Integrated surveillance for antimicrobial resistance in Salmonella from clinical and retail meat sources reveals genetically related isolates harboring quinolone and ceftriaxone resistant determinants

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**Background:** Antimicrobial resistance in foodborne pathogens, including non-typhoidal *Salmonella* (NTS), is a public health concern. Pennsylvania conducts integrated surveillance for antimicrobial resistance in NTS from human and animal sources.

**Methods:** During 2015-2017, clinical laboratories submitted 4,478 NTS isolates from humans and 96 isolates were found in 2,520 retail meat samples. One hundred and nine clinical isolates that shared pulsed-field gel electrophoresis patterns with meat isolates and all strains from meat samples were tested for susceptibility to antimicrobial agents. Six clinical and 96 NTS isolates from meat sources (total 102) were analyzed by whole-genome sequencing (WGS).

**Results:** 28 (25.7 %) of the 109 clinical NTS and 21 (21.9%) of strains from meat sources had resistance to ≥3 antimicrobial drug classes (MDR). Sixteen (15.7%) of the 102 isolates analyzed by WGS had resistance mechanisms that confer resistance to expanded-spectrum cephalosporins (ESCs), such as ceftriaxone. We identified a bla CTX-M-65 in two S. Infantis isolates from clinical and three S. Infantis isolates from meat sources. These five bla CTX-M-65-positive S. Infantis strains carried ≥5 additional resistance genes plus a D87Y mutation in gyrA that encodes fluoroquinolone resistance. WGS showed that isolates from patients and meat samples were within ≤10 and ≤5 alleles for S. Infantis and S. Reading, respectively.
Conclusions: A significant proportion of NTS isolates from human and animal sources were MDR and 16% had genetic mechanisms that confer resistant to ceftriaxone. These results emphasize need for integrated surveillance in healthcare and agricultural settings.

Keywords: *Salmonella*, antibacterial agents, antimicrobial resistance, microbial quality, foodborne pathogens, multi-drug resistant, extended-spectrum β-lactamase–producing, ESBL, CTX-M-65, *bla*<sub>CMY</sub>, ceftriaxone susceptibility.
**Introduction**

Each year, non-typhoidal *Salmonella enterica* (NTS) causes an estimated 93.8 million episodes of acute gastroenteritis worldwide resulting in 90,300 deaths (1-2). About 34% of severe illnesses associated with foodborne pathogens in the United States (US) result from NTS infections (3). There are at least 2,600 NTS serotypes adapted to a variety of ecological niches including intestinal tracts of humans and animals (4). Human infections occur via ingestion of bacteria through consumption of contaminated food of animal origin including poultry meat and pork (5-6).

Although most episodes of NTS gastroenteritis resolve within 4-7 days without treatment, antibiotics can be lifesaving in persons with invasive infections. Antibiotics including ceftriaxone and ciprofloxacin are recommended for those at elevated risk of invasive NTS disease—for example, neonates, immunocompromised people, and those over age 50 with known or suspected atherosclerosis (4,7). While rare, *Salmonella* can cause mycotic aneurysms, especially of the aorta (8). Ceftriaxone is a favored antimicrobial because of its efficacy in treating invasive salmonellosis, safety profile, and convenience of once daily administration (6-9). Ciprofloxacin, with excellent bioavailability, has the advantage of having an oral formulation.

Increasing NTS resistance to multiple antimicrobial classes coupled with a high prevalence of invasive infections in many parts of the world has become a public health concern (10-11). In the US, antimicrobial-resistant NTS cause an estimated 212,500 infections each year resulting in 70 deaths (6). Injudicious use of antimicrobials in agriculture to meet expanding demand for animal protein is considered a driver of the global spread of bacteria with genetic resistance mechanisms; examples include extended...
spectrum beta-lactamases (ESBLs) that hydrolyze β-lactam antibiotics including ceftriaxone (12-15).

PulseNet, a national laboratory network coordinated by the Centers for Disease Control and Prevention (CDC), uses standardized molecular methods to conduct surveillance for NTS and facilitate early detection of outbreaks (16). In addition to participating in PulseNet, Pennsylvania monitors antimicrobial resistance of foodborne bacteria isolated from patients and retail food samples through the National Antimicrobial Resistance Monitoring System (NARMS) coordinated by the CDC and the Food and Drug Administration (FDA) [17]. We use data generated through NARMS and PulseNet to implement a state-based integrated surveillance system that compares NTS isolated from patients and food sources to guide antimicrobial susceptibility testing and outbreak investigation. The ongoing implementation of the 2015 National Plan for Combating Antibiotic-Resistant Bacteria has facilitated the transition from molecular subtyping using pulsed-field gel electrophoresis (PFGE) to whole-genome sequencing (WGS) in surveillance for Salmonella and other human pathogens in the US (18). WGS provides greater discrimination than PFGE and can reveal evolutionary relationships of bacteria and delineate antimicrobial resistance mechanisms (19). Our objective was to characterize NTS from clinical and retail meat sources that had identical PFGE patterns to elucidate antimicrobial resistance and genetic relatedness.
Materials and methods

During 2015-2017, approximately 115 clinical laboratories in Pennsylvania submitted a total of 4,478 NTS isolates from people to the Pennsylvania Department of Health Bureau of Laboratories (BOL) in compliance with communicable disease reporting requirements (20). We concurrently conducted a prospective microbiological survey of NTS contamination in retail meat samples: chicken breasts (n=1,170), ground turkey (n=630), ground beef (n=360), and pork chops (n=360). Samples were purchased from retail outlets located in four counties of southeastern Pennsylvania. We used a NARMS standardized protocol for transporting and processing retail food samples (21). NTS was isolated from retail meat samples using laboratory methods previously described (22-23).

All NTS isolates from meat samples were included in the study. Clinical isolates were included only if they had a PFGE pattern indistinguishable from one of the NTS isolated from a meat sample (Figure 1), since NTS isolates from retail meat and human sources with identical PFGE patterns have an increased likelihood of being genetically related (16). Clinical NTS from stool samples were considered non-invasive, whereas NTS from other sites (including abscess, aspirate, bile fluid, urine and blood) were considered invasive (7, 11).

Characterization of bacterial isolates by PFGE and susceptibility testing.

Salmonella isolates were confirmed and serotyped according to the Kaufmann-White scheme (24). DNA fragments digested with restriction enzymes XbaI and BlnI were separated by PFGE as described (16). The fingerprints captured in gel images were
analyzed with BioNumerics software (version 6.6, Applied Maths). Pattern names were assigned after comparing the fingerprints with those in the national database (16).

We tested clinical isolates and those from meat sources by broth microdilution for susceptibility to 14 antimicrobial agents from nine antimicrobial classes: aminoglycosides (gentamicin, streptomycin), penicillins (ampicillin), β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid), cephalosporins (cefotixin, ceftriaxone), macrolides (azithromycin), phenicols (chloramphenicol), quinolones (nalidixic acid, ciprofloxacin), folate pathway inhibitors (sulfisoxazole, trimethoprim-sulfamethoxazole (TMP-SMX), and tetracyclines (tetracycline). We used Clinical and Laboratory Standards Institute guidelines (CLSI) criteria and NARMS consensus breakpoints to interpret results (25-26). Isolates with decreased susceptibility to ciprofloxacin (DSC; MIC ≥0.12 μg/mL) were categorized as resistant to the quinolone class (26-27). If an isolate was resistant to three or more antimicrobial classes, we classified it as multidrug resistant (MDR). We also examined resistance to the four antibiotics recommended for severe NTS infections by the Infectious Diseases Society of America (IDSA) treatment guidelines: ceftriaxone, ciprofloxacin, TMP-SMX, and amoxicillin (7). Resistance to amoxicillin was based on susceptibility to amoxicillin and clavulanic acid.

Whole genome sequencing, resistance genes, and plasmids. We sequenced a subset of clinical NTS isolates included in the NARMS frequency-based sampling (17, 27) and all NTS from meat sources using version 2 or 3 chemistry with paired-end 2- by 250-bp or 2- by 300-bp reads on the Illumina MiSeq platform. We followed PulseNet standard
protocols in preparation of DNA libraries, purification, and quality controls and previously described methods for isolates from patients and food sources (28-29). De novo assemblies were produced using shovill v.1.0.4 (https://github.com/tseemann/shovill). To enable comparison of predicted genotypic resistance with phenotypic profiles and to identify plasmids, we used bioinformatics to analyze NTS genomic data. We screened assemblies for resistance determinants using staramr v. 0.4.0, which employs the ResFinder database (updated February 11, 2020) and thresholds of 90% identity and 50% gene coverage and the PointFinder scheme for Salmonella spp. (updated August 30, 2019). We used PlasmidFinder version 2.1 Enterobacteriaceae database (updated July 1, 2020) to identify plasmids with 95% identity and 60% gene coverage.

 Genome comparison and phylogenetic analyses.

We analyzed genome assemblies for S. Infantis, Reading, and Thompson isolates on the PulseNet National Salmonella database using BioNumerics software (version 7.6). These three serotypes were selected because they were the most frequently found among clinical isolates included in the study. We compared isolates in each of the three serotypes by core genome multilocus sequence typing (cgMLST), a gene by gene comparison approach used for outbreak cluster detection in PulseNet (16). We further assessed relatedness with single nucleotide polymorphisms (SNPs) analysis using the CFSAN SNP Pipeline on the GalaxyTrakr website as previously described (30). Isolates from human and meat sources were considered closely related if they differed by ≤10 alleles or SNPs (31).
A maximum likelihood phylogenetic tree based on SNPS differences with bootstraps was constructed using PhyML 3.1 in GalaxyTrakr and we visualized it using IcyTree (https://icytree.org/).

WGS and surveillance data. Accession numbers are provided in (SI-Table 1). Sequence short reads for all study isolates were uploaded to the National Center for Biotechnology’s publicly available database (https://www.ncbi.nlm.nih.gov/sra).

Results

NTS strains from patients and retail meat. Of 4,478 Salmonella isolates from patients received by the BOL during the study period, 120 (2.7%) (excluding one duplicate) had PFGE patterns that were indistinguishable from at least one of the patterns in the 96 bacterial strains from contaminated meat (Figure 1). Of these 120 isolates, 109 (91%) were available for the study. Of the 109 PFGE matched isolates, 94 (86.2%) were from stool, 8 (7.3%) from blood, 4 (3.7%) from urine, and 3 (2.8%) from other sources such as bile fluid. The median age of patients with gastroenteritis was 41 years (range 1-93 years) and that for patients with primary bacteremia was 36 years (range 1-81 years). Patients with noninvasive NTS illnesses were similar by age compared with those with invasive Salmonella infections.

During the study period, NTS was detected in 4.0%, 7.0%, and 1.4% of chicken (47/1170), ground turkey (44/630), and pork chop samples (5/360), respectively (Figure 1). No Salmonella was recovered from any of the 360 ground beef samples tested.
Salmonella serotypes and XbaI PFGE patterns. Among the 109 clinical isolates, 15 serotypes were identified. The five most common accounted for 87 (79.8%) of the isolates (Table 1). The 96 isolates from meat sources had 25 distinct serotypes: the five most common accounted for 52 (54.2%) of the isolates (Table 1). Forty-nine (51%) of NTS isolated from meat samples had similar PFGE patterns to those found in clinical isolates. All S. Reading isolates were PFGE pattern JLGX01.0098 (pattern 98). S. Reading, S. Thompson, and S. Infantis were the top three most common serotypes among human isolates with PFGE patterns similar to those from retail meat. Ten (47.6%) of S. Reading isolates from humans were associated with invasive infections. The other serotypes associated with invasive salmonellosis were I 4,5,12:i (n=2), Infantis (n=2), and Thompson (n=1).

Antimicrobial resistance in NTS isolates. Of the five serotypes most commonly recovered from clinical and meat sources, only serovar Enteritidis was susceptible to multiple antibiotic classes (Table 1). Forty (36.7%) of the 109 clinical isolates were resistant to at least one class of antibiotics, and 28 (25.7%) were resistant to at least three classes. Eighteen (16.1%) isolates had resistance to three of the four antibiotics recommended by IDSA for treatment of severe Salmonella infections. Among isolates from humans, MDR (defined as resistance to ≥3 classes) increased during the study period from 6.3% in 2015 to 34.2% in 2017 (Figure 2). Ten (62.5%) of S. Infantis isolates from humans and three (75%) from meat sources were resistant to ceftriaxone and shared the same PFGE pattern (Table 1). Resistance to ceftriaxone in S. Infantis isolates from humans rose from 0% (n=16) in 2015 to 23.7% (9/38) in 2017, and a parallel increase was observed in isolates from meat samples. Two of the above mentioned S. Infantis
were associated with clinical infections in 2017 were resistant to 7 antimicrobial classes, including ceftriaxone plus nalidixic acid, and had R-type ACSSuTCxNalCot (S Table 1 - metadata). ACSSuTCxNalCot refers to resistance to A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; T, tetracycline; Cx, ceftriaxone; Nal, nalidixic acid; Cot, trimethoprim/sulfamethoxazole.

Six MDR S. Reading isolates, including two associated with systemic infections in pediatric patients, shared patterns with strains isolated from meat sources and were resistant to amoxicillin/clavulanate and ceftriaxone. One of the five S. Kentucky meat isolates with resistance to five antimicrobial classes was isolated from a chicken sample and a high-risk patient, within the same geographic region and time frame.

**Genetic mechanisms for antimicrobial resistance.** We searched for antimicrobial resistance mechanisms in genomic sequence data from 102 isolates, six from humans and all 96 from meat samples (Figure 1). We identified a $\text{bla}_{\text{CTX-M-65}}$ in two S. Infantis isolates from clinical samples and in three S. Infantis isolates from meat sources (all were pattern 787). These five $\text{bla}_{\text{CTX-M-65}}$-positive S. Infantis strains carried five to nine additional resistance genes and a mutation in DNA gyrase ($\text{gyrA D87Y}$) that enables bacteria to neutralize fluoroquinolones (Table 2). The resistance genes were previously shown to be carried on the IncFIB(pN55391) mega-plasmid ($\approx$300 kb) [14, 27]. We detected genes encoding β-lactamase derivatives in five S. Reading isolates including three $\text{bla}_{\text{TEM-1C}}$-positive strains, one from a patient. Three meat isolates from meat were $\text{bla}_{\text{HERA-3}}$ positive indicative of resistance to ampicillin, and all had additional genes that confer resistance to streptomycin, sulfisoxazole, tetracycline, and gentamicin (S-Table1). Eleven (14.46%)
isolates from meat sources had the $bla_{CMY-2}$ gene. Seven of the $bla_{CMY-2}$ positive isolates were either S. Typhimurium or S. Typhimurium var 5- while three were S. Kentucky. All $bla_{CMY-2}$-positive strains exhibited resistance to all beta lactams tested including ampicillin, amoxicillin/clavulanate and ceftriaxone.

**Interpretation of WGS to infer relationship between clinical and meat sources.** One S. Infantis clinical isolate (SRR6687365) differed from two isolates found in poultry meat samples (SRR6351071 and SRR6350849) by $\leq 10$ alleles as shown by cgMLST analysis and by $\leq 25$ SNPs as shown by CFSAN Pipeline analysis. Two Infantis isolates from poultry, collected in February and March of 2016, differed by one allele and one SNP (S Table 1). A Reading isolate (SRR10835618) associated with salmonellosis was separated from two strains (SRR8064308 and SRR7653314) found in poultry samples by $\leq 5$ alleles and $\leq 10$ SNPs [S Table 2-3].

Multiple Reading isolates from meat sources had $\leq 5$ allele and $\leq 10$ SNP differences. The maximum likelihood phylogenetic analysis with the substitution model showed a tree with three distinct clades supported by robust bootstrap values (Figure 3). The first clade showed that the single clinical isolate was closely related to two strains recovered from ground turkey that originated from a single facility (P-22000). The two strains were separated by two SNPS and were collected within four months in 2017. Clade 3 had two isolates (SRR6350973 and SRR7907813) that had no SNP differences; these were collected from meat samples produced in the same plant in November 2016 and January 2017 (S-Table1).
Discussion

In this study, we analyzed 109 nontyphoid *Salmonella* strains isolated from clinical samples submitted to our lab during 2015-2017 and that were identical by classical PFGE subtyping to NTS isolates found in meat samples tested over the same period. Among isolates from humans, MDR (defined as resistance to ≥3 classes) increased during the study period from 6.3% in 2015 to 34.2% in 2017. We observed that an estimated 14% and 19% of strains from clinical and food sources, respectively, were resistant to at least one antimicrobial agent (ceftriaxone, ciprofloxacin, TMP-SMX, or amoxicillin) recommended for treatment of severe salmonellosis by the current IDSA practice guidelines (7). The most common serotype detected in patients and contaminated meat purchased in retail outlets was *S. Reading*, and almost half (47%) of the *S. Reading* clinical isolates were associated with invasive disease.

WGS analyses of a subset of clinical isolates and all strains from meat sources identified five serovar *Infantis* isolates (two from patients and three in contaminated meat samples) that had plasmid-mediated *blaCTX-M-65*. This gene encodes an ESBL that hydrolyzes broad-spectrum cephalosporins including ceftriaxone. The *S. Reading* isolates from clinical and meat sources were closely related as shown by high-resolution WGS, suggesting a recent common ancestor.

Our finding that *S. Infantis* strains from patient and meat sources expressed the ESBL *blaCTX-M-65* is consistent with other reports of this resistance mechanism in NTS isolated in the US (29, 32). The IncFIB(pN55391) plasmid was first described in *S. Infantis*...
strains isolated in Israel; a rapid clonal expansion was observed in humans and poultry during 2008-2015 in Israel and later reported in other parts of the world (15, 29, 32-35). The emergence of $bla_{CTX-M-65}$ on a large conjugative mega-plasmid in *S. Infantis* is worrisome because there are limited options for treatment of humans infections, and this mobile genetic element could facilitate dissemination of this resistance mechanism to other bacterial pathogens (29, 34-35). During the study period *S. Infantis* isolates from meat with indistinguishable PFGE patterns from clinical isolates were investigated in multi-state outbreaks including in Pennsylvania. Taken together with previous evidence, WGS comparison of *S. Infantis* strains from patients and meat sources strongly suggests that transmission to humans occurs through the food chain.

Other investigators have documented an increase in ESBLs that appears to be driven by use of cephalosporins in healthcare and agricultural settings (11-13). In the US, ESBLs are common in healthcare settings. In 2017, they caused nearly 200,000 infections resulting in 9,000 deaths and treatment costs in the range of $1.2 billion (6). These findings underscore the need for robust integrated surveillance for antimicrobial resistance in NTS combined with One Health stewardship to preserve ceftriaxone for treatment of severe salmonellosis. The One Health stewardship approach is based on the understanding that antimicrobial resistance is exacerbated by antibiotic use in healthcare, veterinary, agriculture and environmental settings (17). Given the critical need to preserve the effectiveness of these drugs, since 2012 the FDA has prohibited unapproved use of cephalosporins in cattle, swine, chickens, and turkeys (36). It must also be noted that robust surveillance for antimicrobial resistance depend on timely case reporting by
physicians and submission of isolates or other material with the infectious agent (e.g., a patient specimen) by clinical laboratories (20, 32).

In the current study over 21% of all isolates from patients and meat samples purchased from randomly selected grocery stores in Pennsylvania were multi-drug resistant, which is higher than what has been observed in the overall NARMS data (38). This might be because clinical isolates in our study were matched with NTS from meat sources. In *Salmonella* differences over time are influenced by resistance within serotypes, changes in serotype distribution, or both (27). Additionally, NARMSNOW data for *Salmonella* on the CDC website show geographic variation (www.cdc.gov). Surprisingly, in our study, even excluding *S. Infantis*, which is typically multi-drug resistant, 4 clinical and 15 strains from food sources were resistant to ceftriaxone, driven by serovars Heidelberg and Kentucky. These isolates from meat sources had the *bla*$_{CMY-2}$ gene. In the US, this gene in *Salmonella* is typically plasmid-borne (39). The diversity of *Salmonella* serotypes in meat products with a plasmid-mediated resistance mechanism implies that they are widely disseminated, and they serve as a reservoir for drug-resistant human infections.

One clinical *S. Reading* isolate was highly related to two strains from retail meat samples and of the same pattern found in contaminated turkey products linked to two concurrent *S. Reading* outbreaks in the US and Canada during 2017-2019. Of the 300 cases investigated in the US, 132 people were hospitalized and one died (40). Our data suggest that these contaminated poultry products were being sold to consumers starting September 2016, much earlier than previously reported (www.fsis.usda.gov). Since 2012, *S. Reading* has been among the three top three serotypes identified in turkey meat samples tested by Food Safety and Inspection Service, accounting for 25% (8/32) of
Salmonella-positive turkey samples in 2014 (41). Together these data suggest that S. Reading was circulating in poultry prior to the recent multistate outbreaks. Further, these data illustrate the importance of One Health approach in efforts to prevent human infections.

Our study was limited by use of pattern-based criteria for selection of clinical isolates for comparison. The use of pattern-based criteria likely underestimated the number of isolates that were genetically related to Salmonella found in contaminated meat. Sequencing of additional clinical NTS isolates could have further elucidated the relationship between human and animal isolates and antimicrobial resistance mechanisms. Strengths include analysis of susceptibility profiles for all isolates and use of a state-based integrated surveillance database to compliment genomic findings.

Our findings demonstrate that multi-drug resistant Salmonella strains, including ceftriaxone-resistant isolates, are frequently found in meat products sold to consumers. Although we cannot say with certainty that the emergence of ESBL-producing and other drug resistant human pathogens is the result of injudicious use of extended-spectrum cephalosporins in poultry and livestock production, we have demonstrated that meat products are potential sources of antimicrobial resistant Salmonella strains and that similar NTS are found in humans. There is already compelling evidence that widespread use of extended-spectrum cephalosporins in human and veterinary medicine, combined with non-therapeutic use in agriculture, is fueling the spread of antimicrobial resistant genetic mechanisms in foodborne pathogens worldwide (13-12, 42). The results from our study emphasize the need for integrated surveillance to monitor trends in antimicrobial resistance and to detect emergence of clinically consequential pathogens in humans and
food animals (6-7, 11, 32). Finally, these data reinforce the necessity for coordinated local, national, and transnational policies and interventions to promote antimicrobial stewardship in human medicine and in food production as articulated in the global action plan coordinated by the World Organization (43).
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Figure legends

Figure 1. Flow chart of NTS isolates from clinical and retail meat sources Pennsylvania, 2015-2017. Created with an online application: https://app.diagrams.net/

Figure 2. Antimicrobial resistance to selected antibiotics in NTS isolates from clinical samples and retail meat sources — Pennsylvania, 2015-2017. Plotted are percent of NTS resistant to the indicated antibiotic in samples collected in 2015, 2016, and 2017.

Abbreviations: AMC, amoxicillin-clavulanate; MDR, resistance to least $\geq$3 of the nine antimicrobial classes tested. Created using MS Excel

Figure 3. Phylogenetic tree of S. Reading isolates (n=18) from clinical and meat sources constructed using PhyML 3.1 in GalaxyTrakr and visualized using IcyTree. Isolate source is indicated by an icon, along with NCBI accession number and the date isolated. Three well-supported clades are labeled at the left.
References

1. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis 2010; 50:882–9. doi: 10.1086/650733

2. Global Burden of Diarrheal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis 2017; 17:909–48. doi: 10.1016/S1473-3099(17)30276-

3. Tack DM, Ray L, Griffin PM, et al. Preliminary incidence and trends of infections with pathogens transmitted commonly through food — foodborne diseases active surveillance network, 10 U.S. sites, 2016–2019. MMWR Morb Mortal Wkly Rep 2020;69:509–514.

4. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. Front Microbiol 2014;5:391. doi:10.3389/fmicb.2014.00391

5. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. Immunol Cell Biol 2007;85(2):112-8.

6. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2019. Accessed October 13, 2020 at: https://www.cdc.gov/drugresistance/biggest-threats.html

7. Shane AL, Mody RK, Crump JA, et al. Infectious diseases society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis 2017. doi: 10.1093/cid/cix669

8. Guo Y, Bai Y, Yang C, Wang P, Gu L. Mycotic aneurysm due to Salmonella species: clinical experiences and review of the literature. Braz J Med Biol Res 2018;51(9):e6864. doi:10.1590/1414-431X20186864

9. Ceftriaxone. DrugBank. Accessed June 25, 2020 at: https://www.drugbank.ca/drugs,DB01212

10. World Health Organization. Global antimicrobial resistance surveillance system (GLASS) report early implementation, 2016-2017. Accessed October 2, 2020 at: https://www.who.int/docs/default-source/searo/amr/global-antimicrobial-resistance-surveillance-system-(glass)-report-early-implementation-2016-2017.pdfsfvrsn=ea19cc4a_2.
11. GBD 2017 Non-Typhoidal Salmonella Invasive Disease Collaborators. The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 2019;19(12):1312-1324. doi:10.1016/S1473-3099(19)30418-9

12. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18(4):657-686. doi:10.1128/CMR.18.4.657-686.2005

13. Van Boeckel TP, Brower C, Gilbert M, et al. Global trends in antimicrobial use in food animals. PNAS 2015;112 (18): 5649-5654.

14. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 2011;24(4):718-733. doi:10.1128/CMR.00002-11

15. Hindermann D, Gopinath G, Chase H, et al. Salmonella enterica serovar Infantis from food and human infections, Switzerland, 2010-2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage. Front Microbiol 2017;8:1322.

16. Centers for Disease Control and Prevention. PulseNet Methods. Accessed June 21, 2020 at: https://www.cdc.gov/pulsenet/pathogens/index.html

17. Karp BE, Tate H, Plumblee JR, et al. National antimicrobial resistance monitoring system: two decades of advancing public health through integrated surveillance of antimicrobial resistance. Foodborne Pathog Dis 2017;14(10):545-557. doi:10.1089/fpd.2017.2283

18. The White House. National action plan for combating antibiotic-resistant bacteria. Accessed on May 22, 2020 at: https://obamawhitehouse.archives.gov/sites/default/files/docs/national_action_plan_for_combating_antibiotic-resistant_bacteria.pdf

19. Armstrong GL, MacCannell DR, Taylor J, et al. Pathogen genomics in public health. N Engl J Med 2019;381:2569-2580.

20. Commonwealth of Pennsylvania. Pennsylvania code chapter 27, 2002. Reporting of cases by clinical laboratories. Accessed May 11, 2020 at: https://www.pacode.com/secure/data/028/chapter27/subchapBtoc.htm

21. Food and Drug Administration. National antimicrobial resistance monitoring: methods. Accessed June 18, 2020 at https://www.fda.gov/media/101741/download

22. Atlas R, Snyder J. Reagents, Stains, and Media: Bacteriology, p 316-349. In Jorgensen J, Pfaller M, Carroll K, Funke G, Landry M, Richter S, Warnock D (ed), Manual of Clinical Microbiology, Eleventh Edition. ASM Press, Washington, DC, 2015. doi: 10.1128/9781555817381.ch19
23. M’ikanatha NM, Sandt CH, Localio AR, et al. Multidrug-resistant *Salmonella* isolates from retail chicken meat compared with human clinical isolates. *Foodborne Pathog Dis* 2010;7:929-34

24. Popoff MY, Le Minor L. Antigenic formulas of the *Salmonella* serovars, 9th revision. WHO Collaborating Center for Reference and Research on *Salmonella*. Paris: Institut Pasteur; 2007.

25. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Wayne, PA: CLSI, M100S26, 2016.

26. Food and Drug Administration. National antimicrobial resistance monitoring system-enteric bacteria (NARMS): 2012-2013 integrated report. US Department of Health and Human Services, Rockville, MD, 2015.

27. Centers for Disease Control and Prevention. National antimicrobial resistance monitoring system for enteric bacteria (NARMS): Human isolates surveillance report for 2015 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC, 2018.

28. Centers for Disease Control and Prevention. WGS protocols. Accessed October 6, 2020 at https://www.cdc.gov/pulsenet/pathogens/wgs.html.

29. Tate H, Folster JP, Hsu CH, et al. Comparative analysis of Extended-Spectrum-β-Lactamase CTX-M-65-producing *Salmonella enterica* serovar Infantis isolates from humans, food animals, and retail chickens in the United States. *Antimicrob Agents Chemother* 2017;61(7):e00488-17. doi:10.1128/AAC.00488-17

30. Keefer AB, Xiaoli L, M’ikanatha NM, Yao K, Hoffmann M, Dudley EG. Retrospective whole-genome sequencing analysis distinguished PFGE and drug-resistance-matched retail meat and clinical *Salmonella* isolates. *Microbiology (Reading)* 2019;165(3):270-286. doi:10.1099/mic.0.000768

31. Besser JM, Carleton HA, Trees E, et al. Interpretation of Whole-genome sequencing for enteric disease surveillance and outbreak investigation. *Foodborne Pathog Dis* 2019;16(7):504-512. doi:10.1089/fpd.2019.2650

32. Brown AC, Chen JC, Watkins LKF, et al. CTX-M-65 Extended-Spectrum β-Lactamase-producing *Salmonella enterica* serotype Infantis, United States. *Emerg Infect Dis* 2018;24(12):2284-2291. doi:10.3201/eid2412.180500

33. Gestal MC, Zurita J, Paz Y Mino A, Ortega-Paredes D, Alcocer I. Characterization of a small outbreak of *Salmonella enterica* serovar Infantis that harbour CTX-M-65 in Ecuador. *Braz J Infect Dis* 2016;20(4):406-407. doi:10.1016/j.bjid.2016.03.007
34. Hindermann D, Gopinath G, Chase H, et al. *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010-2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage. *Front Microbiol* 2017;8:1322.

35. Cohen E, Rahav G, Gal-Mor O. Genome sequence of an emerging *Salmonella enterica* serovar Infantis and genomic comparison with other *S.* Infantis strains. *Genome Biol Evol* 2020;12:151-159. doi:10.1093/gbe/evaa048

36. Food and Drug Administration. New animal drugs; Cephalosporin drugs; Extralabel animal drug use; Order of prohibition. 21 CFR Part 530 [Docket No. FDA–2008–N–0326]. Federal Register 2012; 77:736-738.

37. World Health Organization. Critically important antimicrobials for human medicine 6th revision, 2018. Accessed September 1, 2020 at: https://apps.who.int/iris/bitstream/handle/10665/312266/9789241515528-eng.pdf?ua=1

38. Food and Drug Administration. 2016-2017 NARMS Integrated summary. Accessed June 18, 2020 at https://www.fda.gov/media/101741/download

39. Folster JP, Pecic G, Singh A, et al. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat, and humans in the United States 2009. *Foodborne Pathog Dis* 2012;9(7):638-645. doi:10.1089/fpd.2012.1130

40. Hassan R, Buck S, Noveroske D, et al. Multistate outbreak of *Salmonella* infections linked to raw turkey products - United States, 2017-2019. *MMWR Morb Mortal Wkly Rep* 2019;68(46):1045-1049. doi:10.15585/mmwr.mm6846a1

41. USDA. Food Safety and Inspection Service. Serotypes profile of *Salmonella* isolates from meat and poultry products, January 1998 through December 2014. Accessed November 17, 2020 at: https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/annual-serotyping-reports

42. Bush K, Bradford PA. Epidemiology of beta-lactamase-producing pathogens. *Clinical Microbiology Review* 2020; 33: e00047-19.

43. World Health Organization. Global action plan on antimicrobial resistance. Accessed October 16,2020 at: https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/
Table 1. Antimicrobial resistance in non-typhoidal *Salmonella* from humans and retail meat sources Pennsylvania, 2015-2017*

| Serovar          | Antibiotic† | No. (%) of isolates resistant to multiple antimicrobial classes |
|------------------|-------------|---------------------------------------------------------------|
|                  | # of isolates | Amc | Amp | Axo | Chl | Cot | Fis | Nal | Tet | ≥1 | ≥3 | ≥5 |
| **Source: Human**|             |     |     |     |     |     |     |     |     |    |    |    |
| Reading          | 21          | 0   | 7   | 0   | 0   | 12  | 0   | 11  | 14  | 6  | 28.6 | 0  |
| Thompson         | 21          | 1   | 1   | 1   | 1   | 0   | 1   | 1   | 2   | 9.5 | 9.5 | 0  |
| Infantis         | 16          | 0   | 11  | 11  | 9   | 10  | 13  | 14  | 14  | 87.5 | 14 | 62.5 |
| I 4,5,12:i:-      | 15          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0  | 0  | 0  |
| Enteritidis      | 14          | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 1   | 7.1 | 0  | 0  |
| **Other**        | 22          | 3   | 5   | 3   | 0   | 0   | 5   | 0   | 9   | 40 | 27.3 | 2 | 9.1 |
| **All serovars‡**| 109         | 4   | 25  | 15  | 9   | 11  | 31  | 14  | 32  | 36.7 | 28 | 11.0 |
| **Source: Meat** |             |     |     |     |     |     |     |     |     |    |    |    |
| Reading          | 18          | 0   | 7   | 0   | 0   | 0   | 0   | 2   | 9   | 50 | 5.6 | 0  |
| Kentucky         | 13          | 5   | 5   | 5   | 0   | 0   | 0   | 11  | 13  | 100 | 5 | 38.5 |
| Heidelberg       | 8           | 1   | 1   | 1   | 0   | 0   | 0   | 3   | 5   | 62.5 | 1 | 12.5 |
| Typhimurium var. 05- | 7       | 4   | 4   | 4   | 0   | 0   | 0   | 6   | 6   | 85.7 | 1  | 57.1 |
| Enteritidis      | 6           | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 1   | 16.7 | 0  | 0  |
| **Other**        | 44          | 4   | 12  | 8   | 4   | 3   | 0   | 4   | 21  | 56.8 | 10 | 22.7 |
| **All serovars‡**| 96          | 14  | 30  | 18  | 4   | 3   | 0   | 4   | 43  | 61.5 | 21 | 17.7 |

* Only serovars with at least 6 isolates are listed individually † CLSI: Clinical and Laboratory Standards Institute ‡ Isolates with decreased susceptibility to ciprofloxacin (DSC; MIC ≥0.12 µg/mL) were categorized as resistant to the quinolone class § Antimicrobial agent abbreviations: AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AXO, ceftriaxone; CHL, chloramphenicol; COT, trimethoprim-sulfamethoxazole; FIS, sulfisoxazole; NAL, nalidixic acid; TET, tetracycline.
Table 2. Resistance phenotypes and genotypes in *Salmonella* Infantis isolates from humans and retail meat sources, Pennsylvania, 2015-2017

| Isolate Id | Source§ | NCBI Accession | Date collected | Total antimicrobial classes resistant* | Resistant to Drugs in IDSA Guidelines | Resistance genes | Plasmids |
|------------|---------|----------------|----------------|----------------------------------------|----------------------------------------|------------------|----------|
| PNUSAS033  | Human   | SRR6687365     | Oct-17         | 7                                      | +                                     | aac(3)-Iva, aadA1, aph(3')-Ia, aph(4)-Ia, blaCTX-M-65, dfrA14, floR, gyrA(87), sul1, tet(A) ant(3'')-Ia, aac(3')-Ia | IncFIB(pN5391)  |
| PNUSAS120  | Human   | SRR10835627    | Sep-17         | 4                                      | +                                     | aac(3)-Iva, aadA1, aph(3')-Ia, ant(3'')-Ia, aac(3')-Ia, dfrA14, gyrA(87), sul1, tet(A) | IncFIB(pN5391)  |
| PNUSAS013  | Human   | SRR5865301     | Jun-17         | 7                                      | +                                     | aac(3)-Iva, aadA1, aph(4)-Ia, blaCTX-M-65, dfrA14, floR | ncFIB(pN55391)  |
| N58033     | Pork Chops | SRR3295615     | Mar-15         | 0                                      |                                       |                  | IncFIB(pN5391)  |
| N16S097    | Chicken Breast | SRR6350849     | Feb-16         | 6                                      | +                                     | aac(3)-IV, ant(3'')-Ia | IncFIB(pN5391)  |
| Isolates with decreased susceptibility to ciprofloxacin (DSC; MIC ≥0.12 μg/mL) were categorized as resistant to the quinolone class. |
|---|
| *Abbreviations for antimicrobial agents are based on Clinical and Laboratory Standards Institute (CLSI). AMC, amoxicillin-clavulanic acid; AXO, ceftriaxone; CIP, Ciprofloxacin; COT, trimethoprim-sulfamethoxazole; §: All clinical isolates are from stool. |

| N16S103 | Chicken Breast | SRR6351 | Mar 16 | 6 | + | + | + | aph(3')-Ia, aph(4')-Ia, bla<sub>CTX-M-65</sub>, dfra14, floR, gyrA(D87Y), sul1, tet(A), aac(3)-IV, ant(3'')-Ia, aph(3')-Ia, aph(4')-Ia, bla<sub>CTX-M-65</sub>, dfra14, floR, fosA3, gyrA(D87Y), sul1, tet(A), aac(3)-IV, aph(3')-Ia, aph(4')-Ia, bla<sub>CTX-M-65</sub>, dfra14, floR, gyrA(D87Y) | IncFIB(pN55391) |
| N17S816 | Chicken Breast | SRR8064 | Apr 16 | 5 | + | + | IncFIB(pN55391) |
Isolates from human source

2,478 non-typhoidal *Salmonella* (NTS) submitted to the Bureau of Lab during 2015-2017

Pulsed-field gel electrophoresis (PFGE) on all NTS isolates from human source

120 NTS isolates from humans sources with indistinguishable PFGE patterns with the 96 strains from meat samples

109 out of the 120 NTS were available for our study. All 109 were tested for AST and 6 were analyzed by WGS

Antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS) on all 96 NTS from meat sources

Genetic mechanism for resistance probed in 6 clinical and 96 meat isolates that underwent WGS. Pairwise comparison for *S. Infantis, S. Reading* and *S. Thompson*. 
Figure 1. Percent of isolates from patients (A) and meat (B) sources that demonstrated resistance to amoxicillin-clavulanate (AMC), ceftriaxone, and resistance to least ≥3 of the nine antimicrobial classes tested (MDR) [18-19]. Among isolates from patients, resistance to ceftriaxone, a third generation cephalosporin preferred for severe infections in children, increased from zero in 2015 to 23.7% in 2017. Overall, MDR increased for isolates from human and animal sources during the study period.
