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Characterization of Multiple-Antimicrobial-Resistant *Salmonella* Serovars Isolated from Retail Meats

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A total of 133 *Salmonella* isolates recovered from retail meats purchased in the United States and the People's Republic of China were assayed for antimicrobial susceptibility, the presence of integrons and antimicrobial resistance genes, and horizontal transfer of characterized antimicrobial resistance determinants via conjugation. Seventy-three (82%) of these *Salmonella* isolates were resistant to at least one antimicrobial agent. Resistance to the following antibiotics was common among the United States isolates: tetracycline (68% of the isolates were resistant), streptomycin (61%), sulfamethoxazole (42%), and ampicillin (29%). Eight *Salmonella* isolates (6%) were resistant to ceftriaxone. Fourteen isolates (11%) from the People's Republic of China were resistant to nalidixic acid and displayed decreased susceptibility to ciprofloxacin. A total of 19 different antimicrobial resistance genes were identified in 30 multidrug-resistant *Salmonella* isolates. The bla_{CMY-2} gene, encoding a class A AmpC β-lactamase, was detected in all 10 *Salmonella* isolates resistant to extended-spectrum β-lactams. Resistance to ampicillin was most often associated with a TEM-1 family β-lactamase gene. Six aminoglycoside resistance genes, *aadA1*, *aadA2*, *aacC2*, *Kn*, *aph*(*3*)-*IIa*, and *aac*(*3*)-*IVa*, were commonly present in the *Salmonella* isolates. Sixteen (54%) of 30 *Salmonella* isolates tested had integrons ranging in size from 0.75 to 2.7 kb. Conjugation studies demonstrated that there was plasmid-mediated transfer of genes encoding CMY-2 and TEM-1-like β-lactamases. These data indicate that *Salmonella* isolates recovered from retail raw meats are commonly resistant to multiple antimicrobials, including those used for treating salmonellosis, such as ceftriaxone. Genes conferring antimicrobial resistance in *Salmonella* are often carried on integrons and plasmids and could be transmitted through conjugation. These mobile DNA elements have likely played an important role in transmission and dissemination of antimicrobial resistance determinants among *Salmonella* strains.

The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern. The use of antimicrobials in any venue, including disease treatment and growth promotion in domestic livestock, can potentially lead to widespread dissemination of antimicrobial-resistant bacteria (16, 29, 34). In recent years, testing of *Salmonella* isolates from the United States and other countries has shown that an increasing proportion are multidrug resistant (7, 15, 18, 27). Of particular concern is the isolation of ceftriaxone- and ciprofloxacin-resistant *Salmonella*, because of the importance of these two agents in treating *Salmonella* infections in children and adults (7, 11, 32), respectively.

Resistance to antimicrobial agents in bacteria is mediated by several mechanisms, including (i) changes in bacterial cell wall permeability, (ii) energy-dependent removal of antimicrobials via membrane-bound efflux pumps, (iii) modification of the site of drug action, and (iv) destruction or inactivation of antimicrobials (3, 26). Acquired antimicrobial resistance phenotypes most often develop via conjugative transfer of plasmids (12, 14, 17). Plasmids may carry class I integrons, which are mobile DNA elements that are important in the proliferation of bacterial multidrug resistance (MDR), especially among the gram-negative enteric species (2, 10, 24, 30). Integrons primarily have been found located within transposons Tn*402* and Tn*21*, which in turn reside on broad-host-range plasmids or the IncF plasmid (6, 31). By incorporating into transposons and plasmids, integrons participate in the capture of resistance genes and dissemination of these genes among bacteria.

Molecular genetic techniques have been used to characterize antimicrobial-resistant salmonellae, especially *Salmonella enterica* serovar Typhimurium DT104 (4, 5, 9, 23, 25). For instance, variant *Salmonella* genomic island 1 (SGI1) MDR regions, consisting of integrons encoding different resistance genes, have been found in the chromosomal DNA of *Salmonella* serovars Typhimurium DT104 and Agona (4). The formation of these MDR clusters is hypothesized to favor expression of a large number of resistance genes and to enhance their transfer to other bacteria. Also, because class I integrons have become integrated into the chromosome in *Salmonella* serovars Typhimurium DT104 and Agona, they are able to persist even in the absence of antimicrobial selection (4, 9) with no

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TABLE 1. Antimicrobial resistance of *Salmonella* isolates from retail meats obtained in the United States and the People's Republic of China

Antimicrobial	Breakpoint concn (μg/ml)	United States isolates, 1998–2000 (n = 89)		People's Republic of China isolates, 1999–2000 (n = 44)	
		% Resistant	% With intermediate susceptibility	% Resistant	% With intermediate susceptibility
β-Lactams					
Ampicillin	32	29	0	39	2
Amoxicillin-clavulanate	32	21	9	0	2
Cephalotin	32	24	4	2	0
Ceftiofur	8	19	1	0	0
Ceftriaxone	64	9	10	0	0
Cefoxitin	32	18	0	0	0
Chloramphenicol	32	11	0	20	2
Tetracycline	16	68	0	43	2
Aminoglycosides					
Amikacin	64	0	0	0	0
Apramycin	32	0	0	0	0
Gentamycin	16	2	0	2	0
Kanamycin	64	6	0	11	0
Streptomycin	64	61	0	27	0
Sulfonamides					
Sulfamethoxazole	512	42	3	16	0
Trimethoprim-sulfamethoxazole	4/76[a]	9	0	9	0
Quinolones and fluoroquinolone					
Nalidixic acid	32	0	0	32	0
Ciprofloxacin	4	0	0	0	0

[a] Breakpoint concentration of trimethoprim/breakpoint concentration of sulfamethoxazole.

apparent fitness cost to the cell. This has led, in the case of *Salmonella* serovar Typhimurium DT104, to a stable and widely disseminated clone of multidrug-resistant *Salmonella* serovar Typhimurium.

The objectives of this study were to determine the antimicrobial susceptibility phenotypes of *Salmonella* strains isolated from retail meats purchased in the Washington, D.C., area in the United States and in the People's Republic of China and to characterize the genetic mechanisms underlying the antimicrobial-resistant phenotypes observed for the isolates. We also examined selected isolates for the ability to donate resistance genes via conjugative transfer of plasmids to *Escherichia coli*. Our goal was to increase our understanding of the molecular genetic mechanisms involved in the emergence and dissemination of antimicrobial-resistant *Salmonella* isolates.

MATERIALS AND METHODS

***Salmonella* isolates.** A total of 133 *Salmonella* isolates were included in the study. Eighty-nine isolates were recovered from retail ground meat samples of chicken, turkey, pork, and beef purchased in the Washington, D.C., area; these isolates included 45 isolates from samples purchased between June and September 1998 and 44 isolates from samples purchased between August 1999 and August 2000. The other 44 *Salmonella* isolates were isolated from samples of pork, beef, chicken, and mutton purchased in 10 provinces in the People's Republic of China from October 1999 to December 2000.

All *Salmonella* isolates were recovered from meats by using methods described in the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (13). The isolates were further identified with API identification kits (Bio Merieux, Marcy, France) and were serotyped with commercial antiserum (Difco, Detroit, Mich.) used according to the manufacturer's instructions.

Antimicrobial susceptibility testing. Antimicrobial MICs for the 133 *Salmonella* isolates were determined by using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and were interpreted by using the National Committee for Clinical Laboratory Standards standards for microdilution broth methods (21, 22). The 17 antimicrobials used and their recommended resistance breakpoints are shown in Table 1. *E. coli* ATCC 25922 and ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms.

DNA isolation, PCR, and gene sequence analysis. Based on serotypes and antimicrobial resistance profiles, 30 multidrug-resistant *Salmonella* isolates were selected for further characterization of antimicrobial resistance genes and class 1 integrons. Chromosomal and plasmid DNA of these bacterial isolates were isolated by using a Wizard genomic DNA purification kit (Promega, Madison, Wis.) and a High plasmid purification kit (Roche, Indianapolis, Ind.), respectively. The quantity of the DNA was determined by using a Smartspect 3000 spectrophotometer (Bio-Rad, Hercules, Calif.). Sixty-one pairs of oligonucleotide primers (Table 2) were designed to target 61 antimicrobial resistance genes that confer resistance to six categories of antimicrobial agents, including β-lactams, aminoglycosides, phenicols, tetracycline, trimethoprim, and sulfonamides. Most primers were designed to differentiate the specific gene sequence of interest; the only exceptions were the $bla_{\text{TEM-1}}$ primers, which amplified the entire family of bla_{TEM} genes. The primers were designed by using the OLIGO 5.0 software program (National Biosciences, Inc., Plymouth, Minn.) and were synthesized commercially (Invitrogen, Carlsbad, Calif.). PCR was performed in 50 μl (total volume) of distilled H_2O containing each deoxyribonucleotide at a concentration of 0.25 mM, 1.5 mM $MgCl_2$, 0.2 U of Gold *Taq* DNA polymerase, and 50 pmol of each primer. The temperature profile included an initial template denaturation step consisting of 95°C for 10 min, followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min and a final step consisting of 72°C for 7 min (8). The presence of class I integrons among the 30 *Salmonella* isolates was determined by PCR by using primers 5′CS (5′-GGCATCCAAGCACAAGC-3′) and 3′-CS (5′-AAGCAGACTTGACTGAT-3′) as previously described (35).

All PCR products were purified with High PCR purification kits (Roche) and were sequenced at the University of Maryland Center of Agriculture Biotechnology, College Park, Md. The resulting DNA sequence data were compared to data in the GenBank database by using the BLAST algorithm (1) available at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

Conjugation experiments. Multidrug-resistant *Salmonella* isolates recovered from retail meats (10 isolates from the Washington, D.C., area and 4 isolates from the People's Republic of China) were used as donor strains in conjugation experiments to study antimicrobial resistance gene transfer. Two nalidixic acid-resistant *E. coli* strains (1003 and 1016) were used as recipient strains. Conjugation was performed by the filter mating method as described previously (8). Briefly, donor and recipient cells (ratio, 1:10) were mixed in Luria-Bertani broth

TABLE 2. Sequences of oligonucleotide primers used in PCR assays for identification of antimicrobial resistance genes in *Salmonella* isolates from retail meats

Antimicrobial(s)	Resistance gene	Oligonucleotide primer sequences		Size (bp)	Accession no.
		Forward primer (5′-3′)	Reverse primer (5′-3′)		
β-Lactams	$bla_{\text{CMY-2}}$	TGG CCG TTG CCG TTA TCT AC	CCC GTT TTA TGC ACC CAT GA	870	X91840
	$bla_{\text{CMY-9}}$	TCA GCG AGC AGA CCC TGT TC	CTG GCC GGG ATG GGA TAG TT	874	AB049588
	$bla_{\text{FOX-1}}$	CAG CCG ATG CTC AAG GAG TA	CAA CCC AGC CCC TGA GTC AT	761	X77455
	$bla_{\text{DHA-1}}$	GCC GGT CAC TGA AAA TAC AC	TAC GGC TGA ACC TGG TTG TC	762	Y16410
	bla_{MI1}	AGC GTC GCC AGT TCT GCA TT	GAC CGG CCA GTT GAG CAT CT	858	M37839
	$bla_{\text{SHV-1}}$	GGC CGC GTA GGC ATG ATA GA	CCC GGC GAT TTG CTG ATT TC	714	F148850
	$bla_{\text{TEM-1}}$	CAG CGG TAA GAT CCT TGA GA	ACT CCC CGT CGT GTA GAT AA	643	AF309824
	$bla_{\text{CTX-M1}}$	AAC CGT CAC GCT GTT GTT AG	TTG AGG CTG GGT GAA GTA AG	766	X92506
	$bla_{\text{CTX-M2}}$	GGC GTT GCG CTG ATT AAC AC	TTG CCC TTA AGC CAC GTC AC	486	X92507
	$bla_{\text{CTM-M14}}$	GCC TGC CGA TCT GGT TAA CT	GCC GGT CGT ATT GCC TTT GA	358	AF252622
	$bla_{\text{VEB-1}}$	TAG CCG TTT TGT CTG AGA TA	TTA CCC CAA CAT CAT TAG TG	543	AF205943
	$bla_{\text{OXA-1}}$	AAT GGC ACC AGA TTC AAC TT	CTT GGC TTT TAT GCT TGA TG	595	J02976
	$bla_{\text{OXA-2}}$	CAA GCC AAA GGC ACG ATA GT	ACG ATT GCC TCC CTC TTG AA	644	X07260
	$bla_{\text{OXA-7}}$	GAA GCC GTC AAT GGT GTT TT	ATG CCC TCA CTT GCC ATG AT	686	X75562
	$bla_{\text{PSE-1}}$	TGC TTC GCA ACT ATG ACT AC	AGC CTG TGT TTG AGC TAG AT	438	AF153200
	$bla_{\text{IMP-1}}$	TGA GGC TTA CCT AAT TGA CA	TCA GGC AAC CAA ACC ACT AC	324	S71932
Aminoglycosides	*aac*(*3*)-*Ia*	TGA GGG CTG CTC TTG ATC TT	ATC TCG GCT TGA ACG AAT TG	436	X15852
	aac(*3*)-*IIa*	CGG CCT GCT GAA TCA GTT TC	AAA GCC CAC GAC ACC TTC TC	439	X13543
	aacC2	GGCAATAACGGAGGCAATTCGA	CTCGATGGCGACCGAGCTTCA	450	X51534
	aacC4	ACTGAGCATGACCTTGCGATGCTCTA	TACCTTGCCTCTCAAACCCCGCTT	436	AJ009820
	aac(*3*)-*IVa*	GAT GGG CCA CCT GGA CTG AT	GCG CTC ACA GCA GTG GTC AT	462	X01385
	aac(*6′*)	TTG GAC GCT GAG ATA TAT GA	GCT CCT TTT CCA GAA TAC TT	476	M18086
	aph(*2″*)	GAC CGT GTT CTT GAA TTC TA	GCG GGA ATC TTT TAG CAT TA	464	M13771
	ant(*3″*)-*Ia*	CGC CGA AGT ATC GAC TCA AC	GCG GGA CAA CGT AAG CAC TA	559	X02340
	aadD	ATATTGGATAAATATGGGGAT	TCCACCTTCCACTCACCGGTT	161	AF051917
	ant(*6*)-*Ia*	GCC GGA GGA TAT GGA ATT AT	TCA GCG GCA TAT GTG CTA TC	666	AF299292
	Kn	ACTGGCTGCTATTGGGCGA	CGTCAAGAAGGCGATAGAAGG	515	U66885
	aph(*3′*)-*IIa*	TCC GGT GCC CTG AAT GAA CT	ACG GGT AGC CAA CGC TAT GT	519	V00618
	aph(*4*)-*Ia*	TCT CGG AGG GCG AAG AAT CT	TTG CCG TCA ACC AAG CTC TG	763	V01499
Tetracycline	*tetA*	GCG CCT TTC CTT TGG GTT CT	CCA CCC GTT CCA CGT TGT TA	831	X00006
	tetB	CCC AGT GCT GTT GTT GTC AT	CCA CCA CCA GCC AAT AAA AT	723	V00611
	tetC	TTG CGG GAT ATC GTC CAT TC	CAT GCC AAC CCG TTC CAT GT	1019	AB023657
	tetD	CTG GGC AGA TGG TCA GAT AA	TGA CCA GCA CAC CCT GTA GT	832	X65876
	tetE	CGT CGC CCT GTA TTG TTA CT	TGG TCA GCA CCC CTT GTA AT	814	M34933
	tetG	AGC AGG TCG CTG GAC ACT AT	CGC GGT GTT CCA CTG AAA AC	623	AF07155
Trimethoprim	*dhfrI*	CGG TCG TAA CAC GTT CAA GT	CTG GGG ATT TCA GGA AAG TA	220	AF382145
	dhfrII	AGT TTG CGC TTC CCC TGA GT	CTT AGG CCA CAC GTT CAA GTG	194	AF083409
	dhfrIII	ACC TGC CGA TCT GCG TCA T	TCG CAG GCA TAG CTG TTC	387	J03306
	dhfrV	TTG GTT GCG GTC CAC ACA TA	CTC CTT CCG GCT CAA TAT C	330	X12868
	dhfrVI	GTT TCC GAG AAT GGA GTA AT	ACT AAA CGC AAC GCA TAG TA	508	K01163
	dhfrVII	AGC AAA AGG TGA GCA GTT AC	GTG CTG GAA CGA CTT GTT AG	419	X58425
	dhfrVIII	TTG GGA AGG ACA ACG CAC TT	ACC ATT TCG GCC AGA TCA AC	382	U10186
	dhfrIX	TCA GAT TCC GTG GCA TGA AC	AAT GGT CGG GAC CTC AGA T	400	X57730
	dhfrX	ACC AGA GCA TTC GGT AAT CA	TTG GAT CAC CTA CCC ATA GA	445	I06418
	dhfrXII	AAA TTC CGG GTG AGC AGA AG	CCC GTT GAC GGA ATG GTT AG	429	Z21672
	dhfrXIII	GCA GTC GCC CTA AAA CAA AG	GAT ACG TGT GAC AGC GTT GA	294	Z50802
	dhfrXV	GCC GTG GGT CGA TGT TTG AT	TTC ACC ACC ACC AGA CAC A	395	Z83311
	dhfrXVI	GCT CTC CCA AAT CGA AAG TA	ATT GCA GGC GCT TGT TAA CT	332	AF077008
Sulfomamides	*sulI*	TCA CCG AGG ACT CCT TCT TC	CAG TCC GCC TCA GCA ATA TC	331	X15024
	sulII	CCT GTT TCG TCC GAC ACA GA	GAA GCG CAG CCG CAA TTC AT	435	M36657
Chloramphenicol	*cat1*	CTT GTC GCC TTG CGT ATA AT	ATC CCA ATG GCA TCG TAA AG	508	M64281
	cat2	AAC GGC ATG ATG AAC CTG AA	ATC CCA ATG GCA TCG TAA AG	547	AJ401047
	cat3	ATC GGC ATC GTT TAC CAT GT	ATC CCC TTC TTG CTG ATA TT	531	AY042185
	cmlA	CGC CAC GGT GTT GTT GTT AT	GCG ACC TGC GTA AAT GTC AC	394	AF078527
	cmlB	ACT CGG CAT GGA CAT GTA CT	ACG GAC TGC GGA ATC CAT AG	840	AF034958
	flo	CTG AGG GTG TCG TCA TCT AC	GCT CCG ACA ATG CTG ACT AT	673	AF252855.

(Difco). The mixture was then collected on a 0.45-μm-pore-size filter and incubated on blood agar plates (BAP) at 37°C overnight. The mating mixture was washed from the filter and spread onto BAP containing a combination of nalidixic acid (60 μg/ml) and streptomycin (50 μg/ml) or a combination of nalidixic acid (100 μg/ml) and kanamycin (50 μg/ml). Bacterial colonies on BAP containing appropriate antibiotics were transferred onto MacConkey agar (Difco) plates and incubated overnight at 37°C. Presumptive *E. coli* transconjugants were confirmed to be *E. coli* by the API test and were assayed for susceptibility to 17 antimicrobial agents. Transfer of antimicrobial resistance genes was confirmed by PCR by using primers shown in Table 2.

RESULTS

Antimicrobial resistance of *Salmonella* isolates. Seventy-three (82%) of the *Salmonella* strains isolated from retail meats purchased in the Washington, D.C., area exhibited resistance to at least one antimicrobial. Resistance to tetracycline (68% of the isolates were resistant), resistance to streptomycin (61%), and resistance to sulfamethoxazole (42%) were observed most often, whereas resistance to β-lactams was observed less frequently (Table 1). Among the β-lactams, resistance was greatest to ampicillin (29% of the isolates were resistant), followed by cephalothin (24%), amoxicillin-clavulanate (21%), ceftiofur (19%), cefoxitin (18%), and ceftriaxone (9%). In addition to eight isolates resistant to ceftriaxone, nine isolates (10%) exhibited intermediate susceptibility to ceftriaxone. All the *Salmonella* isolates that exhibited intermediate susceptibility to ceftriaxone were resistant to the other β-lactams tested. The *Salmonella* isolates also exhibited resistance to chloramphenicol (11% of the isolates were resistant), kanamycin (6%), and gentamicin (2%). All *Salmonella* isolates recovered from retail foods in the Washington, D.C., area were susceptible to amikacin, apramycin, ciprofloxacin, and nalidixic acid (Table 1).

Twenty-eight (64%) *Salmonella* isolates from the People's Republic of China exhibited resistance to at least one antimicrobial. The highest frequencies of resistance were the frequencies of resistance to tetracycline (43% of the isolates were resistant), ampicillin (39%), and streptomycin (32%). Resistance was also observed, but to a lesser extent, for chloramphenicol (20%), sulfamethoxazole (16%), kanamycin (11%), and trimethoprim (9%) (Table 1). None of the isolates exhibited resistance to β-lactams other than ampicillin, except for one isolate that was resistant to cephalothin. In contrast to the United States isolates, approximately one-third of the isolates from the People's Republic of China were quinolone resistant. Fourteen (32%) of the isolates were resistant to nalidixic acid and also had increased MICs of ciprofloxacin. The MIC at which 90% of the isolates tested were inhibited by ciprofloxacin for the isolates from the People's Republic of China was more than 30 times higher (0.5 μg/ml) than the corresponding value for the isolates from the United States (<0.015 μg/ml) (data not shown).

Antimicrobial resistance genes and class 1 integrons. Among the 30 multiple-antimicrobial-resistant *Salmonella* isolates (defined as isolates that were resistant to two or more antimicrobials), 19 resistance genes conferring resistance to six categories of antimicrobials, including β-lactams, aminoglycosides, phenicols, tetracycline, trimethoprim, and sulfonamides, were identified. The PCR results were consistent with the antimicrobial susceptibility phenotypes (Table 3). For example, the *sulI* and/or *sulII* genes were detected in each of the sulfonamide-resistant *Salmonella* isolates; the *tetA* and/or *tetB* genes were detected in each of the tetracycline-resistant isolates; and the dihydrofolate reductase genes, *dhfr1*, *dhfr12*, and *dhfr13*, were detected in each of the trimethoprim-resistant isolates. Either or both of the chloramphenicol acetyltransferase genes, *cat1* and *cat2*, were detected in the chloramphenicol-resistant *Salmonella* isolates from the People's Republic of China, while the *flo* gene was detected in each of the chloramphenicol-resistant *Salmonella* isolates from the United States.

The distribution of aminoglycoside resistance genes in the *Salmonella* isolates was diverse. Six different resistance genes, *aadA1*, *aadA2*, *aacC2*, *Kn*, *aph*(*3*)-*IIa*, and *aac*(*3*)-*IVa*, were detected. The *aadA1* gene was detected most frequently and was present in 17 of the isolates. Three isolates contained *aadA1* and *aadA2*. Isolate CHS31 contained four types of aminoglycoside resistance genes, *aadA1*, *aadA2*, *aacC2*, and *aac*(*3*)-*Iva*. A total of 12 antimicrobial resistance genes were amplified from the DNA of this isolate. The *aac*(*3*)-*IVa* and *aacC2* genes (conferring resistance to gentamicin) and the *aph*(*3*)-*IIa* gene (conferring resistance to kanamycin) were detected in *Salmonlella* isolates from the People's Republic of China.

Three kinds of β-lactamase genes were detected in the *Salmonella* isolates. The $bla_{\text{CMY-2}}$ gene was detected in 10 extended-spectrum β-lactamase-resistant *Salmonella* isolates, 5 of which also contained a $bla_{\text{TEM-1}}$-like gene. Each of the nine ampicillin-resistant isolates from the People's Republic of China contained a $bla_{\text{TEM-1}}$-like gene. Consistent with previous findings (19), the $bla_{\text{PSE-1}}$gene, which was located in a 1.0-kb class 1 integron, was amplified in each of two *Salmonella* serovar Typhimurium DT104 isolates with an ACSSuT antibiogram (Table 3).

Six integron amplicons, which were 0.75, 1, 1.2, 1.5, 2.0, and 2.7 kb long, were detected in 16 (54%) of the 30 *Salmonella* isolates (Table 3). The most common antimicrobial resistance genes carried by these integrons were *aadA1* and *aadA2* conferring resistance to streptomycin and *dhfrXII* conferring resistance to trimethoprim. A 2.7-kb integron in two *Salmonella* serovar Typhimurium DT208 isolates contained an *aadA* gene, as well as a 1.2-kb gene having an unknown function (GenBank accession no. AY204504). A protein BLAST search revealed that the 1.2-kb open reading frame exhibited 56% amino acid homology with a reverse transcriptase from *Serratia marcescens*. No change in antimicrobial susceptibility was observed when this open reading frame was overexpressed as a cloned copy in *E. coli* (data not shown).

Conjugative transfer of resistance genes. The 10 *Salmonella* isolates from retail meats purchased in the Washington, D.C., area transferred their plasmids to *E. coli* at rates ranging from 6.0×10^{-8} to 2.4×10^{-4} transconjugant per recipient cell. Examples of the conjugation study results are shown in Table 4. Transconjugants 1083/1003 and 1290/1003 acquired resistance to 9 and 11 of the antimicrobial agents tested, respectively. Transfer of $bla_{\text{CMY-2}}$ and $bla_{\text{TEM-1}}$-like genes to the recipient *E. coli* strain was confirmed by a PCR assay. Because antimicrobial resistance genes specifying the ACSSuT resistance phenotype have integrated into the *Salmonella* chromosome (4, 5), the two *Salmonella* serovar Typhimurium DT104 isolates did not transfer this phenotype to the *E. coli* recipient strain (Table 4). One of four *Salmonella* isolates from the

TABLE 3. Antimicrobial resistance and resistance gene profiles and class I integrons of *Salmonella* isolates from retail meats obtained in the United States and the People's Republic of China

Strain	Serotype	Meat	Antimicrobial resistance profile[a]	Antimicrobial resistance gene(s)	Size of integron (kb)
1083[b]	Agona	Turkey	Amo, Amp, Cef, Cet, Cep, Fox, Str, Sul, Tet, Tri	bla_{CMY-2}, bla_{TEM-1}, *aadA1*, *dhfr1*, *sulI*, *sulII*, *tetA*	1.2
1089[b]	Agona	Turkey	Amo, Amp, Cef, Cet, Cep, Fox, Str, Sul, Tet, Tri	bla_{CMY-2}, bla_{TEM-1}, *aadA1*, *dhfr1*, *sulI*, *sulII*, *tetA*	1.2
1126[b]	Agona	Turkey	Amo, Amp, Cef, Cet, Cep, Fox, Str, Sul, Tet, Tri	bla_{CMY-2}, bla_{TEM-1}, *aadA1*, *dhfr1*, *sulI*, *sulII*, *tetA*	1.2
1163[b]	Agona	Turkey	Str, Sul, Tet	*aadA1*, *sulI*, *tetB*	1.0
1271[b]	Djugu	Pork	Sul, Tri	*dhfr12*, *dhfr13*, *sulI*	2.0
S34[c]	H:E-2	Chicken	Amo, Amp, Cef, Cep, Fox	bla_{CMY-2}	
S14[b]	Hadar	Turkey	Sul, Tet	*SulI*, *SulII*, *tetA*	
1272[b]	Heidelberg	Pork	Kan, Str, Sul, Tet	*aadA1*, *sulI*, *tetB*	1.0
S31[c]	Infantis	Chicken	Amo, Amp, Cef, Cep, Fox	bla_{CMY-2}	
S33[c]	Infantis	Chicken	Amo, Amp, Cef, Cep, Fox, Sul	bla_{CMY-2}, *sulI*	
S16[c]	Orion	Pork	Sul, Tet	*sulI*, *sulII*, *tetA*	
1189[b]	Typhimurium	Chicken	Sul, Tet, Tri	*dhfr12*, *dhfr13*, *sulI*, *tetA*, *tetB*	0.75
S21[c]	Typhimurium DT104	Pork	Amp, Cml, Str, Sul, Tet	*pse-1*, *flo-1*, *aadA2*, *aadA1*, *sulI*, *sulII*, *tetA*, *tetB*	1.0
S27[c]	Typhimurium	Chicken	Amo, Amp, Cef, Cep, Fox	bla_{CMY-2}	
S29[c]	Typhimurium	Chicken	Amo, Amp, Cef, Cep, Fox	bla_{CMY-2}	
S44[c]	Typhimurium	Chicken	Amo, Amp, Cef, Cep, Cet, Fox	bla_{CMY-2}	
1275[b]	Typhimurium DT104	Pork	Amp, Cml, Ffc, Str, Sul, Tet	*pse-1*, *flo-1*, *aadA2*, *aadA1*, *sulI*, *sulII*, *tetA*, *tetB*	1.0
S43[c]	Typhimurium	Chicken	Amo, Amp, Cef, Cep, Fox, Str, Sul, Tet	bla_{CMY-2}, *aadA1*, *sulI*, *tetB*	
1290[b]	Typhimurium DT208	Chicken	Amo, Amp, Cef, Cet, Cep, Cml, Fox, Gen, Kan, Str, Sul, Tet	bla_{CMY-2}, bla_{TEM-1}, *flo-1*, *aadA1*, *sulII*, *tetA*, *tetB*	2.7
1291[b]	Typhimurium DT208	Chicken	Amo, Amp, Cef, Cep, Cet, Cml, Fox, Gen, Kan, Str, Sul, Tet	bla_{CMY-2}, bla_{TEM-1}, *flo-1*, *aadA1*, *sulII*, *tetA*	2.7
CHS34[d]	Derby	Pork	Amp, Cml, Tri, Sul, Tet	bla_{TEM-1}, *cat1*, *cat2*, *dhfr1*, *sulI*, *tetA*	1.5
CHS36[d]	Derby	Beef	Amp, Cml, Str, Tri, Sul, Tet	bla_{TEM-1}, *cat1*, *cat2*, *aadA1*, *dhfr1*, *sulI*, *tetA*	1.5
CHS38[d]	Derby	Beef	Amp, Cml, Str, Tri, Sul, Tet	bla_{TEM-1}, *cat1*, *cat2*, *aadA1*, *dhfr1*, *sulI*, *tetA*	1.5
CHS32[d]	Derby	Pork	Amp, Cml, Kan, Nal, Str, Tet	bla_{TEM-1}, *cat2*, *aph(3)-IIA*, *aadA1*, *sulI*, *tetA*	
CHS5c[d]	Enteritidis	Chicken	Amp, Str, Sul, Tet	bla_{TEM-1}, *aadA1*, *sulII*, *tetA*	
CHS14[d]	Enteritidis	Chicken	Nal, Str, Sul, Tet	*aadA1*, *sulI*, *sulII*, *tetA*	
CHS45[d]	Enteritidis	Chicken	Amp, Cml, Kan, Nal, Str, Tet	bla_{TEM-1}, *cat2*, *aph(3)-IIA*, *tetA*	
CHS43[d]	Haardt	Chicken	Amp, Cml, Kan, Nal, Str, Tet	bla_{TEM-1}, *cat2*, *aadA1*, *aph(3)-IIA*, *tetA*	1.5
CHS31[d]	Typhimurium	Beef	Amp, Cef, Cml, Gen, Kan, Nal, Str, Tri, Sul, Tet	bla_{TEM-1}, *cat1*, *cat2*, *aadA2*, *aadA1*, *aac(3)-IVA*, *aacC2*, *dhfr12*, *dhfr13*, *sulII*, *tetA*, *tetB*	2.0
CHS46[d]	Untypable	Chicken	Amp, Cml, Kan, Nal, Str, Tet	bla_{TEM-1}, *cat2*, *aadA1*, *aph(3)-IIA*, *tetA*	

[a] Amo, amoxicillin-clavulanic acid; Amp, ampicillin; Cef, ceftiofur; Cet, ceftriaxone; Cep, cephalothin; Fox, cefoxitin; Cml, chloramphenicol; Gen, gentamicin; Kan, kanamycin; Nal, nalidixic acid; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline; Tri, trimethoprim-sulfamethoxazole.
[b] Isolated in the United States in the period from June to September 1998.
[c] Isolated in the United States in the period from August 1999 to August 2000.
[d] Isolated in the People's Republic of China in the period from October 1999 to December 2000.

People's Republic of China transferred the ampicillin resistance phenotype to *E. coli* 1016. The transfer of other resistance phenotypes could not be measured because *E. coli* 1016 had these phenotypes prior to the conjugation experiment (Table 4).

DISCUSSION

In this study, we examined *Salmonella* isolates recovered from retail meats purchased in the United States and the People's Republic of China to determine their antimicrobial susceptibility phenotypes and genotypes. In general, our findings are similar to those described in previous studies showing that *Salmonella* isolates in retail meats are commonly resistant to multiple antimicrobials, including tetracycline, sulfamethoxazole, and streptomycin (20, 32). Our findings also showed that the frequencies of antimicrobial resistance among *Salmonella* strains isolated from retail meats purchased in the People's Republic of China were lower than the frequencies of antimicrobial resistance among *Salmonella* strains isolated from retail meats purchased in the United States. Further studies involving larger sample sizes are necessary to more precisely determine if there are differences in antimicrobial resistance between *Salmonella* isolates from the two countries.

Resistance to ceftriaxone is a concern because of the importance of this agent for treatment of salmonellosis in children. Ceftriaxone resistance in *Salmonella* is largely due to the AmpC β-lactamase (bla_{CMY-2}) gene, and reports of this resistance have been increasing in the United States (11, 32, 33). Strains of *Salmonella* carrying bla_{CMY-2} were first isolated from human, animal, and food samples in the United States in 1996 (11, 36). In this study, 19% of *Salmonella* isolates from retail

TABLE 4. Antimicrobial susceptibility profiles of donors, recipients, and transconjugants in the conjugation experiments

Strain	Type	MIC (μg/ml) of[a]:												Conjugation rate	Resistance gene(s)
		Fox	Cml	Tet	Cet	Amo[b]	Cip	Gen	Nal	Cef	Sul	Cep	Tri	Kan	Amp	Str		
E. coli 1003	Recipient	4	8	<4	<0.25	1/0.5	**>4**	0.5	**>32**	0.25	16	8	0.12	<16	2	32		
1083[c]	Donor	**>16**	<4	**>32**	**>64**	**>32/16**	<0.01	1	<4	**8**	**>512**	**>32**	**>4**	<16	**>32**	**>64**		$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
1083/1003	Transconjugant	**>16**	8	**>32**	16	**>32/16**	**>4**	1	**>32**	**8**	**>512**	**>32**	**>4**	<16	**>32**	**>64**	2.4×10^{-4}	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
DT208 strain 1290[d]	Donor	**>16**	**>32**	**>32**	32	**>32/16**	<0.01	**16**	<4	**>8**	**>512**	**>32**	<0.12	**>64**	**>32**	**>64**		$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
1290/1003	Transconjugant	**>16**	**>32**	**>32**	32	**>32/16**	**>4**	**16**	**>32**	**>8**	**>512**	**>32**	<0.12	**>64**	**>32**	**>64**	6.0×10^{-8}	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1}}$
DT104 strain 1275[d]	Donor	<0.50	**>32**	**32**	<0.25	16/8	<0.01	<0.25	<4	0.25	**>512**	4	0.25	<16	**>32**	**>64**		
1275/1003	Transconjugant	2	2	<4	<0.25	16/8	**>4**	0.5	**>32**	0.25	**>512**	2	0.25	<16	2	**>64**	6.0×10^{-8}	
DT104 strain S21[d]	Donor	2	**>32**	**>32**	<0.25	16/8	<0.01	<0.25	<4	0.25	**>512**	4	<0.12	<16	**>32**	**>64**		
S21/1003	Transconjugant	2	2	<4	<0.25	16/8	**>4**	0.5	**>32**	0.25	16	2	<0.12	<16	2	**>64**	6.0×10^{-8}	
E. coli 1016	Recipient	4	**>32**	**>32**	<0.25	1/5	0.25	**>16**	**>32**	0.25	**>512**	8	**>4**	**>64**	4	**>64**		
CHS5[e]	Donor	2	4	**>32**	<0.25	8/4	<0.01	<0.25	<4	0.25	**>512**	8	0.25	<16	**>32**	**>64**		$bla_{\text{TEM-1}}$
CHS5/1016	Transconjugant	4	**>32**	**>32**	<0.25	8/4	0.25	**>16**	**>32**	0.25	**>512**	16	**>4**	**>64**	**>32**	**>64**	8.0×10^{-5}	$bla_{\text{TEM-1}}$

[a] Amo, amoxicillin-clavulanic acid; Amp, ampicillin; Cef, ceftiofur; Cet, ceftriaxone; Cep, cephalothin; Cip, ciprofloxacin; Fox, cefoxitin; Cml, chloramphenicol; Gen, gentamicin; Kan, kanamycin; Nal, nalidixic acid; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline; Tri, trimethoprim-sulfamethoxazole. Resistance is indicated by boldface type.

[b] MIC of amoxicillin/MIC of clavulanic acid.

[c] *Salmonella* serovar Agona.

[d] *Salmonella* serovar Typhimurium.

[e] *Salmonella* serovar Enteritidis.

meats purchased in the United States were resistant or exhibited intermediate susceptibility to ceftriaxone and harbored the $bla_{\text{CMY-2}}$ gene. Conversely, all of the *Salmonella* isolates from the People's Republic of China were susceptible to ceftriaxone (and other cephalosporins), and none harbored $bla_{\text{CMY-2}}$. A possible explanation for these observations is that ceftriaxone-resistant *Salmonella* strains in meats have arisen due to cross-resistance between ceftriaxone and ceftiofur, a cephalosporin used in food animals (29, 33). Ceftiofur, the only cephalosporin approved for therapeutic use in cattle, has been approved for use in the United States since 1988, whereas it was approved for use in the People's Republic of China in 2002 (www.agri.gov.cn/blgg/t20021219_36976.htm).

Quinolones and fluoroquinolones have been used in veterinary medicine in the People's Republic of China since the 1980s. In contrast, they were not approved for therapeutic use in animals in the United States until 1995. The differences in fluoroquinolone susceptibility between isolates from the United States and isolates from the People's Republic of China likely reflect the different approval dates in the two countries. Thirty-two of the *Salmonella* isolates from the People's Republic of China were resistant to nalidixic acid and had increased MICs of ciprofloxacin, while all of the isolates from the United States were susceptible to these drugs. Nevertheless, the relatively high frequency of increased MICs of ciprofloxacin among the isolates from the People's Republic of China warrants continued surveillance to detect emerging ciprofloxacin-resistant phenotypes.

Two *Salmonella* serovar Typhimurium DT104 strains (1275 and S21) isolated from pork within a 1-year span in the Washington, D.C., area displayed very similar antimicrobial resistance phenotypes, genotypes, and pulsed-field gel electrophoresis patterns. Both of these isolates had the classical ACSSuT resistance phenotype and, accordingly, were found to contain the $bla_{\text{PSE-1}}$, *flo-1*, *aadA2*, *sulI*, and *tetA* genes. These genes are known constituents of the SGI1 MDR region (4, 23). In addition, three more resistance genes, *sulII*, *aadA1*, and *tetB*, were detected in these isolates, suggesting that *Salmonella* may contain multiple genes that specify resistance to similar drugs (5, 9). In *Salmonella* serovar Typhimurium DT104, the resistance genes known to be constituents of SGI1 were not transferred to *E. coli*, whereas the *aadA1* gene specifying the streptomycin-resistant determinant is encoded in a conjugal plasmid, which can be transferred to *E. coli* by conjugation. In contrast to the antimicrobial resistance determinants in *Salmonella* serovar Typhimurium DT104, most of the antimicrobial resistance determinants in other *Salmonella* isolates were encoded in a transferable plasmid and could be transferred to *E. coli* by conjugation. Furthermore, the molecular mechanisms of antimicrobial resistance in these isolates were also different from SGI1 MDR in *Salmonella* serovar Typhimurium DT104. The reason for the widespread dissemination of SGI1 MDR among *Salmonella* serovar Typhimurium DT104 isolates is not clear.

Most of the resistance genes, including $bla_{\text{CMY-2}}$ and the genes contained in integrons, were located on plasmids in the *Salmonella* isolates in this study. Plasmids carrying $bla_{\text{CMY-2}}$ resistance were readily transferred under the selective pressure of β-lactam antibiotics; they were also cotransferred by selection with other antibiotics on the same plasmid (e.g., strepto-

mycin). The *E. coli* recipient cells acquired 9 to 11 antimicrobial resistance phenotypes by receiving the plasmid from *Salmonella* serovar Agona and *Salmonella* serovar Typhimurium DT208 via conjugation. These findings indicated that conjugal plasmids play a significant role in the dissemination of multiple-antimicrobial-resistant bacteria.

A better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads should enable us in the future to design intervention strategies to reduce its progression. Because antimicrobial-resistant bacteria may be transferred to humans through the food chain (28, 34), selection of novel antimicrobial resistance mechanisms in *Salmonella* in animals (28), which specify resistance to antibiotics used in humans, is troubling. Efforts that include further implementation of hazard analysis of critical control point programs in food production are needed to reduce the incidence of *Salmonella* in food. The judicious use of antibiotics, including cephalosporins and fluoroquinolones in food animals, is also critical to control the rapid spread of antimicrobial-resistant bacteria.

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