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The Western United States has Greater Antibiotic Resistance Among Salmonella Recovered from Intestinal Cecal Samples of Food Animals.

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Running head: RESISTANT *SALMONELLA* FROM ANIMAL CECAL

**The Western United States has Greater Antibiotic Resistance Among *Salmonella*
Recovered from Intestinal Cecal Samples of Food Animals.**

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Abstract

As part of the National Antimicrobial Resistance Monitoring System (NARMS) activities, the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) collected cecal samples from food animal slaughter facilities throughout the country between 2014 and 2018. Of the 26,780 cecal samples from cattle, swine, chicken and turkey, 6,350 (23.71%) tested positive for *Salmonella*. NARMS tested *Salmonella* for susceptibility to aminoglycosides, folate pathway inhibitors, macrolides, phenicols, quinolones, beta lactams, and tetracyclines. Using the regional subdivisions defined in the USDA Office of Investigation, we used chi-square test to assess potential association between the region from which the samples were collected and both *Salmonella* prevalence and susceptibility. The results show a significant association between region and *Salmonella* prevalence, when accounting for source and establishment size, with the southeast region having the highest probability of finding *Salmonella*. However, the western region had the highest resistance probability across all antimicrobial classes except for macrolides, which showed no regional association. This association between region and resistance was strongest among isolates from cattle. Analysis of whole-genome sequencing data indicated that a significantly higher prevalence of *Salmonella* Newport in cattle in the western region (accounting for 9.52% of cattle isolates, compared to 3.44% in other regions) may account for the greater resistance to multiple drug classes. Approximately 90% of *Salmonella* Newport in the west exhibited the MDR-AmpC phenotype encoded by *aph(3'')-Ib/aph(6)-Id*, *bla_{CMY-2}*, *floR*, *sul2*, and *tetA*. Thus, differences in resistance across regions may be due to geographical differences in the prevalence of specific *Salmonella* serotypes and their accompanying resistance genes.

Key words: Antimicrobial resistance, *Salmonella* Newport, NARMS

Highlights

- *Salmonella* was significantly more prevalent in the southeast region.
- The prevalence of *Salmonella* was significantly higher in samples from small establishments.
- Antimicrobial resistance was significantly higher in the western region.
- Serotype, specifically *Salmonella* Newport, was likely influencing the association between regions and resistance to antimicrobial classes

In livestock farming, antibiotics can be used for various approved claims, including disease treatment of animals, as well as disease prevention and control (7, 8, 24). Of concern to human health is that most of the antibiotic classes administered to food animals are also used in human medicine (19), leading to cross-resistance in bacterial pathogens affecting both animals and humans (14, 15). Antimicrobial Resistance (AMR) is a global health problem that contributes to tens of thousands of deaths per year (17). *Salmonella* resistant to medically important antimicrobials, including cephalosporins and fluoroquinolones, has been deemed by the Centers for Disease Control and Prevention (CDC) as a serious threat to public health (2). Surveillance of antimicrobial use and resistance is critical in the development of science-based policies to help reduce the public health burden of AMR. The National antimicrobial resistance monitoring system (NARMS) monitors antimicrobial resistance among various foodborne pathogens, including *Salmonella*, isolated from food-producing animals, retail meats, and humans in the United States (21). These data provide an understanding of the factors leading to

antimicrobial resistance emergence and spread, as well as insight into the transmission of foodborne pathogens from farm to fork.

While antibiotic use practices in both animals and humans draw the greatest attention with regards to selection for antimicrobial resistance, it is possible that regional factors may play a role, including differences in agricultural practices and climate. Few studies have investigated this subject, although some studies have shown that the prevalence of *Salmonella* varies by geographical region and this prevalence-regional association may have some impact on regional variations in resistance findings (4, 7, 22, 11). This study examined AMR among 14 antimicrobials classified into 7 drug classes, in *Salmonella* recovered from food animal cecal contents to determine whether there were any significant variations among distinct regions within the United States.

Materials and Methods

Cecal Samples. The samples result from a joint FSIS-FDA non-regulatory project that conducted nationally representative, randomized sampling of intestinal animal contents throughout the USA at various time points between 2014-2018. *Salmonella* were isolated from samples collected from federally inspected slaughter and processing plants. The Public Health Veterinary (PHV) collected samples of cecal contents from the large intestines of cattle (dairy cows, beef cows, steers, and heifers), poultry (young chicken and turkey), and swine (market swine and sows). Livestock and poultry slaughter establishments were eligible for the NARMS sampling program based on data in the FSIS Public Health Information System (PHIS): by establishment size; the animal classes slaughtered; and annual slaughter volumes. The PHV randomly selects from the lots of animals presented for slaughter on the scheduled sampling day

that have passed ante-mortem inspection. Along with establishment management PHV identified the point in the slaughter process where the viscera/large intestines will be retrieved for sampling. Other information about the owner/producer name and address, and animal identification information were recorded. The samples were shipped to the FSIS lab for testing. During each sampling task, the number of samples collected was, 5, 5, 4, and 2 for chicken, turkeys, cattle, and swine respectively, (20).

Susceptibility testing. Isolates were forwarded to FSIS where they underwent broth microdilution susceptibility testing against a panel of 14 antibiotics using published methods (21). Antibiotics tested were from 7 drug classes: aminoglycosides (gentamicin and streptomycin), folate pathway inhibitors (sulfisoxazole and trimethoprim-sulfamethoxazole), macrolides (azithromycin), phenicols (chloramphenicol), quinolones (ciprofloxacin and nalidixic acid), tetracyclines (tetracycline), and beta lactams (amoxicillin-clavulanate, ceftiofur, ceftriaxone, ampicillin). These were tested on the NARMS Gram-negative panels CMV3AGNF and CMV4AGNF (Trek Diagnostic Systems, Westlake, OH). When available, interpretive criteria were used and interpreted per the Clinical and Laboratory Standards Institute (3) MIC Interpretive Standards, except for streptomycin, ceftiofur, and azithromycin, where NARMS provisional breakpoints were used.

Regions. Geographic regions were divided per the United States Department of Agriculture (USDA) Office of Investigation, Enforcement and Audit map.

Western Region (Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming, Guam, American Samoa, and the Mariana Islands).

Southwest Region (Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas).

Northeast Region (Connecticut, Indiana, Maine, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, and Wisconsin).

Southeast Region (Alabama, Arkansas, Delaware, District of Columbia, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, West Virginia, Puerto Rico, and the Virgin Islands).

Statistical Analysis. We treated *Salmonella* growth, *Salmonella* susceptibility, region, establishment size, and source of isolates as categorical variables which take outcomes: Yes (*Salmonella* growth) or No (no *Salmonella* growth); resistance, susceptible (not resistant); western, southwest, northeast, southeast; large, small, very small; chicken, cattle, swine, turkey; respectively. We used chi-square test of association to assess the independence between regions and *Salmonella* prevalence or *Salmonella* resistance to AMR classes (1, 10, 13). To quantify the association, we used multivariate logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) that quantify the association of antimicrobial class resistance with region adjusting for sample source, and establishment size (9, 16, 17, 18, 23). All statistical analyses were conducted using Statistical Analysis Software (SAS, Version 9.4). All chi-square tests of association were 2-sided, and a p-value of < 0.05 was considered statistically significant.

Analysis of sequenced data. Sequencing data were downloaded from the NCBI Isolates Browser, analyzing for the presence of resistance genes in different commodities, regions, and *Salmonella* serotypes for isolates collected from 2014 thru 2018. Resistance genes were identified in NCBI by AMRFinder (5). Search results were performed on February 19, 2020.

Results

***Salmonella* isolates.** Between 2014 and 2018 the USDA's FSIS collected 26,780 cecal samples as part of NARMS surveillance. The 8 sample sources were combined into cattle, swine, chicken, and turkey and distributed as follows: 15,333 (57.26%), 6,507 (24.30%), 3,399 (12.69%), and 1,541 (5.75%), respectively. The samples were collected from all 50 states and in establishments that FSIS classified as large (41.61%), small (34.84%), and very small (23.55%). 14 samples were not assigned to any establishment (2 cattle, 11 swine, 1 turkey). Among regions, the samples were distributed as follows: 8,968 (33.49%), 6,652 (24.84%), 6,390 (23.86%), 4,770 (17.81%) were from the southwest, the northeast, the southeast, and the west, respectively. Overall, 6,350 (23.71%) samples tested positive for *Salmonella* and among those positive samples, the 6 most common serotypes were Anatum (12.24%), Montevideo (7.44%), Cerro (6.90%), Infantis (6.85%), Derby (5.90%), and Typhimurium (5.23%).

Prevalence of *Salmonella* by regions and sources. The overall prevalence by source was: 47.63%, 31.89%, 14.80%, 12.65% for swine, chicken, turkey, and cattle respectively. The test of association between regions and *Salmonella* prevalence indicated that the southeast was the leading region for *Salmonella* positive samples. In chickens, most of the samples were from the southeast (77.02%); the west and the northeast had lower proportions of chicken samples (2.79%) and (3.06%) respectively, (Figure 1). The southeast was also associated with a significantly higher prevalence of *Salmonella* (33.34%) and the west and the northeast were associated with the lowest prevalence, 23.15% and 22.12% respectively (Figure 2). The odds of finding a sample with *Salmonella* contamination were 0.58[0.36, 0.95], 0.80[0.65, 0.97], and 0.53[0.33, 0.85] for west, southwest and northeast respectively as compared to the southeast.

In swine, the sample numbers were similar across regions (35.16%, 28%, 27.14% for southwest, northeast, and southeast respectively), except for the west, where there were significantly fewer ($p < .001$) samples (9.7%) (Figure 1). The southeast was associated with significant higher prevalence of *Salmonella* from swine isolates. The odds of finding a sample with *Salmonella* contamination were 0.66[0.55, 0.79], 0.71[0.63, 0.81], 0.56[0.49, 0.64] for the west, the southwest, and the northeast respectively as compared to the southeast.

In cattle, the southeast was associated with the lowest proportion of cattle samples (10.07%), (Figure 1). The west was associated with significantly lower prevalence as compared to the southwest, the northeast and the southeast, the odds were 0.67[0.59, 0.76], 0.75[0.65, 0.87], 0.76[0.64, 0.92], respectively, (Figure 2).

The highest proportion of turkey samples were in the southwest (34.91%) ($p = .003$), which also had the highest *Salmonella* prevalence for turkeys (18.96%) (Figure 1, Figure 2). The odds ratios of finding a turkey sample with *Salmonella* contamination were 0.68[0.48, 0.97], 0.48[0.33, 0.69], for the northeast and the southeast respectively as compared to the southwest.

Prevalence of *Salmonella* by establishments size. The distribution of samples by establishment size were as follow: 41.61%, 34.84%, and 23.55% for large, small and very small establishments respectively. Fourteen samples lacked establishment classification. The majority (93.67%) of chicken samples were from large establishments ($p < .001$), however *Salmonella* prevalence was not statistically significantly different among different establishment sizes. (Figure 3, 4). In cattle, most of the samples were from small establishments (40.59%), with no significant differences in *Salmonella* prevalence across different establishment sizes. In swine, the proportion of samples from very small (39.94%) and small (38.39%) establishments were each significantly higher than the proportion of samples from large establishments (21.5%) (p

<.001). However, the prevalence of *Salmonella* was significantly higher in small (59.61%) establishment as compared to very small (35.86%) and large (48.25%) ones ($p < .001$). In turkey, most of the samples were from large (68.98%) establishments versus 29.2% and 1.75% for small and very small establishments ($p < .001$). *Salmonella* prevalence was balanced among different establishment sizes, (Figure 4).

Prevalence of *Salmonella* by serotypes and regions. 6334 *Salmonella* isolates were distributed among 128 different serotypes. There were 16 *Salmonella* isolates with no available serotype information. Table 1 shows the distribution of *Salmonella* serotypes by regions and possible regions association among 32 serotypes which were observed 30 or more isolates each. The test of association indicates that some serotypes were predominant in one region. That was the case for 12 *Salmonella* serotypes that were predominant in the southwest (6,7:g,m,s:e,n,z15(95%), Adelaide (42.31%), Agona (45.56%), Anatum(44.26%), Uganda (40.16%), Reading (47.56%), Muenchen(48.85%), Montevideo(39.7%), Meleagridis(43.04%), Mbandaka(43.01%), I 4,[5],12:i:-(46.05%), Derby (45.99%)) northeast was leading in 2 *Salmonella* serotypes (Cerro(52.86%), Saintpaul(43.24%)), the southeast was leading in 6 (Enteritidis (70.17%), Infantis(47.47%), Kentucky(60.21%), Typhimurium(56.5%), Worthington(41.38%), Schwarzengrund(75.74%)). The test revealed that these 5 serotypes, Altona, Hadar, Heidelberg, Ohio, and Senftenberg, were equally dominant between southeast and southwest regions ; Brandenburg, Newport, and Muenster serotypes were equally dominant between west and southwest regions ; Eko serotype was equally dominant southeast and northeast regions ; and Johannesburg, London serotypes were equally dominant between southeast, southwest, and northeast regions, (Table 1).

Resistance of *Salmonella* to antimicrobial classes. There was association between regions and resistance to all antimicrobial classes tested except for macrolides. Overall, the west had samples with higher odds of resistance against 6 classes of 7 tested.

Within sources of isolates, the probability of finding a cattle isolate resistant against tetracyclines, aminoglycosides, folate pathway inhibitors and beta lactams was significantly higher in the west as compared to other regions. The odds ratios for the west compared to the southwest, northeast, and southeast were: ns(no significant), 1.78[1.29, 2.45], 2.72[1.85, 4.02] for tetracyclines; 3.95[2.63, 5.91], 5.36[3.17, 9.05], 5.36[3.17, 9.05], 2.87[1.47, 5.60] for aminoglycosides; 2.96[2.03, 4.31], 4.88[2.96, 8.03], 2.68[1.41, 5.12] for folate pathway inhibitors; and 4.94[3.22,7.58], 10.1[5.19,19.31], 5.58[2.36, 13.17] for beta lactams. In chicken isolates, the west exhibited significant high odds of resistance to tetracyclines as compared to southwest, 7.84[2.71,22.69], and the southeast, 3.55[1.28, 9.84]. Swine isolates from the southwest had a high likelihood to resist tetracyclines. The odds ratios were: 1.49[1.21, 1.85], 1.36[1.13, 1.65] for the northeast and southeast respectively. However, swine isolates from the west were likely to resist quinolones, aminoglycosides and folate pathway inhibitors. The odds ratios of the west compared to the southwest, northeast, and southeast were: 3.52[1.79, 6.93], 6.5[2.44, 17.32], 8.08[3.44, 18.96] for quinolones; ns(no significant), 1.71[1.19, 2.47], 2.43[1.70, 3.47] for aminoglycosides; and ns(no significant), 1.82[1.26, 2.61], 2.56[1.80, 3.66] for folate pathway inhibitors. Turkey isolates from all 3 regions except the northeast were dominantly resisting against tetracyclines, aminoglycosides, and folate pathway inhibitor. The odds ratios of the west as compared to the northeast were 4.48[1.45, 13.84] for tetracyclines; 5.30[1.72, 16.32] for aminoglycosides; and 7.78[2.14,28.29] for folate pathway inhibitor.

Genomic sequence data analysis. By downloading results from the NCBI isolate browser, we were able to identify potential genomic explanations for differences in resistance prevalence across regions and sources. The most notable findings included increased resistance prevalence in the west for isolates from cattle, including for drugs ampicillin, amoxicillin-clavulanate, ceftriaxone, sulfisoxazole, streptomycin, and tetracycline. We found that a higher prevalence of resistant *Salmonella* Newport in the west may have accounted for this, as 31 Newport in the west (of 34 Newport total with sequencing data) had the genes *aph(3'')*-*Ib/aph(6)-Id*, *bla_{CMY-2}*, *floR*, *sul2*, and *tetA*. In contrast, the other regions had a combined 23 isolates of *Salmonella* Newport, of which only seven had all the above resistance genes. Overall, *Salmonella* Newport accounted for 5.47% (40/730) of isolates in the west but only 0.91% (51/5620) in the other regions. Removing *Salmonella* Newport from the dataset resulted in substantially lower resistance prevalence among cattle isolates across each of the drugs for which the west was significantly higher than the other regions (Figure 5). In many cases, these resulted in resistance prevalence being similar across the west and other regions, indicating that resistant *Salmonella* Newport was largely responsible for the differences observed.

We also sought a genomic explanation for another of the more prominent results, with quinolone resistance being higher in swine in the west. While we found no significant differences in the regional prevalence of *qnr* genes, their recent emergence in swine has resulted in increased resistance to fluoroquinolones (NARMS, 2014). We found that for isolates with genomic data, 2.41% of isolates in the west (7/291) had *qnr* genes, compared with 2.63% of isolates in the other regions (74/2808). This does not appear to explain why quinolone resistance would be higher in the west in this source, so we subsequently analyzed for the presence of *gyrA* mutations, the other most common quinolone resistance mechanism. These mutations were

present in 4.12% (12/291) of isolates in the west, compared to 0.35% (10/2808) of isolates in the other regions. Upon further analysis, we found that eight of the twelve isolates in the west with *gyrA* mutations were *Salmonella* Brandenburg, all in the small NCBI SNP cluster PDS000011941.23 which contained a variety of swine and pork isolates, along with several human clinical isolates; only one isolate from the other regions was in this cluster. Thus, only a small collection of related isolates appears to be responsible for the relatively higher quinolone resistance observed in the west among swine isolates.

Discussion

We tested the association between geographic region and *Salmonella* prevalence and resistance in food animal cecal samples collected between 2014 and 2018. The overall prevalence of *Salmonella* was 23.71%. Isolates from swine were associated with the highest prevalence (47.63%), and those from cattle were associated with the lowest prevalence (12.65%) of *Salmonella*. Furthermore, the results show a significantly higher prevalence in *Salmonella* from animals processed in the southeast region. In terms of establishment size, a significantly higher proportion (41.61%) of samples was from large establishments, and very small establishments had the lowest proportion (23.55%). However, the prevalence of *Salmonella* was significantly higher ($p < .001$) in samples from small establishments: 26.02% versus 22.94% or 21.66% for large and very small establishment respectively.

This study revealed that the 6334 *Salmonella* isolates were distributed among 128 different serotypes. The test of association on serotypes that were available in 30 or more isolates identified that 12 *Salmonella* serotypes were predominant in the southwest

(6,7:g,m,s:e,n,z15, Adelaide, Agona, Anatum, Uganda, Reading, Muenchen, Montevideo, Meleagridis, Mbandaka, I 4,[5],12:i:-, Derby). 6 *Salmonella* serotypes were predominant in the southeast (Enteritidis, Infantis, Kentucky, Typhimurium, Worthington, Schwarzengrund). 2 *Salmonella* serotypes (Cerro, Saintpaul) were predominant in the northeast. 5 serotypes, Altona, Hadar, Heidelberg, Ohio, and Senftenberg, were equally dominant between the southeast and the southwest regions. Brandenburg, Newport, and Muenster serotypes were equally dominant between the west and the southwest regions. Eko serotype was equally dominant between the southeast and the northeast regions, and Johannesburg, London serotypes were equally dominant between southeast, southwest, and northeast regions, (Table 1). It is interesting to note that none of the 32 serotypes with at least 30 isolates counts is equally distributed among all 4 regions, an indication that region associations are driven by *Salmonella* serotypes.

For antimicrobial resistance, there is an association between region and individual drugs grouped into drug classes. Overall, isolates from the west had a higher probability to be resistant against drug classes/individual drugs as compared to isolates from other regions. Particularly, cattle isolates had significantly higher resistance to most of the drug classes tested: tetracyclines, aminoglycosides, folate pathway inhibitor, and beta- lactams. Analysis of whole-genome sequencing data indicates that a significantly higher prevalence of *Salmonella* Newport in cattle in the western region (accounting for 9.52% (38/399) of cattle isolates, compared to 3.31% (51/1540) in other regions) may account for the greater resistance to multiple drug classes. The identified genotype is associated with the MDR-AmpC/ACSSuT phenotype that has previously been found in *Salmonella* Newport (6, 19). It is unclear if there are any underlying factors such as agricultural practices or antimicrobial use driving these differences in *Salmonella* Newport prevalence, or if they simply result from the circulating strains in a given area. The analysis of

retail meat data collected by NARMS between 2002-2017, (11) revealed that the northeast was the most influential region when estimating the national prevalence of *Salmonella* and *Salmonella* resistance to antimicrobials, with *Salmonella* serotype Typhimurium driving the differences. In this study of animals at slaughter, the southeast was the leading region for *Salmonella* prevalence, and that the western region had more resistance to antimicrobials. This appeared to be largely driven by the prevalence of multidrug-resistant *Salmonella* serotype Newport despite the finding that it was not among the 6 most common *Salmonella* serotypes in this dataset.

Although studies have shown a significant association between regions and the prevalence of *Salmonella* (4, 7, 15, 11), the results of the current study should be interpreted with caution due to unequal distribution between regions of certain sample source from different animal species with significantly higher prevalence. Chicken had the second highest prevalence (31.89%) after swine, and 77.02% of chicken samples were from the southeast, (Figure 1). Also, swine had the highest prevalence (47.63%) of *Salmonella* and more than a quarter (27%) of all swine samples were from the southeast. Oversampling may also explain the significantly higher ($p < .001$) prevalence of *Salmonella* in samples from small establishments. The prevalence of *Salmonella* was significantly higher in swine. However, swine samples were not balanced with establishment sizes. In fact, large establishments accounted for a significantly lower proportion of swine samples as compared to small and very small establishments, (Figure 3). This study highlights the importance of genomics analysis when comparing regional differences in resistance prevalence as small differences in serotype prevalence can result in relatively large statistical differences across regions. These may not be reflective of broad differences in

antimicrobial usage or agricultural practices across regions and is consistent with previous work on retail meats (11).

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Supplemental Material

Supplemental material associated with this article can be found online at: [URL to be completed by the publisher].

Reference

1. Bewick, V., L. Cheek, J. Ball. 2004. Statistics review 8: *Qualitative data - tests of association*. *Crit Care*. 8:46-53.
2. Center for Disease Control. Antibiotic Resistance Threats in the United States. 2013. CDC website. September 16, 2013. Available at: www.cdc.gov/drugresistance/threat-report-2013
3. Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing, 29th ed. Clinical 7 and Laboratory Standards Institute, Wayne, PA.

4. Elnekave, E., S. Hong, A. E. Mather, D. Boxrud, A. J. Taylor, V. Lappi, T. J. Johnson, F. Vannucci, P. Davies, C. Hedberg, A. Perez, J. Alvarez. 2018. *Salmonella enterica* Serotype 4,[5],12:i:- in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 66(6), 877–885.
<https://doi.org/10.1093/cid/cix909>
5. Feldgarden, M., V. Brover, D.H. Haft, A.B. Prasad, D.J. Slotta, I. Tolstoy. 2019. Validating the NCBI AMRFinder Tool and Resistance Gene Database Using Antimicrobial Resistance Genotype-Phenotype Correlations in a Collection of NARMS Isolates. *Antimicrob Agents Chemother.* 63(11): doi: 10.1128/AAC.00483-19
6. Greene, S. K., A. M. Stuart, F. M. Medalla, J. M. Whichard, R. M. Hoekstra, T. M. Chiller. 2008. Distribution of multidrug-resistant human isolates of MDR-ACSSuT *Salmonella* Typhimurium and MDR-AmpC *Salmonella* Newport in the United States, 2003-2005. *Foodborne pathogens and disease*, 5(5), 669–680.
<https://doi.org/10.1089/fpd.2008.0111>
7. Hao, H., G. Cheng, Z. Iqbal, X. Ai, H. I. Hussain, L. Huang, M. Dai, Y. Wang, Z. Liu, Z. Yuan. 2014. Benefits and risks of antimicrobial use in food-producing animals. *Front. Microbiol.*, 5:288. doi: 10.3389/fmicb.2014.00288.
8. Hong, P. Y., N. Al-Jassim, M.I., Ansari, R. I Mackie, R. I. 2013. Environmental and Public Health Implications of Water Reuse: Antibiotics, Antibiotic Resistant Bacteria, and Antibiotic Resistance Genes. *Antibiotics (Basel, Switzerland)*, 2(3), 367–399.
<https://doi.org/10.3390/antibiotics2030367>

9. Hosmer, D.W., S. Lemeshow, J. Wiley, InterScience (Online Service) 2000. Applied logistic regression (2nd ed). Wiley, New York
10. McHugh, M.L. 2013. “The chi-square test of independence.” *Biochemia medica*. 2: 143-9
11. Nyirabahizi, E., G. H. Tyson, H. Tate, C. Kabera, E. Crarey, S. Ayers, E. Strain. 2020. Northeastern U.S. *Salmonella* Strains from Retail Meat Are More Prevalent and More Resistant to Antimicrobials. *J Food Prot*. 2020;83(5):849- 857. doi:10.4315/JFP-19-549
12. Saiful Islam K.B.M., Shiraj-Um-Mahmuda S., Hazzaz-Bin-Kabir M. 2016. Antibiotic usage patterns in selected broiler farms of Bangladesh and their public health implications. *J. Public Health Dev. Ctries*. 2:276–284
13. Scott, M., D. Flaherty, J. Currall. 2013. Statistics: Dealing with categorical data. *J Small Anim Pract*. 54:3-8.
14. Smith, D. L., J. Dushoff, J. G. Morris. 2005. Agricultural antibiotics and human health. *PLoS Med*. 2:e232
15. Smith, D. L., A. D. Harris, J. A. Johnson, E. K. Silbergeld, J. G. Morris. Jr. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci. USA* 99:6434–39
16. Szklo, M., F. J. Nieto. 2007. Epidemiology: Beyond the basics. 2nd edition. Sudbury, MA: Jones and Bartlett Publishers

17. Szumilas, M. 2010. Explaining odds ratios. *Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie canadienne de psychiatrie de l'enfant et de l'adolescent*. 19: 227-229
18. Tack, D. M., E. P. Marder, P. M. Griffin, et al. Preliminary Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2015–2018. *MMWR Morb Mortal Wkly Rep* 2019; 68:369–373.
DOI: <http://dx.doi.org/10.15585/mmwr.mm6816a2external> icon.
19. Tyson, G.H., H. P Tate, S. Zhao, C. Li, U. Dessai, M. Simmons, P. F Mc Dermott. Identification of Plasmid-Mediated Quinolone Resistance in *Salmonella* Isolated from Swine Ceca and Retail Pork Chops in the United States. *Antimicrob Agents Chemother*. 2017 Sep 22;61(10). pii: e01318-17. doi: 10.1128/AAC.01318-17. Print 2017 Oct.
20. U. S. Department of Agriculture, Food Safety and Inspection Service. Cecal sampling under the National antimicrobial resistance monitoring system (NARMS) surveillance program, 2014. Available at: <https://www.fsis.usda.gov/wps/wcm/connect/056b7ec7-5456-4325-ae55-1a73ddd6f348/10100.1.pdf?MOD=AJPERES>
21. U. S. Food and Drug Administration. National antimicrobial Resistance Monitoring System integrated report: 2014. The National Antimicrobial Resistance Monitoring System: enteric bacteria. Available at: <https://www.fda.gov/media/101511/download>
22. U. S. Food and Drug Administration. The National Antimicrobial Resistance Monitoring System. Available at: <https://www.fda.gov/media/108304/download>

23. Vittinghoff, E., D. V. Glidden, S.C. Shiboski, & C. E. McCulloch. 2005. *Statistics for biology and health. Regression methods in biostatistics: Linear, logistic, survival, and repeated measures models*. New York, NY, US: Springer Publishing Co.
24. You, Y., E. K. Silbergeld. Learning from agriculture: Understanding low-dose antimicrobials as drivers of resistome expansion. *Front Microbiol.* 2014; 5:284

Figure Legends

Figure 1: The distribution of cecal samples by region and source

Figure 2: The prevalence of *Salmonella* by source and region.

Figure 3: The distribution of cecal samples by establishments size and source

Figure 4: The prevalence of *Salmonella* by source and establishment size.

Figure 5. Resistance prevalence of cattle *Salmonella* with and without *S. Newport*

Table 1: Serotypes by regions

Serotypes	West	Southwest	Northeast	Southeast	Total	Leading regions
6,7:g,m,s:e,n,z15	1(2.27)	42(95.45)	1(2.27)	0	44	SW
Adelaide	13(16.67)	33(42.31)	12(15.38)	20(25.64)	78	SW
Agona	13(7.69)	77(45.56)	37(21.89)	42(24.85)	169	SW
Altona	5(16.13)	14(45.16)	4(12.9)	8(25.81)	31	SW and SE
Anatum	68(8.77)	343(44.26)	142(18.32)	222(28.65)	775	SW
Brandenburg	20(47.62)	13(30.98)	3(7.14)	6(14.29)	42	W and SW
Cerro	31(7.09)	104(23.8)	231(52.86)	71(16.25)	437	NE
Derby	35(9.36)	172(45.99)	73(19.52)	94(25.13)	374	SW
Eko	10(11.24)	19(21.35)	26(29.21)	34(38.2)	89	NE and SE
Enteritidis	8(4.42)	34(18.78)	12(6.63)	127(70.17)	181	SE
Give	5(13.16)	13(34.21)	16(42.11)	4(10.53)	38	SW and NE
Hadar	5(7.58)	28(42.42)	15(22.73)	18(27.27)	66	SW and SE
Heidelberg	2(3.92)	22(43.14)	4(7.84)	23(45.1)	51	SW and SE
I 4,[5],12:i:-	23(10.7)	99(46.05)	45(20.93)	48(22.33)	215	SW
Infantis	35(8.06)	124(28.57)	69(15.9)	206(47.47)	434	SE
Johannesburg	17(5.63)	89(29.47)	88(29.14)	108(35.76)	302	SW, NE, and SE
Kentucky	25(6.46)	92(23.77)	37(9.56)	233(60.21)	387	SE
London	6(5)	33(27.5)	35(29.17)	46(38.33)	120	SW, NE, and SE
Mbandaka	13(13.98)	40(43.01)	13(13.98)	27(29.03)	93	SW
Meleagridis	11(13.92)	34(43.04)	14(17.72)	20(25.32)	79	SW
Montevideo	111(23.57)	187(39.7)	130(27.6)	43(9.13)	471	SW
Muenchen	16(12.21)	64(48.85)	20(15.27)	31(23.66)	131	SW
Muenster	26(31.71)	26(31.71)	15(18.29)	15(18.29)	82	W and SW
Newport	40(43.96)	31(34.07)	7(7.69)	13(14.29)	91	W and SW
Ohio	2(3.39)	22(37.29)	8(13.56)	27(45.76)	59	SW and SE
Reading	7(8.54)	39(47.56)	14(17.07)	22(26.83)	82	SW
Saintpaul	5(6.76)	22(29.73)	32(43.24)	15(20.27)	74	NE
Schwarzengrund	9(6.62)	10(7.35)	14(10.29)	103(75.74)	136	SE
Senftenberg	20(17.09)	39(33.33)	16(13.68)	42(35.09)	117	SW and SE
Typhimurium	56(16.92)	56(16.92)	32(9.67)	187(56.5)	331	SE

SW: Southwest, SE: Southeast, NE: Northeast, W: West

Only serotypes with at least 30 total isolates are included in this table

The number in parentheses indicates the equivalent percentage.

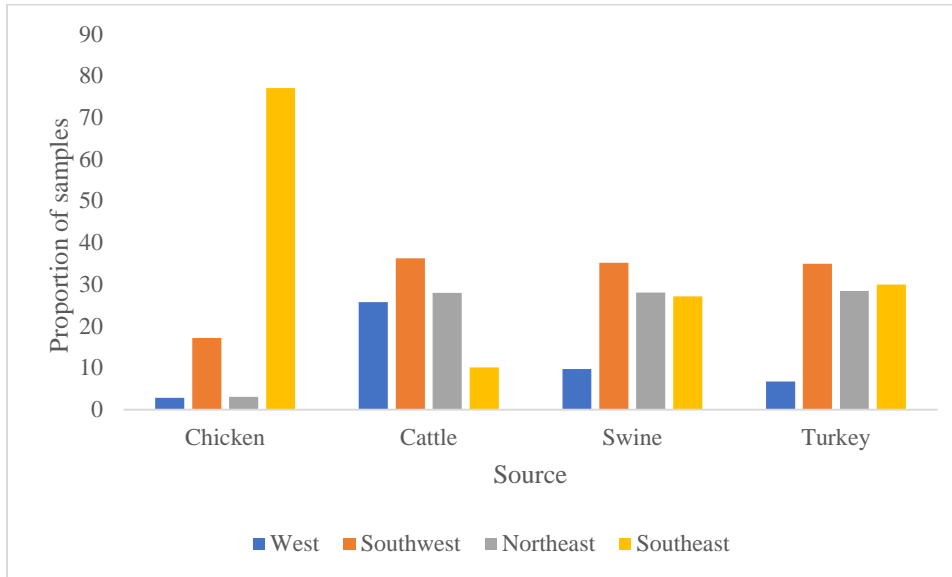
Uganda	13(10.66)	49(40.16)	31(25.41)	29(23.77)	122	SW
Worthington	7(12.07)	14(24.14)	13(22.41)	24(41.38)	58	SE

SW: Southwest, SE: Southeast, NE: Northeast, W: West

Only serotypes with at least 30 total isolates are included in this table

The number in parentheses indicates the equivalent percentage.

Figure 1: The distribution of cecal samples by region and source (n=26766). 77.02% of all chicken samples were from the southeast.



*14 samples (2 cattle, 11 swine, 1 turkey) missed region assignment as the state where they were collected was missing.

Figure 2: The prevalence of *Salmonella* by source and region, (n=6350). The prevalence of *Salmonella* from swine was the highest among all commodities and within regions, the southeast was leading.

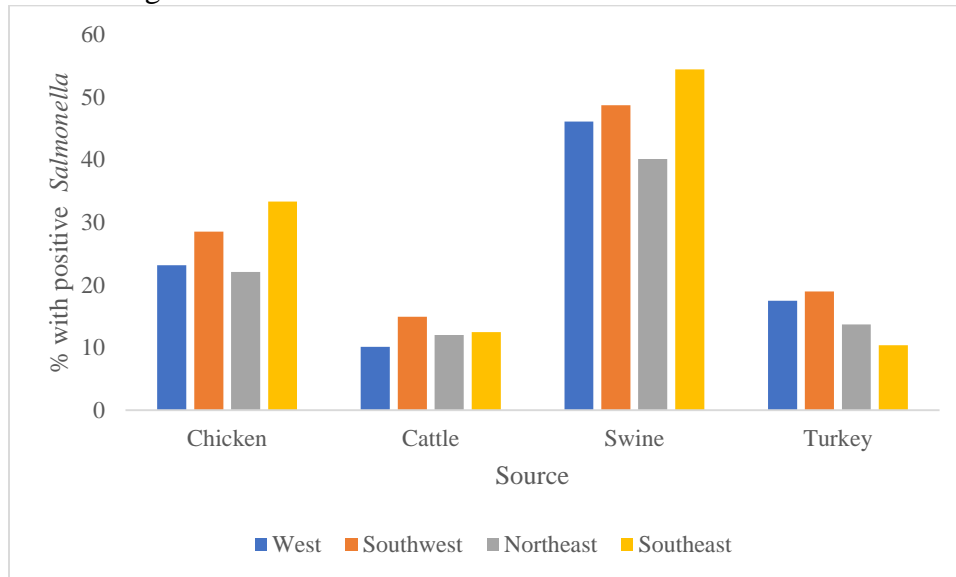


Figure 3: The distribution of cecal samples by establishments size and source, (n=26766). 93.67% of chicken samples were from large establishments.

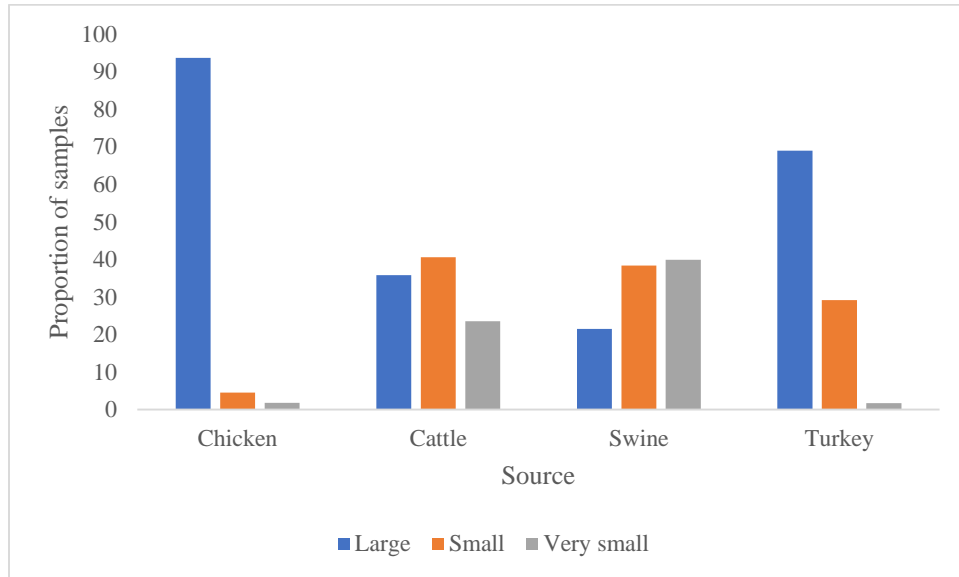


Figure 4: The prevalence of *Salmonella* by source and establishment size, (n=6530).

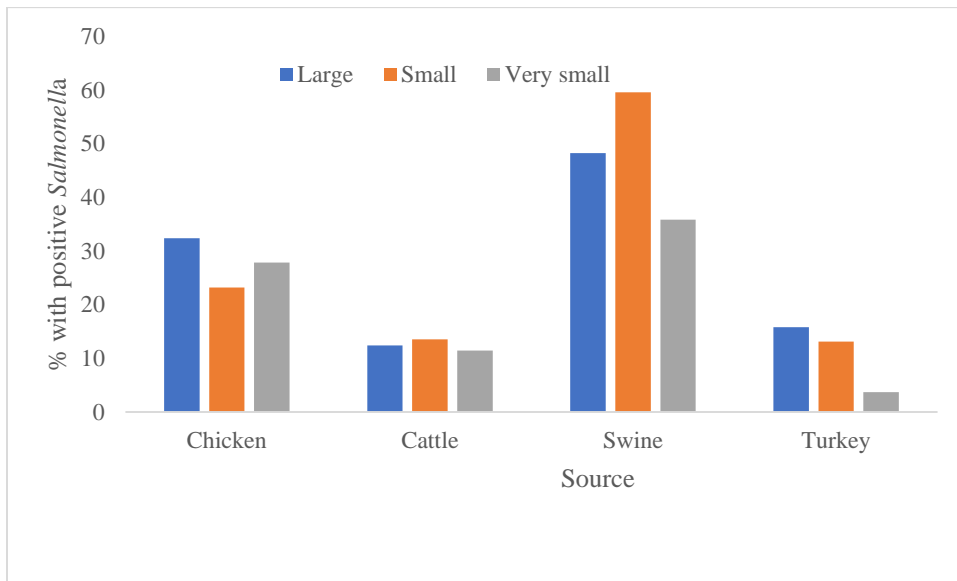
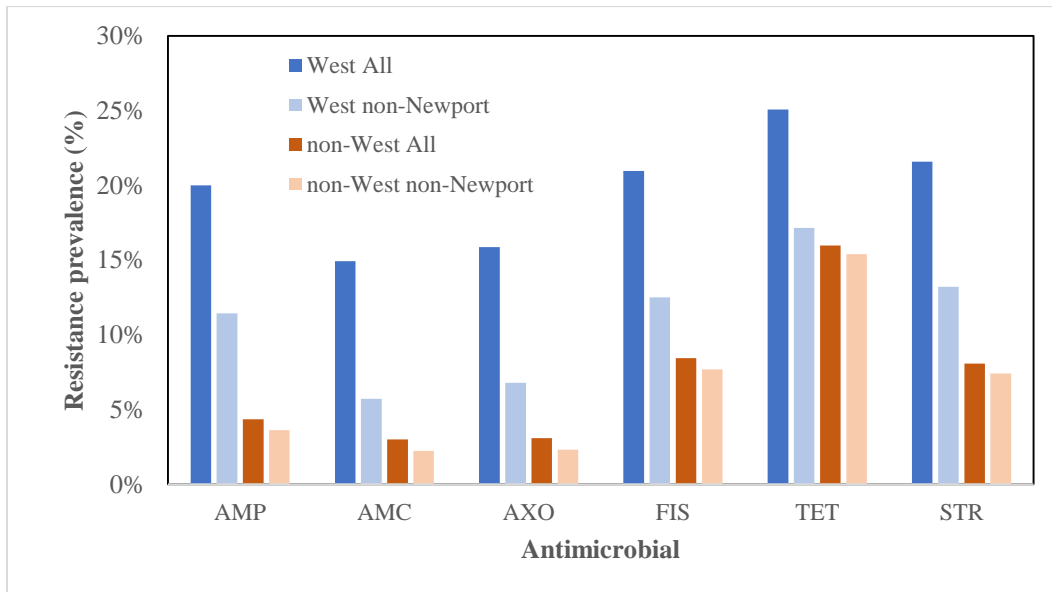


Figure 5. Resistance prevalence of cattle *Salmonella* with and without *Salmonella* Newport, (n=91). The resistant *Salmonella* Newport was largely responsible for the differences observed.







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