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The Prevalence of Multidrug Resistance Is Higher among Bovine than Human *Salmonella enterica* Serotype Newport, Typhimurium, and 4,5,12:i:− Isolates in the United States but Differs by Serotype and Geographic Region▽†

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Salmonella **represents an important zoonotic pathogen worldwide, but the transmission dynamics between humans and animals as well as within animal populations are incompletely understood. We characterized *Salmonella* isolates from cattle and humans in two geographic regions of the United States, the Pacific Northwest and the Northeast, using three common subtyping methods (pulsed-field gel electrophoresis [PFGE], multilocus variable number of tandem repeat analysis [MLVA], and multilocus sequence typing [MLST]). In addition, we analyzed the distribution of antimicrobial resistance among human and cattle *Salmonella* isolates from the two study areas and characterized *Salmonella* persistence on individual dairy farms. For both *Salmonella enterica* subsp. *enterica* serotypes Newport and Typhimurium, we found multidrug resistance to be significantly associated with bovine origin of isolates, with the odds of multidrug resistance for Newport isolates from cattle approximately 18 times higher than for Newport isolates from humans. Isolates from the Northwest were significantly more likely to be multidrug resistant than those from the Northeast, and susceptible and resistant isolates appeared to represent distinct *Salmonella* subtypes. We detected evidence for strain diversification during *Salmonella* persistence on farms, which included changes in antimicrobial resistance as well as genetic changes manifested in PFGE and MLVA pattern shifts. While discriminatory power was serotype dependent, the combination of PFGE data with either MLVA or resistance typing data consistently allowed for improved subtype discrimination. Our results are consistent with the idea that cattle are an important reservoir of multidrug-resistant *Salmonella* infections in humans. In addition, the study provides evidence for the value of including antimicrobial resistance data in epidemiological investigations and highlights the benefits and potential problems of combining subtyping methods.**

Salmonella is an important human and animal pathogen worldwide. In the United States, *Salmonella* causes an estimated 1.4 million human cases, 15,000 hospitalizations, and more than 400 deaths each year (44, 75). Human infections can be acquired through contact with animals or humans shedding *Salmonella* or through contaminated environments, but the majority of human infections are food-borne, and a large number of human outbreaks have been linked to foods of animal origin (20). Beef represents one well-recognized source of human infection (71). In addition, a number of human cases have been linked to dairy products or cattle contact, for instance at state fairs or on dairy farms (for example, see references 25, 35, and 61).

Salmonella enterica subsp. *enterica* serotypes Typhimurium and Newport are commonly isolated from human cases, including those linked to cattle (20, 61). In 2006, *Salmonella* serotypes Typhimurium and Newport were isolated from 17 and 8% of reported human salmonellosis cases in the United States, respectively, making them the first and third most common human disease-associated serotypes in the United States (15). *S. enterica* serotype 4,5,12:i:− is both genetically and antigenically closely related to *Salmonella* serotype Typhimurium, of which it represents a monophasic variant (62). *Salmonella enterica* serotype 4,5,12:i:− is characterized by a deletion of flagellar genes *fliA* and *fliB*, which prevents expression of the phase 2 flagellar antigen (60). In the United States, the prevalence of *Salmonella* serotype 4,5,12:i:− has increased considerably over the past 10 years, and in 2006, *Salmonella* serotype 4,5,12:i:− represented the sixth most commonly isolated serotype from humans in the United States (15, 60).

Salmonella serotype Newport represents two distinct clonal groups or lineages—one predominantly associated with isolates from cattle (i.e., Newport lineage A) and one associated with isolates from birds (i.e., Newport lineage B) (1, 33). Mem-

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bers of both lineages cause human infections (1, 33). The two Newport lineages can be clearly distinguished by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), and some correlation between genetic lineage and antimicrobial resistance profile seems to exist (1, 33). In general, Newport lineage B isolates are pansusceptible or resistant to only a few antimicrobial drugs. In contrast, lineage A is strongly associated with multidrug resistance and includes a Newport subtype commonly referred to as Newport MDR-AmpC (1, 33).

The prevalence of antimicrobial resistance among *Salmonella* serotype Newport and Typhimurium isolates has increased worldwide during the last 2 decades, predominantly as a result of emerging multidrug-resistant (MDR) strains (14, 52, 65). During the 1990s, *Salmonella* serotype Typhimurium phage type DT104 with pentaresistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) increased considerably in prevalence around the world, and some isolates acquired resistance to additional antimicrobial agents, including trimethoprim or ciprofloxacin (52). MDR *Salmonella* serotype Typhimurium DT104 has been isolated from a wide variety of host species and caused numerous large human outbreaks around the world (65). *Salmonella* serotype Newport MDR-AmpC, characterized by resistance to ACSSuT and carrying a plasmid encoding resistance to amoxicillin-clavulanic acid, cefoxitin, ceftiofur, and cephalothin emerged in the United States during the late 1990s, where it quickly became widespread among humans and cattle, leading to several large human outbreaks (14).

Whether antimicrobial drug use in animals facilitates the emergence of MDR human pathogens is still subject to debate. Some studies report a temporal association between the introduction of new antimicrobial agents in veterinary medicine and the emergence of antimicrobial resistance (for instance, see references 22 and 58), but questions regarding the underlying evolutionary mechanisms, the origin and distribution of naturally occurring resistance genes, and the role of antimicrobial usage among humans remain (for example, see references 2 and 66 for reviews on this topic). Moreover, some studies report a higher prevalence of antimicrobial resistance among *Salmonella* isolates from farm animals than humans. Gebreyes et al. (26), for instance, found a higher prevalence of antimicrobial resistance among *Salmonella* isolates from pigs than humans, but potential effects attributable to differences in serotype distribution are difficult to assess in this study. In recent years, risk factors for MDR have received considerable attention. Infections with MDR *Salmonella* strains can lead to treatment failures, may be of longer duration, and may result in more severe clinical disease. Hence, such infections lead more often to hospitalization or death than infections with susceptible *Salmonella* strains, but serotype or subtype differences between resistant and susceptible *Salmonella* strains complicate the interpretation of clinical data (34, 41, 68).

Subtyping methods allow characterization of *Salmonella* isolates and include phenotypic methods (e.g., serotyping or phage typing) as well as molecular subtyping methods, such as pulsed-field gel electrophoresis (PFGE), ribotyping, multilocus variable number of tandem repeat analysis (MLVA), and multilocus sequence typing (MLST) (5). PFGE is widely used and robust, and rigorous standardization allows comparison between laboratories (5). However, the method is time-intensive and laborious, requires careful standardization and analysis, does not allow phylogenetic inference, and can in rare cases be affected by endogenous nucleases or DNA methylation (for a review of this topic, see reference 5). MLVA and MLST are rapid, allow for easy data exchange between laboratories, and provide some phylogenetic information (5). MLVA is highly discriminatory but subject to rapid diversification and therefore most appropriate for the analysis of closely related isolates. While MLST lacks discriminatory power within *Salmonella* serotypes, it is highly reproducible and allows for phylogenetic analysis of more distantly related isolates (1, 5, 33). PFGE and MLST can be performed regardless of serotype, but MLVA protocols are serotype specific and have so far only been validated for a limited number of *Salmonella* serotypes. Moreover, MLVA can be complicated by inaccurate sizing of DNA fragments, and the degree of reliability can be considerably influenced by nucleotide composition and fragment length (5). Overall, these subtyping methods differ considerably in discriminatory power and sometimes yield conflicting results, and the most appropriate subtyping method or combination thereof strongly depends on serotype and chosen application (19, 56, 72, 76). Other genetic or phenotypic characteristics, such as antimicrobial resistance patterns or the presence of specific plasmids, have also been used successfully for subtyping in outbreak investigations and other epidemiological studies and can provide valuable additional information (7, 8, 40, 63, 64).

Here we describe the distribution and subtype diversity of *Salmonella* serotypes Newport, 4,5,12:i:−, and Typhimurium among cattle and humans in two geographic regions of the United States, and we assess common risk factors for multidrug resistance. In addition, we utilize three *Salmonella* subtyping methods (PFGE, MLVA, and MLST), analyze their usefulness for characterizing isolates representing three common human-associated *Salmonella* serotypes, and compare the combined discriminatory power of PFGE and MLVA to that of PFGE and antimicrobial resistance patterns.

MATERIALS AND METHODS

Isolate selection. Nontyphoidal *Salmonella enterica* subsp. *enterica* isolates from bovine ($n = 222$) and human ($n = 203$) sources representing *S. enterica* serotypes Typhimurium ($n = 190$), 4,5,12:i:− ($n = 40$), and Newport ($n = 195$) were included in this study (see Table S1 in the supplemental material). These isolates were collected between January 2004 and May 2005 in two geographic regions of the United States, the Pacific Northwest and the Northeast, and human isolates were obtained from the Wadsworth Center, New York State Department of Health ($n = 73$) or from the Washington State Department of Health Public Health Laboratories ($n = 130$). Human-source *Salmonella* isolates specifically originated from reported cases among residents of New York State (69 isolates, including 2 isolates with unknown county of origin, 1 isolate from New York City, and 66 isolates from New York State) and New Jersey ($n = 4$); isolates from New York City and New Jersey residents represent exceptional cases where isolates were submitted to the New York State Department of Health. For the Northwest, human isolates were obtained from Washington State ($n = 130$) residents. The main source populations for human isolates thus are New York State (except New York City) and Washington State with approximate human populations of 11 and 6.6 million residents, respectively (70).

Northeastern bovine-source *Salmonella* isolates ($n = 154$) were collected from dairy cattle on 57 farms in New York ($n = 113$) and Vermont ($n = 41$) as part of a previously reported study to determine *Salmonella* incidence in cattle herds in the northeastern United States (18) and obtained via the New York State Animal Health Diagnostic Center. Pacific Northwest bovine isolates ($n = 68$) were collected in Washington ($n = 58$), Idaho ($n = 8$), and Oregon ($n = 2$) from

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cattle on 39 farms, primarily via the Washington Animal Diagnostic Laboratory. The majority of isolates from cattle were isolated from clinical cases, except for 17 cattle isolates from the Pacific Northwest, which were obtained from cattle without clinical signs of salmonellosis, and originated from previous field studies (see Table S1 in the supplemental material). Of the 227 isolates collected in the Northeast (Table S1), 152 have been described and characterized previously using PFGE and MLST based on a three-gene MLST scheme (based on the *manB*, *fimA*, and *mdh* genes) (1, 60). Isolate designation of these previously characterized isolates is identical to their original designation to allow comparison between the studies.

Serotyping of all bovine and human isolates included in this study was performed according to the Kaufman-White scheme using the standard procedure described by Ewing et al. (24). Where discrimination between *Salmonella* serotypes 4,5,12:i:− and Typhimurium appeared questionable, serotype designation was confirmed by PCR amplification of the phase 2 flagellin gene *fljB* as described previously (21, 60).

PFGE analysis. PFGE analysis with restriction enzyme XbaI (Invitrogen, Carlsbad, CA) was performed according to the CDC PulseNet protocol (55) using *Salmonella* serovar Braenderup isolate H9812 (Centers for Disease Control and Prevention) as the reference strain as described previously (37). PFGE patterns were analyzed using BioNumerics software package version 2.5 (Applied Maths, Austin, TX). Similarity analyses were based on Dice coefficients with a maximum space tolerance of 1.5%, and cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) algorithm in BioNumerics. Clusters were inspected visually to ascertain correct classification.

MLVA typing analysis. Isolates were stored at −80°C, and single colonies were subcultured on LB agar plates overnight prior to MLVA analysis. MLVA analysis was performed as described previously, with analysis of *Salmonella* serotype Typhimurium and 4,5,12:i:− isolates based on previously described variable number of tandem repeat (VNTR) loci STTR5, STTR6, STTR9, and STTR10pl, while *Salmonella* serotype Newport isolates were characterized using VNTR loci STTR5, STTR6, NEWPORTA, NEWPORTB, NEWPORTL, and NEWPORTM, also as described previously (19, 42). Briefly, boiled-lysate suspensions of single bacterial colonies were added to 25-μl PCR mixtures and subjected to denaturation (15 min at 94°C), followed by 25 cycles, with 1 cycle consisting of 30 s at 94°C, 1 min at 55°C, and 90 s at 72°C. Following a final extension step of 10 min at 72°C, size standard (LIZ600; Applied Biosystems, Foster City, CA) and Hi Di formamide were added to the PCR mixtures, and the mixtures were loaded into a 3730 DNA analyzer with Pop-7 polymer (Applied Biosystems) for capillary electrophoresis. Electropherograms were subsequently analyzed using GeneMarker (Softgenetics LCC, State College, PA), and MLVA patterns were assigned on the basis of the composite data for the respective VNTR loci (see Table S3 in the supplemental material). Fragment sizes for each VNTR locus were analyzed as categorical data in BioNumerics. Minimum spanning trees (MSTs) based on the MLVA data were constructed for *Salmonella* serotype Newport as well as *Salmonella* serotype Typhimurium and 4,5,12:i:− isolates using categorical coefficients and allowing for hypothetical nodes.

Data from MLVA (categorical coefficients) and PFGE (Dice similarity coefficients) were combined using the "average from experiments" function in BioNumerics and the UPGMA algorithm. Clusters were identified in the resulting dendrograms by choosing an arbitrary level of similarity at 75% to define a cluster, and cluster numbers were assigned to clusters containing two or more isolates. Correspondence between the clustering based on MLVA and PFGE analysis was assessed using Kendall's tau rank correlation coefficient and Pearson's correlation coefficient, again using BioNumerics.

Antimicrobial resistance patterns. Susceptibility to a panel of antimicrobial agents was determined for all isolates using a disc diffusion method as described previously (4). The antimicrobial agents tested belonged to the following drug classes: beta-lactams (ampicillin [10 μg], ceftazidime [30 μg], and amoxicillin-clavulanic acid [20 and 10 μg, respectively]), quinolones (nalidixic acid [30 μg]), phenicols (chloramphenicol [30 μg]), aminoglycosides (kanamycin [30 μg], gentamicin [10 μg], and streptomycin [10 μg]), sulfonamides and dihydrofolate reductase inhibitors (triple-sulfa [sulfadiazine, sulfamethazine, and sulfamerazine] [300 μg] and trimethoprim-sulfamethoxazole [1.25 and 23.75 μg, respectively]), and tetracyclines (tetracycline [30 μg]). Resistance breakpoints were as reported by the Clinical and Laboratory Standards Institute (46, 47). To facilitate data analysis, isolates classified as intermediate were analyzed as susceptible. Multidrug resistance (MDR) was defined as resistance to more than two antimicrobial drug classes.

MLST analysis. Isolates with highly prevalent PFGE patterns were further analyzed by sequence typing using a 7-gene MLST scheme (targeting *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) as described previously (38). A single representative isolate was selected at random (using the random generator available at www.random.org) for each PFGE pattern for characterization by MLST analysis. Bacterial genomic DNA was prepared using the Qiagen genomic DNA purification kit (Qiagen, Valencia, CA) and amplified by PCR, followed by Sanger sequencing of PCR products. Consensus sequences were assembled using the DNAStar software package (Lasergene, Madison, WI) and submitted to the MLST database at the Environmental Research Institute (ERI), University College Cork (http://mlst.ucc.ie/) for assignment of MLST allele numbers. Consensus sequences were subsequently trimmed to span the allelic region of interest specified previously (38), and concatenated sequences spanning all 7 genes were constructed using SEAL (http://tree.bio.ed.ac.uk/software/seal/). Sequences were aligned using SEAL, and phylogenetic trees based on individual genes or the concatenated sequences were inferred as described below. In addition, representative sequences for each inferred allelic type were aligned with Newport and Typhimurium sequences available in the MLST database (see Table S3 in the supplemental material), choosing one representative sequence for each allelic type present in the MLST database.

Unrooted maximum likelihood (ML) phylogenetic trees were estimated using PAUP*, with the best-fitting model of nucleotide substitution determined using Modeltest (53) (all parameter values available from the authors upon request). Support for individual nodes on the trees was assessed through bootstrap values based on 1,000 neighbor-joining trees using PAUP*.

Statistical analysis. To avoid overrepresentation of *Salmonella* strains persisting on individual farms, a single representative isolate was chosen at random from groups of isolates that originated from the same farm, had indistinguishable PFGE and MLVA patterns, and showed identical antimicrobial resistance patterns (see Table S1 in the supplemental material). This reduced data set (n = 366) was used to describe the distribution of resistance against individual antimicrobial drugs and to compare the discriminatory power of the different subtyping methods. Simpson's index of diversity (D) was calculated as described previously to assess the serotype-specific discriminatory power of the different analytical methods (36, 57). D can vary between 0 and 1, with 1 indicating infinite diversity and 0 indicating no diversity. To assess the statistical significance of differences between individual D values, 95% confidence intervals (95% CIs) based on normal approximation were calculated as described previously (30).

Ninety-five percent confidence intervals for the proportion of isolates resistant to individual antimicrobial agents were calculated using the normal approximation to the binominal distribution. Due to the low number of *Salmonella* serotype 4,5,12:i:− isolates included in this study, these isolates were excluded from subsequent analysis of multidrug resistance, resulting in a second reduced data set (n = 338). The frequency distributions of *Salmonella* serotype Newport and Typhimurium isolates were analyzed using Fisher's exact test, with P values of <0.05 deemed statistically significant. To estimate the odds ratios for risk factors associated with multidrug resistance, logistic regression analysis was performed, with model selection performed using a backward stepwise selection algorithm. Overall model fit was assessed on the basis of deviance estimates and inspection of studentized residuals, and 95% Wald confidence intervals for odds ratios were estimated. All statistical analyses were performed using SAS version 9.2 (SAS, Cary, NJ).

To further analyze the distribution of *Salmonella* subtypes associated with PFGE patterns that were shared between humans and cattle (or between the two geographic regions in the United States), patterns with a frequency of <2 were excluded, since the probability of these patterns being shared equaled zero, and the remaining isolates were classified by the presence or absence of a shared PFGE pattern, resulting in a data set of 234 isolates comprising 116 *Salmonella* serotype Newport isolates and 118 *Salmonella* serotype Typhimurium isolates.

Nucleotide sequence accession number. The newly determined *Salmonella enterica* serotype Typhimurium *hemD* sequence was submitted to GenBank and assigned accession no. GU784796.

RESULTS

PFGE and MLVA subtype diversity. The initial data set of 425 isolates included 190 *Salmonella enterica* serotype Typhimurium isolates, which we classified into 90 PFGE patterns and 135 MLVA patterns, and 195 *Salmonella enterica* serotype Newport isolates, which we classified into 54 PFGE patterns and 59 MLVA patterns (see Table S1 in the supplemental material). Among the 40 *Salmonella* serotype 4,5,12:i:− isolates, we detected 13 PFGE and 15 MLVA patterns. Two

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TABLE 1. Distribution of resistance to individual antimicrobial agents among sources and *Salmonella enterica* serotypes

Antimicrobial agent or drug resistance category	Distribution of resistance to antimicrobial agent among:									
	All isolates		Bovine isolates		Human isolates		Serotype Newport isolates[a]		Serotype Typhimurium isolates[a]	
	No. of isolates[b]	% of isolates (95% CI)[c]	No. of isolates	% of isolates (95% CI)	No. of isolates	% of isolates (95% CI)	No. of isolates	% of isolates (95% CI)	No. of isolates	% of isolates (95% CI)
Antimicrobial agents										
Triple-sulfa[d]	288	79 (74–83)	143	88 (83–93)	145	71 (65–78)	137	88 (83–93)	139	76 (70–83)
Tetracycline	238	65 (60–70)	139	85 (80–91)	99	49 (42–56)	122	78 (72–85)	111	61 (54–68)
Ampicillin	233	64 (59–69)	142	87 (82–92)	91	45 (38–52)	120	77 (70–84)	106	58 (51–65)
Streptomycin	229	63 (58–68)	128	79 (72–85)	101	50 (43–57)	121	78 (71–84)	100	55 (48–62)
Chloramphenicol	187	51 (46–56)	110	67 (60–75)	77	38 (31–45)	98	63 (55–70)	83	46 (38–53)
Ceftazidime	153	42 (37–47)	104	64 (56–71)	49	24 (18–30)	106	68 (61–75)	46	25 (19–32)
Amoxicillin-clavulanic acid	119	33 (28–37)	86	53 (45–60)	33	16 (11–21)	79	51 (43–58)	34	19 (13–24)
Kanamycin	82	22 (18–27)	53	33 (25–40)	29	14 (9–19)	19	12 (7–17)	62	34 (27–41)
Sulfoxazole-trimethoprim	43	12 (8–15)	21	13 (8–18)	22	11 (7–15)	13	8 (4–13)	30	16 (11–22)
Gentamicin	8		0		8		1		5	
Nalidixic acid	4		0		4		2		2	
Drug resistance category										
Pansusceptible	74	20 (16–25)	20	12 (8–17)	54	27 (21–33)	19	12 (7–17)	39	21 (16–28)
Intermediate resistance	54	15 (11–18)	4	3 (0–5)	50	25 (19–31)	15	10 (5–14)	35	19 (14–25)
Multidrug resistant	238	65 (60–70)	139	85 (80–91)	99	49 (42–56)	122	78 (72–85)	108	59 (52–67)

[a] Data for *Salmonella* serotype 4,5,12:i:− isolates not shown.
[b] Number of isolates showing resistance to the specific antimicrobial drug out of 366 isolates included in the reduced data set.
[c] Percentage of isolates resistant to the specific antimicrobial drug. The 95% binominal confidence interval (95% CI) based on normal approximation is shown in parentheses.
[d] Triple-sulfa is sulfadiazine, sulfamethazine, and sulfamerazine.

PFGE patterns (L1 and L6) were detected among *Salmonella* serotype 4,5,12:i:− and Typhimurium isolates. To avoid overrepresentation of persisting *Salmonella* subtypes on farms, we selected a single representative for each combination of PFGE, MLVA, and antimicrobial resistance pattern on a given farm, resulting in a reduced data set of 182 *Salmonella* serotype Typhimurium isolates, 156 *Salmonella* serotype Newport isolates, and 28 *Salmonella* serotype 4,5,12:i:− isolates (see Table S1 in the supplemental material).

Distribution of antimicrobial resistance. Among the 203 human *Salmonella* isolates and 163 independent bovine *Salmonella* isolates, 74 isolates were susceptible to all antimicrobial drugs tested (i.e., pansusceptible), representing 39 *Salmonella* serotype Typhimurium isolates, 19 *Salmonella* serotype Newport isolates, and 16 *Salmonella* serotype 4,5,12:i:− isolates (Table 1). These isolates included 5 of the 91 (6%) bovine *Salmonella* serotype Newport isolates, 14 of the 65 (22%) human *Salmonella* serotype Newport isolates, 11 of the 62 (18%) bovine *Salmonella* serotype Typhimurium isolates, and 28 of the 120 (23%) human *Salmonella* serotype Typhimurium isolates. In addition, 4 of the 10 (40%) bovine *Salmonella* serotype 4,5,12:i:− isolates and 12 of the 18 (67%) human *Salmonella* serotype 4,5,12:i:− isolates were pansusceptible. For most antimicrobial agents, resistance prevalence was significantly higher among cattle isolates than human isolates, while significantly more human isolates than cattle isolates were pansusceptible (Table 1). Resistance to gentamicin and nalidixic acid was detected only among human isolates and at a low frequency. In general, resistance prevalence was higher among *Salmonella* serotype Newport isolates than *Salmonella* serotype Typhimurium isolates, but resistance to kanamycin was significantly more prevalent among *Salmonella* serotype Typhimurium isolates than *Salmonella* serotype Newport isolates (Table 1).

One hundred twenty-two of 156 (78%) *Salmonella* serotype Newport isolates, 9 of 28 (32%) *Salmonella* serotype 4,5,12,:i:− isolates, and 108 of 182 (59%) *Salmonella* serotype Typhimurium isolates were multidrug resistant, defined as resistant to more than 2 classes of antimicrobial agents (Table 1). For both *Salmonella* serotype Newport and Typhimurium, multidrug resistance was significantly ($P < 0.05$) associated with origin from cattle (data not shown). To estimate odds ratios, a logistic regression model was fit to the data. Using a backward stepwise selection approach, we constructed a final model consisting of the predictors "source," "serotype," "geographic origin," and the interaction term "source × serotype." The year of isolation was not significant and therefore not included in the final model. In our data set, the odds of multidrug resistance were approximately 18 times higher for bovine *Salmonella* serotype Newport isolates than for human Newport isolates and more than 3 times higher for bovine *Salmonella* serotype Typhimurium isolates than for human Typhimurium isolates (Table 2). Among both human and bovine isolates, the odds of multidrug resistance were higher for *Salmonella* serotype Newport isolates than for *Salmonella* serotype Typhimurium isolates, even though the differences were only statistically significant among isolates from cattle (Table 2). Moreover, in the United States, the odds of multidrug resistance were approximately twice as high for isolates that originated from the Northwest compared to isolates from the Northeast.

***Salmonella* diversity on farms.** The median number of *Salmonella* isolates collected per farm equaled 1 and ranged from 1 to 32. Both *Salmonella* serotype Newport and Typhimurium were isolated from 2 farms in the Northeast and 5 farms in the Northwest, while both serotypes Newport and 4,5,12:i:− were isolated from 2 farms in the Northeast and none in the Northwest

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TABLE 2. Odds ratio estimates for odds of multidrug resistance based on logistic regression model[a]

Comparison of isolates	Odds ratio	95% CI[b]
Bovine vs. human serotype Newport	18.2	6.3–52.6
Bovine vs. human serotype Typhimurium	3.5	1.7–7.1
Bovine serotype Newport vs. bovine Typhimurium	6.1	2.1–17.9
Human serotype Newport vs. human serotype Typhimurium	1.2	0.6–2.2
Northwest vs. Northeast	2.0	1.2–3.4

[a] The odds of multidrug resistance in *S. enterica* isolates of different serotypes, from different hosts, and from different geographic areas of the United States were calculated. The overall model fit was satisfactory as determined on the basis of deviance estimates ($P = 0.41$) and visual inspection of studentized residuals.
[b] 95% Wald confidence interval for odds ratio.

(see Table S2 in the supplemental material). On average, 1.3 PFGE patterns, 1.5 MLVA patterns, and 1.3 antimicrobial resistance patterns were detected among *Salmonella* isolates from the same farm in the Northeast (ranges, 1 to 3, 1 to 7, and 1 to 4, respectively; median, 1), and 1.2 PFGE patterns, 1.4 MLVA patterns, and 1.5 antimicrobial resistance patterns were detected among isolates from the same farm in the Northwest (ranges, 1 to 5, 1 to 5, and 1 to 7, respectively; median, 1).

On 27 of the 96 farms, representing 17 of 57 (30%) farms in the Northeast and 10 of 39 (26%) farms in the Northwest, multiple isolates with indistinguishable PFGE patterns were isolated (see Table S2 in the supplemental material). On the farms where the same PFGE pattern was detected more than once, the predominant PFGE pattern was shared by between 40% and 100% of isolates (median, 100%; average, 83%). The average number of isolates that were indistinguishable by PFGE, MLVA, and antimicrobial resistance profiles equaled 1.7 isolates per farm (range, 1 to 19 isolates; median, 1), and on average, 3.3 different combinations of PFGE, MLVA, and antimicrobial resistance patterns were isolated from these farms (range, 1 to 12; median, 1). *Salmonella* isolates that were indistinguishable by MLVA, PFGE, and antimicrobial resistance patterns were isolated from the same farm over time periods ranging from less than 1 month to more than 7 months; however, isolation dates were missing for some isolates, and longer durations are therefore possible.

On 7 of the 27 farms, all isolates shared a single PFGE and MLVA pattern, while on another 8 farms, the isolates shared a single PFGE pattern but had MLVA patterns that differed from each other at one locus (see Table S2 in the supplemental material). On 10 of the 15 farms with isolates with a single PFGE pattern, all isolates shared the same antimicrobial resistance pattern, while on each of the remaining 5 farms, two closely related antimicrobial resistance profiles were detected. On farm NE152, all 4 isolates shared the same MLVA pattern but differed in PFGE pattern. On several of the remaining farms, some of the isolates shared PFGE and others shared MLVA patterns. For instance, among the 19 *Salmonella* serotype 4,5,12:i:− isolates from farm NE261, 5 PFGE patterns and 2 MLVA patterns were detected. These isolates could be classified into 2 clusters based on PFGE, with clusters differing by 4 bands (Fig. 1). One cluster represented susceptible isolates, and the other represented multidrug-resistant isolates, yet isolates from both clusters shared identical MLVA patterns, likely indicating close evolutionary relatedness. Most of the isolates shared MLVA pattern Z, but two isolates, collected in August 2004, shared MLVA pattern bE and yet belonged to two different PFGE clusters (Fig. 1). PFGE patterns for one multidrug-resistant isolate and one susceptible isolate (isolates S5-580 and S5-759) shared most bands with one cluster but some with the other, and were isolated in July and

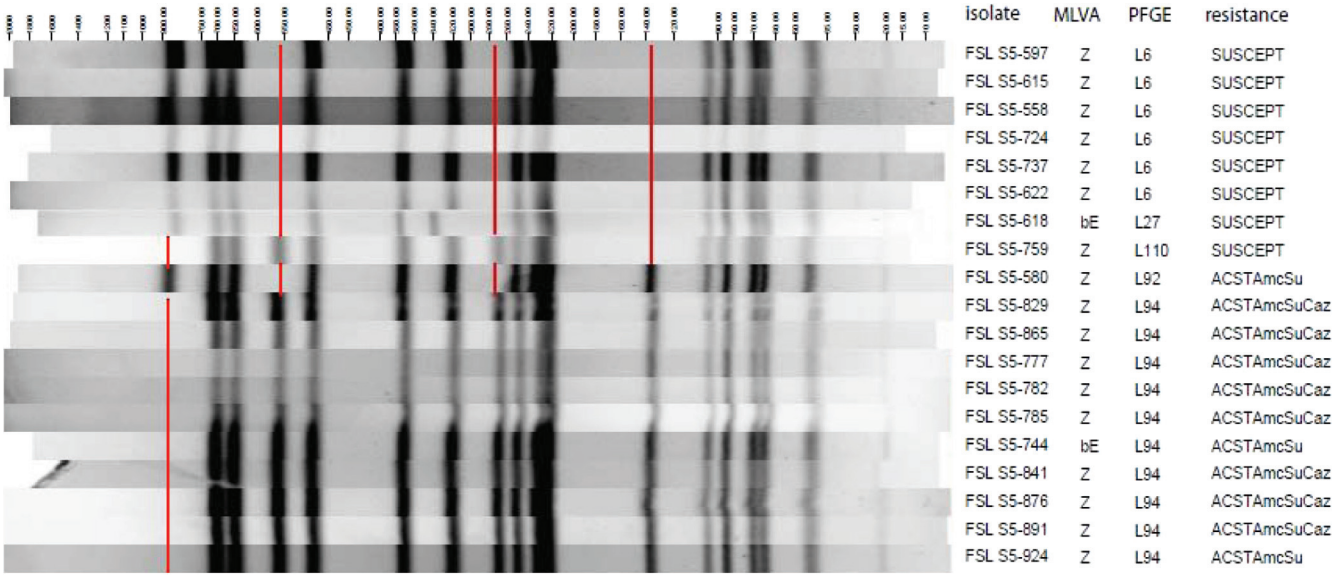


FIG. 1. Characterization of *Salmonella enterica* serotype 4,5,12:i:− isolates from farm NE299 by PFGE, MLVA, and antimicrobial resistance pattern. The isolate designations, MLVA pattern, PFGE pattern, and antimicrobial resistance pattern are shown to the right of the figure. The antimicrobial resistance pattern is shown as follows: SUSCEPT, susceptible; ACSTAmcSu, resistance to ampicillin, chloramphenicol, streptomycin, tetracycline, amoxicillin-clavulanic acid, and sulfonamides; ACSTAmcSuCaz, resistance to ampicillin, chloramphenicol, streptomycin, tetracycline, amoxicillin-clavulanic acid, sulfonamides, and ceftazidime. Absent PFGE bands distinguishing the two clusters of isolates are indicated by red lines.

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TABLE 3. Simpson's index of diversity scores among the three *Salmonella* serotypes by different *Salmonella* subtyping methods

Isolate (n)[a]	Simpson's index of diversity[b] for the following subtyping method(s):			
	PFGE[c]	MLVA[d]	MLVA and PFGE (combined)	PFGE and antimicrobial resistance (combined)[e]
Serotype 4,5,12:i:− (n = 28)	**0.833** (0.711–0.955)	**0.910** (0.843–0.977)	**0.971** (0.940–1.00)	**0.844** (0.717–0.971)
Serotype Typhimurium (n = 182)	**0.967** (0.953–0.981)	**0.993** (0.989–0.996)	**0.997** (0.996–0.999)	**0.988** (0.980–0.994)
Serotype Newport (n = 156)	**0.930** (0.911–0.948)	**0.913** (0.886–0.941)	**0.980** (0.970–0.990)	**0.972** (0.961–0.982)
Bovine isolates (n = 163)	**0.947** (0.933–0.960)	**0.950** (0.932–0.968)	**0.984** (0.976–0.992)	**0.979** (0.971–0.987)
Human isolates (n = 203)	**0.986** (0.980–0.992)	**0.994** (0.991–0.997)	**0.999** (0.997–0.999)	**0.992** (0.987–0.997)
Bovine serotype Typhimurium (n = 62)	**0.938** (0.910–0.966)	**0.976** (0.959–0.993)	**0.992** (0.984–0.999)	**0.980** (0.967–0.994)
Human serotype Typhimurium (n = 120)	**0.975** (0.960–0.990)	**0.994** (0.990–0.999)	**0.997** (0.994–1.00)	**0.990** (0.979–0.997)
Bovine serotype Newport (n = 91)	**0.859** (0.830–0.889)	**0.856** (0.811–0.900)	**0.954** (0.931–0.977)	**0.943** (0.922–0.964)
Human serotype Newport (n = 65)	**0.976** (0.959–0.994)	**0.968** (0.947–0.988)	**0.995** (0.990–1.00)	**0.985** (0.971–0.999)

[a] The number of isolates included in the final data set (n) (i.e., reduced data set of 366 isolates) is shown.
[b] The mean estimates are shown in boldface type, and the 95% confidence intervals are shown in parentheses.
[c] *Salmonella* serotype 4,5,12:i:−, Typhimurium, and Newport isolates exhibited 13, 90, and 54 PFGE patterns, respectively.
[d] *Salmonella* serotype 4,5,12:i:−, Typhimurium, and Newport isolates exhibited 15, 135, and 59 MLVA patterns, respectively.
[e] *Salmonella* serotype 4,5,12:i:−, Typhimurium, and Newport isolates exhibited 7, 41, and 22 antimicrobial resistance patterns, respectively.

September 2004, respectively. All other susceptible isolates were collected between June and August of that year, while the remaining multidrug-resistant isolates were obtained between 31 August and December 2004.

On 9 farms, some isolates with identical MLVA and PFGE patterns but different antimicrobial resistance profiles were isolated (see Table S2 in the supplemental material). On 5 of these 9 farms, information on sample collection dates allowed inference as to whether resistance was likely acquired or lost. On 3 of the farms, isolates appeared to have gained additional resistance to ceftazidime, while on 1 farm, isolates seemed to have lost resistance to sulfa-trimethoprim (Table S2). On farm NE510, some isolates seem to have gained resistance to ceftazidime, while other isolates later appeared to have lost resistance to kanamycin or triple-sulfa. Conclusions on resistance loss or gain are difficult to draw though, as isolates with specific resistance patterns may have coexisted in a given farm or may have been present at a given time period without being detected. Further studies are therefore needed to determine the stability of resistance patterns on farms.

Discriminatory power of *Salmonella* subtyping methods. Comparisons of *Salmonella* subtyping methods were based on the reduced data set comprising a total of 366 *Salmonella* isolates. Overall, a combination of MLVA and PFGE showed the highest discriminatory power (Table 3), as judged by Simpson's index of diversity (D). For *Salmonella* serotype Typhimurium, the discriminatory power of MLVA was significantly higher than that of PFGE, while no significant differences were detected for *Salmonella* serotype Newport or *Salmonella* serotype 4,5,12:i:− (Table 3). For *Salmonella* serotype Newport, the combination of PFGE with antimicrobial resistance patterns yielded a significantly higher discriminatory power than either PFGE or MLVA alone. For *Salmonella* serotype Typhimurium, this combination yielded a higher discriminatory power than PFGE, which was comparable to that of MLVA.

Analysis of serotype and source diversity. Simpson's index of diversity estimates for *Salmonella* isolates from humans were significantly higher than those for isolates obtained from cattle, regardless of the subtyping method (Table 3). This trend was consistent among both *Salmonella* serotype Typhimurium and Newport isolates, but the differences were significant only for *Salmonella* serotype Newport isolates. Among the isolates from cattle, *Salmonella* serotype Typhimurium isolates were significantly more diverse than *Salmonella* serotype Newport isolates, while the differences between the two serotypes were considerably less pronounced among the human isolates (Table 3).

Analysis of correspondence in clustering based on MLVA and PFGE. To estimate the correlation between similarity coefficients based on MLVA and those based on PFGE, we use BioNumerics to calculate Kendall's tau rank correlation coefficient and Pearson's correlation coefficient. For *Salmonella* serotype Typhimurium, Kendall's tau rank correlation coefficient equaled 0.11 (95% CI, 0.105 to 0.119) while Pearson's correlation coefficient equaled 0.19. For *Salmonella* serotype Newport, the estimates were 0.46 (95% CI, 0.456 to 0.470) and 0.61, respectively, suggesting a better correspondence between the subtyping methods for *Salmonella* serotype Newport, possibly due to the more genetically homogeneous population structure of *Salmonella* serotype Newport.

Genetic relationship between isolates. To further describe the phylogenetic relationship between the isolates, we analyzed 22 *Salmonella* isolates (2 *Salmonella* serotype 4,5,12:i:− isolates, 12 *Salmonella* serotype Typhimurium isolates, and 8 *Salmonella* serotype Newport isolates), representing the most common PFGE types, using a 7-gene MLST scheme (Table 4). All analyzed Newport isolates represented the same sequence type (ST) and clearly clustered separately from the *Salmonella* serotype Typhimurium and 4,5,12:i:− isolates (see Fig. S1 in the supplemental material). The *Salmonella* serotype Typhimurium isolates represented two STs; the more frequent ST was identical to that of the two *Salmonella* serotype 4,5,12:i:− isolates analyzed. The other ST was shared by two *Salmonella* serotype Typhimurium isolates (S5-511 and S5-831) and differed from the first ST in a single nucleotide in *hemD*. Isolate S5-511 was isolated from a human case, and isolate S5-831 was isolated from cattle; both isolates were collected in New York State and were pansusceptible. However, the isolates differed

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TABLE 4. MLST alleles of representative *Salmonella* isolates with PFGE patterns detected at high frequency[a]

Isolate	*Salmonella* serotype	PFGE pattern	No. of isolates with the same PFGE pattern[b]	Antimicrobial resistance pattern[c]	Host species	Allele no. for the following gene by MLST scheme[d]						
						hisD	*hemD*	*sucA*	*thrA*	*purE*	*dnaN*	*aroC*
S5-597[e]	4,5,12:i:−	L6	13	Suscept	Cattle	9	12	9	2	5	7	10
S5-777	4,5,12:i:−	L94	4	ACSTAmcSuCaz	Cattle	9	12	9	2	5	7	10
R6-027	Typhimurium	L154	6	AKSTSu	Cattle	9	12	9	2	5	7	10
R6-104	Typhimurium	L42	4	AKSTSuCaz	Human	9	12	9	2	5	7	10
R6-107	Typhimurium	L2	12	ACKSTSuCaz	Human	9	12	9	2	5	7	10
R6-108	Typhimurium	L40	4	ACSxtSTSuCaz	Human	9	12	9	2	5	7	10
R6-114	Typhimurium	L46	9	S	Human	9	12	9	2	5	7	10
R8-3079	Typhimurium	L21	6	SSu	Human	9	12	9	2	5	7	10
R8-3130[e]	Typhimurium	L1	5	Suscept	Human	9	12	9	2	5	7	10
S5-509	Typhimurium	L25	26	ACSTAmcSu	Human	9	12	9	2	5	7	10
S5-511	Typhimurium	L96	4	Suscept	Human	9	New1[f]	9	2	5	7	10
S5-640	Typhimurium	L93	4	Suscept	Human	9	12	9	2	5	7	10
S5-679	Typhimurium	L104	9	AKSTSu	Human	9	12	9	2	5	7	10
S5-831	Typhimurium	L97	3	Suscept	Cattle	9	New1[f]	9	2	5	7	10
R6-051	Newport	L75	18	ACKSTSuCaz	Cattle	14	21	12	12	15	7	10
R6-054	Newport	L73	9	ASTSuCaz	Cattle	14	21	12	12	15	7	10
R6-112	Newport	L77	4	ACSTSuCaz	Human	14	21	12	12	15	7	10
R8-3128	Newport	L71	20	ACSTAmcSuCaz	Human	14	21	12	12	15	7	10
S5-446	Newport	L107	5	Suscept	Human	14	21	12	12	15	7	10
S5-518	Newport	L105	4	ACSTAmcSuCaz	Human	14	21	12	12	15	7	10
S5-545	Newport	L108	20	ACSTAmcSuCaz	Cattle	14	21	12	12	15	7	10
S5-562	Newport	L70	23	ACSTAmcSu	Cattle	14	21	12	12	15	7	10

[a] *hemD* sequencing of an additional 18 pansusceptible *Salmonella* serotype Newport isolates, representing PFGE pattern L107 (4 isolates) as well as 15 other PFGE patterns that were each detected once in this study (see Table S1 in the supplemental material) identified allelic type (AT) *hemD21* among the 5 isolates from cattle and ATs *hemD21* and *hemD45* among 5 and 8 human isolates, respectively.

[b] Based on the final, reduced data set.

[c] The antimicrobial resistance pattern is shown as follows: Suscept, susceptible; ACSTAmcSuCaz, resistance to ampicillin, chloramphenicol, streptomycin, tetracycline, amoxicillin-clavulanic acid, sulfonamides, and ceftazidime; AKSTSu, resistance to ampicillin, kanamycin, streptomycin, tetracycline, and sulfonamides; and Sxt, resistance to sulfoxazole-trimethoprim.

[d] Allele numbers were assigned using the MLST database (http://mlst.ucc.ie/).

[e] PFGE pattern detected among both *Salmonella* serotype Typhimurium and 4,5,12:i:− isolates.

[f] Newly described AT.

in two MLVA loci (STTR5 and STTR6) and by more than three bands in their PFGE patterns.

We subsequently compared the concatenated nucleotide sequences for each sequence type to previously reported MLST types for *Salmonella* serotype Newport and Typhimurium isolates (see Table S3 and Fig. S1 in the supplemental material). The *Salmonella* serotype Newport isolates analyzed in this study were identical to 145 of the 389 published Newport sequences, thereby representing the most frequent Newport ST in the MLST database (http://mlst.ucc.ie/). Similarly, the predominant *Salmonella* serotype Typhimurium ST in our study exactly matched the ST for 251 of the 412 published serotype Typhimurium sequences, representing the most common Typhimurium ST in the MLST database. The less frequent *Salmonella* serotype Typhimurium ST in our study had no exact matches in the database, but it is very closely related to the other serotype Typhimurium ST detected in this study and clearly clustered with these isolates in phylogenetic analyses (see Fig. S1 in the supplemental material).

Relationship between the genetic similarity of isolates and the resistance type. In the minimum spanning tree (MST) based on MLVA data for *Salmonella* serotype Typhimurium and 4,5,12:i:− isolates, multidrug-resistant and susceptible isolates occasionally clustered together, and resistant and susceptible isolates sometimes shared identical MLVA patterns (Fig. 2A). Among the *Salmonella* serotype Newport isolates, multidrug-resistant and susceptible isolates never shared identical MLVA patterns but sometimes showed similar MLVA patterns that clustered together. For example, three pansusceptible bovine *Salmonella* serotype Newport isolates (A4-496, S5-919, and S5-900), which were isolated from the same farm (NE299) between December 2004 and February 2005 and clustered together (Fig. 2B), also clustered with a susceptible human isolate (R8-3109) and with a bovine isolate (R6-028) resistant to six antimicrobials (ampicillin, chloramphenicol, streptomycin, tetracycline, sulfonamides, and ceftazidime [ACSTSuCaz]). Another pansusceptible bovine *Salmonella* serotype Newport isolate from farm NE299, S5-970, differed in MLVA locus STTR6 and clustered separately from the other 3 pansusceptible isolates from the same farm, even though the 4 isolates were indistinguishable by PFGE.

To further characterize the genetic relationship between the pansusceptible *Salmonella* serotype Newport isolates, we sequenced *hemD* in all pansusceptible serotype Newport isolates (Table 4); analysis of this gene has been shown to clearly classify Newport isolates into the two recognized lineages (33). Analysis of *hemD* sequences indeed grouped our isolates into the two separate lineages. One lineage contained pansusceptible *Salmonella* serotype Newport isolates from cattle and humans; these isolates had the same *hemD* allelic type (AT)

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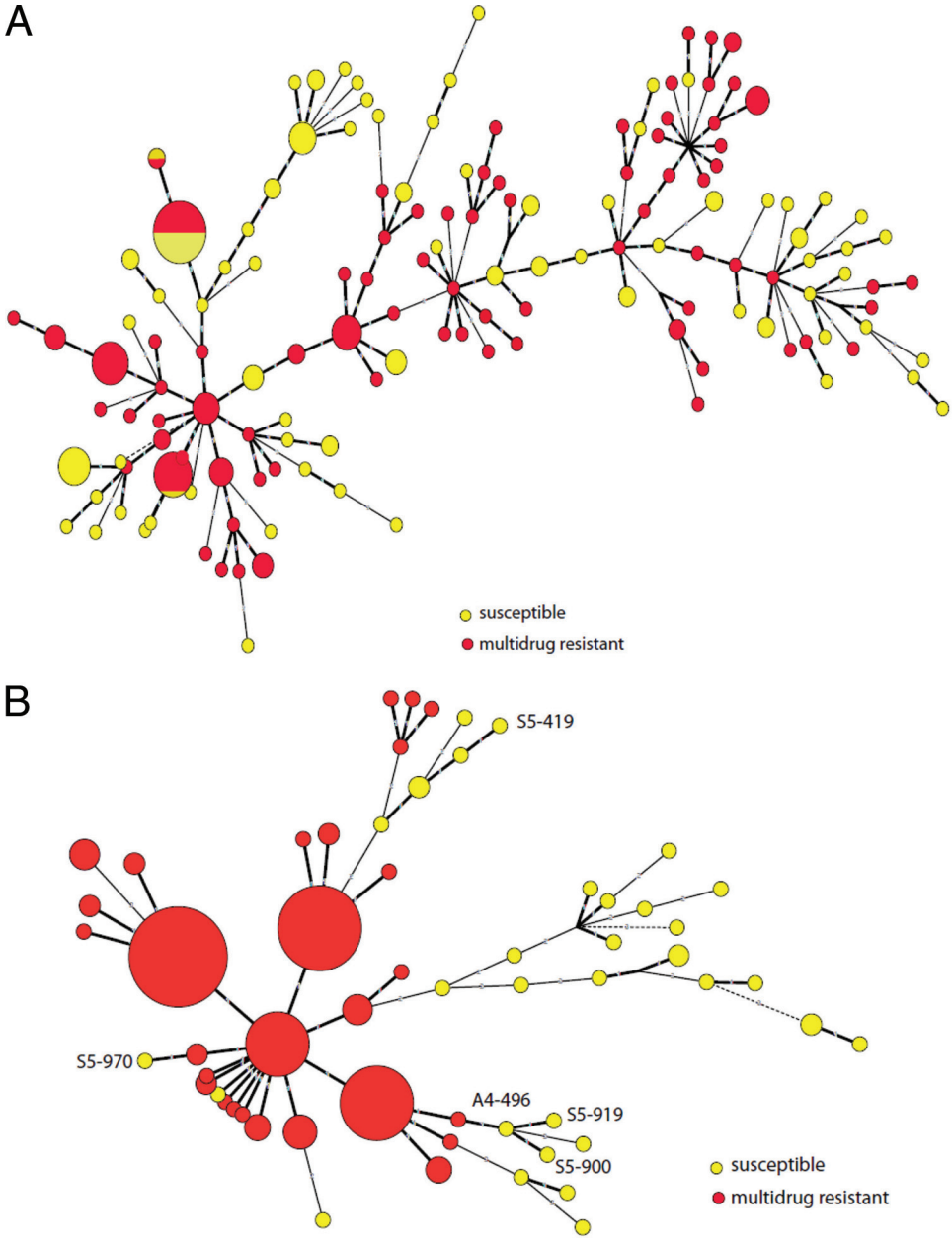


FIG. 2. Minimum spanning tree (MST) based on MLVA data for *Salmonella enterica* serotype Typhimurium isolates (A) and *Salmonella enterica* serotype 4,5,12:i:− or Newport isolates (B) included in this study. The size of the circle is proportional to the number of isolates represented by each node, and the length of the branch is based on categorical coefficients and proportional to the number of differences between nodes. Hypothetical nodes are indicated by broken lines. Nodes encompassing multidrug-resistant isolates are shown in red, and those encompassing susceptible isolates are shown in yellow. Pansusceptible *Salmonella* serotype Newport isolates from farm NE299, which are characterized in the text, are indicated by their respective accession number in the tree. Interpretation of central nodes as ancestral is dependent on linear relationship between phylogenetic relationship and difference in the number of repeats, an assumption that might not be valid in all cases, particularly for isolates from *Salmonella* serotype Newport lineages A and B.

(*hemD21*) as the multidrug-resistant bovine serotype Newport isolates characterized by MLST analysis in our study. The isolates belonging to the other lineage, all obtained from humans, shared AT *hemD45*; on the basis of the MLST database, this AT represented a perfect match with a large number of serotype Newport isolates that have been collected from reptiles, humans, birds, and a small number of domestic animals (see Table S4 in the supplemental material). Six of 14 (43%) pansusceptible human *Salmonella* serotype Newport isolates analyzed here belong to AT *hemD21*, contributing 6 of 11 (55%) pansusceptible serotype Newport isolates with AT *hemD21*; this AT was also associated with the multidrug-resistant bovine serotype Newport isolates analyzed by 7-gene MLST in our study, indicating it is associated with the cattle-associated Newport lineage. Isolates that grouped together based on *hemD* AT also were clearly distinct by PFGE analysis,

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corroborating previous findings that the two *Salmonella* serotype Newport lineages represent distinct subgroups (1, 33) (data not shown).

Distribution of isolates between sources. To better characterize the distribution of related isolates, we performed a cluster analysis based on the combination of PFGE and MLVA results. On the basis of a clustering cutoff at 75% similarity, among the *Salmonella* serotype Newport isolates, we identified 10 combined PFGE and MLVA clusters that contained multiple isolates as well as 7 unique isolates (see Fig. S2 in the supplemental material). The largest cluster included 89 of the 93 bovine isolates and 31 of the 65 human isolates; 2 of the remaining clusters also contained bovine and human serotype Newport isolates (see Fig. S2A in the supplemental material). Among the *Salmonella* serotype Typhimurium isolates, we detected 28 unique isolates as well as 29 combined PFGE and MLVA clusters; 17 of these clusters contained human and cattle isolates (Fig. S2B).

To further characterize the subtype distribution, we analyzed the frequency distribution of isolates with PFGE patterns that were detected among both bovine and human isolates. We identified 17 PFGE patterns, 7 among *Salmonella* serotype Newport isolates and 10 among *Salmonella* serotype Typhimurium isolates, that were detected among both human and bovine isolates. Among the multidrug-resistant isolates, 13 PFGE patterns were shared between cattle and humans, 6 PFGE patterns among *Salmonella* serotype Newport isolates, and 7 patterns among *Salmonella* serotype Typhimurium isolates, representing a total of 139 isolates. Among the susceptible isolates, 8 PFGE patterns, representing 17 isolates, were shared between humans and cattle.

Distribution of isolates between the Pacific Northwest and the northeastern United States. To better understand the subtype distribution between the two geographic regions in the United States, we analyzed the distribution of isolates with PFGE patterns that were detected in both geographic regions. Two of the 10 *Salmonella* serotype Newport combined PFGE and MLVA clusters and 18 of the 29 *Salmonella* serotype Typhimurium combined PFGE and MLVA clusters contained isolates from both geographic regions (see Fig. S2 in the supplemental material). Five PFGE patterns were identified among isolates from both geographic regions, including 4 patterns associated with *Salmonella* serotype Newport isolates and 1 pattern associated with *Salmonella* serotype Typhimurium isolates (data not shown). All PFGE patterns that were detected in both geographic regions were also associated with both isolates from cattle and humans, indicating that these *Salmonella* subtypes are probably widely distributed among cattle and humans across the United States.

Among the *Salmonella* serotype Typhimurium isolates, 5 PFGE patterns were shared between resistant and susceptible isolates (i.e., L15, L2, L21, L25, and L36) including one PFGE pattern (L25) found among susceptible and multidrug-resistant isolates in both geographic regions. Three PFGE patterns associated with multidrug-resistant *Salmonella* serotype Newport isolates (L70, L71, and L90) and one PFGE pattern associated with a susceptible *Salmonella* serotype Newport isolate (L90) were detected in both geographic regions.

DISCUSSION

A total of 425 *Salmonella* isolates from two geographic regions in the United States, the Pacific Northwest and the Northeast, were characterized by PFGE and MLVA analysis to obtain a better understanding of genetic diversity, epidemiology, and antimicrobial resistance among *Salmonella* serotype Newport, Typhimurium, and 4,5,12:i:− isolates from humans and cattle. Overall, our data showed the following. (i) Multidrug resistance is significantly more prevalent among isolates associated with cattle than isolates associated with humans but differs considerably by serotype and geographic region of the United States. (ii) *Salmonella* subtypes persist on farms, although diversification of molecular subtypes and resistance types may potentially occur over short time periods. (iii) Genetic diversity differs considerably among *Salmonella* serotypes and between *Salmonella* isolates from human and bovine host populations. (iv) The combination of PFGE with MLVA or antimicrobial resistance patterns can increase subtype discrimination for selected *Salmonella* serotypes.

Multidrug resistance is significantly more prevalent among cattle-associated isolates than human-associated isolates, but it differs considerably by serotype and geographic region of the United States. The high frequency of antimicrobial resistance among *Salmonella* isolates from predominantly clinically sick cattle observed here is consistent with previous reports (18). These trends are also comparable to those reported by the National Antimicrobial Resistance Monitoring System (NARMS) in the United States, but the frequency of antimicrobial resistance observed in our study was considerably higher than that reported by NARMS, especially for human isolates (45). NARMS reported that approximately 62% of human *Salmonella* serotype isolates and 32% of cattle *Salmonella* serotype Typhimurium isolates, as well as 83% of human and 17% of cattle Newport isolates collected in 2006 were susceptible to all tested antimicrobial drugs (45). By comparison, we found much lower frequencies of pansusceptible isolates, with 23 and 18% of human and cattle *Salmonella* serotype Typhimurium isolates and 22 and 6% of human and cattle *Salmonella* serotype Newport isolates being pansusceptible, respectively. Most likely, these differences represent differences in the geographic origin of isolates between the NARMS report and our study. Geographic differences in the prevalence of antimicrobial resistance among *Salmonella* isolates have been described previously, and our study characterized isolates from only two states, while NARMS data are based on isolates from all 50 U.S. states. This is important, as Greene et al. (29) reported a positive correlation between cattle density per acre and the prevalence of multidrug resistance among human *Salmonella* serotype Newport isolates, and New York and Washington specifically represent two states with high cattle density and comparatively high prevalence of MDR as reported by Greene et al. (29). Furthermore, *Salmonella* isolates originating from New York City residents are generally submitted to the New York City Department of Health and were therefore only very rarely included in this study, potentially resulting in sampling biased toward rural human populations. In addition to different sampling schemes, differences in the antimicrobial drugs tested and the testing method used may also have contributed to the high frequency of drug resistance reported here. Impor-

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tantly, a considerable number of human isolates in our study were resistant only to triple-sulfa, a combination of sulfonamides not tested for by NARMS. These isolates would hence likely be reported as pansusceptible by NARMS. While our study and NARMS used different resistance testing methods (i.e., broth microdilution versus disc diffusion), a previous study has reported good correspondence between resistance typing results based on these two methods, at least for *Salmonella* serotype Heidelberg, despite minor differences, suggesting a limited effect of these methodological differences (48).

Resistance to ACSSuT as well as to amoxicillin-clavulanic acid and ceftiofur was particularly prevalent in our study, but resistance to other antimicrobial agents, such as kanamycin or the combination of sulfisoxazole-trimethoprim, was also relatively common, indicating that the MDR subtypes acquired additional antimicrobial resistance genes, a phenomenon that has been described previously (27). The high prevalence of resistance to ceftazidime, an expanded-spectrum cephalosporin, reported in previous studies and confirmed here, is of particular concern, as extended-spectrum cephalosporins are the drugs of choice for treatment of nontyphoidal salmonellosis in humans if fluoroquinolones are contraindicated (31, 54).

In our study, MDR and susceptible isolates were generally characterized by distinct MLVA and PFGE subtypes, particularly for *Salmonella* serotype Newport. Multidrug resistance was significantly more common among bovine than human isolates, especially for *Salmonella* serotype Newport, with the odds of multidrug resistance approximately 18 times higher for bovine than human Newport isolates. While differences in sampling and surveillance between human and cattle isolates have to be considered, these factors are unlikely to have distorted the study significantly, as both human and cattle isolates originated almost exclusively from cases with clinical signs typical of salmonellosis. Rather, our findings suggest a significantly higher frequency of multidrug resistance among cattle than human *Salmonella* isolates, supporting the hypothesis that cattle may represent one possible reservoir for multidrug-resistant *Salmonella* and that cattle may serve as a source for emerging MDR strains (see references 2 and 66 for a review of this topic). This conclusion is corroborated by findings of other studies which indicate that consumption of beef or dairy products, as well as dairy farm contact, represent important risk factors for human *Salmonella* serotype Newport MDR-AmpC infection (74). Moreover, a number of human *Salmonella* serotype Newport MDR-AmpC outbreaks have been linked to beef or dairy product consumption, and a role of cattle as reservoir for multidrug-resistant *Salmonella* serotype Newport has been proposed previously (14). Bovine *Salmonella* serotype Typhimurium isolates, however, were also significantly more likely to be multidrug resistant than human isolates. Similar observations have also been reported by Busani et al. (9), who reported a significantly lower prevalence of resistance to ACSSuT among *Salmonella* serotype Typhimurium isolates from humans than from nonhuman sources and specifically detected a comparably high prevalence of the ACSSuT resistance type among serotype Typhimurium isolates from cattle. Differences in antimicrobial resistance prevalence between isolates from humans and animals were not formally tested in that study though, and resistance prevalence was highest among environmental samples. Somewhat higher resistance prevalence among animal than human *Salmonella* serotype Typhimurium isolates for some antimicrobial drugs has also been reported in other studies, and a role of cattle and other domestic animals as reservoir for human infection with multidrug-resistant *Salmonella* serotype Typhimurium strains has been proposed repeatedly (12, 13, 51, 52).

We also detected a significant effect of geographic region, with the odds of multidrug resistance approximately twice as high for isolates from the Pacific Northwest as for isolates from the Northeast. Among the cattle isolates, sampling differences between the two study sites may have contributed to this observation, with cattle isolates from the Northeast originating from herds enrolled in an active surveillance and sampling project, thereby potentially increasing the likelihood of detecting milder clinical cases or including animals exhibiting signs caused by other diseases (18). However, our findings are consistent with a previous study which reported an association between prevalence of multidrug resistance among human *Salmonella* serotype Newport isolates and cattle density per acres of farmland, even though in this previous study (29), the highest resistance prevalence was observed in the northeastern United States, particularly in Maine and Connecticut. *Salmonella* prevalence in dairy herds is known to vary considerably by geographic region and two 2002 studies found a considerably lower *Salmonella* prevalence in dairy herds in the northeastern United States compared to the western United States, potentially due to smaller average herd sizes in the Northeast (3, 11). While our data thus suggest a possible correlation between *Salmonella* prevalence and antimicrobial resistance prevalence, further studies will be needed to test this hypothesis and to better define risk factors for high prevalence of multidrug-resistant *Salmonella*.

***Salmonella* subtypes persist on farms, although diversification of molecular subtypes and resistance types may potentially occur over short time periods.** In our study, specific *Salmonella* strains appear to have persisted on farms for considerable amounts of time, with isolates characterized by indistinguishable MLVA, PFGE, and resistance profiles isolated from the same farm over periods of more than 7 months. *Salmonella* persistence on farms has been a consistent finding, but the observed duration of persistence varies (17). In our study, all but 17 isolates were collected from cattle with clinical signs of salmonellosis. As differences between herds with and without clinical signs of salmonellosis (for instance, differences in serotype distribution or *Salmonella* prevalence) have been reported, differences in duration of persistence also appear likely (18). The generalizability of our results may therefore be restricted to herds with clinical cases of salmonellosis.

On some farms, we collected isolates with different molecular patterns, typically closely related PFGE, MLVA, or antimicrobial resistance patterns. For example, *Salmonella* isolates from farm NE261 could be grouped into two clusters based on PFGE and antimicrobial resistance patterns, while MLVA results indicated that isolates from both clusters were probably closely related. The molecular methods applied in this study do not allow for definitive inferences concerning the underlying genetic events. However, the fact that prior to August 2004, the predominant persistent *Salmonella* serotype 4,5,12:i:− strain on farm NE261 was multidrug resistant, while a pansusceptible 4,5,12:i:− strain subsequently appeared to become most prev-

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alent on this farm, paired with the observation that the new strain was characterized by a PFGE pattern that differed by the absence of one band and the presence of three new bands compared to the initial strain could be explained by the loss of a mobile element encoding multidrug resistance determinants. Importantly, one isolate from the same farm, collected in early September, was also pansusceptible but contained two bands unique to the cluster of resistant isolates while lacking one band unique to the pansusceptible cluster and one band unique to the multidrug-resistant cluster, consistent with a second molecular process, potentially the insertion or excision of a bacteriophage. The possibility that this and other similar observations represent a reintroduction of closely related *Salmonella* subtypes onto a given premise or that multiple closely related subtypes coexisted on the same farm without being detected initially cannot be excluded. However, this example illustrates the possibility of considerable diversification of *Salmonella* strains during persistence on farms or in production environments, which can complicate molecular epidemiological investigations (for instance, see references 43 and 69).

Genetic diversity differs considerably among *Salmonella* serotypes and between *Salmonella* isolates from human and bovine host populations. In this study, *Salmonella* serotype Typhimurium isolates seemed more genetically diverse than *Salmonella* serotype Newport isolates, and differences were particularly pronounced among isolates from cattle. These observations agree with previous studies, which report a clonal nature of *Salmonella* serotype Newport subtype MDR-AmpC (7, 31, 33). *Salmonella* serotype Newport MDR-AmpC appears predominantly associated with cattle but has also been isolated from humans and a variety of other host species (see reference 10 for a review of this topic). In general, we found cattle isolates to be less genetically diverse than human isolates regardless of the serotype analyzed. These observations may be due to sampling differences, especially since cattle isolates originated from a limited number of farms, but the results appear to agree with previous study results, which show somewhat lower genetic diversity among animal *Salmonella* isolates than human *Salmonella* isolates, likely reflecting the fact that human infections can occur from a variety of sources, which are expected to harbor a considerable variety of *Salmonella* subtypes (28, 51, 59). However, future studies are needed to reliably capture the genetic diversity in *Salmonella* populations circulating among humans and cattle.

Combination of PFGE with MLVA or antimicrobial resistance patterns can increase subtype discrimination for selected *Salmonella* serotypes. In our study, PFGE showed a high discriminatory power for all serotypes tested, supporting the value of this subtyping method (6, 16, 33). However, for *Salmonella* serotype Typhimurium, the discriminatory power of MLVA was significantly higher than that of PFGE, while for *Salmonella* serotype Newport, the discriminatory power of both methods was comparable, consistent with previous reports (19, 42, 67).

We detected moderate correspondence between isolate clustering based on PFGE and MLVA, also consistent with previous studies (19, 76). PFGE patterns can be impacted by a variety of genetic events, including plasmid gain or loss, transposition, integration, or loss of mobile genetic elements, such as bacteriophages, and point mutation (16). MLVA, in contrast, detects copy number differences in tandem repeat DNA motifs, which result from slipped-strand mispairing during DNA replication (73). VNTR mutation rates seem to depend on a variety of factors, including the number of tandem repeats and nucleotide composition, and variability appears to differ between VNTR loci (39, 49). MLVA and PFGE patterns are therefore affected by different evolutionary mechanisms, and results have to be interpreted accordingly. Clustering confirmed by both subtyping methods provides strong evidence for true genetic relatedness, while discordant results can be difficult to interpret. Similar to our study which suggested diversification of both PFGE and MLVA patterns on individual farms, the diversification of PFGE and MLVA patterns has been reported during human salmonellosis outbreaks, even though diversification rates are difficult to estimate (23, 50, 64, 67). In such instances, the use of additional information, e.g., epidemiologic data, antimicrobial resistance patterns, or prior knowledge of subtype diversity, can be very useful for inferring relationships between isolates. In fact, while in our study both antimicrobial resistance and MLVA patterns seemed to diversify during *Salmonella* persistence on farms, resistance patterns appeared to be less variable, and among isolates from the same farm and of the same PFGE type, resistance patterns varied in at most one antimicrobial drug. Moreover, in our study, PFGE and antimicrobial resistance-based clustering was occasionally more consistent with epidemiologic data than MLVA-based clustering, as seen for farm NE229. Combining PFGE with antimicrobial resistance profiles also yielded a considerable gain in discriminatory power over that of PFGE alone. Antimicrobial resistance data have contributed useful information in several epidemiologic investigations, but to our knowledge, our study is the first to formally quantify the gain in discriminatory power for three common *Salmonella* serotypes (7, 8, 64). In our analysis as well as in previous studies, however, the combination of PFGE and MLVA provided the highest discriminatory power (19, 42, 67). Overall, these observations further reinforce the need to choose the *Salmonella* subtyping methods most appropriate for a given study goal, while additional factors, such as subtype diversity or the availability of serotype-specific protocols, have to be taken into consideration (8, 67).

Even though MLST does not generally provide high discriminatory power within serotypes, we found sequence typing of a single gene sufficient to distinguish between the two *Salmonella* serotype Newport lineages (5). Importantly, MLST-based lineage attribution corresponded well with PFGE-based lineage attribution, corroborating previous findings (1, 33). Since *Salmonella* serotype Newport lineages A and B are associated with different animal hosts, lineage determination can provide useful insights for serotype Newport source attribution, thereby potentially allowing source attribution for the important fraction of human *Salmonella* infections for which the source currently remains unknown (32, 71). The ability to predict *Salmonella* serotype Newport lineage based on single-gene sequencing or PFGE as shown here can thus potentially contribute to more cost-effective and rapid *Salmonella* serotype Newport source attribution.

Conclusions. Our study provides support for the hypothesis that cattle may represent a reservoir for multidrug-resistant *Salmonella* and may serve as a source for emerging MDR

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strains. However, the importance of that role may depend on a variety of factors, including serotype and geographic origin. Our data show that *Salmonella* can persist on individual dairy farms for prolonged periods of time, potentially with diversification of some subtype patterns over time. In some instances, new antimicrobial resistance may be acquired or lost during persistence, and interpretation of epidemiologic links based exclusively on subtyping results can be complicated. While PFGE and MLVA are highly reliable and generally extremely useful, they can be affected by a variety of genetic events, and discordant results must be interpreted carefully. Some *Salmonella* subtypes appear to be widespread among cattle and humans across the United States, but most appear to be associated with a specific geographic area and, to a lesser extent, host species.

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