FSIS National Antimicrobial Resistance Monitoring System Cecal Sampling Program, 2014 *Salmonella* Report

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Summary

The Food Safety and Inspection Service isolates bacteria from the ceca (beginning of the large intestine) of swine (market swine and sows), cattle (heifers, steers, beef cows, and dairy cows), and poultry (young chickens and young turkeys) for the presence of *Salmonella spp.*, *Campylobacter spp.*, generic *Escherichia coli*, and *Enterococcus spp.* This is a descriptive report of antimicrobial resistance results for *Salmonella* during calendar year 2014, the first full year of cecal sampling. This report complements the findings described in the 2014 NARMS Integrated Report. The isolate level data described in this report are available on the Food and Drug Administration (FDA) NARMS website. Additional information on the food animal component of NARMS can be found on the on the FSIS NARMS web page.

Key Findings

Overview

- In 2014, *Salmonella* was isolated from 21.5% (1,077/5,001) of cecal samples
- Sixty six percent of the *Salmonella* isolates were pansusceptible (i.e. not resistant to any antimicrobial drug tested).
- Thirteen percent of the *Salmonella* isolates were multidrug resistant (MDR) (i.e. resistant to 3 or more antimicrobial classes).
- Less than 5% of the *Salmonella* isolates were resistant to each of the antimicrobial drugs classified by FDA as critically important for human clinical use.
- Among antimicrobial drugs classified by FDA as highly important, tetracycline (28.8%) and streptomycin (17.9%) had the highest percentage of resistance among the *Salmonella* isolates.
- Looking at *Salmonella* serotypes, a notably high proportion of resistant and MDR isolates was found for *Salmonella* I 4,[5],12:i:- (89.5% resistant; 68.4% MDR) and *Salmonella* Typhimurium (85.5%; 51.6%).

By Commodity

Poultry

- The highest proportion of resistant *Salmonella* isolates was identified in turkey (76%).
- The highest proportion of resistant *Salmonella* Typhimurium isolates was obtained from chicken (49%).
- All ten *Salmonella* Heidelberg isolates were obtained from poultry with seven pansusceptible chicken isolates and three MDR turkey isolates.

Swine (Market Swine, Sows)

- All *Salmonella* I 4,[5],12:i:- isolates (nine isolates) from market swine were resistant to at least one antimicrobial drug with 89% having the ampicillin/streptomycin/sulfonamide/tetracycline (ASSuT) resistance profile. Half of the sow *Salmonella* I 4,[5],12:i:- isolates (10 isolates) exhibited this resistance profile. In some cases, these isolates were resistant to other antimicrobial drugs in addition to ASSuT.

Cattle (Dairy Cow, Beef Cow, Steers, Heifer)

- Nineteen percent of all cattle isolates were resistant to at least one antimicrobial drug.
- The two most common serotypes among cattle isolates were *Salmonella* Newport and *Salmonella* Typhimurium. At least half of the cattle isolates for these serotypes exhibited resistance (50% and 71%, respectively) and all resistant isolates were MDR.

Introduction

Antimicrobial drugs are used in food-producing animals to prevent illness, treat disease, and limit the spread of infection already present in a herd or flock. Animal exposure to antimicrobial drugs can promote selection for resistant subpopulations of bacteria that can spread to other animals, the environment and humans. FSIS has made it a priority to track changes in antibiotic susceptibility of meat and poultry products to reduce the potential of human exposure to harmful resistant pathogens.

FSIS collects data for the animal arm of NARMS through two programs including: the Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) verification program and the cecal sample program. For PR/HACCP verification sampling, select classes of livestock carcasses (cow/bulls, steers/heifers, market hogs, broilers) and raw ground/comminuted product (beef, chicken, and turkey) are sampled from federally inspected slaughter and processing plants throughout the United States.[1] Carcass samples consist of swabs (beef, turkey, hogs), or rinsates (chicken), and raw ground/comminuted product samples collected during processing, wherein in-plant antimicrobial interventions may have been applied to reduce bacterial loads either during slaughter or further processing, or a combination of both. While this approach allows NARMS surveillance to be efficiently and economically coupled with FSIS PR/HACCP verification sampling, the historical sampling design (risk-based sample collection from 2006-2015) and varying types of product classes targeted under

[1] Of note, FSIS suspended scheduling cows/bulls from sampling in 2011 and market hogs, steer, and heifers in 2012 because of the low number of positive samples.

PR/HACCP sampling presented important limitations to analyzing trends and drawing conclusions about the occurrence of antibiotic resistance in food animals. In 2014, FSIS began to move from a risk-based sampling approach to routine sampling of all establishments that produce commodities included in the PR/HACCP verification programs (81 FR at 7285; February 11, 2016). This change enables the Agency to better evaluate prevalence and contamination trends over time in these commodities.

The World Health Organization (WHO) recommends that if on-farm sampling is not possible, samples from healthy animals at slaughter may be used to estimate bacterial resistance in food animals.[2] Cecal samples collected immediately after slaughter may reflect microbial characteristics prior to the application of in-plant processing interventions, making them better indicators of pre-harvest antimicrobial resistance. Microbiota in the animal cecum also may be affected by the time spent in transport and in holding pens prior to slaughter. Therefore, sampling results may reflect gut colonization by microorganisms present in these environments.

Since March 2013, FSIS's cecal sampling has provided a means for monitoring antimicrobial resistance in intestinal (cecal) bacteria at slaughter from four food animal species (chickens, turkeys, swine, and cattle) and associated slaughter classes (young chicken, young turkey, beef cow, dairy cow, steer, and heifer, market swine and sow). In contrast to historical PR/HACCP verification sampling , cecal sampling is routinely conducted using a statistical model based on establishment production volume and provides more nationally representative data on antimicrobial susceptibility among four targeted bacteria (*Salmonella, Campylobacter, Escherichia coli* and *Enterococcus*)[3] prior to slaughter.

Salmonella isolates described in this report were recovered by FSIS from cecal samples collected in CY2014 as described in FSIS Directive 10,100.1. As with other data collected for NARMS, data generated from cecal samples may: facilitate detection of trends in antimicrobial resistance or emerging antimicrobial resistance profiles of concern from food animals in the United States; support the identification of mitigation steps to limit the spread of resistance to antimicrobial drugs classified as critically important; improve the scientific basis for food animal antibiotic use policies and regulations; and enable risk analysis of foodborne antimicrobial resistant hazards when evaluating a new animal antibiotic for safety.[4]

The following descriptions of resistance are used in the results section of this report. Pansusceptible isolates are susceptible to all antimicrobial drugs included in the NARMS testing panel. Multidrug resistance (MDR) is reported as resistance to three or more of the nine antimicrobial classes included in the NARMS testing panel. Isolates that are resistant to all or all but one of these classes are considered extremely drug resistant (XDR).[5] Bacteria that are classified as XDR are epidemiologically significant not

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[2] World Health Organization (2017). Integrated surveillance of antimicrobial resistance: guidance from a WHO Advisory Group. Retrieved from: http://apps.who.int/iris/bitstream/10665/255747/1/9789241512411-eng.pdf?ua=1.

[3] CDC, FDA, FSIS (2016). NARMS Integrated Report, 2014. Retrieved from http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM528861.pdf.

[4] FDA, Antimicrobial Resistance, About NARMS, updated 11/18/2016. Retrieved from: http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/ucm059089.htm#Data_usage

[5] CDC, FDA, FSIS (2016). NARMS Integrated Report, 2014. Retrieved from http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM528861.pdf.

only because of resistance to multiple antimicrobial drugs, but also because of the likelihood of being resistant to all, or almost all, clinically approved antimicrobial drugs.

Results

Resistance by Slaughter Class

Salmonella was isolated from 21.5% (1,077/5,001) of samples, with percent positive ranging within the slaughter classes from 2.8% (15/540) in beef cows to 58.1% (328/565) in sows. All isolates were analyzed for antimicrobial resistance and 65.6% (707/1,077) exhibited no resistance, while 34.4% (370/1,077) were resistant to one or more antimicrobial drugs, and 13.4% (144/1,077) were MDR. XDR was found in 0.4% (4/1,077) isolates. Figure 1 shows the percentage of cecal samples that were positive for *Salmonella* for each slaughter class.

Figure 1. Percent cecal samples (N = 5,001 total samples) *Salmonella* positive, by production class, 2014.

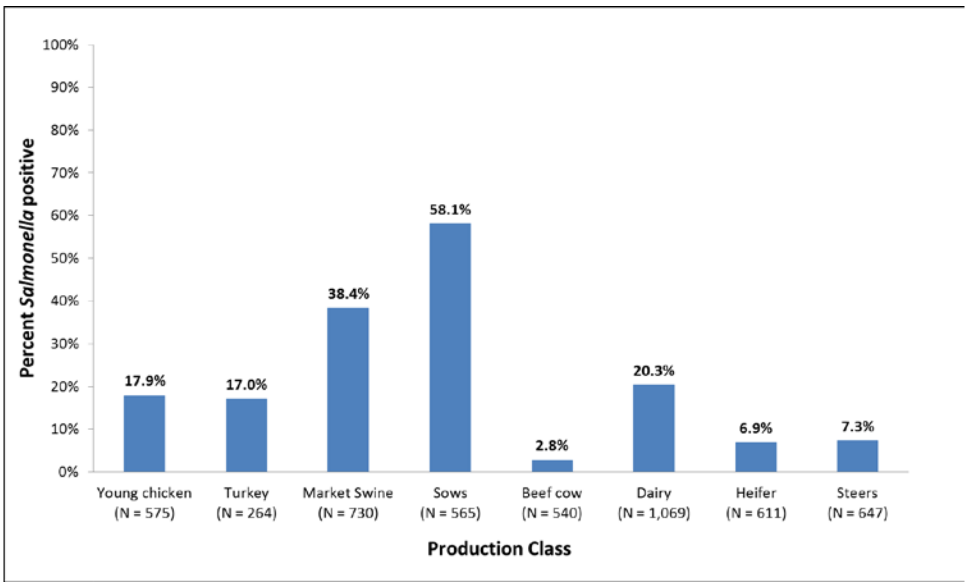
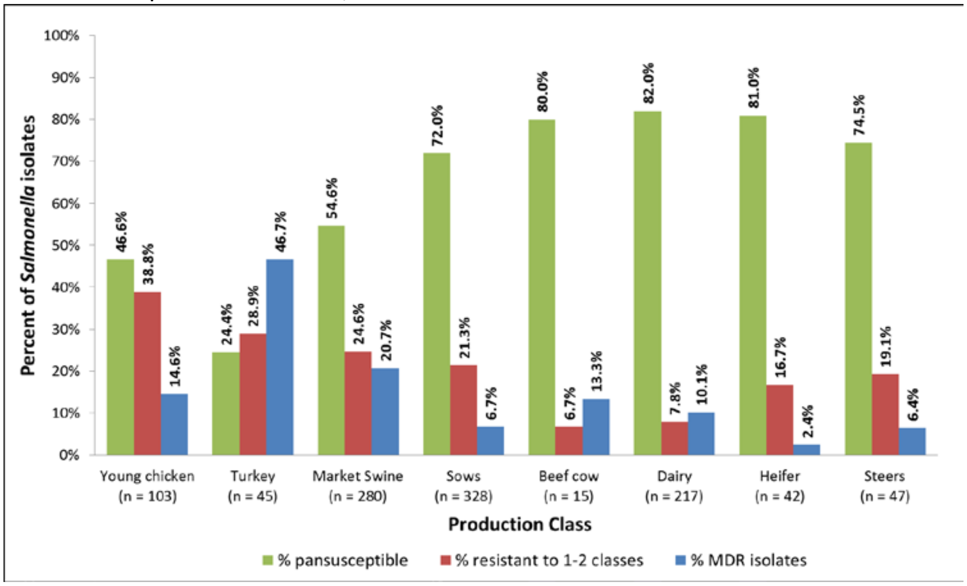


Figure 2 represents the *Salmonella* isolates by resistance (pansusceptible, resistant to 1-2 antimicrobial drug classes, or MDR) within each slaughter class. The highest proportion of resistant isolates was identified in turkey (75.6% [34/45] resistant), followed by chicken (53.4% [55/103]), swine (36.0% [219/608]), and cattle (19.3% [62/321]). MDR isolates were identified in less than 20% of isolates in all slaughter classes except for turkeys (46.7% [21/45]) and market swine (20.7% [58/280]).

Figure 2. Percent cecal *Salmonella* isolates (n = 1,077 total isolates) pansusceptible, resistant, and MDR, within each production class, 2014.



Resistance by *Salmonella* Serotype

Salmonella serotypes varied by slaughter class. Table 1 summarizes the top 10 serotypes from all classes, the proportion of isolates resistant to at least one antimicrobial drug, the proportion of isolates that are MDR, and the slaughter class with the most isolates for each serotype. For the following top 10 serotypes, over 75% of isolates were exclusive to one species: *Salmonella* Cerro (84.4% [76/90] from cattle), *Salmonella* Derby (96.1% [75/78] from swine), *Salmonella* Johannesburg (97.2% [70/72] from swine), *Salmonella* Infantis (95.1% [58/61] swine), and *Salmonella* Montevideo (86.7% [52/60] from cattle).

Table 1. Top 10 *Salmonella* serotypes isolated from cecal samples (serotype totals highlighted in grey).

Serotype	No. of isolates (% total isolates§)	No. of resistant isolates* (% serotype isolates)	No. of MDR isolates (% serotype isolates)	Primary slaughter class of serotype (# of isolates, % of serotype)
Anatum	142 (13.2%)	58 (40.8%)	0 (0.0%)	Sows (68, 47.9%)
Cerro	90 (8.4%)	5 (5.6%)	0 (0.0%)	Dairy (70, 77.8%)
Derby	78 (7.2%)	52 (66.7%)	30 (38.5%)	Market Swine (49, 62.8%)
Johannesburg	72 (6.7%)	9 (12.5%)	2 (2.8%)	Sows (42, 58.3%)
Typhimurium	62 (5.8%)	53 (85.5%)	32 (51.6%)	Chicken (27, 43.5%)
Infantis	61 (5.7%)	3 (4.9%)	2 (3.3%)	Sows (36, 59.0%)
Montevideo	60 (5.6%)	10 (16.7%)	2 (3.3%)	Dairy (38, 63.3%)
Agona	50 (4.6%)	24 (48.0%)	13 (26.0%)	Market Swine (17, 34.0%)
Kentucky	41 (3.8%)	25 (61.0%)	6 (14.6%)	Chicken (27, 65.9%)
Muenchen	23 (2.1%)	12 (52.2%)	0 (0.0%)	Sows (9, 39.1%)

§Total isolates: n = 1,077; *Includes MDR isolates

Table 2 shows the *Salmonella* serotypes with the highest proportion of isolates exhibiting resistance to at least one antimicrobial drug. The table includes the percentage of all isolates, percentage of resistant isolates, percentage of MDR isolates, and the slaughter class where the majority of each serotype was identified. The serotypes with higher proportions of resistant isolates include some serotypes that were less common overall. Serotypes with a high proportion of resistance in general and MDR included *Salmonella* I 4,[5],12:i:- (89.5% [17/19] resistant; 68.4% [13/19] MDR) and *Salmonella* Typhimurium (85.5% [53/62]; 51.6% [32/62]).

Table 2. *Salmonella* serotypes with highest proportion of resistant isolates (serotypes with ≥ 10 isolates) (resistant isolate totals highlighted in grey).

Serotype	No. of isolates (% total isolates§)	No. of resistant isolates* (% serotype isolates)	No. of MDR isolates (% serotype isolates)	Primary slaughter class of serotype (# of isolates, % of serotype)
Hadar	11 (1.0%)	10 (90.9%)	4 (36.4%)	Turkey (7, 63.6%)
I 4,[5],12:i:-	19 (1.8%)	17 (89.5%)	13 (68.4%)	Sows (10, 52.6%)
Typhimurium	62 (5.8%)	53 (85.5%)	32 (51.6%)	Chicken (27, 43.5%)
Schwarzengrund	17 (1.6%)	12 (70.6%)	3 (17.7%)	Chicken (8, 47.1%)
Derby	78 (7.2%)	52 (66.7%)	29 (37.2%)	Market Swine (49, 62.8%)
Kentucky	41 (3.8%)	25 (61.0%)	6 (14.6%)	Chicken (27, 65.9%)
Muenchen	23 (2.1%)	12 (52.2%)	0 (0.0%)	Sows (9, 39.1%)
Agona	50 (4.6%)	24 (48.0%)	13 (26.0%)	Market Swine (17, 34.0%)
Newport	22 (2.0%)	10 (45.5%)	9 (40.9%)	Dairy (15, 68.2%)
Reading	14 (1.3%)	6 (42.9%)	3 (21.4%)	Turkey (6, 42.9%)

§Total isolates: n = 1,077; *Includes MDR isolates

Table 3 shows the *Salmonella* serotypes with the highest proportion of isolates exhibiting MDR. Only two serotypes had the majority of isolates identified as MDR: *Salmonella* I 4,[5],12:i:- and *Salmonella* Typhimurium (68.4% and 51.6% MDR, respectively).

Table 3. *Salmonella* serotypes with highest proportion of MDR isolates (serotypes with ≥ 10 isolates) (MDR isolate totals highlighted in grey).

Serotype	No. of isolates (% total isolates[§])	No. of resistant isolates* (% serotype isolates)	No. of MDR isolates (% serotype isolates)	Primary slaughter class of serotype (# of isolates, % of serotype)
I 4,[5],12:i:-	19 (1.8%)	17 (89.4%)	13 (68.4%)	Sows (10, 52.6%)
Typhimurium	62 (5.8%)	53 (85.5%)	32 (51.6%)	Chicken (27, 43.5%)
Newport	22 (2.0%)	10 (45.5%)	9 (40.9%)	Dairy (15, 68.2%)
Derby	78 (7.2%)	52 (66.7%)	30 (38.5%)	Market Swine (49, 62.8%)
Hadar	11 (1.0%)	10 (90.9%)	4 (36.4%)	Turkey (7, 63.6%)
Heidelberg	10 (0.9%)	3 (30.0%)	3 (30.0%)	Chicken (7, 70.0%)
Agona	50 (4.6%)	24 (48.0%)	13 (26.0%)	Market Swine (17, 34.0%)
Reading	14 (1.3%)	6 (42.9%)	3 (21.4%)	Turkey (6, 42.9%)
Saintpaul	22 (2.0%)	8 (36.4%)	4 (18.2%)	Market Swine (9, 40.9%)
Schwarzengrund	17 (1.6%)	12 (70.6%)	3 (17.6%)	Chicken (8, 47.1%)

[§]Total isolates: n = 1,077; *Includes MDR isolates

Salmonella serotypes identified among cecal isolates from each slaughter class varied. For example, the majority of chicken isolates were one of two serotypes, *Salmonella* Typhimurium or *Salmonella* Kentucky (both 26.2% [27/103]), while market swine and sow isolates showed greater diversity. *Salmonella* Typhimurium and *Salmonella* Kentucky isolates from chicken were often resistant (74.1% [20/27] and 96.3% [26/27], respectively), but less often MDR (18.5% [5/27] and 29.6% [8/27]). For dairy cattle, only two common serotypes (with ≥ 10 isolates) exhibited resistance in a majority of isolates: *Salmonella* Newport and *Salmonella* Typhimurium (53.3% [8/15] and 90.0% [9/10] MDR, respectively).

Salmonella Resistance Profiles

All *Salmonella* isolates with resistance (34.4% [370/1,077]) were resistant to one or more of the antimicrobial drugs classified by FDA as highly important or critically important. Figure 3 shows the percentage of isolates resistant to each antimicrobial drug. In some cases, a single isolate was resistant to more than one of these drugs and is counted more than once in the figure. Streptomycin (17.9% [193/1,077]) and tetracycline (28.8% [310/1,077]) were the only antimicrobial drugs classified as highly important to which more than 10% of all isolates exhibited resistance. Resistance to each of the antimicrobial drugs classified as critically important was less than 5%. Less than 1% of isolates were resistant to azithromycin (0.6% [7/1,077]). Table 4 shows the cecal *Salmonella* serotypes most frequently isolated with resistance to each highly important or critically important antimicrobial drugs.

Figure 3. Percent of cecal *Salmonella* isolates (n = 1,077) resistant to each of the highly important and critically important antimicrobial drugs, 2014. Note that of the 370 isolates represented in this figure, 220 were resistant to more than one of these drugs and thus are counted more than once.

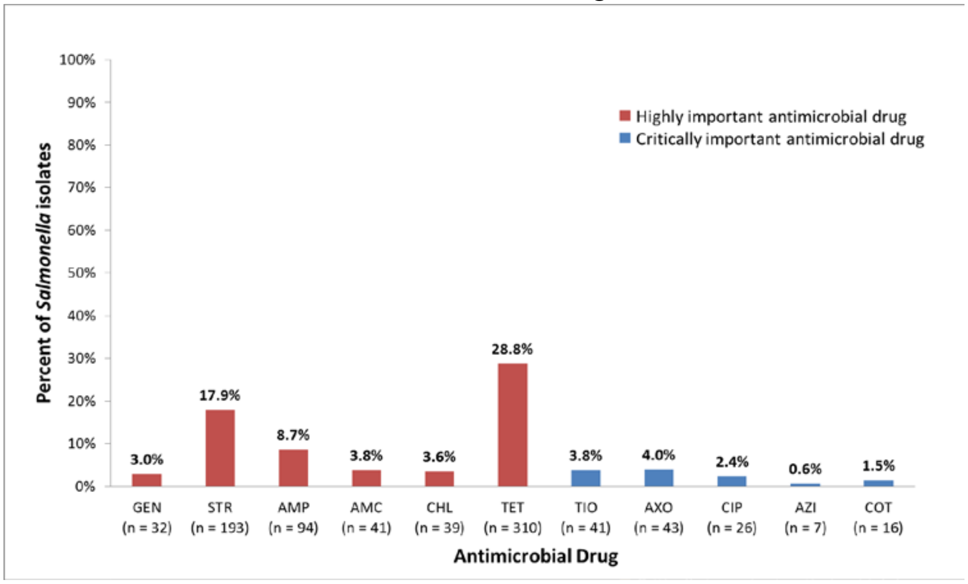
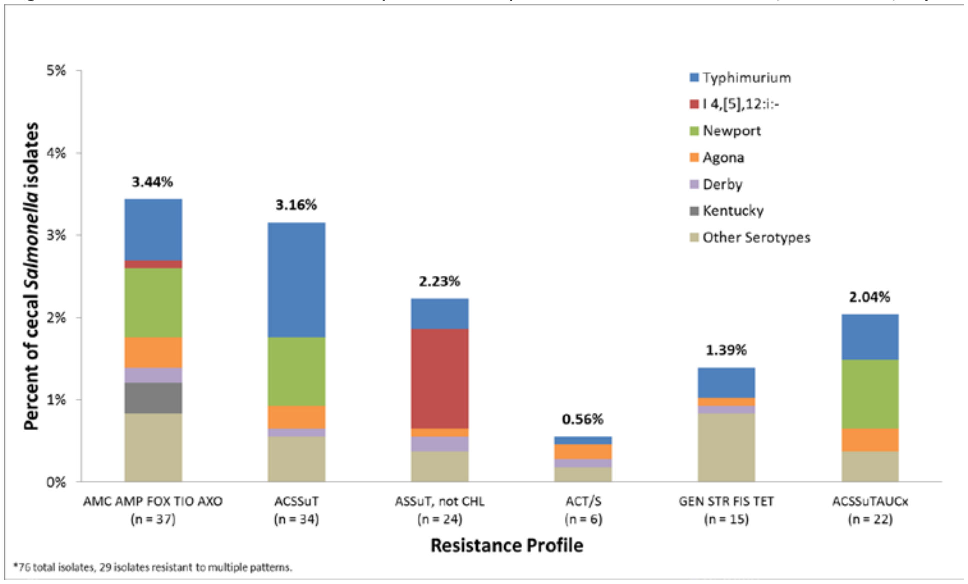


Table 4. *Salmonella* serotypes resistant to highly important and critically important antimicrobial drugs.

Antimicrobial drug	Number of resistant isolates	Most common serotype (# resistant, % of resistant isolates)	Second most common serotype (# resistant, % of resistant isolates)
TIO	41	Typhimurium (11, 26.8%)	Newport (9, 22.0%)
AXO	43	Typhimurium (11, 25.6%)	Newport (9, 20.9%)
CIP	26	Muenchen (9, 34.6%)	Anatum (6, 23.1%)
AZI	7	Typhimurium (2, 28.6%)	Agona, Brandenburg, Heidelberg, Montevideo, Schwarzengrund (1 isolate each, 14.3% each)
COT	16	Agona (3, 18.8%)	Kentucky, Senftenberg (2 isolates each, 12.5% each)
GEN	32	Schwarzengrund, Senftenberg (5 isolates each, 15.6% each)	Saintpaul, Typhimurium (4 isolates each, 12.5% each)
STR	193	Derby (33, 17.1%)	Typhimurium (28, 14.5%)
AMP	94	Typhimurium (26, 27.7%)	I 4,[5],12:i:- (13, 13.8%)
AMC	41	Typhimurium (11, 26.8%)	Newport (9, 22.0%)
CHL	39	Typhimurium (16, 41.0%)	Newport (9, 23.1%)
TET	310	Anatum (56, 18.1%)	Derby, Typhimurium (49 isolates each, 15.8% each)

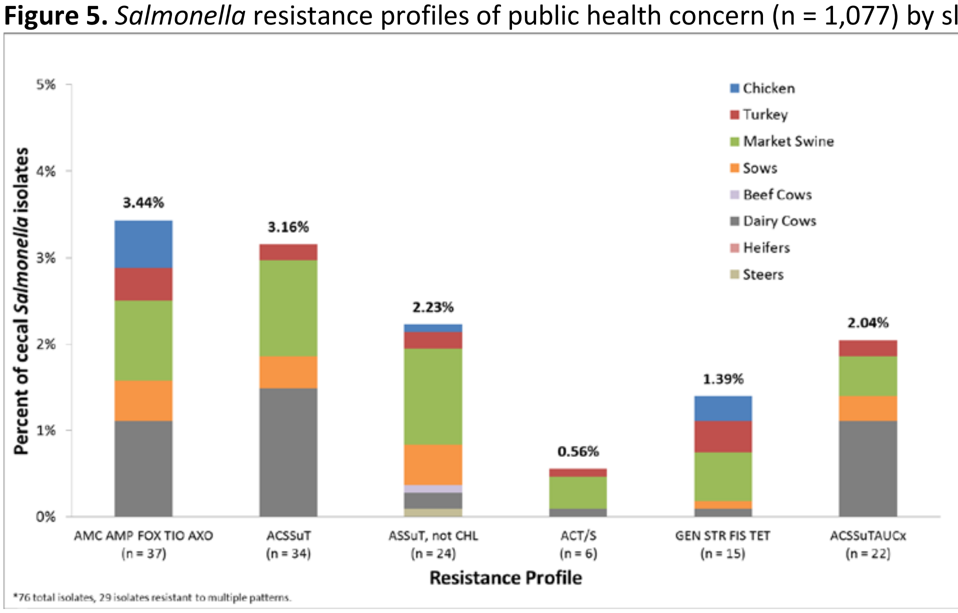
Figure 4 shows the serotypes identified as exhibiting resistance profiles that are of public health concern to FSIS. These resistance profiles are listed in Appendix Table 4. They are of interest in part because they indicate multidrug resistance, often to multiple critically important or highly important antimicrobial drugs. Some have not been previously seen frequently in the United States, however others such as ACSSuT and ASSuT have been historically associated with *Salmonella* Typhimurium DT104 and *Salmonella* I 4,[5],12:i:- respectively. For several of these profiles, the highest proportion of isolates was in one or two serotypes. The majority of isolates with ASSuT resistance are *Salmonella* I 4,[5],12:i:- (54.2% [13/24]), while the majority of isolates with ACSSuT resistance were *Salmonella* Typhimurium (44.1% [15/34]) or *Salmonella* Newport (26.5% [9/34]). In some cases, the resistance profile of a single isolate contained more than one MDR profile of concern, and is counted more than once.

Figure 4. *Salmonella* resistance profiles of public health concern (n = 1,077) by serotype, 2014*.



Similar data are presented in Figure 5, except by slaughter class. Beef cattle and steers each had only one cecal isolate with the ASSuT resistance profile, and no cecal isolates from heifers exhibited resistance to any of the profiles of public health concern. Market swine and dairy cow cecal samples made up the highest proportion of isolates with any of the resistance profiles of concern (35.5% [27/76] and 25.0% [19/76] of the isolates described here, respectively).

Figure 5. *Salmonella* resistance profiles of public health concern (n = 1,077) by slaughter class, 2014*.



Resistance in *Salmonella* Serotypes Associated with Human Illness

During 2011-2014, *Salmonella* Enteritidis, *Salmonella* Heidelberg, *Salmonella* I 4,[5],12:i:-, *Salmonella* Typhimurium, *Salmonella* Infantis, and *Salmonella* Newport were serotypes that were associated with

FSIS foodborne illness investigations. A review of these serotypes found that 89.5% (17/19) of *Salmonella* Enteritidis isolates were from chicken and all were pansusceptible. All *Salmonella* Heidelberg isolates (n = 10) were isolated from poultry. Seven of ten (70%) were from chicken and all were pansusceptible. The remaining three (30%) were from turkey and were MDR.

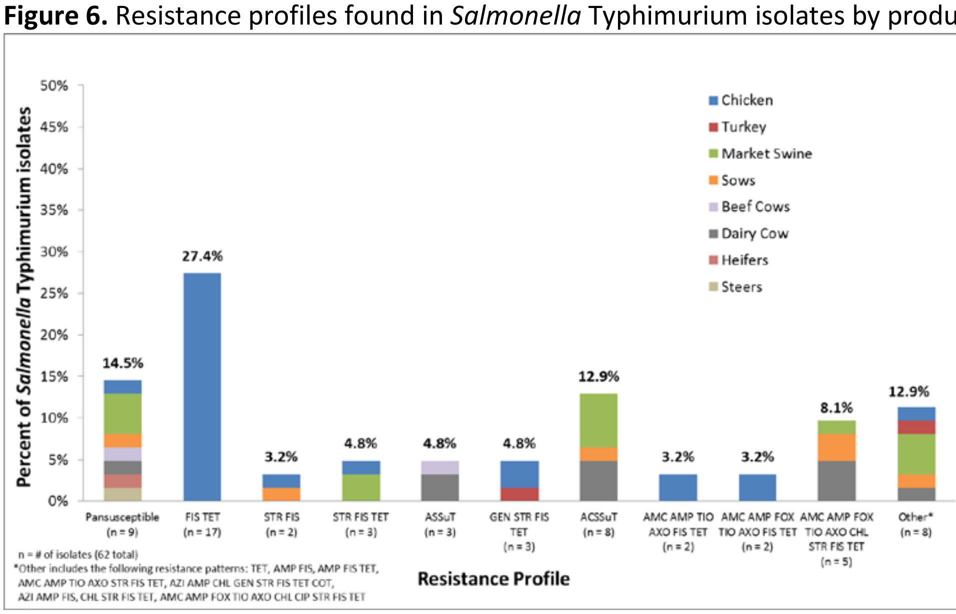
The three turkey *Salmonella* Heidelberg isolates exhibited resistance to the following antimicrobial drug classes:

1. ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), folate pathway inhibitors (FIS), and tetracyclines (TET)
2. ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), aminoglycosides (GEN, STR), and folate pathway inhibitors (FIS)
3. Macrolides (AZI), ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), phenicols (CHL), aminoglycosides (GEN, STR), folate pathway inhibitors (FIS, COT), and tetracyclines (TET).

Salmonella I 4,[5],12:i:- isolates were recovered from both swine slaughter classes. All market swine *Salmonella* I 4,[5]12:i:- isolates were resistant to at least one antimicrobial drug, and most were MDR with the ASSuT profile (88.9% [8/9]) (some isolates were resistant to additional antimicrobial drugs as well). In contrast, 50.0% (5/10) of sow *Salmonella* I 4,[5],12:i:- isolates were resistant to antimicrobial drugs included in the ASSuT profile.

Salmonella Typhimurium isolates were identified across all slaughter classes (Figure 6) with a high proportion of isolates resistant or MDR (85.5% [53/62] and 51.6% [32/62], respectively). Heifers and steers were the only slaughter classes where all *Salmonella* Typhimurium isolates were pansusceptible. Chicken, market swine, and dairy cows accounted for a majority of resistant isolates (49.1% [26/53], 18.9% [10/53], and 17.0% [9/53], respectively).

Figure 6. Resistance profiles found in *Salmonella* Typhimurium isolates by product class, 2014.



Salmonella Infantis was isolated from chicken, market swine, sows, and dairy cow cecal samples. Only 4.9% (3/61) of *Salmonella* Infantis isolates exhibited resistance and all were from market swine. The resistance profiles for market swine identified were: FIS TET (one isolate) and AMP STR TET (two isolates).

Salmonella Newport was recovered primarily from market swine and dairy cow cecal samples. Eight of 15 (53.3%) *Salmonella* Newport isolates from dairy cow samples exhibited MDR, all with the ACSSuTAuCx resistance profile plus resistance to two additional cephems (FOX, TIO). Two of five (40.0%) market swine *Salmonella* Newport isolates were resistant; one with the MDR profile found in dairy cattle isolates and one resistant to tetracycline. The remaining *Salmonella* Newport isolates from dairy cattle and market swine isolates were pansusceptible.

Additionally, *Salmonella* Dublin is a serotype of concern that appears to have increasing resistance to ceftriaxone in clinical, PR/HACCP ground beef and retail ground beef isolates.[6] Yet in 2014, only one *Salmonella* Dublin isolate was recovered from FSIS cecal sampling; this isolate was pansusceptible.

Extremely Resistant *Salmonella* Isolates

Only 4/1,077 (0.4%) of isolates exhibited an XDR resistance profile. These isolates and their resistance are as follows:

- Market swine *Salmonella* Agona isolate and turkey *Salmonella* Heidelberg isolate: Macrolides (AZI), ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), phenicols (CHL), aminoglycosides (GEN, STR), folate pathway inhibitors (FIS, COT), and tetracyclines (TET)
- Dairy cow *Salmonella* Montevideo isolate: Macrolides (AZI), ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), phenicols (CHL), aminoglycosides (STR), folate pathway inhibitors (FIS, COT), and tetracyclines (TET)
- Dairy cow *Salmonella* Typhimurium isolate: ß-lactam/ß-lactamase inhibitor combinations (AMC), penicillins (AMP), cephems (FOX, TIO, AXO), phenicols (CHL), quinolones (CIP), aminoglycosides (STR), folate pathway inhibitors (FIS), and tetracyclines (TET)

Conclusion

Cecal sampling represents a major addition to NARMS and has resulted, for the first time, in a random and nationally representative sampling of antimicrobial susceptibility of all bacteria targeted by NARMS, within all four major food animal species. The cecal sampling program establishes representative sampling that reflects microbial characteristics prior to the application of in-plant processing interventions, and that may serve as indicators of pre-harvest antimicrobial resistance. FSIS PR/HACCP regulations require establishments to lower levels of bacteria by ensuring process controls in production of animal products.

This report covers the first full year of cecal sampling and provides initial data on antimicrobial resistant *Salmonella* in cecal samples from animals slaughtered in federally inspected establishments. Notable findings include: the high level of resistance in turkey cecal isolates; the isolation of minimal *Salmonella* Dublin isolates; the majority of ASSuT resistance being in swine isolates; and, the low number of XDR

[6] CDC, FDA, FSIS (2016). NARMS Integrated Report, 2014. Retrieved from http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM528861.pdf.

isolates. Ongoing analysis of isolates collected in this program and the addition of more advanced microbial characterization, especially through whole genome sequencing, will enable FSIS to assess how AMR profiles in food animals compare with AMR profiles of public health concern. Additionally, information from cecal sampling allows surveillance of antimicrobial resistance in isolates recovered from different points in the food production system. These analyses will be used to inform sampling programs, guidelines, and policies; and support policies designed to reduce AMR bacteria in the food chain. Through these activities, FSIS is uniquely positioned to contribute science based expertise to promote a better understanding of the characteristics and movement of resistant bacteria throughout the food chain.

Appendix

Sampling Protocols

Cecal sampling within slaughter establishments is scheduled at a frequency based on establishment size, animal classes slaughtered, and annual slaughter volumes. Establishments are sampled in a tiered, randomized fashion based on slaughter volume, resulting in a scheme representative of overall national production.

As described in FSIS Directive 10100.1, Section III.A,[7] sampling tasks for the NARMS sampling program are assigned at frequencies indicated in Appendix Table 1, using the previous 12 months of slaughter data.

Appendix Table 1. NARMS sampling frequencies.

Slaughter Volume	Maximum number of sampling tasks per month per sampling project
Top 25% of Slaughter Establishments	4
Second 25% of Slaughter Establishments	2
Lowest 50% of Slaughter Establishments	1

An algorithm sets the number of cecal samples collected per slaughter class each month to reach an annual target. Appendix Table 2 shows the maximum numbers of samples that can be assigned per NARMS project per month.

Appendix Table 2. NARMS sampling algorithm (number of samples scheduled per class).

Class	Annual	Monthly¶
Young Chickens§	600	55 (275 birds)
Young Turkeys§	300	28 (140 birds)
Dairy Cattle	1200	110
Beef Cattle*	2000	184
Market Hogs	800	74
Sows	800	74

§ Five cecal samples are pooled as one sample in young chickens and young turkeys
*samples divided between steer, heifer, adult beef equally
¶ Including about a 10% overage

Antimicrobial Susceptibility Testing

Minimal Inhibitory Concentrations (MICs) are determined for each isolate. MICs are defined as the lowest concentration of an antimicrobial drug that will inhibit the visible growth of a microorganism

[7] FSIS (2014). FSIS Directive 10,100.1 FSIS Sampling for the National Antimicrobial Resistance Monitoring System (NARMS). Retrieved from: https://www.fsis.usda.gov/wps/wcm/connect/056b7ec7-5456-4325-ae55-1a73ddd6f348/10100.1.pdf?MOD=AJPERES.

after overnight incubation. Breakpoints are set by FDA and Clinical and Laboratory Standards Institute (CLSI) to categorize bacteria as susceptible or resistant.

When breakpoint changes occur, historical isolates' MICs are re-evaluated using current criteria to facilitate comparisons with historical data. This report uses breakpoints and categories that were implemented in January 2014 (Appendix Table 3).

Appendix Table 3. Interpretive criteria used for antimicrobial susceptibility testing of *Salmonella*[1].

Antimicrobial Class	Antimicrobial Drug	Breakpoints (µg/ml)		
		Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin (GEN)	≤ 4	8	≥ 16
	Streptomycin (STR)[1]	≤ 32	N/A	≥ 64
ß-Lactam/ß-Lactamase Inhibitor Combinations	Amoxicillin–Clavulanic Acid (AMC)	≤ 8 / 4	16 / 8	≥ 32 / 16
Cephems	Cefoxitin (FOX)	≤ 8	16	≥ 32
	Ceftiofur (TIO)	≤ 2	4	≥ 8
	Ceftriaxone (AXO)	≤ 1	2	≥ 4
Folate Pathway Inhibitors	Sulfisoxazole (FIS)	≤ 256	N/A	≥ 512
	Trimethoprim–Sulfamethoxazole (COT)	≤ 2 / 38	N/A	≥ 4 / 76
Macrolides	Azithromycin (AZI)[1]	≤ 16	N/A	≥ 32
Penicillins	Ampicillin (AMP)	≤ 8	16	≥ 32
Phenicols	Chloramphenicol (CHL)	≤ 8	16	≥ 32
Quinolones	Ciprofloxacin[2] (CIP)	≤ 0.06	N/A	≥ 0.12
	Nalidixic acid (NAL)	≤ 16	N/A	≥ 32
Tetracyclines	Tetracycline (TET)	≤ 4	8	≥ 16

[1] Breakpoints were adopted from CLSI M100-S26 document (2014), except for streptomycin and azithromycin, which have no CLSI breakpoints.

[2] In previous reports, resistance to nalidixic acid has been used as a surrogate for quinolone resistance. However with the 2012 Clinical Laboratory Standards Institute (CLSI) decision to widen the intermediate susceptibility range for ciprofloxacin, we now use decreased susceptibility to ciprofloxacin (DSC, MIC >= 0.12 ug/ml) as an indicator of emerging quinolone resistance. For the 2015 report, DSC is incorporated into the definition of multidrug resistance (MDR, resistance to 3 or more classes of antimicrobials). Although DSC encompasses both resistant and intermediate susceptibility populations, it is more clinically relevant than nalidixic acid resistance. In this report, the new MDR definition is retrospectively applied to all NARMS *Salmonella* data collected before 2015(Internal Communication).

Appendix Table 4. Multidrug resistance profile descriptions.

Profile Abbreviation	Resistance Description
AMC AMP FOX TIO AXO	Resistance to amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, and ceftriaxone, with or without resistance to other antimicrobial drugs
GEN STR FIS TET	Resistance to gentamicin, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline, with or without resistance to other antimicrobial drugs
ACSSuT	Resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline, with or without resistance to other antimicrobial drugs
ASSuT	Resistance to at least ampicillin, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline, with or without resistance to other antimicrobial drugs, except chloramphenicol
ACSSuTAuCx	Resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, tetracycline, amoxicillin/clavulanic acid, and ceftriaxone, with or without resistance to other antimicrobial drugs
ACT/S	Resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, with or without resistance to other antimicrobial drugs

Categorization of the importance of Antimicrobial Drugs[8]

Five criteria are used by FDA to rank the importance of antimicrobial drugs in human medicine. The criteria are ranked 1 to 5 from most important to least important (Appendix Table 5).

Appendix Table 5. Criteria to rank importance of antimicrobial drugs.

Criterion 1:	Antimicrobial drugs used to treat enteric pathogens that cause foodborne disease
Criterion 2:	Sole therapy or one of few alternatives to treat serious human disease or drug is essential component among many antimicrobial drugs in treatment of human disease
Criterion 3:	Antimicrobial drugs used to treat enteric pathogens in non-foodborne disease
Criterion 4:	No cross-resistance within drug class and absence of linked resistance with other drug classes
Criterion 5:	Difficulty in transmitting resistance elements within or across genera and species of organisms

The categories of importance for antimicrobial drugs based on these criteria are defined in Appendix Table 6, and Appendix Table 7 shows the categorization of important antimicrobial drugs included in AST for *Salmonella*.

[8] FDA (2003). Guidance for Industry #152, Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052519.pdf.

Appendix Table 6. Antimicrobial drug categories of importance.

Critically important (C):	Antimicrobial drugs which meet BOTH criteria 1 and 2 in Appendix A of the FDA Guidance for Industry #152
Highly important (H):	Antimicrobial drugs which meet EITHER criterion 1 or 2 in Appendix A of the FDA Guidance for Industry #152
Important (I):	Antimicrobial drugs which met EITHER criterion 3 and/or 4 and/or 5 in Appendix A of the FDA Guidance for Industry #152
Not classified (NC):	Antimicrobial drugs which are not given a classification in FDA's Guidance for Industry #152

Appendix Table 7. FDA categorization for antimicrobial drugs of importance highlighted in NARMS antimicrobial susceptibility analyses.

Antimicrobial Class	Antimicrobial Drug	Abbreviation	FDA Classification
Cephems	Ceftiofur	TIO	***Critically important (C)***
	Ceftriaxone	AXO	
Quinolones	Ciprofloxacin	CIP	
Macrolides	Azithromycin	AZI	
Folate Pathway Inhibitors	Trimethoprim-Sulfamethoxazole	COT	
Aminoglycosides	Gentamicin	GEN	***Highly Important (H)***
	Streptomycin	STR	
Penicillins	Ampicillin	AMP	
Beta-Lactam/Beta-Lacatamase Inhibitor Combinations	Amoxicillin-Clavulanic Acid	AMC or AUG	
Phenicols	Chloramphenicol	CHL	
Tetracyclines	Tetracycline	TET	
Cephems	Cefoxitin	FOX	***Important (I)***
Quinolones	Nalidixic Acid	NAL	
Folate Pathway Inhibitors	Sulfamethoxazole-Sulfisoxazole	FIS	***Not Classified (NC)***