β -Lactam Resistance in *Salmonella* Strains Isolated from Retail Meats in the United States by the National Antimicrobial Resistance Monitoring System between 2002 and 2006[▽]

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Ampicillin-resistant (Amp^r) *Salmonella enterica* isolates ($n = 344$) representing 32 serotypes isolated from retail meats from 2002 to 2006 were tested for susceptibility to 21 other antimicrobial agents and screened for the presence of five beta-lactamase gene families (bla_{CMY} , bla_{TEM} , bla_{SHV} , bla_{OXA} , and bla_{CTX-M}) and class 1 integrons. Among the Amp^r isolates, 66.9% were resistant to five or more antimicrobials and 4.9% were resistant to 10 or more antimicrobials. Coresistance to other β -lactams was noted for amoxicillin-clavulanic acid (55.5%), ceftiofur (50%), cefoxitin (50%), and ceftazidime (24.7%), whereas less than 5% of isolates were resistant to piperacillin-tazobactam (4.9%), cefotaxime (3.5%), ceftriaxone (2%), and aztreonam (1.2%). All isolates were susceptible to cefepime, imipenem, and cequinome. No *Salmonella* producing extended-spectrum beta-lactamases was found in this study. Approximately 7% of the isolates displayed a typical multidrug-resistant (MDR)-AmpC phenotype, with resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, plus resistance to amoxicillin-clavulanic acid, cefoxitin, and ceftiofur and with decreased susceptibility to ceftriaxone (MIC ≥ 4 μ g/ml). Pulsed-field gel electrophoresis results showed that several MDR clones were geographically dispersed in different types of meats throughout the five sampling years. Additionally, 50% of the isolates contained bla_{CMY} , 47% carried bla_{TEM-1} , and 2.6% carried both genes. Only 15% of the isolates harbored class I integrons carrying various combinations of *aadA*, *aadB*, and *dfrA* gene cassettes. The bla_{CMY} , bla_{TEM} , and class I integrons were transferable through conjugation and/or transformation. Our findings indicate that a varied spectrum of coresistance traits is present in Amp^r *Salmonella* strains in the meat supply of the United States, with a continued predominance of bla_{CMY} and bla_{TEM} genes in β -lactam-resistant isolates.

Nontyphoid *Salmonella enterica* is one of the most important food-borne pathogens and represents a significant public health hazard worldwide. It is estimated that 1.4 million people in the United States are infected with non-Typhi *Salmonella* annually, resulting in 15,000 hospitalizations and more than 400 deaths (28). *Salmonella* infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs, milk, seafood, and produce (10). Salmonellosis following direct contact with animals and dog treats has also been reported (3, 6, 7). Human salmonellosis usually results in a self-limiting diarrhea that does not require antimicrobial therapy. However, in severe cases of enteritis and systemic infections, fluoroquinolones and extended-spectrum cephalosporins such as ceftriaxone (AXO) are used as first-line therapeutics (12, 27).

Multidrug-resistant (MDR) *Salmonella* strains have been detected in many serotypes, such as *S. enterica* serotype Typhimurium (9, 26), *S. enterica* serotype Agona, *S. enterica* serotype Anatum, *S. enterica* serotype Choleraesuis, *S. enterica* serotype Dublin, *S. enterica* serotype Heidelberg, *S. enterica* serotype Kentucky, *S. enterica* serotype Newport, *S. enterica* serotype Schwarzengrund, *S. enterica* serotype Senftenberg, and *S. enterica* sero-

type Uganda, among others (14, 33, 35) (<http://internet-dev/cvm/2005NARMSExeRpt.htm>). The most common MDR pattern, which first emerged in *S. Typhimurium*, has been a pattern of resistance to ampicillin (AMP), chloramphenicol (CHL), streptomycin (STR), sulfonamides, and tetracycline (TET) (ACSSuT). More recently, strains exhibiting the ACSSuT pattern also have acquired MDR plasmids carrying the bla_{CMY} gene and others (30) that can spread readily to different members of the *Enterobacteriaceae*. The strains demonstrate extensive resistances, which, in addition to the ACSSuT phenotype, may include resistance to amoxicillin-clavulanic acid (AUG), cefoxitin (FOX), and ceftiofur (TIO) and decreased susceptibility to AXO (MIC ≥ 4 μ g/ml). TIO is a third-generation cephalosporin that was approved for use in animals in 1998. Tio^r *Salmonella* isolates often show resistance or decreased susceptibility to AXO (also a third-generation cephalosporin used to treat human infections). Some strains may also display resistance to gentamicin (GEN), kanamycin (KAN), and trimethoprim-sulfamethoxazole ([SMX] COT) as well as resistance to disinfectants and heavy metals. Resistance to third-generation cephalosporins in *Salmonella* strains is of interest because these are the drugs of choice for treating salmonellosis in children, where fluoroquinolones are contraindicated (13).

To date, more than 340 beta-lactamases have been described (11). The most common genes, such as bla_{TEM} , bla_{SHV} , bla_{CTX-M} , bla_{OXA} , bla_{PER} , bla_{PSE} , and bla_{CMY} , have been detected in *Salmonella*, with the prevalence of these genes vary-

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ing by region (32). Extended-spectrum beta-lactamases (ESBLs) are less prevalent in *Salmonella* strains than in other gram-negative bacteria such as *Klebsiella*, *Escherichia coli*, and *Proteus*. The ESBLs are β -lactamases capable of conferring bacterial resistance to the penicillins; to first-, second-, and third-generation cephalosporins; and to aztreonam (ATM) (but not to the cephamycins or carbapenems) by hydrolysis of these antibiotics, which are inhibited by β -lactamase inhibitors such as clavulanic acid (21). Most ESBL-carrying *Salmonella* strains have been reported in Latin America, the Western Pacific, and Europe (32), with only a few reports from North America. In the United States the first case was reported in 1994, when *bla*_{CTX-5} was detected in an *S. Typhimurium* var. Copenhagen strain from an infant adopted from Russia (25). Additional ESBL *Salmonella* strains have been reported recently, one from a horse (*bla*_{SHV-12}) and another from a 3-month-old child (*bla*_{CTX-M-5}) (23, 25). Carbapenem resistance in *Salmonella* is also rare in the United States but has been detected in *S. enterica* serotype Cubana associated with a plasmid-mediated *bla*_{KPC-2} gene (18). In contrast to the low prevalence of ESBL-carrying *Salmonella* strains in the United States, AmpC resistance mediated by *bla*_{CMY} has been emerging in both humans and food animals. The *bla*_{CMY} encodes a cephalomycinase that exhibits extended resistance to many beta-lactams, including first-, second-, and third-generation cephalosporins (36).

The objectives of this study were to determine the genetic basis of beta-lactam resistance and to examine the extent of coresistance to other antimicrobials among 344 Amp^r *Salmonella* isolates obtained from retail meats. We screened for the presence of five beta-lactam resistance gene families (*bla*_{CMY}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, and *bla*_{CTX-M}) and the presence of class I integrons. The range of resistance phenotypes borne on plasmids was examined by filter mating and electroporation, and all isolates were characterized for genetic relatedness using pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

Bacterial isolates. All Amp^r *Salmonella* isolates ($n = 344$), representing 32 serotypes, recovered from retail meats from 2002 to 2006 by the National Antimicrobial Resistance Monitoring System (NARMS) were used in this study. Among the 344 Amp^r isolates, 28 (8.1%) were isolated in 2002, 66 (19.2%) were from 2003, 81 (23.5%) were from 2004, 94 (27.3%) were from 2005, and 75 (21%) were from 2006. Nearly all of the isolates were recovered from poultry meats, with 162 (47%) from ground turkey and 158 (45.9%) from chicken breast; 13 (3.8%) were from ground beef, and 11 (3.2%) were from pork chops. Among the 32 serotypes, the most five common serotypes were *S. Typhimurium* (including *S. Typhimurium* var. 5-; $n = 84$; 24.4%), *S. Heidelberg* ($n = 56$; 16.3%), *S. enterica* serotype Saintpaul ($n = 53$; 15.4%), *S. Kentucky* ($n = 45$; 13.1%), and *S. Senftenberg* ($n = 16$; 4.7%). Detailed information on sampling, isolation, identification, and serotyping can be found at <http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm>. Bacteria were stored in Trypticase soy broth containing 15% glycerol at -80°C until use.

Antimicrobial susceptibility testing. Antimicrobial MICs were determined using a Sensititre automated antimicrobial susceptibility system in accordance with the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH). Initially, all strains were tested using a panel designed for NARMS, which included AXO, TIO, AUG, AMP, FOX, ciprofloxacin (CIP), nalidixic acid (NAL), amikacin (AMI), GEN, STR, KAN, SMX, COT, TET, and CHL. All Amp^r strains were tested with a secondary panel of β -lactam antimicrobials that included ATM, ceftazidime (CZM), imipenem (IMI), cefepime (FEP), piperacillin-tazobactam (P/T), ceftazidime-clavulanic acid (T/C), cefotaxime (FOT), and cefotaxime-clavulanic acid (F/C). Results were interpreted

in accordance with CLSI criteria with the exception of STR (resistance breakpoint, ≥ 64 $\mu\text{g/ml}$) and CZM (resistance breakpoint, ≥ 32 $\mu\text{g/ml}$) (5). *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in the antimicrobial MIC determinations.

DNA isolation, PCR, and DNA sequencing. The presence of *bla*_{CMY}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, *bla*_{CTX-M}, and class I integrons was detected by PCR using previously published methods (1, 24, 34). DNA templates were prepared using MoBio Ultraclean DNA isolation kits (MoBio Laboratories, Inc., Carlsbad, CA) following the manufacturer's instructions. Amplifications were carried out using 200 ng of DNA template, 250 μM of each deoxynucleoside triphosphate, 1.5 mM MgCl_2 , 50 pmol of each primer, and 1 U of AmpliTaq Gold *Taq* polymerase (Applied Biosystems, Foster City, CA). The amplified products were separated by gel electrophoresis on 1.0% agarose gels and stained with ethidium bromide. The gels were visualized under UV light. Each positive PCR product was sequenced using an ABI 3730 automated sequencer. Sequences were analyzed using the National Center for Biotechnology Information's BLAST network service (<http://www.ncbi.nlm.nih.gov/BLAST/>). Each gene was identified by comparison to sequences in the GenBank database of accession numbers AB126601, DQ238100, DQ388125, DQ069244, AJ809407, AB282997, and EU113221.

Conjugation and transformation. To assess the transfer mechanism and the extent of resistance phenotypes carried on plasmids, conjugations and transformations were carried out by filter mating and electroporation, respectively. Nineteen *bla*_{CMY}-positive *Salmonella* isolates representing 14 serotypes from different sources were selected as donors, and ElectroMax DH5 α *E. coli* cells (Invitrogen, Carlsbad, CA) were used as recipients (Table 1). TIO and NAL were used as the selective agents for the donor and recipient strains, respectively. Filter mating was carried out as previously described (4). For electroporation, plasmids were prepared using Qiagen HiSpeed plasmid purification kits (Qiagen, Valencia, CA). In a microcentrifuge tube, 20 μl of thawed DH5 α cells was added to 200 ng (2 μl) of plasmid DNA. The mixture was pipetted into a chilled cuvette and electroporated at 2.0 kV, 200 Ω , and 25 μF . After electroporation, 1 ml of SOC medium (Sigma-Aldrich, St. Louis, MO) was added, and the suspension was incubated at 37°C with shaking for 1 h. The mixture was streaked on selective plates containing 4 $\mu\text{g/ml}$ of TIO and examined for bacterial growth after 24 h of incubation at 37°C .

PFGE. PFGE was performed according to the protocol developed by the Centers for Disease Control and Prevention (<http://www.cdc.gov/pulsenet/protocols.htm>), using *S. enterica* serovar Braenderup H9812 as the control strain. Agarose-embedded DNA was digested with 50 U of XbaI or BlnI (Boehringer Mannheim, Indianapolis, IN) for least 4 h in a water bath at 37°C . The restriction fragments were separated by electrophoresis in 0.5 $\times$ Tris-borate-EDTA buffer (Invitrogen, Carlsbad, CA) 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. Isolates showing DNA smears were retested using plugs digested with XbaI or BlnI and electrophoresis buffer containing 50 μM thiourea in 0.5 $\times$ Tris-borate-EDTA buffer. The gels were stained with ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad). PFGE results were analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium), and banding pattern similarity was compared using an average of the results of two enzymes with a 1.5% band position tolerance. All PFGE profiles generated from this study were submitted to the national CDC PulseNet database for comparison with isolates from clinical human salmonellosis cases.

RESULTS

Antimicrobial susceptibility profiles. A wide range of susceptibility patterns were noted among the Amp^r isolates, with 66.9% of isolates resistant to at least 5 antimicrobials and 4.9% resistant to at least 10 antimicrobials. In addition to resistance to AMP, cross-resistance to other beta-lactams was noted for AUG (55.5%), TIO (50%), FOX (50%), and TAZ (24.7%), whereas less than 5% of isolates were resistant to P/T (4.9%), FOT (3.5%), AXO (2%), and ATM (1.2%). All isolates were susceptible to FEP, IMI, and CZM. For aminoglycosides, the most common coresistance was to STR (55.5%), followed by KAN (30.8%) and GEN (24.4%). All isolates were susceptible to AMI. For quinolones and fluoroquinolones, 3.3% were resistant to NAL, and none were resistant to CIP. Additionally,

TABLE 1. *Salmonella* isolates selected as donors in conjugation and electroporation experiments^a

Isolate no.	<i>Salmonella</i> serotype	Source	Year of isolation	Resistance profile ^b
21380	Heidelberg	Ground turkey	2002	FOX/TET/AUG/GEN/TIO/FIS/KAN/AMP/STR
23742	Dublin	Ground beef	2003	FOX/CHL/TET/AUG/TIO/FIS/AMP/STR
29440	Enteritidis	Ground beef	2003	FOX/CHL/TET/AXO/AUG/TIO/FIS/AMP/STR
30654	Agona	Ground turkey	2003	FOX/TET/AUG/TIO/FIS/AMP
30661	Enteritidis	Chicken breast	2003	FOX/AUG/TIO/AMP
32413	Kentucky	Chicken breast	2003	FOX/AUG/TIO/AMP/STR
N187	Bredeney	Ground turkey	2004	FOX/TET/AUG/GEN/TIO/FIS/AMP/STR
N418	Heidelberg	Ground turkey	2004	FOX/CHL/TET/AUG/GEN/TIO/FIS/KAN/AMP/STR
N499	Typhimurium var. Copenhagen	Chicken breast	2004	FOX/TET/AUG/TIO/FIS/KAN/AMP
N518	Kentucky	Chicken breast	2004	FOX/TET/AUG/TIO/FIS/KAN/AMP/STR
N635	Newport	Ground beef	2004	FOX/CHL/TET/AUG/TIO/FIS/AMP/STR
N1571	Derby	Ground turkey	2004	FOX/TET/AUG/TIO/AMP
N4534	I 4,5,12: nonmotile	Chicken breast	2005	FOX/TET/AUG/TIO/FIS/AMP
N4646	Typhimurium	Ground turkey	2005	FOX/AUG/TIO/AMP
N5389	I 4,12:r: -	Ground turkey	2005	FOX/CHL/TET/AUG/TIO/FIS/AMP/STR
N5396	Anatum	Chicken breast	2005	FOX/TET/AUG/TIO/AMP/STR
N6312	I 3,10: nonmotile	Ground turkey	2005	FOX/TET/AUG/TIO/FIS/KAN/AMP/STR
N6328	Bredeney	Ground turkey	2005	FOX/TET/AXO/AUG/TIO/FIS/AMP/STR
N6430	Typhimurium	Chicken breast	2005	FOX/TET/AUG/TIO/FIS/KAN/AMP

^a The recipient strain was *E. coli* DH5 α (Nal^r) (Invitrogen). All 19 isolates showed decreased susceptibility (MIC, 8 to 32 μ g/ml) or resistance (MIC $\geq$ 64 μ g/ml) to AXO.

^b FIS, sulfisoxazole.

57.8% of isolates were resistant to TET, 55.5% to sulfonamides, 10.8% to CHL, and 1.7% to COT (Table 2). Based on susceptibility to clavulanic acid with TAZ and FOT, no ESBL-producing *Salmonella* strains were detected in this study.

MDR patterns varied within and between serotypes. For example, the most prevalent MDR pattern in *S. Typhimurium* was AMP/AUG/FOX/TIO/SMX/TET ($n = 25$; 30%), followed by AMP/AUG/FOX/TIO ($n = 19$; 23%), AMP/AUG/FOX/TIO/KAN/SMX/TET ($n = 17$; 20%), and ACSSuT (AMP/CHL/STR/SMX/TET) ($n = 8$; 10%). By contrast, the dominant MDR pattern in *S. Kentucky* was AMP/AUG/FOX/TIO/STR/TET ($n = 35$; 78%), followed by AMP/AUG/FOX/TIO/CHL/GEN/KAN/STR/SMX/TET ($n = 4$; 9%); all nine strains of *S. Newport* displayed the typical MDR-AmpC phenotype with resistance to ACSSuT, plus resistance to AUG/FOX/TIO and decreased susceptibility to AXO (MIC $\geq$ 4 μ g/ml). Four *S. Newport* isolates showed additional resistance to COT. The top MDR profiles among Tio^r isolates are listed in Table 3. Some serotypes such as *S. Heidelberg* showed very diverse resistance patterns such that 20 resistance patterns were observed among 56 Amp^r isolates. Overall, 7% of the isolates displayed the typical MDR-AmpC phenotype, and several of these showed resistance also to P/T, ATM, TAZ, and FOT, as well as other drug classes represented by GEN, KAN, and COT. The typical MDR-AmpC phenotype was present in *S. Dublin*, *S. enterica* serotype Enteritidis, *S. Heidelberg*, *S. enterica* serotype I 4,12:r: -, *S. Kentucky*, *S. Newport*, *S. Saintpaul*, and *S. Typhimurium* var. Copenhagen. Additional information regarding antimicrobial resistance and serotypes of *Salmonella* from retail meats is available at the NARMS website (<http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm>).

Beta-lactam resistance genes and class 1 integrons. For the beta-lactam resistance genes, 50% ($n = 163$) of the Amp^r isolates carried *bla*_{CMY}, 47% ($n = 151$) carried *bla*_{TEM-1}, and

TABLE 2. Antimicrobial resistance phenotypes of Amp^r *Salmonella* isolates from NARMS retail meats from 2002 to 2006

Class and/or antimicrobial	Resistance breakpoint (μ g/ml) ^a	% of isolates showing resistance ($n = 344$)
β-Lactams		
AUG	$\geq 32/16^c$	55.5
AMP	≥ 32	100
ATM	≥ 32	1.2
FEP	≥ 32	0
FOT	≥ 64	3.5
FOX	≥ 32	50
CQN ^b	≥ 32	0
TAZ	≥ 32	24.7
TIO	≥ 8	50
AXO	≥ 64	2
IMP	≥ 16	0
P/T	$\geq 128/4^d$	4.9
Quinolones and fluoroquinolones		
CIP	≥ 4	0
NAL	≥ 32	3.3
Aminoglycosides		
AMI	≥ 64	0
GEN	≥ 16	24.4
KAN	≥ 64	30.8
SRT ^b	≥ 64	55.5
Sulfonamide or potentiated sulfonamide		
SMX	≥ 512	55.5
COT	$\geq 4/76^e$	1.7
TET	≥ 16	57.8
CHL	≥ 32	10.8

^a MIC (μ g/ml) determined via microdilution broth methods in accordance with CLSI standards.

^b No CLSI breakpoint.

^c Values are for amoxicillin-clavulanic acid, respectively.

^d Values are for piperacillin-tazobactam, respectively.

^e Values are for trimethoprim-SMX, respectively.

TABLE 3. Most common TIO resistance profiles of *Salmonella* isolated from retail meats^a

Resistance profile	No. of isolates
AMP/AUG/AXO/FOX/TIO.....	37
AMP/AUG/AXO/FOX/TIO/STR/TET.....	35
AMP/AUG/AXO/FOX/TIO/SMX/TET.....	27
AMP/AUG/AXO/FOX/TIO/KAN/SMX/TET.....	19
AMP/AUG/AXO/FOX/TIO/CHL/STR/SMX/TET.....	11
AMP/AUG/AXO/FOX/TIO/CHL/STR/SMX/TET/COT.....	7
AMP/AUG/AXO/FOX/TIO/CHL/GEN/KAN/STR/SMX/TET.....	6

^a All Tio^r isolates showed decreased susceptibility (MIC, 8 to 32 µg/ml) or resistance to AXO.

2.6% ($n = 9$) carried both genes. No strains were found to carry *bla*_{SHV}, *bla*_{OXA}, or *bla*_{CTX-M}. All *bla*_{CMY}-positive isolates were Tio^r with decreased susceptibility or resistance to AXO, whereas all isolates carrying *bla*_{TEM-1} and not *bla*_{CMY} were susceptible to TIO. All isolates carrying both *bla*_{TEM-1} and *bla*_{CMY} displayed MDR phenotypes with resistance to 8 to 11 antimicrobials of different classes, except for one *S. Typhimurium* var. Copenhagen isolate from chicken breast that showed resistance only to beta-lactams (AMP/AUG/FOX/TIO/TAZ). Twenty-one Amp^r isolates did not contain any of the five beta-lactam resistance genes tested. Fifteen percent of isolates ($n = 52$) contained class I integrons carrying various combinations of *aadA*, *aadB*, and *dfrA* gene cassettes encoding resistance to STR, GEN/KAN, and COT, respectively.

Conjugation and transformation. Nineteen *bla*_{CMY}-positive *Salmonella* isolates representing 14 serotypes were selected as donor strains in conjugation and transformation based on the combination of resistance profile, serotype, and source and year of isolation (Table 1). The results showed that the *bla*_{CMY} gene can be transferred by both filter mating and electroporation. However, filter mating showed more transfer than electroporation. Sixty-eight percent (13/19) of isolates successfully transferred *bla*_{CMY} by filter mating and only 37% (7/19) by electroporation. Other resistance phenotypes such as resistance to CHL, TET, GEN, SMX, KAN, and STR were also cotransferred in some isolates by filter mating but in no cases by electroporation. Six isolates including serotypes *S. Anatum*, *S. Agona*, *S. Derby*, *S. Kentucky*, *S. Typhimurium*, and *S. I 4,5,12: nonmotile* successfully transferred *bla*_{CMY} by both filter mating and electroporation, whereas four isolates comprised of *S. Dublin*, *S. Enteritidis*, *S. Newport*, and *S. I 4,12:r:-* did not transfer *bla*_{CMY} by either technique. PCR confirmed that all 13 transconjugates carried *bla*_{CMY}; one also carried a class 1 integron, and another carried both *bla*_{TEM-1} and a class 1 integron. Seven transformants showed the presence of only *bla*_{CMY}.

PFGE profiles. Genomic DNA digestion with XbaI and BlnI generated a total of 249 PFGE profiles among the 344 Amp^r isolates (data not shown). PFGE showed a good correlation with *Salmonella* serotypes among most isolates, where isolates of the same serotype clustered together. PFGE profiles showed that some serotypes were genetically more diverse than others (data not shown). For example, some isolates of *S. Typhimurium* and *S. enterica* serotype Bredeney formed multiple distinct clusters with 50 to 60% pattern similarity.

Among the 249 strain types identified by PFGE, some were recovered repeatedly from different states over the 5-year sampling time period (Fig. 1). Most clones were seen in only one type of meat product (Fig. 1, clusters A1, A2, B, and C), but several were seen from different meat products. In clone D, a beef isolate of *S. Saintpaul* shared the same PFGE profile with 12 turkey *S. Saintpaul* isolates (Fig. 1). Similarly, in clone C1 (Fig. 2), one turkey *S. Newport* isolate showed the same PFGE profile as two pork *S. Newport* isolates. This may be the result of cross-contamination at the retail outlet, as suggested by sampling time and place. Some PFGE clusters displayed good correlation with their antimicrobial resistance profiles (Fig. 2). For example, 21 *S. Typhimurium* isolates formed three clusters, each containing strains sharing 90 to 100% PFGE pattern similarity (Fig. 1, clusters A1, A2, and A3) and specific resistance profiles (AMP/AUG/FOX/TIO/KAN/SMX/TET, AMP/AUG/FOX/TIO/SMX/TET, and AMP/CHL/STR/SMX/TET). Such correlations were also seen in serotypes Kentucky, Newport, and Reading (Fig. 2, clusters B, C, and D).

DISCUSSION

Because of the importance of extended-spectrum cephalosporins for treating salmonellosis, resistance to this class of compounds in *Salmonella* is monitored. According to CDC NARMS data, resistance to extended-spectrum cephalosporins in human clinical isolates increased from 0.2% in 1996 to 4.9% in 2003 but declined to 2.9% in 2005 (<http://www.cdc.gov/narmsAnnualReport2005.pdf>). Our analysis of NARMS retail meat monitoring data showed that 50% of all Amp^r *Salmonella* isolates were resistant to extended-spectrum cephalosporins, which accounted for 12.7% of all *Salmonella* recovered during 2002 to 2006. By source, *Salmonella* isolated from chicken breast showed the highest overall prevalence of Tio^r (10% to 25.3%) during the 5 years of sampling, followed by ground turkey (5% to 8.1%). In addition, certain serotypes were more likely to display Tio^r than others, such as *S. Newport*, *S. Typhimurium*, *S. Kentucky*, *S. Heidelberg*, *S. Dublin*, *S. Agona*, and *S. Uganda* (<http://www.fda.gov/cvm/2006NARMSAnnualRpt.htm>) (33). Due to the limited number of isolates from ground beef and pork chops, it was difficult to assess the prevalence of Tio^r in these two commodities. However, there were no Tio^r *Salmonella* strains detected from 27 ground beef isolates and 17 pork chop isolates recovered in 2005 and 2006. Compared with USDA NARMS data on *Salmonella* from food animals during the same period (2002 to 2006), Tio^r was most prevalent in cattle isolates (13.3% to 21.6%), followed by chicken (9.8% to 12.8%), turkey (2.5% to 5.3%), and swine (2% to 4.3%) (<http://www.ars.usda.gov/Main/docs.htm?docid=17320>). The difference in the prevalence of Tio^r *Salmonella* between retail meats and food animals was most likely due to the sampling scheme. The NARMS animal program took samples from both slaughter house and veterinary clinics. As the Tio^r phenotype was often related to *Salmonella* serotypes and as specific serotypes such as *S. Dublin*, *S. Agona*, *S. Reading*, and *S. Uganda*, with high percentages of the Tio^r phenotype, were often isolated from sick cattle, the prevalence of Tio^r was likely greater in *Salmonella* isolates from clinical samples than from retail meats.

FIG. 1. XbaI/BlnI PFGE profiles of selected of Amp^r clones from *Salmonella* serotypes Heidelberg, Kentucky, Saintpaul, and Typhimurium.

No ESBLs were found in *Salmonella* isolates from this study. However, all Tio^r isolates showed cross-resistance or decreased susceptibility to other beta-lactams. Some were core-resistant to other classes of antimicrobials, including aminoglycosides, sulfonamides, COT, TET, and CHL, with 67% of isolates showing resistance to at least 5 antimicrobials and 5% of isolates showing resistance to at least 10 antimicrobials. Many of the conjugative plasmids in our study showed that they are MDR plasmids; in addition to carrying bla_{CMY}, they also carried resistance to combinations of CHL, TET, GEN, SMX, KAN, and STR. These plasmids are often large, which makes them difficult to transfer by electroporation. A study by Welch et al. (30) showed that MDR-AmpC plasmid (pSN254) from *S. Newport* was larger than 170 kb and contained 12 resistance genes belonging to six different classes, including beta-lactams, aminoglycosides, SMX, TET, phenicols, and quaternary ammonium compounds. Sequence data showed that the plasmid contains two copies each of bla_{CMY}, sul, and sugE. The sul and sugE genes encode resistance to SMX and to a subset of toxic quaternary ammonium compounds (including cetylpyridinium chloride), respectively. The plasmid backbone of pSN254 showed 99% DNA sequence homology with the IncA/C plasmid backbone commonly present in many members of the *Enterobacteriaceae* such as *Yersinia*, *Aeromonas*, and *Vibrio* (17, 20). This plasmid appears to be nearly universally

present in Tio^r meat isolates of *Salmonella* (29) and is also present in MDR *E. coli* and *Klebsiella* from retail meats (30). The IncA/C backbone is also similarly detected in Tio^r *Salmonella* isolated from varieties of animal species (16).

Our study showed that the bla_{CMY} and bla_{TEM-1} genes were the major contributors to beta-lactam resistance in food isolates of *Salmonella*. A similar finding was reported by Frye et al. using USDA NARMS cattle isolates from 2000 to 2004 (8), where the IncA/C plasmid also predominated. Screening human clinical non-Typhi *Salmonella* isolates with decreased susceptibility to quinolones and extended-spectrum cephalosporins recovered from 1996 to 2004, Whichard et al. reported the presence of bla_{SHV} and bla_{OXA} in addition to bla_{CMY} and bla_{TEM-1} (31). In our study, 21 Amp^r isolates from retail meats did not contain any of the five beta-lactam resistance genes tested. This may be due to the presence of other mechanisms such as efflux pumps (2), cell wall changes (22), or other undetected β-lactamases. It is not surprising that no ESBL-carrying *Salmonella* were identified since they are relatively rare in North America compared to other species in the *Enterobacteriaceae* family (19). Previous studies demonstrated that bla_{CMY-2} confers resistance to a spectrum of beta-lactam compounds and is responsible for resistance to first-, second-, and third-generation cephalosporins (36). It has been noticed in our study that bla_{CMY} and bla_{TEM-1} are almost mutually ex-

FIG. 2. PFGE and antimicrobial resistance profiles of selected Amp^r *Salmonella* isolates. A black box in the antibiogram column indicates resistance to a particular antimicrobial. AMC, amoxicillin-clavulanic acid; SMX/FIS, SMX-sulfisoxazole.

clusive, with only 2.6% isolates carrying both genes. We observed a 100% correlation between the presence of *bla*_{CMY} and resistance to TIO, FOX, and AUG plus resistance/decreased susceptibility to AXO (4 to 64 µg/ml), TAZ (16 to 64 µg/ml), and FOT (8 to 64 µg/ml). The MIC differences for AXO, TAZ, and FOT beta-lactams among individual *bla*_{CMY}-positive strains could be due to allelic differences. Among the 43 *bla*_{CMY} alleles identified so far, relatively few have been characterized for their role is resistance to different beta-lactams (15). Alternatively, these differences may be due to synergistic or additive effects between *bla*_{CMY} and other loci or undetected β-lactamases.

Our data suggest that *Salmonella* contamination of food can occur at different stages of meat production and processing. PFGE using XbaI and BlnI digestion generated 249 patterns among the 344 Amp^r isolates examined. Several clones were identified from chicken breasts (Fig. 1, clones A1, B, and C; Fig. 2, clusters A1 to A3 and B1 and B2) collected from different stores in different states from 2002 to 2006, suggesting that they may come from a common source at slaughter or in a processing plant or on a farm. Similar observations were made regarding isolates from ground turkey (Fig. 1, clone A2, and Fig. 2, clone D) and from ground beef (Fig. 2, clone C2). Additionally, two *S. Saintpaul* clones, one from ground turkey

and one from ground beef (Fig. 1, clone D), as well as a clone of *S. Newport* in ground turkey and pork chops (Fig. 2, clone C1), were found in meat samples from the same store during the same sampling month, suggesting that cross-contamination occurred during processing at the retail outlet. Similar findings were seen in our previous study of *S. Heidelberg* (35).

In summary, resistance to AMP and extended-spectrum cephalosporins in *Salmonella* is not uncommon in the U.S. retail meat supply, and its occurrence is due largely to the presence of TEM and CMY enzymes. CMY continues to be the only apparent explanation for extended-spectrum cephalosporin resistance. Ongoing testing of domestically produced meat commodities will help provide information needed to better understand risks associated with antimicrobial resistance of enteric pathogens in the domestic retail meat supply, including the detection of new resistant genotypes should they arise.

REFERENCES

1. Brinas, L., M. Zarazaga, Y. Saenz, F. Ruiz-Larrea, and C. Torres. 2002. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. *Antimicrob. Agents Chemother.* **46**:3156–3163.
2. Chen, S., S. Cui, P. F. McDermott, S. Zhao, D. G. White, I. Paulsen, and J. Meng. 2007. Contribution of target gene mutations and efflux to decreased

- susceptibility of *Salmonella enterica* serovar Typhimurium to fluoroquinolones and other antimicrobials. *Antimicrob. Agents Chemother.* **51**:535–542.
3. Clark, C., J. Cunningham, R. Ahmed, D. Woodward, K. Fonseca, S. Isaacs, A. Ellis, C. Anand, K. Ziebell, A. Muckle, P. Sockett, and F. Rodgers. 2001. Characterization of *Salmonella* associated with pig ear dog treats in Canada. *J. Clin. Microbiol.* **39**:3962–3968.
 4. Clewell, D. B., F. Y. An, B. A. White, and C. Gawron-Burke. 1985. *Streptococcus faecalis* sex pheromone (cAM373) also produced by *Staphylococcus aureus* and identification of a conjugative transposon (Tn918). *J. Bacteriol.* **162**:1212–1220.
 5. Clinical and Laboratory Standards Institute. 2005. Performance standards for antimicrobial susceptibility testing; 15th information supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
 6. Dunne, E. F., P. D. Fey, P. Kludt, R. Reporter, F. Mostashari, P. Shillam, J. Wicklund, C. Miller, B. Holland, K. Stamey, T. J. Barrett, J. K. Rasheed, F. C. Tenover, E. M. Ribot, and F. J. Angulo. 2000. Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with *ampC* beta-lactamase. *JAMA* **284**:3151–3156.
 7. Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs. 2000. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *N. Engl. J. Med.* **342**:1242–1249.
 8. Frye, J. G., P. J. Fedorka-Cray, C. R. Jackson, and M. Rose. 2008. Analysis of *Salmonella enterica* with reduced susceptibility to the third-generation cephalosporin ceftriaxone isolated from U.S. cattle during 2000–2004. *Microb. Drug Resist.* **14**:251–258.
 9. Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo. 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N. Engl. J. Med.* **338**:1333–1338.
 10. Gomez, T. M., Y. Motarjemi, S. Miyagawa, F. K. Kaferstein, and K. Stohr. 1997. Foodborne salmonellosis. *World Health Stat. Q.* **50**:81–89.
 11. Hasman, H., D. Mevius, K. Veldman, I. Olesen, and F. M. Aarestrup. 2005. β -Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J. Antimicrob. Chemother.* **56**:115–121.
 12. Helms, M., P. Vastrup, P. Gerner-Smidt, and K. Molbak. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg. Infect. Dis.* **8**:490–495.
 13. Hohmann, E. L. 2001. Nontyphoidal salmonellosis. *Clin. Infect. Dis.* **32**:263–269.
 14. Hsueh, P. R., L. J. Teng, S. P. Tseng, C. F. Chang, J. H. Wan, J. J. Yan, C. M. Lee, Y. C. Chuang, W. K. Huang, D. Yang, J. M. Shyr, K. W. Yu, L. S. Wang, J. J. Lu, W. C. Ko, J. J. Wu, F. Y. Chang, Y. C. Yang, Y. J. Lau, Y. C. Liu, C. Y. Liu, S. W. Ho, and K. T. Luh. 2004. Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and *Choleraesuis* from pigs to humans, Taiwan. *Emerg. Infect. Dis.* **10**:60–68.
 15. Jacoby, G. A. 2009. AmpC β -lactamases. *Clin. Microbiol. Rev.* **22**:161–182.
 16. Lindsey, R. L., P. J. Fedorka-Cray, J. G. Frye, and R. J. Meinersmann. 2009. Inc A/C plasmids are prevalent in multidrug-resistant *Salmonella enterica* isolates. *Appl. Environ. Microbiol.* **75**:1908–1915.
 17. McIntosh, D., M. Cunningham, B. Ji, F. A. Fekete, E. M. Parry, S. E. Clark, Z. B. Zalinger, I. C. Gilg, G. R. Danner, K. A. Johnson, M. Beattie, and R. Ritchie. 2008. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an Inc A/C plasmid similar to the *Salmonella enterica* plasmid pSN254. *J. Antimicrob. Chemother.* **61**:1221–1228.
 18. Miriagou, V., L. S. Tzouveleakis, S. Rossiter, E. Tzelepi, F. J. Angulo, and J. M. Whichard. 2003. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. *Antimicrob. Agents Chemother.* **47**:1297–1300.
 19. Mulvey, M. R., G. Soule, D. Boyd, W. Demczuk, and R. Ahmed. 2003. Characterization of the first extended-spectrum beta-lactamase-producing *Salmonella* isolate identified in Canada. *J. Clin. Microbiol.* **41**:460–462.
 20. Pan, J. C., R. Ye, H. Q. Wang, H. Q. Xiang, W. Zhang, X. F. Yu, D. M. Meng, and Z. S. He. 2008. *Vibrio cholerae* O139 multiple-drug resistance mediated by *Yersinia pestis* pIP1202-like conjugative plasmids. *Antimicrob. Agents Chemother.* **52**:3829–3836.
 21. Paterson, D. L., and R. A. Bonomo. 2005. Extended-spectrum beta-lactamases: a clinical update. *Clin. Microbiol. Rev.* **18**:657–686.
 22. Poole, K. 2002. Outer membranes and efflux: the path to multidrug resistance in gram-negative bacteria. *Curr. Pharm. Biotechnol.* **3**:77–98.
 23. Rankin, S. C., J. M. Whichard, K. Joyce, L. Stephens, K. O'Shea, H. Aceto, D. S. Munro, and C. E. Benson. 2005. Detection of a *bla*_{SHV} extended-spectrum β -lactamase in *Salmonella enterica* serovar Newport MDR-AmpC. *J. Clin. Microbiol.* **43**:5792–5793.
 24. Saladin, M., V. T. Cao, T. Lambert, J. L. Donay, J. L. Herrmann, Z. Ould-Hocine, C. Verdet, F. Delisle, A. Philippon, and G. Arlet. 2002. Diversity of CTX-M beta-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. *FEMS Microbiol. Lett.* **209**:161–168.
 25. Sjolund, M., J. Yam, J. Schwenk, K. Joyce, F. Medalla, E. Barzilay, and J. M. Whichard. 2008. Human *Salmonella* infection yielding CTX-M beta-lactamase, United States. *Emerg. Infect. Dis.* **14**:1957–1959.
 26. Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int. J. Food Microbiol.* **62**:1–5.
 27. Travers, K., and M. Barza. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. *Clin. Infect. Dis.* **34**(Suppl. 3):S131–S134.
 28. 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.
 29. Welch, T. J., J. Evenhuis, D. G. White, P. F. McDermott, H. Harbottle, R. A. Miller, M. Griffin, and D. Wise. 2009. IncA/C plasmid-mediated florfenicol resistance in the catfish pathogen *Edwardsiella ictaluri*. *Antimicrob. Agents Chemother.* **53**:845–846.
 30. Welch, T. J., W. F. Fricke, P. F. McDermott, D. G. White, M. L. Rosso, D. A. Rasko, M. K. Mammel, M. Eppinger, M. J. Rosovitz, D. Wagner, L. Rahallison, J. E. Leclerc, J. M. Hinshaw, L. E. Lindler, T. A. Cebula, E. Carniel, and J. Ravel. 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. *PLoS One* **2**:e309.
 31. Whichard, J. M., K. Gay, J. E. Stevenson, K. J. Joyce, K. L. Cooper, M. Omondi, F. Medalla, G. A. Jacoby, and T. J. Barrett. 2007. Human *Salmonella* and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. *Emerg. Infect. Dis.* **13**:1681–1688.
 32. Winokur, P. L., R. Canton, J. M. Casellas, and N. Legakis. 2001. Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clin. Infect. Dis.* **32**(Suppl. 2):S94–103.
 33. Zhao, S., P. F. McDermott, D. G. White, S. Qaiyumi, S. L. Friedman, J. W. Abbott, A. Glenn, S. L. Ayers, K. W. Post, W. H. Fales, R. B. Wilson, C. Reggiardo, and R. D. Walker. 2007. Characterization of multidrug resistant *Salmonella* recovered from diseased animals. *Vet. Microbiol.* **123**:122–132.
 34. 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.
 35. Zhao, S., D. G. White, S. L. Friedman, A. Glenn, K. Blickenstaff, S. L. Ayers, J. W. Abbott, E. Hall-Robinson, and P. F. McDermott. 2008. Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. *Appl. Environ. Microbiol.* **74**:6656–6662.
 36. Zhao, S., D. G. White, P. F. McDermott, S. Friedman, L. English, S. Ayers, J. Meng, J. J. Maurer, R. Holland, and R. D. Walker. 2001. Identification and expression of cephamycinase *bla*_{CMY} genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrob. Agents Chemother.* **45**:3647–3650.