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Comparison of Multilocus Sequence Typing, Pulsed-Field Gel Electrophoresis, and Antimicrobial Susceptibility Typing for Characterization of *Salmonella enterica* Serotype Newport Isolates

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In the United States, multidrug-resistant phenotypes of *Salmonella enterica* serotype Newport (commonly referred to as MDR-AmpC) have emerged in animals and humans and have become a major public health problem. Although pulsed-field gel electrophoresis (PFGE) is the current "gold standard" typing method for *Salmonella*, multilocus sequence typing (MLST) may be more relevant to investigations exploring evolutionary and population biology relationships. In this study, 81 *Salmonella enterica* serotype Newport isolates from humans, food animals, and retail foods were examined for antimicrobial susceptibility and characterized using PFGE and MLST of seven genes, *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. Forty-nine percent of the isolates were resistant to nine or more of the tested antimicrobials. *Salmonella* isolates displayed resistance most often to sulfamethoxazole (57%), streptomycin (56%), tetracycline (56%), ampicillin (52%), and ceftiofur (49%) and, to a lesser extent, to kanamycin (19%), trimethoprim-sulfamethoxazole (17%), and gentamicin (11%). A total of 43 PFGE patterns were generated using XbaI, indicating a genetically diverse population. The largest PFGE cluster contained isolates from clinically ill swine, cattle, and humans. MLST resulted in 12 sequence types (STs), with one type encompassing 62% of the strains. Ten new sequence types and one novel allele type were identified. Furthermore, MLST typing showed that strains closely related by PFGE clustered in major STs, whereas more distantly related strains were separated into two clusters by PFGE. The results of this study demonstrated that the MLST scheme employed here clustered *S. enterica* serovar Newport isolates in distinct molecular populations, and strain discrimination was enhanced by combining PFGE, antimicrobial susceptibility, and MLST results.

Salmonella enterica serovars are responsible for approximately 1.4 million gastroenteritis cases each year in the United States and represent a major pubic health problem worldwide (33, 56). The U.S. FoodNet surveillance program reported that *Salmonella* serotypes were the second leading cause of bacterial food-borne infections in 2004, with five serotypes accounting for 59% of the *Salmonella* infections. The top three serotypes were identified as Typhimurium, Enteritidis, and Newport (5). An increase in the incidence of *Salmonella enterica* serotype Newport infections was initially reported by the CDC in 2000. Many of these strains exhibited a multidrug-resistant phenotype (commonly referred to as *S. enterica* serovar Newport MDR-AmpC) characterized by resistance to nine antimicrobials: ampicillin, amoxicillin-clavulanic acid, cefoxitin, cephalothin, ceftiofur, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition to the characteristic resistance to nine antimicrobials, these strains also exhibited decreased susceptibility to ceftriaxone (MIC range, 16 to 32 μg/ml) (6). These strains are of particular clinical concern, as they possess plasmid-mediated AmpC β-lactamases (e.g., bla_{CMY}), which confer decreased susceptibility to a wide range of beta-lactams, including ceftriaxone, the drug of choice for treating complicated salmonellosis in children (22, 59). *S. enterica* serovar Newport MDR-AmpC strains have been linked to human exposure via dairy cattle and to food-borne transmission to humans by the consumption of contaminated ground beef, pork, and other food products (22, 59).

DNA-based strain-typing methods for bacterial pathogens play a crucial role in understanding infectious-disease transmission, tracking, and response and have been used widely to distinguish *Salmonella* clinical isolates recovered from animals, food-borne disease, and nosocomial infections (21, 30, 53, 58). Pulsed-field gel electrophoresis (PFGE) and antimicrobial susceptibility typing (AST) are two commonly used methods for studying microbial epidemiology and trends in the antibiotic resistance of bacteria. PFGE is currently used by the CDC PulseNet surveillance program and is generally accepted as the "gold standard" for molecular typing of *Salmonella* (7, 18, 34, 41, 57). Due to its limitations, many studies have compared PFGE to other genetic typing methods in attempts to identify more powerful tools for epidemiological investigations and evolutionary analyses. Comparisons have been made with various typing schemes, including serotyping and phage typing, repetitive-element PCR, multilocus variable-number tandem repeat analysis, amplified fragment length polymorphism, antibiotic susceptibility typing, and DNA sequence typing (4, 14, 19, 21, 22, 29, 32, 44, 45, 59).

Multilocus sequence typing (MLST) is based on allelic dif-

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ferences in the nucleotide sequences of housekeeping or virulence genes among bacterial strains (31). MLST methods and databases have been developed for a growing number of clinically important bacterial pathogens, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Salmonella* serotypes (1, 10, 14, 26, 29, 39, 52, 54, 55). MLST has shown various degrees of utility as a discriminatory typing method for several bacterial pathogens, including *E. coli* O157:H7 and several *Salmonella* serotypes (14, 29, 39, 40, 52, 54). Fewer MLST data are available for *S. enterica* serovar Newport, as only three publications have reported on a limited number of isolates (29, 52, 54). In this study, 81 *Salmonella enterica* serotype Newport isolates from clinically ill animals, animal-derived foods, and human infections were analyzed by antimicrobial susceptibility typing, PFGE, and MLST, in order to compare the discriminatory powers of the methods.

MATERIALS AND METHODS

Bacterial strains. Eighty-one *Salmonella enterica* serotype Newport isolates, comprised of 20 isolates from human patients, 51 isolates from clinically ill food animals, and 10 isolates from retail meat products, were used in this study. Of the 61 animal and retail meat isolates, 15 isolates originated from turkeys (including 7 from retail ground-turkey samples from FoodNet sites in Maryland, California, Iowa, and Tennessee), 20 isolates were from cattle, 16 isolates were from swine (including 3 from retail pork chop samples from FoodNet sites in Maryland and Oregon), and 10 isolates originated from chickens. The *S. enterica* serotype Newport isolates used in this study were identified in 27 different states from 2001 to 2003. Of the retail meat samples, five isolates were obtained from two sites, while the rest of the retail isolates were obtained from independent sites. Human isolates were received from the Stanford University Medical Center (Stanford, CA) and the Iowa Department of Public Health (Des Moines, IA) as anonymous samples. Clinical animal isolates were received from the National Veterinary Services Laboratory (Ames, IA) and were chosen according to the animal source of infection and for variation of the state of isolation in order to achieve a wide geographical representation among the isolates (59). Isolates were confirmed as *Salmonella* using VITEK gram-negative identification cards (bioMérieux Inc., Hazelwood, MO) and were serotyped with commercial Difco antisera (Becton Dickinson and Company, Sparks, MD). *Salmonella enterica* serotype Newport isolates were grown on Trypticase soy agar plates (TSA II) supplemented with 5% defibrinated sheep blood (Becton Dickinson Microbiology Systems, Sparks, Md.) and were stored in Trypticase soy broth (Becton Dickinson Microbiology Systems, Sparks, Md.) containing 15% glycerol at −80°C until needed.

Antimicrobial susceptibility testing. Antimicrobial MICs were determined using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and interpreted using the CLSI (formerly NCCLS) standards (37, 38). The antimicrobials tested included amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. The quality control organisms used included *E. coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 to ensure that all antimicrobial agents were appropriately quality controlled, except for streptomycin, for which official quality control standards have not been set (37, 38). Chi-square analysis and logistical-regression analysis were performed to indicate significant differences.

PFGE. Pulsed-field gel electrophoresis was performed according to the procedures developed by the CDC (35) and as previously described (59). Briefly, agarose-embedded DNA was digested with 50 U of XbaI (Boehringer Mannheim, Indianapolis, IN) overnight in a water bath at 37°C. The restriction fragments were separated by electrophoresis in 0.5× Tris-borate-EDTA buffer at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16 to 63.8 s. The gels were stained with ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad). *Salmonella enterica* serotype Newport *am01144* was used as the control strain (59). Isolates presenting DNA smear patterns were retested using plugs digested with XbaI and subjected to electrophoresis in buffer containing 50 μM of thiourea in 0.5× Tris-borate-EDTA buffer. Interpretation of DNA fingerprint patterns was accomplished using Bionumerics 4.0 software (Applied Maths, Austin, TX). The banding patterns were compared using Dice coefficients with a 1.5% band position tolerance. Isolate relatedness was determined using the unweighted pair group method using arithmetic averages (UPGMA). Simpson's index of diversity (*D*) was used as an indicator of the discriminatory power of each method and is calculated according to the following formula: $D = 1 - (\Sigma n(n-1)/N(N-1))$, where *D* is the diversity, *N* is the total number of strains in the sample, and *n* is the number of strains in each type (25).

MLST. Genomic-DNA templates were prepared using the UltraClean Microbial DNA Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Seven genes were chosen for MLST: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*, to allow comparison to an existing *Salmonella* MLST database (http://web.mpiib-berlin.mpg.de/mlst). Amplification protocols detailed in the database were used in this study, including primer sequences and annealing temperatures. Amplifications for all genes were carried out with approximately 0.2 μg DNA template, 250 μM (each) deoxynucleoside triphosphates, 2.5 mM $MgCl_2$, 25 pmol of primers, and 1 U of Gold *Taq* polymerase (Perkin-Elmer, Foster City, Calif.) in 50-μl reaction mixtures. PCR cycling conditions were a 10-min hold at 94°C, followed by 34 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. Products were separated by 1.5% agarose gel electrophoresis and visualized with ethidium bromide staining and UV illumination with a gel documentation system (Gel Doc 2000; Bio-Rad, Hercules, Calif).

Amplification products were purified using a 96-well Millipore Multi-screen Filter plate according to the manufacturer's recommendations (Millipore, Billerica, MA). Amplicons were resuspended in 50 μl of nuclease-free water, and DNA sequences were determined using the BigDye Terminator v3.1 cycle-sequencing kit (Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. Reaction mixtures contained 20 mM of primer, approximately 20 ng of DNA template, 1× BigDye Terminator v3.1 sequencing buffer, and 1 μl of terminator ready-reaction mixture in a 5-μl total volume. Cycle-sequencing conditions were a 96°C hold for 1 min and 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. After the cycling was completed, the sequenced products were precipitated with 20 μl 75% ethanol for 30 min and centrifuged for 45 min in a Centra CL3 centrifuge (Thermo Electron Corporation, Waltham, Mass.) at 4,000 × *g*. Dried DNA pellets were resuspended in 7 μl of Hi-Di Formamide (Applied Biosystems, Foster City, Calif.), and the products were analyzed on an ABI PRISM DNA analyzer 3700 (Applied Biosystems, Foster City, Calif.).

MLST data analysis. Sequences were edited, and complementary sense and antisense fragments were aligned using Bionumerics 4.0 software (Applied Maths, Austin, TX). The sequences were submitted to the MLST database website (http://web.mpiib-berlin.mpg.de/mlst) and assigned existing or novel allele type numbers and sequence type numbers defined by the database. This multimicroorganism database defines a novel allele type if it contains one or more nucleotide changes from existing allele sequences. Composite sequence types (STs) are assigned based on the set of allele types derived from each of the seven loci. STs were analyzed for relatedness using the eBURST v3 program (http://eburst.mlst.net; 15).

RESULTS

Antimicrobial susceptibility phenotypes. The susceptibility profiles of the 81 *Salmonella enterica* serotype Newport isolates were determined using a broth microdilution method in accordance with CLSI standards. All isolates were susceptible to amikacin, nalidixic acid, and ciprofloxacin (Table 1). Fifty-nine percent ($n = 48/81$) of *S. enterica* serovar Newport isolates exhibited resistance to at least one antimicrobial. Forty of these isolates were resistant to nine or more of the tested antimicrobial agents. *Salmonella* isolates most often displayed resistance to sulfamethoxazole (57%), streptomycin (56%), tetracycline (56%), ampicillin (52%), amoxicillin-clavulanic acid (49%), cefoxitin (49%), and ceftiofur (49%) and to a lesser extent to kanamycin (19%), trimethoprim-sulfamethoxazole (17%), and gentamicin (11%). Kanamycin resistance was most often observed in *S. enterica* serovar Newport isolates recovered from clinically ill cattle (40%)

TABLE 1. Antimicrobial resistance phenotypes of *Salmonella* Newport isolates from different animal and food types

Antimicrobial agent	Resistance breakpoint[a]	% Resistant isolates (*n*)							
		Human (*n* = 20)	Cattle (*n* = 20)	Swine (*n* = 13)	Chicken (*n* = 10)	Turkey (*n* = 8)	Ground turkey (*n* = 7)	Pork chop (*n* = 3)	Total (*n* = 81)
Ampicillin	≥32	50 (10)	80 (16)	54 (7)	40 (4)	13 (1)	14 (1)	100 (3)	52
Amoxicillin/clavulanic acid	≥32	50 (10)	80 (16)	54 (7)	30 (3)	0	14 (1)	100 (3)	49
Cefoxitin	≥32	50 (10)	80 (16)	54 (7)	30 (3)	0	14 (1)	100 (3)	49
Cephalothin	≥32	50 (10)	80 (16)	54 (7)	40 (4)	0	14 (1)	100 (3)	51
Ceftiofur	≥8	50 (10)	80 (16)	54 (7)	30 (3)	0	14 (1)	100 (3)	49
Ceftriaxone	≥64	5 (1)	5 (1)	0	0	0	0	0	3
Chloramphenicol	≥32	50 (10)	85 (17)	46 (6)	40 (4)	0	14 (1)	100 (3)	51
Tetracycline	≥16	50 (10)	95 (19)	62 (8)	40 (4)	0	14 (1)	100 (3)	56
Amikacin	≥32	0	0	0	0	0	0	0	0
Kanamycin	≥64	5 (1)	40 (8)	23 (3)	20 (2)	13 (1)	0	0	19
Gentamicin	≥16	0	20 (4)	15 (2)	10 (1)	13 (1)	14 (1)	0	11
Streptomycin[b]	≥64	50 (10)	90 (18)	62 (8)	40 (4)	0	14 (1)	100 (3)	56
Sulfamethoxazole	≥512	50 (10)	90 (18)	62 (8)	40 (4)	13 (1)	29 (2)	100 (3)	57
Trimethoprim/sulfamethoxazole	≥4	30 (6)	15 (3)	15 (2)	0	0	14 (1)	67 (2)	17
Nalidixic acid	≥32	0	0	0	0	0	0	0	0
Ciprofloxacin	≥4	0	0	0	0	0	0	0	0

[a] MIC (μg/ml) determined via broth microdilution methods in accordance with CLSI standards (37, 38).
[b] Interpretive criteria have not been established by the CLSI.

compared to other sources, although the difference was not significant ($P = \leq 0.10$).

Among the 81 *S. enterica* serovar Newport isolates, 20 susceptibility phenotypes were identified (Fig. 1). Thirteen of the phenotypes were represented by a single isolate. Forty-eight percent ($n = 39$) of the collection were identified as MDR-AmpC and were resistant to ampicillin, amoxicillin-clavulanic acid, cefoxitin, cephalothin, ceftiofur, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, with decreased susceptibility to ceftriaxone (MIC ≥ 16 μg/ml). These isolates were comprised of 16 strains from cattle (41%), 10 strains from human infections (26%), 6 strains from swine (15%), 3 strains from chickens (8%), and 1 strain from a ground turkey meat sample. None of the strains obtained from clinically ill turkeys exhibited the MDR-AmpC phenotype. The high rate of resistance among the cattle isolates and the high rate of susceptibility among the turkey isolates were significantly different at a level of 0.05 (since the confidence interval at 95% did not contain 1). The most common resistance profile was represented by 22% of the collection ($n = 18$). The indicator of discrimination as computed using Simpson's index of diversity was 0.78, where a score of 0.90 or greater is considered a high level of diversity.

PFGE profiles of *S. enterica* serovar Newport isolates. Using digestion with XbaI, a total of 43 PFGE patterns were identified, which were grouped into three major clusters (A, B, and C) with 62% pattern similarity (Fig. 1). Although some PFGE patterns were seen only among *S. enterica* serovar Newport isolates from certain animal species or retail foods, many patterns were shared by isolates from multiple origins. The largest PFGE pattern was identified in cluster A and contained isolates from clinically ill swine, cattle, and humans recovered from seven states (California, Florida, Iowa, Maryland, Missouri, Texas, and Utah). Excluding one *S. enterica* serovar Newport isolate (CVM21548; chloramphenicol susceptible), all of the strains in this pattern exhibited resistance to ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline.

Certain PFGE pattern clusters correlated well with antimicrobial resistance phenotypes. For example, cluster A was almost exclusively comprised of isolates exhibiting the MDR-AmpC phenotype (34/39 isolates) from all animal origins, with only one isolate originating from turkey (CVM 17015) (Fig. 1). This cluster differed markedly from cluster C, containing 22 *S. enterica* serovar Newport isolates, 19 (86%) of which were susceptible to all tested antimicrobials. With regard to specific PFGE patterns associated with *S. enterica* serovar Newport isolates recovered from different animals and retail meats, 13 patterns were generated from 20 human isolates, 14 patterns from 20 cattle isolates, 11 patterns from 15 turkey/ground-turkey isolates, 10 patterns from 16 swine/pork chop isolates, and 6 patterns from 10 chicken isolates.

When PFGE profile and antimicrobial susceptibility phenotypes were analyzed together by UPGMA, two major clusters were identified that displayed 31% profile and pattern similarity (data not shown). Simpson's index of diversity, when calculated for the PFGE method, resulted in a score of 0.97, which was the highest diversity observed in this study when one method was analyzed. The index of diversity was slightly increased when antimicrobial susceptibility typing was combined with PFGE in the diversity equation, resulting in a score of 0.978.

MLST profiles. In order to compare single nucleotide polymorphisms against the whole genome profile provided by PFGE, MLST was conducted on all 81 isolates comparing a partial DNA sequence of seven genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*). Sequence data from both strands were assembled using Bionumerics 4.0 software (Applied Maths, Austin, TX) and entered into the *Salmonella enterica* MLST database (http://web.mpiib-berlin.mpg.de/mlst) for comparison to existing allele types. Between two and four alleles were identified among the 81 *S. enterica* serovar Newport isolates (Table 2). One novel allele type was identified in the *sucA* allele set. Overall, the seven-gene MLST scheme defined 12 sequence types, with one ST (ST 45) encompassing 61.7% of the *S. enterica* serovar Newport collection in this

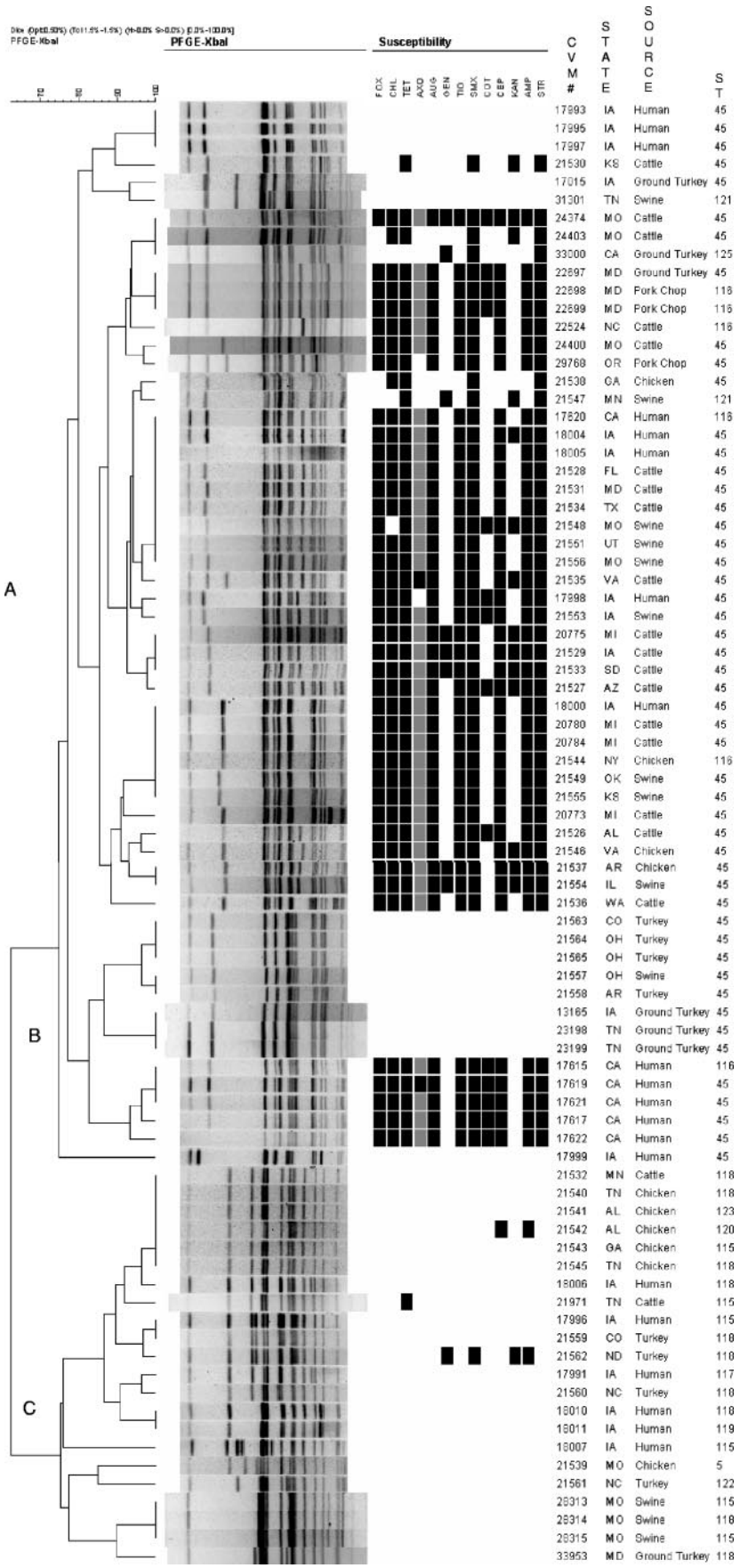
PFGE-XbaI
Susceptibility
FOX CHL TET AXO AUG GEN TIO SMX CIP CEP KAN AMP STR
CVM # STATE SOURCE ST
17993 IA Human 45
17995 IA Human 45
17997 IA Human 45
21530 KS Cattle 45
17015 IA Ground Turkey 45
31301 TN Swine 121
24374 MO Cattle 45
24403 MO Cattle 45
33000 CA Ground Turkey 125
22697 MD Ground Turkey 45
22698 MD Pork Chop 116
22699 MD Pork Chop 116
22524 NC Cattle 116
24400 MO Cattle 45
29768 OR Pork Chop 45
21530 GA Chicken 45
21547 MN Swine 121
17820 CA Human 116
18004 IA Human 45
18005 IA Human 45
21528 FL Cattle 45
21531 MD Cattle 45
21534 TX Cattle 45
21548 MO Swine 45
21551 UT Swine 45
21556 MO Swine 45
21535 VA Cattle 45
17998 IA Human 45
21553 IA Swine 45
20775 MI Cattle 45
21529 IA Cattle 45
21533 SD Cattle 45
21527 AZ Cattle 45
18000 IA Human 45
20780 MI Cattle 45
20784 MI Cattle 45
21544 NY Chicken 116
21549 OK Swine 45
21555 KS Swine 45
20773 MI Cattle 45
21526 AL Cattle 45
21546 VA Chicken 45
21537 AR Chicken 45
21554 IL Swine 45
21536 WA Cattle 45
21563 CO Turkey 45
21564 OH Turkey 45
21565 OH Turkey 45
21557 OH Swine 45
21558 AR Turkey 45
13165 IA Ground Turkey 45
23198 TN Ground Turkey 45
23199 TN Ground Turkey 45
17615 CA Human 116
17619 CA Human 45
17621 CA Human 45
17617 CA Human 45
17622 CA Human 45
17999 IA Human 45
21532 MN Cattle 118
21540 TN Chicken 118
21541 AL Chicken 123
21542 AL Chicken 120
21543 GA Chicken 115
21545 TN Chicken 118
18006 IA Human 118
21971 TN Cattle 115
17996 IA Human 115
21559 CO Turkey 118
21562 ND Turkey 118
17991 IA Human 117
21560 NC Turkey 118
18010 IA Human 118
18011 IA Human 119
18007 IA Human 115
21539 MO Chicken 5
21561 NC Turkey 122
28313 MO Swine 115
28314 MO Swine 118
28315 MO Swine 115
33953 MD Ground Turkey 118
A
B
C

TABLE 2. ST definitions based on allele type for each of seven loci sequenced and assigned by the *Salmonella enterica* database[a]

ST	Allele type							No. of isolates	% of total
	aroC (2)[b]	*thrA* (2)	*sucA* (3)	*dnaN* (3)	*hisD* (2)	*hemD* (4)	*purE* (2)		
5	16	42	39	43	43	45	36	1	1.2
45	10	12	12	7	14	21	15	50	61.7
115*[c]	16	42	12	2	43	45	36	6	7.4
116*	10	12	39	7	14	21	15	6	7.4
117*	16	42	12	2	43	45	15	1	1.2
118*	16	42	39	2	43	45	36	10	12.3
119*	16	42	12	2	43	18	36	1	1.2
120*	16	42	39	2	43	40	36	1	1.2
121*	10	12	12	7	14	21	36	2	2.5
122*	16	42	39	2	43	45	15	1	1.2
123*	10	42	12	2	43	21	36	1	1.2
125*	10	12	53*	7	14	21	15	1	1.2

[a] http://web.mpiib-berlin.mpg.de/mlst.
[b] The number of alleles is given in parentheses.
[c] Asterisks denote novel alleles and novel sequence types.

study (Table 2). The second most common sequence type was ST 118, which included 12.3% of the isolates, followed by STs 115 and 116 (7.4% each). eBURST v3 analysis separated the STs into two groups and one singleton (ST 123), with ST 45 as the founder of complex 1 and ST 118 as the founder of complex 2 (Fig. 2). The founders of both complexes were heptalocus variants and therefore were complexed separately and indicate evolutionary distance. The singleton, ST 123, was not grouped with either complex, as it shared only three loci with ST 45 and four loci with ST 118, so it was not closely enough related to either complex to belong. This ST appears to represent a combination of the two complexes; however, it was not represented by enough strains within the sequence type to substantiate this suggestion.

UPGMA analysis of PFGE fingerprints of ST 45 strains resulted in 75% similarity (data not shown). Similar analysis of PFGE fingerprints for strains comprising ST 118, ST 115, and ST 116 showed similarities of 77.3%, 71.7%, and 79.6%, respectively (data not shown). UPGMA analysis of PFGE fingerprints for all sequence types that were not ST 45, including STs 118, 115, and 116, showed 63.6% percent similarity (data not shown). The index of diversity for MLST, as computed using Simpson's diversity test, was 0.61, the lowest score achieved compared to PFGE and antimicrobial susceptibility typing when a single method was analyzed. When Simpson's index of diversity was used to analyze all three methods simultaneously, the highest index of diversity was achieved, with a score of 0.986.

Concordance of antimicrobial susceptibility typing, PFGE, and MLST. Composite analysis of all three methods revealed a relationship between the pulsed-field fingerprint, the antimicrobial susceptibility phenotype, and the ST (Fig. 1). For example, the largest cluster of isolates (cluster A) was made up largely of MDR-AmpC-positive strains and was comprised mainly of strains with ST 45 and ST 116 (Fig. 1). In fact, eight of nine isolates comprising the largest PFGE pattern in cluster A were identified as ST 45, with the remaining human isolate being ST 116. These two STs are single-locus variants at the *sucA* locus.

Cluster B consisted of a subcluster of 14 isolates from cluster A originating from clinically ill turkey, swine, humans, and retail ground-turkey samples. All cluster B *S. enterica* serovar Newport isolates were ST 45, with the exception of one human isolate from California (ST 116). Cluster C (Fig. 1) was made up almost entirely of pansusceptible isolates and contained mostly ST 118 and ST 115. However, the second largest PFGE pattern in cluster C contained six isolates recovered from either clinically ill chickens (five) or cattle (one) but comprised four different sequence types (ST 118, ST 123, ST 120, and ST 115). STs 118 and 115 are single-locus variants at the *sucA* locus, just as STs 45 and 116 are single-locus variants at that locus (Fig. 2). However, the two groups (STs 45 and 116 versus STs 118 and 115) are widely different by MLST type and differ from each other at six of the seven loci, reinforcing the evolutionary distance between the isolates in clusters A and B and those in cluster C.

DISCUSSION

Given the important public health hazard posed by *Salmonella enterica* serotype Newport, the ability to track antimicrobial susceptibility phenotypes, as well as to identify molecular-fingerprint types, is important in characterizing outbreaks and guiding anti-infective therapy. Numerous reports have documented the highly discriminatory nature of PFGE in successfully tracking infections of different *Salmonella* serotypes to certain geographic areas, including particular farms, and have subsequently linked human illness to exposure to animals or contaminated foods (4, 16, 17, 28, 36, 42, 48, 49). However, a more discriminating method may be required to understand the evolutionary relatedness of epidemiologically unrelated populations (9, 11, 24, 29, 43, 46).

MLST is increasingly being used as an evolutionary and epidemiological tool, with schemes being developed for a number of bacterial pathogens (1, 52, 54, 55). MLST has been used for numerous evolutionary analyses of large and small populations, such as identifying highly clonal lineages of bacteria, as in the case of *Mycobacterium tuberculosis* (51). Epidemiological analyses have also been conducted using large and small bacterial populations and have often been focused on the emergence of virulent phenotypes as identified by MLST (27) and/or the emergence of antibiotic-resistant phenotypes of a

FIG. 1. UPGMA analysis of PFGE profiles of *S. enterica* serovar Newport isolates (identified by unique CVM numbers) showing PFGE fingerprints (62% similar), state of origin (STATE), source of the isolate (SOURCE), antimicrobial susceptibility type, and ST. Major clusters are marked A, B, and C. Resistance in the AST is denoted by a black box. Intermediate resistance in the AST is denoted by a gray box, and susceptibility is denoted by blank space. Antimicrobial abbreviations are as follows: amoxicillin-clavulanic acid, AUG; ampicillin, AMP; cefoxitin, FOX; ceftiofur, TIO; ceftriaxone, AXO; cephalothin, CEP; chloramphenicol, CHL; gentamicin, GEN; kanamycin, KAN; streptomycin, STR; sulfamethoxazole, SMX; tetracycline, TET; and trimethoprim-sulfamethoxazole, COT.

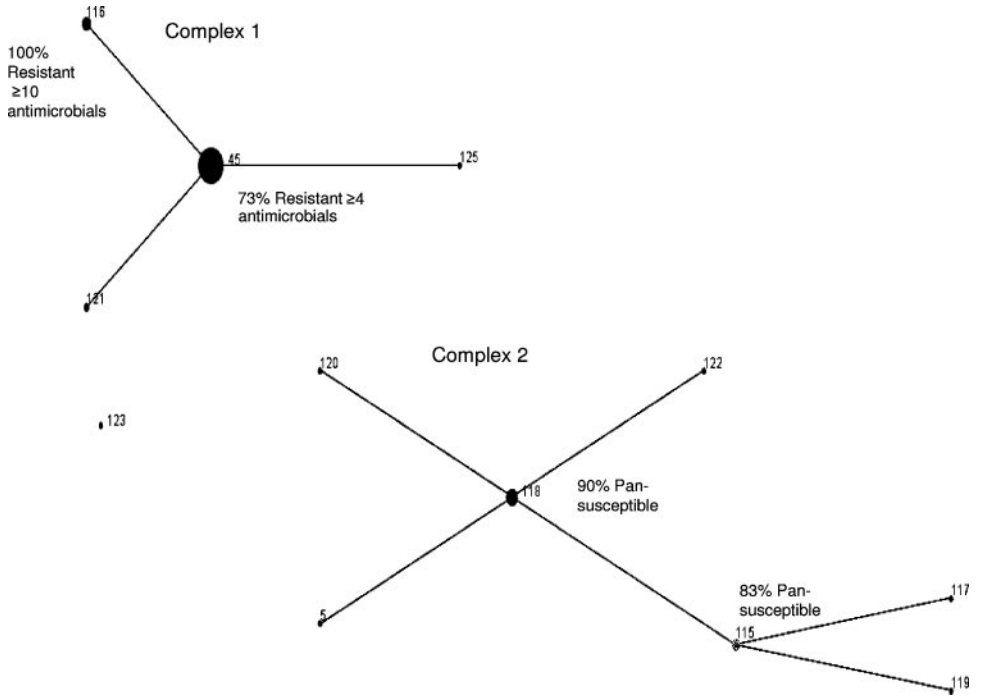

FIG. 2. eBURST v3 diagram of 81 *S. enterica* serovar Newport sequence types. The size of the circle marking each ST indicates the relative number of isolates belonging to that ST. Single-locus variants of each ST are connected by one line. The founder of each complex is defined as the ST that is related to the greatest number of STs in the population that differs at a single locus. The founder of complex 1 is ST 45, and the founder of complex 2 is ST 118. Complex 2 has a subgroup founder (ST 115), or an ST that is not the founder that has at least two single-locus variants. AST information for ST 45, ST 118, ST 116, and ST 115 is listed.

particular bacterial population (23). Although it has been reported that MLST of several housekeeping genes provides a satisfactory level of discrimination among diverse *Salmonella* isolates (29), recent studies suggest that it may not be suitable for distinguishing closely related strains within a particular serovar, due to high sequence identity and slow accumulation of variations of their housekeeping genes (8, 13, 14, 47, 52). We therefore characterized a diverse collection of 81 *S. enterica* serovar Newport isolates originating from a variety of clinically ill animals, humans, and retail meats via MLST and compared the results to those of two classic typing schemes, antimicrobial susceptibility typing and PFGE.

The majority of *S. enterica* serovar Newport isolates were either susceptible to all tested antimicrobials ($n = 33$) or characterized as the MDR-AmpC phenotype, exhibiting resistance to at least 9 of the 16 antimicrobials tested ($n = 39$). No correlation could be determined between specific antimicrobial susceptibility phenotypes and *S. enterica* serovar Newport origin. The majority of both pansusceptible and MDR-AmpC isolates were recovered from all of the different foods and animal types, including humans. However, two interesting observations were noted. *S. enterica* serovar Newport isolates recovered from either clinically ill turkeys ($n = 8$) or retail ground turkey ($n = 7$) were considerably more susceptible than isolates from other animals. In contrast, 85% (17/20) of *S. enterica* serovar Newport isolates from ill cattle displayed the MDR-AmpC phenotype. These data support previous studies of the epidemiology of *S. enterica* serovar Newport MDR-AmpC infections in humans and links with cattle or beef products (3, 22, 45, 50, 59).

Pulsed-field gel electrophoresis was used to assess genetic relatedness and revealed 43 distinct genetic patterns and three major clusters (A to C) among the 81 *S. enterica* serovar Newport isolates. Forty-eight percent (39/81) of all isolates grouped in cluster A, which was also genetically diverse, comprising 19 different PFGE patterns. Certain PFGE clusters showed good correlation with the antimicrobial susceptibility phenotypes. For example, the majority of MDR-AmpC strains grouped together in cluster A ($n = 34/39$), whereas the majority of pansusceptible strains grouped in cluster C ($n = 22/33$). The demarcation of PFGE patterns and clusters between the MDR-AmpC isolates and pansusceptible isolates has been previously noted (3, 22, 59) and suggests that the recent emergence of MDR-AmpC *S. enterica* serovar Newport is due to the clonal expansion of a limited number of genetically related strains that have acquired plasmid-mediated resistance genes. A similar finding was recently reported by Alcaine et al., who postulated that plasmid-mediated ceftiofur-resistant *Salmonella* evolved by independent emergence and clonal spread (2).

Twelve sequence types were defined with MLST, with the majority of isolates (61.7%) grouped in ST 45. The next most common sequence types included ST 118 (12.3%) and STs 115 and 116 (7.4% each). Ninety-seven percent of MDR-AmpC *S. enterica* serovar Newport isolates were characterized as either ST 45 ($n = 32/39$) or ST 116 ($n = 6/39$) and were found only in PFGE clusters A and B. These two sequence types are

single-locus variants at the *sucA* locus, and because they differ in only one of the seven loci sequenced, are therefore closely related. MLST also provided more discriminatory power among *S. enterica* serovar Newport isolates recovered from retail meats on two occasions than did PFGE. *S. enterica* serovar Newport CVM 33000, isolated from a retail ground-turkey sample from California, was indistinguishable by PFGE from two *S. enterica* serovar Newport isolates recovered from clinically ill cattle from Missouri but differed by MLST (ST 125 versus ST 45, respectively). *S. enterica* serovar Newport CVM 22697, isolated from a retail ground-turkey sample from Maryland, was indistinguishable by PFGE from two *S. enterica* serovar Newport isolates recovered from retail pork chops from the same grocery store and collection time, suggesting contamination of meats, possibly at the retail establishment. Nevertheless, MLST resolved the ground-turkey *S. enterica* serovar Newport isolate as ST 45, whereas the two pork chop isolates were characterized as ST 116. ST 45 is different from ST 116 at the *sucA* locus, and sequence analysis showed that the different alleles at this locus (*sucA12* and *sucA39*) differ at 7 bp throughout the allele. Therefore, a spontaneous mutation event causing the change in allele type and ST is unlikely. On the other hand, MLST did not resolve the 40% of clinically ill cattle isolates that were resistant to kanamycin in addition to four or more antimicrobials. All eight were identified as ST 45, and all but three isolates varied in their AST and PFGE types. Therefore, AST phenotype and PFGE resolved these isolates into widely scattered subtypes within cluster A, while MLST grouped them in the same complex.

In contrast to early work by Kotetishvili et al. (29), the seven-gene MLST scheme did not further discriminate either animal origins or geographic locations among our *S. enterica* serovar Newport collection compared with PFGE results. This lack of discrimination versus PFGE has been recently reported with other *Salmonella* serotypes as well (13, 14, 40, 52). Recently, Torpdahl et al. (54) presented MLST data on 25 serotypes of *S. enterica* using the same seven-gene MLST scheme used in this study and found that overall, discrimination was not improved within a serotype compared with PFGE and amplified fragment length polymorphism data. This study included only three *S. enterica* serovar Newport isolates from veterinary and human sources, and upon MLST analysis, they belonged to sequence types 31, 45, and 46, in contrast to the current study, where over 60% of the *S. enterica* serovar Newport isolates belonged to ST 45. It is important to remember, even in light of the recent study analyzing 110 isolates of 25 different serotypes (54), that only a limited number of the over 2,500 *Salmonella* serovars and isolates within them have been characterized using MLST, and most published reports have used a different set of housekeeping genes in their respective MLST schemes (2, 14, 29, 47). As this is the first report characterizing a particular *Salmonella* serovar using the seven genes selected for the global MLST *Salmonella* database housed at the Max-Planck Institut für Infektionsbiologie (http://web.mpiib-berlin.mpg.de/mlst/), further study using this scheme with larger numbers of isolates and additional serovars is warranted to fully explore the utility of MLST for epidemiological purposes.

Combining the results obtained from all three methods yielded more information than any of the methods alone and elicited a higher index of diversity (0.986, or 98.6%), indicating that two randomly selected samples would be identified as different types more often when all three typing methods were employed and that identical isolates would be identified as the same type. The partitioning of *S. enterica* serovar Newport isolates into two major PFGE clusters showed an interesting association with antimicrobial susceptibility and MLST sequence type, as shown in Fig. 1. Based on UPGMA clustering of PFGE fingerprints, sequence types were separated into two groups, which were very closely related intracluster (STs 45 and 116, single-locus variants) but more distantly related intercluster (ST 45 versus ST 118, different at every locus, or heptalocus variants). This study supports previous findings that PFGE can predict clonal-complex designations and that these two DNA-based methods give rise to very similar clustering results (20). MLST revealed that the separation of this population into two major groups was characterized by the relatedness of these isolates and could be identified using seven highly conserved housekeeping genes. This partnership of MLST and PFGE or antimicrobial susceptibility type has not been extensively applied to food-borne pathogens, although it has been applied to methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus* (12). Here, it was shown that antimicrobial susceptibility phenotypes were associated with specific MLST types, and particular sequence types were proposed as predictors of whether *S. aureus* isolates would exhibit resistance to methicillin. Similar associations between the antimicrobial susceptibility phenotype and MLST types were also observed in our current study, where particular sequence types displayed a multidrug resistance phenotype and others were represented by an almost completely pansusceptible phenotype. This observation showed the relationship between these two methods, which previously had been used as separate components instead of as tiers in the levels of epidemiological classification. Although MLST did not add substantial discriminatory power to this study (97.8% typeability using PFGE and AST versus 98.6% typeability using all three methods), the relationship between the three methods provides a phylogenetic aspect to a pure discriminatory-power study.

MLST separated 81 diverse strains from 27 states and five sources into two discrete complexes and one "intermediate" singleton, which shared three or four alleles with each founder. In this study, where 88% of the isolates were recovered from clinically ill animals, polygenetic data that separate related versus distantly related isolates based on conserved sequences give more information about the further expansion of those pathogens than simple banding-pattern differences alone. This, coupled with the fact that the two complexes were associated with antimicrobial resistance or almost complete susceptibility, shows that MLST is a useful polyphyletic and epidemiological tool for tracking pathogens of veterinary or human importance. Due to the moderate to slow accumulation of mutations within the chosen seven housekeeping genes, discrimination between very closely related isolates, such as those within a PFGE cluster, has been shown to be low, but it can provide the information for reliable evolutionary relationships on a more global scale (8). However, for applications in local epidemiological outbreaks, where tracking of particular isolates is imperative, the higher discriminatory power of PFGE may be more useful.

This study is part of our long-range goal to identify strain-typing schemes that provide the analytical power needed to help ascertain the origins of strains for which the vehicle or vector is unknown. While MLST does provide unambiguous data that are easily comparable among different laboratories and provides phylogenetic-relationship inferences that PFGE data cannot provide, the discriminatory ability of MLST may be somewhat problematic due to the high sequence conservation of the genes used to characterize *S. enterica* serovar Newport isolates in this study.

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REFERENCES

1. **Aanensen, D. M., and B. G. Spratt.** 2005. The multilocus sequence typing network: mlst.net. Nucleic Acids Res. **33:**W728–W733.
2. **Alcaine, S. D., S. S. Sukhnanand, L. D. Warnick, W. L. Su, P. McGann, P. McDonough, and M. Wiedmann.** 2005. Ceftiofur-resistant *Salmonella* strains isolated from dairy farms represent multiple widely distributed subtypes that evolved by independent horizontal gene transfer. Antimicrob. Agents Chemother. **49:**4061–4067.
3. **Berge, A. C., J. M. Adaska, and W. M. Sischo.** 2004. Use of antibiotic susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multi-drug-resistant *Salmonella enterica* subsp. *enterica* serovar Newport. Appl. Environ. Microbiol. **70:**318–323.
4. **Botteldoorn, N., L. Herman, N. Rijpens, and M. Heyndrickx.** 2004. Phenotypic and molecular typing of *Salmonella* strains reveals different contamination sources in two commercial pig slaughterhouses. Appl. Environ. Microbiol. **70:**5305–5314.
5. **Centers for Disease Control and Prevention.** 2003. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—selected sites, United States. Morb. Mortal. Wkly. Rep. **53:** 338–343.
6. **Centers for Disease Control and Prevention.** 2005. National antimicrobial resistance monitoring system: enteric bacteria, 2000 annual report. [Online.] http://www.cdc.gov/narms/annual/2000/narms_2000_annual_a.htm.
7. **Cimons, M.** 2000. Rapid food-borne pathogen ID system is making a difference. ASM News **66:**617–619.
8. **Cooper, J. E., and E. J. Feil.** 2004. Multilocus sequence typing—what is resolved? Trends Microbiol. **12:**373–377.
9. **Demczuk, W., G. Soule, C. Clark, H. W. Ackermann, R. Easy, R. Khakhria, F. Rodgers, and R. Ahmed.** 2003. Phage-based typing scheme for *Salmonella enterica* serovar Heidelberg, a causative agent of food poisonings in Canada. J. Clin. Microbiol. **41:**4279–4284.
10. **Dingle, K. E., F. M. Colles, D. Falush, and M. C. Maiden.** 2005. Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. J. Clin. Microbiol. **43:**340–347.
11. **Dunman, P. M., W. Mounts, F. McAleese, F. Immermann, D. Macapagal, E. Marsilio, L. McDougal, F. C. Tenover, P. A. Bradford, P. J. Petersen, S. J. Projan, and E. Murphy.** 2004. Uses of *Staphylococcus aureus* GeneChips in genotyping and genetic composition analysis. J. Clin. Microbiol. **42:**4275–4283.
12. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
13. **Ethelberg, S., M. Lisby, M. Torpdahl, G. Sorensen, J. Neimann, P. Rasmussen, S. M. Bang, U. Stamer, H. B. Hansson, K. Nygard, D. L. Baggesen, E. M. Nielsen, K. Molbak, and M. Helms.** 2005. Prolonged restaurant-associated outbreak of multidrug resistant *Salmonella typhimurium*—secondary publication. Ugeskr. Laeger. **167:**764–766.
14. **Fakhr, M. K., L. K. Nolan, and C. M. Logue.** 2005. Multilocus sequence typing lacks the discriminatory ability of pulsed-field gel electrophoresis for typing *Salmonella enterica* serovar Typhimurium. J. Clin. Microbiol. **43:**2215–2219.
15. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518–1530.
16. **Foley, S. L., S. Simjee, J. Meng, D. G. White, P. F. McDermott, and S. Zhao.** 2004. Evaluation of molecular typing methods for *Escherichia coli* O157:H7 isolates from cattle, food, and humans. J. Food Prot. **67:**651–657.
17. **Fontana, J., A. Stout, B. Bolstorff, and R. Timperi.** 2003. Automated ribotyping and pulsed-field gel electrophoresis for rapid identification of multidrug-resistant *Salmonella* serotype Newport. Emerg. Infect. Dis. **9:**496–499.
18. **Garaizar, J., N. Lopez-Molina, I. Laconcha, B. D. Lau, A. Rementeria, A. Vivanco, A. Audicana, and I. Perales.** 2000. Suitability of PCR fingerprinting, infrequent-restriction-site PCR, and pulsed-field gel electrophoresis, combined with computerized gel analysis, in library typing of *Salmonella enterica* serovar Enteritidis. Appl. Environ. Microbiol. **66:**5273–5281.
19. **Gorman, R., and C. C. Adley.** 2004. Characterization of *Salmonella enterica* serotype Typhimurium isolates from human, food, and animal sources in the Republic of Ireland. J. Clin. Microbiol. **42:**2314–2316.
20. **Grundmann, H., S. Hori, M. C. Enright, C. Webster, A. Tami, E. J. Feil, and T. Pitt.** 2002. Determining the genetic structure of the natural population of *Staphylococcus aureus*: a comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. J. Clin. Microbiol. **40:**4544–4546.
21. **Gudmundsdottir, S., H. Hardardottir, and E. Gunnarsson.** 2003. Subtyping of *Salmonella enterica* serovar Typhimurium outbreak strains isolated from humans and animals in Iceland. J. Clin. Microbiol. **41:**4833–4835.
22. **Gupta, A., J. Fontana, C. Crowe, B. Bolstorff, A. Stout, S. Van Duyne, M. P. Hoekstra, J. M. Whichard, T. J. Barrett, and F. J. Angulo.** 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J. Infect. Dis. **188:**1707–1716.
23. **Homan, W. L., D. Tribe, S. Poznanski, M. Li, G. Hogg, E. Spalburg, J. D. Van Embden, and R. J. Willems.** 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. J. Clin. Microbiol. **40:**1963–1971.
24. **Hudson, C. R., M. Garcia, R. K. Gast, and J. J. Maurer.** 2001. Determination of close genetic relatedness of the major *Salmonella enteritidis* phage types by pulsed-field gel electrophoresis and DNA sequence analysis of several *Salmonella* virulence genes. Avian Dis. **45:**875–886.
25. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
26. **Jefferies, J. M., A. Smith, S. C. Clarke, C. Dowson, and T. J. Mitchell.** 2004. Genetic analysis of diverse disease-causing pneumococci indicates high levels of diversity within serotypes and capsule switching. J. Clin. Microbiol. **42:** 5681–5688.
27. **Jolley, K. A., J. Kalmusova, E. J. Feil, S. Gupta, M. Musilek, P. Kriz, and M. C. Maiden.** 2000. Carried meningococci in the Czech Republic: a diverse recombining population. J. Clin. Microbiol. **38:**4492–4498.
28. **Kirk, M. D., C. L. Little, M. Lem, M. Fyfe, D. Genobile, A. Tan, J. Threlfall, A. Paccagnella, D. Lightfoot, H. Lyi, L. McIntyre, L. Ward, D. J. Brown, S. Surnam, and I. S. Fisher.** 2004. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport—sharing molecular information to solve international outbreaks. Epidemiol. Infect. **132:** 571–577.
29. **Kotetishvili, M., O. C. Stine, A. Kreger, J. G. Morris, Jr., and A. Sulakvelidze.** 2002. Multilocus sequence typing for characterization of clinical and environmental *Salmonella* strains. J. Clin. Microbiol. **40:**1626–1635.
30. **Kubota, K., T. J. Barrett, M. L. Ackers, P. S. Brachman, and E. D. Mintz.** 2005. Analysis of *Salmonella enterica* serotype Typhi pulsed-field gel electrophoresis patterns associated with international travel. J. Clin. Microbiol. **43:**1205–1209.
31. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
32. **Martinez-Urtaza, J., E. Liebana, L. Garcia-Migura, P. Perez-Pineiro, and M. Saco.** 2004. Characterization of *Salmonella enterica* serovar Typhimurium from marine environments in coastal waters of Galicia (Spain). Appl. Environ. Microbiol. **70:**4030–4034.
33. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
34. **Murase, T., T. Okitsu, R. Suzuki, H. Morozumi, A. Matsushima, A. Nakamura, and S. Yamai.** 1995. Evaluation of DNA fingerprinting by PFGE as an epidemiologic tool for *Salmonella* infections. Microbiol. Immunol. **39:**673–676.
35. **National Center for Infectious Diseases, CDC; Division of Bacterial and Mycotic Diseases, Foodborne and Diarrheal Diseases Branch, Public Health Practice Program Office, CDC, Division of Laboratory Services; and Association of Public Health Laboratories.** 2002. Standardized molecular subtyping of foodborne bacterial pathogens by pulsed-field gel electrophoresis. Centers for Disease Control and Prevention, Atlanta, Ga.
36. **Nayak, R., T. Stewart, R. F. Wang, J. Lin, C. E. Cerniglia, and P. B. Kenney.** 2004. Genetic diversity and virulence gene determinants of antibiotic-resistant *Salmonella* isolated from preharvest turkey production sources. Int. J. Food Microbiol. **91:**51–62.
37. **NCCLS.** 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard, 2nd ed. NCCLS document M31-A2. NCCLS, Wayne, Pa.
38. **NCCLS.** 2003. Methods for dilution antimicrobial susceptibility tests for

bacteria that grow aerobically. Approved standard, 6th ed. NCCLS document M7-A6. NCCLS, Wayne, Pa.

39. **Nemoy, L. L., M. Kotetishvili, J. Tigno, A. Keefer-Norris, A. D. Harris, E. N. Perencevich, J. A. Johnson, D. Torpey, A. Sulakvelidze, J. G. Morris, Jr., and O. C. Stine.** 2005. Multilocus sequence typing versus pulsed-field gel electrophoresis for characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates. J. Clin. Microbiol. **43:**1776–1781.
40. **Noller, A. C., M. C. McEllistrem, O. C. Stine, J. G. Morris, Jr., D. J. Boxrud, B. Dixon, and L. H. Harrison.** 2003. Multilocus sequence typing reveals a lack of diversity among *Escherichia coli* O157:H7 isolates that are distinct by pulsed-field gel electrophoresis. J. Clin. Microbiol. **41:**675–679.
41. **Olsen, J. E., M. N. Skov, E. J. Threlfall, and D. J. Brown.** 1994. Clonal lines of *Salmonella enterica* serotype Enteritidis documented by IS*200*-, ribo-, pulsed-field gel electrophoresis and RFLP typing. J. Med. Microbiol. **40:** 15–22.
42. **Pitout, J. D., M. D. Reisbig, M. Mulvey, L. Chui, M. Louie, L. Crowe, D. L. Church, S. Elsayed, D. Gregson, R. Ahmed, P. Tilley, and N. D. Hanson.** 2003. Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC beta-lactamase, CMY-2. J. Clin. Microbiol. **41:**4578–4582.
43. **Prager, R., W. Rabsch, W. Streckel, W. Voigt, E. Tietze, and H. Tschape.** 2003. Molecular properties of *Salmonella enterica* serotype Paratyphi B distinguish between its systemic and its enteric pathovars. J. Clin. Microbiol. **41:**4270–4278.
44. **Ramisse, V., P. Houssu, E. Hernandez, F. Denoeud, V. Hilaire, O. Lisanti, F. Ramisse, J. D. Cavallo, and G. Vergnaud.** 2004. Variable number of tandem repeats in *Salmonella enterica* subsp. *enterica* for typing purposes. J. Clin. Microbiol. **42:**5722–5730.
45. **Rankin, S. C., H. Aceto, J. Cassidy, J. Holt, S. Young, B. Love, D. Tewari, D. S. Munro, and C. E. Benson.** 2002. Molecular characterization of cephalosporin-resistant *Salmonella enterica* serotype Newport isolates from animals in Pennsylvania. J. Clin. Microbiol. **40:**4679–4684.
46. **Reche, M. P., M. A. Echeita, J. E. los Rios, M. A. Usera, P. A. Jimenez, A. M. Rojas, J. Colas, and I. Rodriguez.** 2003. Comparison of phenotypic and genotypic markers for characterization of an outbreak of *Salmonella* serotype Havana in captive raptors. J. Appl. Microbiol. **94:**65–72.
47. **Ross, I. L., and M. W. Heuzenroeder.** 2005. Discrimination within phenotypically closely related definitive types of *Salmonella enterica* serovar Typhimurium by the multiple amplification of phage locus typing technique. J. Clin. Microbiol. **43:**1604–1611.
48. **Seno, M., M. Sakaki, and H. Ogawa.** 2004. Genotypic diversity of *Salmonella* Enteritidis isolates from sporadic patients in limited area during one year. J. Infect. **49:**291–296.
49. **Sivapalasingam, S., E. Barrett, A. Kimura, S. Van Duyne, W. De Witt, M. Ying, A. Frisch, Q. Phan, E. Gould, P. Shillam, V. Reddy, T. Cooper, M. Hoekstra, C. Higgins, J. P. Sanders, R. V. Tauxe, and L. Slutsker.** 2003. A multistate outbreak of *Salmonella enterica* serotype Newport infection linked to mango consumption: impact of water-dip disinfestation technology. Clin. Infect. Dis. **37:**1585–1590.
50. **Spika, J. S., S. H. Waterman, G. W. Hoo, M. E. St Louis, R. E. Pacer, S. M. James, M. L. Bissett, L. W. Mayer, J. Y. Chiu, and B. Hall.** 1987. Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms. A major persisting source of human salmonellosis in California. N. Engl. J. Med. **316:**565–570.
51. **Sreevatsan, S., X. Pan, K. E. Stockbauer, N. D. Connell, B. N. Kreiswirth, T. S. Whittam, and J. M. Musser.** 1997. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc. Natl. Acad. Sci. USA **94:**9869–9874.
52. **Sukhnanand, S., S. Alcaine, L. D. Warnick, W. L. Su, J. Hof, M. P. Craver, P. McDonough, K. J. Boor, and M. Wiedmann.** 2005. DNA sequence-based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes. J. Clin. Microbiol. **43:**3688–3698.
53. **Swaminathan, B., T. J. Barrett, S. B. Hunter, and R. V. Tauxe.** 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. **7:**382–389.
54. **Torpdahl, M., M. N. Skov, D. Sandvang, and D. L. Baggesen.** 2005. Genotypic characterization of *Salmonella* by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism. J. Microbiol. Methods **63:**173–184.
55. **Urwin, R., and M. C. Maiden.** 2003. Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol. **11:**479–487.
56. **Voetsch, A. C., T. J. Van Gilder, F. J. Angulo, M. M. Farley, S. Shallow, R. Marcus, P. R. Cieslak, V. C. Deneen, and R. V. Tauxe.** 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis. **38**(Suppl. 3)**:**S127–S134.
57. **Weide-Botjes, M., B. Kobe, C. Lange, and S. Schwarz.** 1998. Molecular typing of *Salmonella enterica* subsp. *enterica* serovar Hadar: evaluation and application of different typing methods. Vet. Microbiol. **61:**215–227.
58. **Yong, D., Y. S. Lim, J. H. Yum, H. Lee, K. Lee, E. C. Kim, B. K. Lee, and Y. Chong.** 2005. Nosocomial outbreak of pediatric gastroenteritis caused by CTX-M-14-type extended-spectrum beta-lactamase-producing strains of *Salmonella enterica* serovar London. J. Clin. Microbiol. **43:**3519–3521.
59. **Zhao, S., S. Qaiyumi, S. Friedman, R. Singh, S. L. Foley, D. G. White, P. F. McDermott, T. Donkar, C. Bolin, S. Munro, E. J. Baron, and R. D. Walker.** 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol. **41:**5366–5371.