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MAJOR ARTICLE

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 nontyphoidal *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 4478 NTS isolates from humans and 96 isolates were found in 2520 retail meat samples. One hundred 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. Twenty-eight (25.7%) of the 109 clinical NTS and 21 (21.9%) of strains from meat sources had resistance to ≥3 antimicrobial drug classes (multidrug resistance). Sixteen of the 102 (15.7%) isolates analyzed by WGS had resistance mechanisms that confer resistance to expanded-spectrum cephalosporins, such as ceftriaxone. We identified $bla_{\text{CTX-M-65}}$ in 2 *S.* Infantis isolates from clinical and 3 *S.* Infantis isolates from meat sources. These 5 $bla_{\text{CTX-M-65}}$–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 multidrug resistant and 16% had genetic mechanisms that confer resistant to ceftriaxone. These results emphasize need for integrated surveillance in healthcare and agricultural settings.

Keywords. antibacterial agents; antimicrobial resistance; *bla*CMY; ceftriaxone susceptibility; CTX-M-65; ESBL; extended-spectrum β-lactamase–producing; foodborne pathogens; microbial quality; multidrug resistant; *Salmonella*.

Each year, nontyphoidal *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 2600 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

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for animal protein is considered a driver of the global spread of bacteria with genetic resistance mechanisms; examples include extended-spectrum β-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 US 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 4478 NTS isolates from humans to the Pennsylvania Department of Health Bureau of Laboratories 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 = 1170), ground turkey (n = 630), ground beef (n = 360), and pork chops (n = 360). Samples were purchased from retail outlets located in 4 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 1 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 noninvasive, 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 previously [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 9 antimicrobial classes: aminoglycosides (gentamicin, streptomycin), penicillins (ampicillin), β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid), cephalosporins (cefoxitin, ceftiofur, 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 criteria and NARMS consensus breakpoints to interpret results [25, 26]. Isolates with decreased susceptibility to ciprofloxacin (minimum inhibitory concentration ≥0.12 μg/mL) were categorized as resistant to the quinolone class [26, 27]. If an isolate was resistant to 3 or more antimicrobial classes, we classified it as multidrug resistant. We also examined resistance to the 4 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- × 250-bp or 2- × 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 version 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 version 0.4.0, which employs the ResFinder database (updated 11 February 2020) and thresholds of 90% identity and 50% gene coverage and the PointFinder scheme for *Salmonella* species (updated 30 August 2019). We used the PlasmidFinder version 2.1

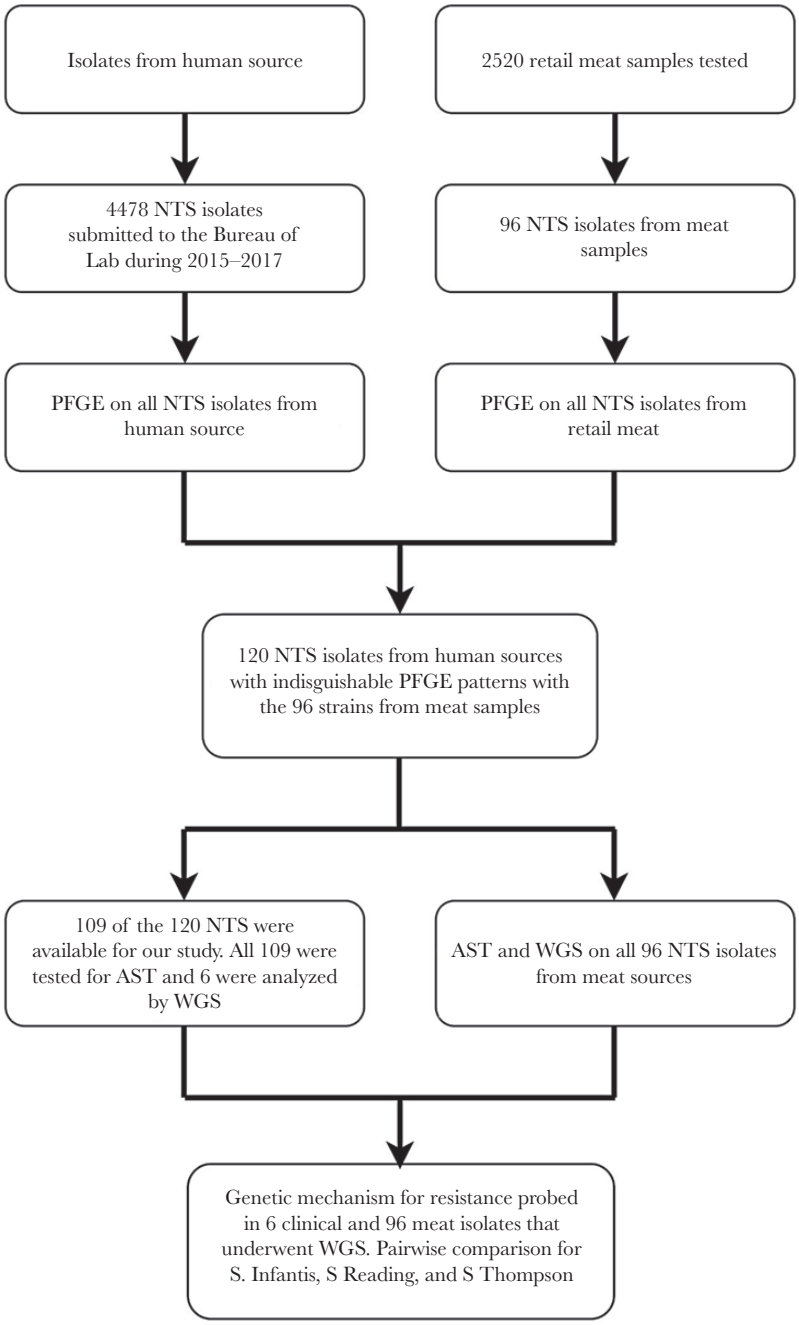

Figure 1. Flowchart of nontyphoidal *Salmonella* isolates from clinical and retail meat sources, Pennsylvania, 2015–2017. Created with an online application: https://app.diagrams.net/. Abbreviations: AST, antimicrobial susceptibility testing; NTS, nontyphoidal *Salmonella*; PFGE, pulsed-field gel electrophoresis; WGS, whole-genome sequencing.

Enterobacteriaceae database (updated 1 July 2020) to identify plasmids with 95% identity and 60% gene coverage.

Genome Comparison and Phylogenetic Analyses

We analyzed genome assemblies for *S. enterica* serotype Infantis, Reading, and Thompson isolates on the PulseNet National *Salmonella* database using BioNumerics software (version 7.6). These 3 serotypes were selected because they were the most frequently found among clinical isolates included in the study. We compared isolates in each of the 3 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 polymorphism (SNP) analysis using the FDA Center for Food Safety and Applied Nutrition (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 SNP 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 (Supplementary Table 1). Sequence short reads for all study isolates were uploaded to the National Center for Biotechnology's (NCBI) publicly available database (https://www.ncbi.nlm.nih.gov/sra).

Ethical Considerations

Access to clinical *Salmonella* isolates data in the surveillance databases was approved by CDC NARMS state-based program activities. Patient consent to participate was not applicable. Only anonymous isolate-level data publicly available on the CDC NARMS website are reported, as are genomic data uploaded to the NCBI repository.

RESULTS

NTS Strains From Patients and Retail Meat

Of 4478 *Salmonella* isolates from patients received by the Pennsylvania Department of Health Bureau of Laboratories during the study period, 120 (2.7%) (excluding 1 duplicate) had PFGE patterns that were indistinguishable from at least 1 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 5 most common accounted for 87 (79.8%) of the isolates (Table 1). The 96 isolates from meat sources had 25 distinct serotypes; the 5 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). *Salmonella* Reading, *S.* Thompson, and *S.* Infantis were the 3 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), *S.* Infantis (n = 2), and *S.* Thompson (n = 1).

Antimicrobial Resistance in NTS Isolates

Of the 5 serotypes most commonly recovered from clinical and meat sources, only *S* Enteritidis was susceptible to multiple antibiotic classes (Table 1). Forty (36.7%) of the 109 clinical isolates were resistant to at least 1 class of antibiotics, and 28 (25.7%) were resistant to at least 3 classes. Eighteen (16.1%) isolates had resistance to 3 of the 4 antibiotics recommended by IDSA for treatment of severe *Salmonella* infections. Among isolates from humans, multidrug resistance (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 the *S.* Infantis isolates from humans and 3 (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 isolates were associated with clinical infections in 2017 were resistant to 7 antimicrobial classes, including ceftriaxone plus nalidixic acid, and had R-type ACSSuTCxNalCot (Supplementary Table 1, metadata) (ACSSuTCxNalCot refers to resistance to A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; T, tetracycline; Cx, ceftriaxone; Nal, nalidixic acid; Cot, TMP-SMX).

Six multidrug-resistant *S.* Reading isolates, including 2 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 5 *S.* Kentucky meat isolates with resistance to 5 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: 6 from humans and all 96 from meat samples (Figure 1). We identified a $bla_{\text{CTX-M-65}}$ in 2 *S.* Infantis isolates from clinical samples and in 3 *S.* Infantis isolates from meat sources (all were pattern 787). These 5 $bla_{\text{CTX-M-65}}$–positive *S.* Infantis strains carried 5 to 9 additional resistance genes and a mutation in DNA gyrase (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 (≈300 kb) [14, 27]. We detected genes encoding β-lactamase derivatives in 5 *S.* Reading isolates including 3 $bla_{\text{TEM-1C}}$-positive strains, 1 from a patient. Three isolates from meat were $bla_{\text{HERA-3}}$ positive, indicative of resistance to ampicillin, and all had additional genes that confer resistance to streptomycin, sulfisoxazole, tetracycline, and gentamicin (Supplementary Table 1). Eleven (14.46%) isolates from meat sources had the $bla_{\text{CMY-2}}$ gene. Seven of the $bla_{\text{CMY-2}}$-positive isolates were either *S.* Typhimurium or *S.* Typhimurium var 5-, whereas 3 were *S.* Kentucky. All $bla_{\text{CMY-2}}$-positive strains exhibited resistance to all β-lactams tested including ampicillin, amoxicillin-clavulanate, and ceftriaxone.

Table 1. Antimicrobial Resistance in Nontyphoidal Salmonella From Humans and Retail Meat Sources, Pennsylvania, 2015–2017

Salmonella enterica Serotype[a]	No. of Isolates	Antibiotic								Isolates Resistant to Multiple Antimicrobial Classes, No. (%)[b]		
		AMC	AMP	AXO	CHL	COT	FIS	NAL	TET	≥1	≥3	≥5
Source: Human												
Reading	21	0	7	0	0	0	12	0	11	14 (66.7)	6 (28.6)	0
Thompson	21	1	1	1	0	1	1	0	1	2 (9.5)	2 (9.5)	0
Infantis	16	0	11	11	9	10	13	14	13	14 (87.5)	14 (87.5)	10 (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	0	1 (7.1)	0	0
Other	22	3	5	3	0	0	5	0	7	9 (40.9)	6 (27.3)	2 (9.1)
All serotypes	109	4	25	15	9	11	31	14	32	40 (36.7)	28 (25.7)	12 (11.0)
Source: Meat												
Reading	18	0	7	0	0	0	0	0	2	9 (50.0)	1 (5.6)	0
Kentucky	13	5	5	5	0	0	0	0	11	13 (100.0)	5 (38.5)	5 (38.5)
Heidelberg	8	1	1	1	0	0	0	0	3	5 (62.5)	1 (12.5)	1 (12.5)
Typhimurium var 5-	7	4	4	4	0	0	0	0	6	6 (85.7)	4 (57.1)	4 (57.1)
Enteritidis	6	0	1	0	0	0	0	0	0	1 (16.7)	0	0
Other	44	4	12	8	4	3	0	4	21	25 (56.8)	10 (22.7)	7 (15.9)
All serotypes	96	14	30	18	4	3	0	4	43	59 (61.5)	21 (21.9)	17 (17.7)

Antibiotic abbreviations are from the Clinical and Laboratory Standards Institute.

Abbreviations: AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AXO, ceftriaxone; CHL, chloramphenicol; COT, trimethoprim-sulfamethoxazole; FIS, sulfisoxazole; NAL, nalidixic acid; TET, tetracycline.

[a]Only serotypes with at least 6 isolates are listed individually.

[b]Isolates with decreased susceptibility to ciprofloxacin (minimum inhibitory concentration ≥0.12 μg/mL) were categorized as resistant to the quinolone class.

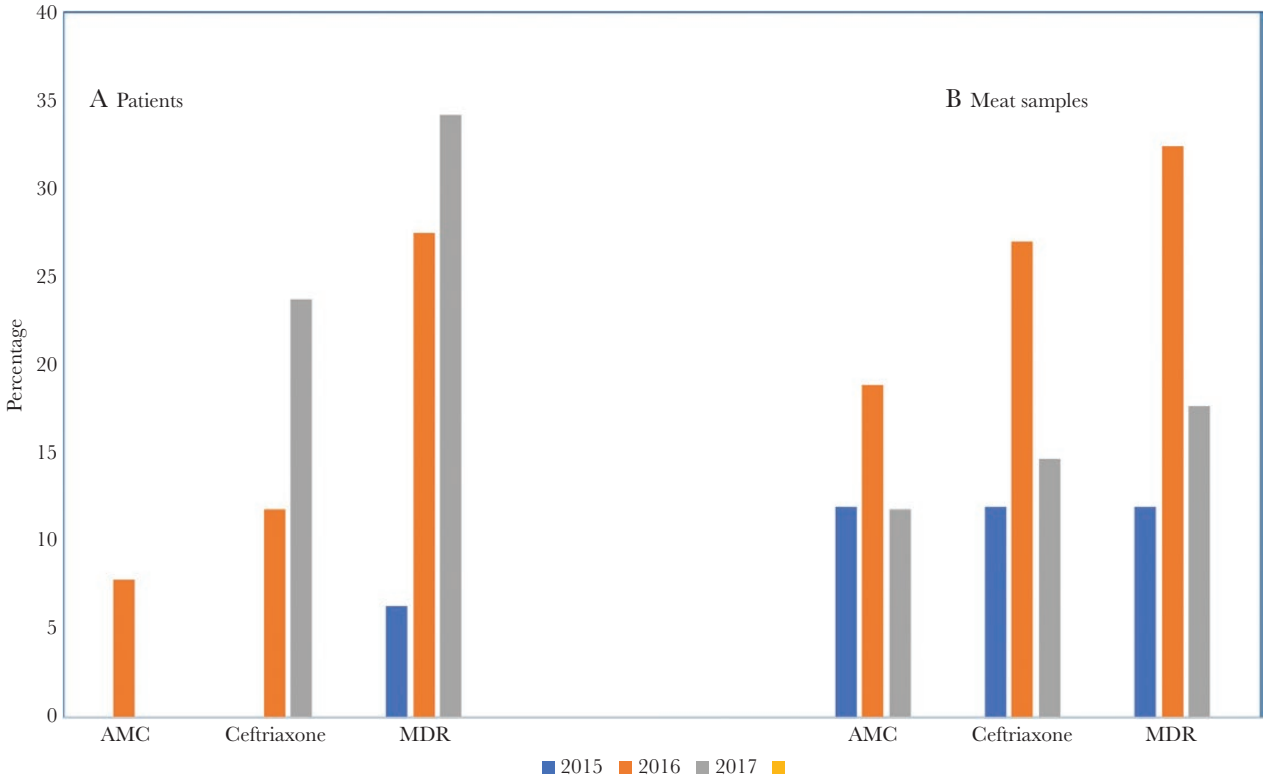

Figure 2. Antimicrobial resistance to selected antibiotics in nontyphoidal *Salmonella* (NTS) isolates from clinical samples (n = 105, *A*) and retail meat sources (n = 96, *B*), Pennsylvania, 2015–2017. Plotted is the percentage of NTS isolates resistant to the indicated antibiotic in samples collected in 2015, 2016, and 2017. Among isolates from patients, resistance to cetriaxone, a third-generation cephalosporin preferred for severe infections in children, increased from zero in 2015 to 23.7% in 2017. Overall multidrug resistance increased for isolates from human animal sources during the study period. Abbreviations: AMC, amoxicillin-clavulanate; MDR, resistance to ≥3 of the 9 antimicrobial classes tested.

Interpretation of WGS to Infer Relationship Between Clinical and Meat Sources

One *S.* Infantis clinical isolate (SRR6687365) differed from 2 isolates found in poultry meat samples (SRR6351071 and SRR6350849) by ≤10 alleles as shown by cgMLST analysis and by ≤25 SNPs as shown by CFSAN Pipeline analysis. Two Infantis isolates from poultry, collected in February and March of 2016, differed by 1 allele and 1 SNP (Supplementary Table 1). An *S.* Reading isolate (SRR10835618) associated with salmonellosis was separated from 2 strains (SRR8064308 and SRR7653314) found in poultry samples by ≤5 alleles and ≤10 SNPs (Supplementary Tables 2 and 3).

Multiple *S.* Reading isolates from meat sources had ≤5 allele and ≤10 SNP differences. The maximum likelihood phylogenetic analysis with the substitution model showed a tree with 3 distinct clades supported by robust bootstrap values (Figure 3). The first clade showed that the single clinical isolate was closely related to 2 strains recovered from ground turkey that originated from a single facility (P-22000). The 2 strains were separated by 2 SNPs and were collected within 4 months in 2017. Clade 3 had 2 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 (Supplementary Table 1).

DISCUSSION

In this study, we analyzed 109 NTS strains isolated from clinical samples submitted to our laboratory 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, multidrug resistance (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 1 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 5 *S.* Infantis isolates (2 from

Table 2. Resistance Phenotypes and Genotypes in Salmonella Infantis Isolates From Humans and Retail Meat Sources, Pennsylvania, 2015–2017

Isolate Identifier	Source[a]	NCBI Accession	Date Collected	Total Antimicrobial Classes Resistant[b]	Resistant to Drugs in IDSA Guidelines				Resistance Genes	Plasmids
					AMC	AXO	CIP	COT		
PNUSAS033127	Human	SRR6687365	2017 Oct	7	…	+	…	+	*Aac (3)-Iva, aadA1, aph(3′)-Ia, aph (4)-Ia, bla*$_{CTX-M-65}$*, dfrA14,* floR, *gyrA*(87), *sul1, tet(A)*	IncFIB(pN55391)
PNUSAS127011	Human	SRR10835627	2017 Sep	4	…	…	+	+	*ant(3″)-Ia, aph(3′)-Ia, dfrA14, gyrA*(87), *sul1, tet(A)*	IncFIB(pN55391)
PNUSAS017891	Human	SRR5865301	2017 Jun	7	…	+	…	+	*aac (3)-Iva, aadA1, aph (4)-Ia, bla*$_{CTX-M-65}$*, dfrA14, floR*	ncFIB(pN55391)
N58033	Pork chops	SRR3295615	2015 Mar	0	…	…	…	…		
N16S097	Chicken breast	SRR6350849	2016 Feb	6	…	+	+	+	*aac (3)-IV, ant(3″)-Ia, aph(3′)-Ia, aph (4)-Ia, bla*$_{CTX-M-65}$*, dfrA14, floR, gyrA*(D87Y), *sul1, tet(A)*	IncFIB(pN55391)
N16S103	Chicken breast	SRR6351071	2016 Mar	6	…	+	+	+	*aac (3)-IV, ant(3″)-Ia, aph(3′)-Ia, aph (4)-Ia, bla*$_{CTX-M-65}$*, dfrA14, floR, fosA3, gyrA*(D87Y), *sul1, tet(A)*	IncFIB(pN55391)
N17S816	Chicken breast	SRR8064300	2016 Apr	5	…	+	+	…	*aac (3)-IV, aph(3′)-Ia, aph (4) -Ia, bla*$_{CTX-M-65}$*, dfrA14, floR, gyrA*(D87Y)	IncFIB(pN55391)

Antibiotic abbreviations are from the Clinical and Laboratory Standards Institute.

Abbreviations: AMC, amoxicillin-clavulanic acid; AXO, ceftriaxone; CIP, ciprofloxacin; COT, trimethoprim-sulfamethoxazole; IDSA, Infectious Diseases Society of America; NCBI, National Center for Biotechnology Information.

[a]All clinical isolates are from stool.

[b]Isolates with decreased susceptibility to ciprofloxacin (minimum inhibitory concentration ≥0.12 µg/mL) were categorized as resistant to the quinolone class.

patients and 3 in contaminated meat samples) that had plasmid-mediated *bla*$_{CTX-M-65}$. 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 *bla*$_{CTX-M-65}$ 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 multistate 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 9000 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, 18]. 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 depends on timely case reporting by physicians and submission of isolates or other material with the infectious agent (eg, a patient specimen) by clinical laboratories [20, 32, 37].

In the current study, >21% of all isolates from patients and meat samples purchased from randomly selected grocery stores in Pennsylvania were multidrug 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, NARMS Now data for *Salmonella* on the CDC website show geographic

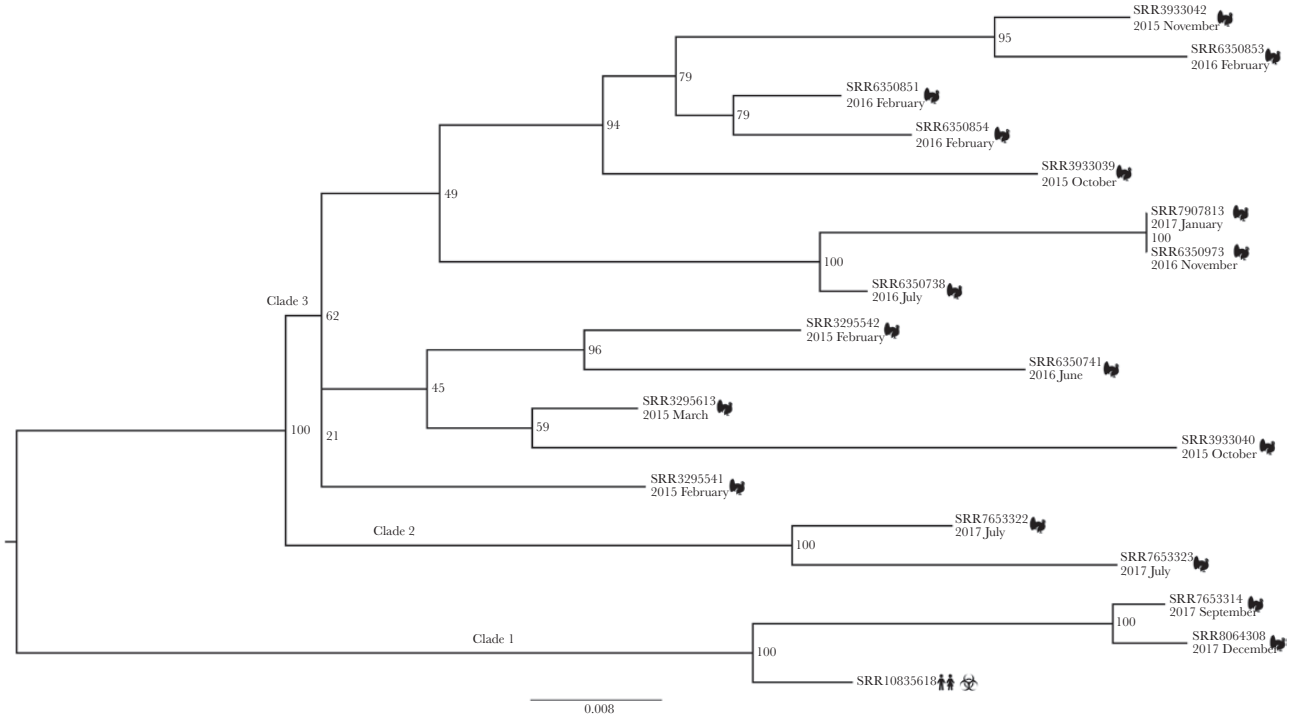

Figure 3. Phylogenetic tree of *Salmonella enterica* serotype Reading isolates (n = 18) from clinical and meat sources constructed using PhyML 3.1 in GalaxyTrakr and visualized using IcyTree. Isolate source is indicated by an icon, along with the National Center for Biotechnology Information accession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

variation (wwwn.cdc.gov). Surprisingly, in our study, even excluding *S.* Infantis, which is typically multidrug resistant, 4 clinical and 15 strains from food sources were resistant to ceftriaxone, driven by serotypes 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 serve as a reservoir for drug-resistant human infections.

One clinical *S.* Reading isolate was highly related to 2 strains from retail meat samples and of the same pattern found in contaminated turkey products linked to 2 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 1 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 top 3 serotypes identified in turkey meat samples tested by the US 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 the 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 complement genomic findings.

Our findings demonstrate that multidrug-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 nontherapeutic use in agriculture, is fueling the spread of antimicrobial-resistant genetic mechanisms in foodborne pathogens worldwide [12, 13, 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 Health Organization [43].

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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