accessed 12 January 2020).

Grimont, P. A., and F. Weill. 2007. Antigenic formulae of the Salmonella serovars. 9th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, France.

Liljebjelke, K. A., C. L. Hofacre, D. G. White, S. Ayers, M. D. Lee, and J. J. Maurer. 2017. Diversity of antimicrobial resistance phenotypes in Salmonella isolated from commercial poultry farms. Front in Vet. Sci. 4:96. https://doi.org/10.3389/fvets.2017.00096.

Moore, P. J. A, J. R. Rao, B. C Millar, C. E. Goldsmith, W. A. Coulter, and J. E. Moore. 2013. Antimicrobial resistance to 14 antimicrobials in marine waters around Northern Ireland: Use of the novel Relative Resistance Index as a marker of ecological status. J. Mar. Res. 71:389–398. https://doi.org/10.1357/002224013812587591.

NARMS. 2018. About NARMS. U.S. Food and Drug Administration. https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/about-narms. (Updated 9 March 2018; Accessed 12 January 2020).

NARMS. 2019a. 2016-2017 NARMS integrated summary. U.S. Food and Drug Administration. https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/2016-2017-narms-integrated-summary. (Updated 22 November 2019; Accessed 12 January 2020).

NARMS. 2019b. NARMS Now: Integrated data. U.S. Food and Drug Administration. https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/narms-now-integrated-data. (Updated 22 November 2019; Accessed 12 January 2020).

Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry.

J. Food Protect. 70:2466–2472. https://doi.org/10.4315/0362-028X-70.11.2466.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17:7–15. https://doi.org/10.3201/eid1701.P11101.

Shah, D. H., N. C. Paul, W. C. Sischo, R. Crespo, and J. Guard. 2017. Population dynamics and antimicrobial resistance of the most prevalent poultry-associated Salmonella serotypes. Poultry Sci. 96:687–702. https://doi.org/10.3382/ps/pew342.

WHO. 2018. Critically important antimicrobials for human medicine. 6th Revision. World Health Organization, Geneva, Switzerland.