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## THE ISOLATION OF ANTIBIOTIC-RESISTANT SALMONELLA FROM RETAIL GROUND MEATS

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### ABSTRACT

**Background** Salmonella is a leading cause of food-borne illness. The emergence of antimicrobial-resistant salmonella is associated with the use of antibiotics in animals raised for food; resistant bacteria can be transmitted to humans through foods, particularly those of animal origin. We identified and characterized strains of salmonella isolated from ground meats purchased in the Washington, D.C., area.

**Methods** Salmonella was isolated from samples of ground chicken, beef, turkey, and pork purchased at three supermarkets. The isolates were characterized by serotyping, antimicrobial-susceptibility testing, phage typing, and pulsed-field gel electrophoresis. The polymerase chain reaction and DNA sequencing were used to identify resistance integrons and extended spectrum  $\beta$ -lactamase genes.

**Results** Of 200 meat samples, 41 (20 percent) contained salmonella, with a total of 13 serotypes. Eighty-four percent of the isolates were resistant to at least one antibiotic, and 53 percent were resistant to at least three antibiotics. Sixteen percent of the isolates were resistant to ceftriaxone, the drug of choice for treating salmonellosis in children. Bacteriophage typing identified four isolates of *Salmonella enterica* serotype typhimurium definitive type 104 (DT104), one of DT104b, and two of DT208. Five isolates of *S. enterica* serotype agona had resistance to 9 antibiotics, and the two isolates of serotype typhimurium DT208 were resistant to 12 antibiotics. Electrophoretic patterns of DNA that were indistinguishable from one another were repeatedly found in isolates from different meat samples and different stores. Eighteen isolates, representing four serotypes, had integrons with genes conferring resistance to aminoglycosides, sulfonamides, trimethoprim, and  $\beta$ -lactams.

**Conclusions** Resistant strains of salmonella are common in retail ground meats. These findings provide support for the adoption of guidelines for the prudent use of antibiotics in food animals and for a reduction in the number of pathogens present on farms and in slaughterhouses. National surveillance for antimicrobial-resistant salmonella should be extended to include retail meats. (N Engl J Med 2001; 345:1147-54.)

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FOODBORNE diseases caused by nontyphoid salmonella represent an important public health problem worldwide. Nearly 1.4 million cases of salmonellosis occur each year in the United States.<sup>1</sup> Most salmonella infections in humans result from the ingestion of contaminated poultry, beef, pork, eggs, and milk.<sup>2</sup> Intestinal salmonellosis typically resolves in five to seven days and does not require treatment with antibiotics. However, bacteremia occurs in 3 to 10 percent of reported, culture-confirmed cases and is particularly common among patients at the extremes of age and those who are immunocompromised. When infection spreads beyond the intestinal tract, appropriate antimicrobial therapy (e.g., ciprofloxacin in adults and ceftriaxone in children) can be lifesaving.<sup>3,4</sup>

The use of antimicrobial agents in any environment creates selection pressures that favor the survival of antibiotic-resistant pathogens. According to the infectious-disease report that was released by the World Health Organization in 2000, such organisms have become increasingly prevalent worldwide.<sup>5</sup> The routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans through the food chain.<sup>6,7</sup> Most infections with antimicrobial-resistant salmonella are acquired by eating contaminated foods of animal origin.<sup>8,9</sup>

There is now widespread dissemination of multidrug-resistant *Salmonella enterica* serotype typhimurium, particularly definitive type 104 (DT104).<sup>4</sup> Recent studies have documented a ceftriaxone-resistant salmonella infection in a child that was acquired

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through exposure to cattle<sup>9</sup> and the emergence of ceftriaxone-resistant salmonella infections in humans in the United States.<sup>10</sup> The sources of salmonella infections are often unknown, but they most likely originate in contaminated food of animal origin. We isolated and characterized salmonella strains from ground meats obtained in retail markets in the greater Washington, D.C., area and determined the antimicrobial-resistance phenotypes of the isolates.

## METHODS

### Collection of Retail Meat Samples and Isolation of Salmonella

Two hundred samples of ground meat (51 samples of chicken, 50 of beef, 50 of turkey, and 49 of pork) were purchased at three retail stores representing three supermarket chains in the greater Washington, D.C., area between June and September 1998: 98 at store 1, 54 at store 2, and 48 at store 3. All poultry and pork samples were processed and packaged at one of four poultry-processing plants and one pork-processing plant, respectively, whereas all samples of beef were ground in the store and then packaged. Salmonella was isolated from ground meats with the use of methods described in the *Bacteriological Analytical Manual* of the Food and Drug Administration.<sup>11</sup>

### Serotyping, Phage Typing, and Pulsed-Field Gel Electrophoresis

Salmonella serotypes were determined with the use of commercial antiserum (Difco, Detroit), according to the manufacturer's instructions. Eight isolates of *S. enterica* typhimurium that were resistant to at least five antibiotics were selected for phage-typing analysis, conducted at the National Veterinary Services Laboratories of the Department of Agriculture in Ames, Iowa.

Pulsed-field gel electrophoresis was used for separation of DNA fragments resulting from digestion by restriction enzymes. The resulting genomic-DNA profiles, or "fingerprints," were interpreted according to established guidelines.<sup>12-14</sup> To be considered part of a cluster, the DNA patterns could not differ from each other by more than 30 percent. Patterns that were the same size and had the same numbers of bands were considered to be the same strain (e.g., type A). Patterns that differed by fewer than four bands were considered to represent subtypes within the main group (e.g., A1, A2, and A3). Patterns that differed from the main pattern by four or more bands were considered to represent different strains (e.g., type A, B, or C).

### Testing for Antimicrobial Susceptibility

Salmonella isolates were assayed for susceptibility to 17 antibiotics used by the National Antimicrobial Resistance Monitoring System.<sup>15</sup> Minimal inhibitory concentrations were determined by the broth-microdilution method with use of the Sensititre system (Trek Diagnostic Systems, Westlake, Ohio) and recommended quality-control organisms. The results were interpreted in accordance with the standards of the National Committee for Clinical Laboratory Standards, when available.<sup>16,17</sup>

### Amplification

Since resistance to sulfa antimicrobial agents is characteristic of class I integrons, sulfamethoxazole-resistant salmonella isolates were screened for the presence of such integrons. In addition, isolates that demonstrated resistance to the extended-spectrum cephalosporins ceftiofur and ceftriaxone were examined for the presence of the extended-spectrum  $\beta$ -lactamase gene *bla*<sub>CMY-2</sub>. Class I integrons were amplified with the use of the polymerase chain reaction (PCR) and primers 5'-CS (5'GGCATCCAAGCACAAAGC3') and 3'-CS (5'AAGCAGACTTGACTGAT3').<sup>18</sup> The *bla*<sub>CMY-2</sub> gene

was amplified with the use of primers *cmv*-F (5'GACAGCCTCT-TTCTCCACA3') and *cmv*-R (5'TGGAACGAAGGCTACGTA3').<sup>19</sup> Amplifications were carried out as described previously.<sup>18,19</sup>

### Nucleotide-Sequencing Analysis

PCR products of integrons and the *bla*<sub>CMY</sub> genes were purified with a kit (Boehringer Mannheim, Indianapolis). The DNA sequences were determined at the University of Maryland, College Park, and compared with use of the Basic Local Alignment Search Tool (National Center for Biotechnology Information, Bethesda, Md.).<sup>20</sup>

## RESULTS

### Serotypes

Salmonella isolates were recovered from 41 of 200 samples of ground meat (20 percent); 4 samples each yielded two strains of salmonella. Salmonella was isolated more frequently from poultry (35 percent of chicken samples and 24 percent of turkey samples) than from pork (16 percent of samples) or beef (6 percent of samples). Thirteen serotypes were identified among the 45 salmonella isolates (Table 1); *S. enterica* serotype Istanbul (28 percent) and *S. enterica* serotype agona (22 percent) were isolated most frequently. All 13 isolates of *S. enterica* serotype Istanbul were recovered from chicken purchased from two stores on various sampling dates. In contrast, *S. enterica* serotype agona was isolated from all four types of ground meat, with turkey being the most frequent source (7 of 10 samples). Four of the eight isolates of *S. enterica* serotype typhimurium were from chicken, and four were from pork.

On three occasions, two different serotypes were isolated from the same sample (Table 1). For example, *S. enterica* serotype chomedei was also isolated from one of the three pork samples from which *S. enterica* serotype typhimurium DT104 was recovered. Serotypes typhimurium DT104b and Derby were both identified in the same pork sample from store 3, and serotypes agona and Kentucky were isolated from the same chicken sample from store 2.

### Antimicrobial Resistance

Eighty-four percent of isolates (38 of 45) displayed resistance to at least one antibiotic, and 53 percent (24 of 45) displayed resistance to at least three antibiotics. Among multidrug-resistant isolates, resistance to streptomycin, sulfamethoxazole, and tetracycline was most often observed (Table 1). The 10 isolates of *S. enterica* serotype agona displayed three resistance phenotypes, with 5 isolates exhibiting resistance to nine antibiotics (including ceftriaxone). The 13 isolates of *S. enterica* serotype Istanbul were all resistant to streptomycin and tetracycline, and 6 of the 13 were also resistant to sulfamethoxazole. Seven of the eight isolates of *S. enterica* serotype typhimurium displayed resistance to at least five antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline — the typical resistance profile

of serotype typhimurium DT104. Bacteriophage typing identified four of these isolates as DT104, one as DT104b, and two as DT208. The two DT208 isolates, which were recovered from samples of ground chicken, showed similar patterns on pulsed-field gel electrophoresis, and both displayed resistance to the same 12 antimicrobial agents, including ceftriaxone.

All strains of salmonella were susceptible to amikacin, apramycin, ciprofloxacin, and nalidixic acid (Table 2). The isolates were most likely to be resistant to tetracycline (80 percent of isolates), streptomycin (73 percent), sulfamethoxazole (60 percent), and to a lesser extent, ampicillin (27 percent). In addition, 16 percent of the isolates displayed resistance to florfenicol, chloramphenicol, amoxicillin-clavulanic acid, cephalothin, ceftiofur, and ceftriaxone (Table 2). Isolates recovered from ground turkey and beef were susceptible to chloramphenicol, florfenicol, and kanamycin, whereas the two gentamicin-resistant isolates were both recovered from ground chicken. Ceftiofur and ceftriaxone-resistant strains were isolated from ground turkey, chicken, and beef but not from ground pork.

#### Antibiotic-Resistance Integrons and *bla*<sub>CMY</sub> Genes

Eighteen of 27 sulfamethoxazole-resistant isolates encompassing four serotypes (agona, chomedey, djugu, and typhimurium) possessed class 1 integrons (Table 1). The sizes of these integrons ranged from 0.75 to 2.7 kb. The five isolates of *S. enterica* serotype agona that were resistant to nine antibiotics had an integron of 1.2 kb, whereas the three isolates that were resistant to streptomycin, sulfamethoxazole, and tetracycline had an integron of 1.0 kb. The 2.0-kb integrons identified in *S. enterica* serotypes chomedey and djugu contained two genes: aminoglycoside adenyltransferase A2 (*aadA2*), which confers resistance to streptomycin and spectinomycin, and dihydrofolate reductase XII (*dfrXII*), which confers resistance to trimethoprim (Table 1). It is interesting to note that these isolates were phenotypically susceptible to streptomycin, which suggests that the *aadA2* gene is not being expressed. The 1.0-kb and 1.2-kb integrons characterized in isolates of *S. enterica* serotype agona contained the *aadA1* gene, which confers resistance to streptomycin.

All eight isolates of *S. enterica* serotype typhimurium possessed integrons. Four DT104 isolates and one DT104b isolate possessed a 1.0-kb integron containing *aadA2* and a 1.2-kb integron containing the  $\beta$ -lactamase gene *bla*<sub>PSE-13</sub>, which confers resistance to ampicillin. The largest integrons (2.7 kb) were identified in the two isolates of *S. enterica* serotype typhimurium DT208, which were resistant to 12 of the 17 antimicrobial agents tested. DNA-sequence analysis identified the *aadA* gene and three open reading frames that have yet to be characterized. In addition, the 0.75-kb integron identified in the remaining *S. en-*

*terica* serotype typhimurium isolate contained the *dfrXIII* gene, which confers resistance to trimethoprim. The five isolates of *S. enterica* serotype agona and the two isolates of *S. enterica* serotype typhimurium DT208 that were resistant to ceftiofur and ceftriaxone had a plasmid-mediated *bla*<sub>CMY-2</sub>  $\beta$ -lactamase gene (Table 1).

#### Results of Pulsed-Field Gel Electrophoresis

Of the 45 isolates, 36 were differentiated with the use of pulsed-field gel electrophoresis. One isolate of *S. enterica* serotype agona was untypable. Overall, eight pulsed-field gel electrophoresis strain types (A through H) and six clusters were identified (Fig. 1). The nine typable isolates of *S. enterica* serotype agona were from three strains (types A, B, and C) and had five electrophoretic patterns. The five type A isolates of *S. enterica* serotype agona were resistant to the same nine antibiotics, whereas the three type C isolates were resistant to only three. These three isolates had identical electrophoretic patterns and were recovered from one sample of ground pork and two samples of ground turkey purchased on different sampling dates from different grocery stores. One isolate of *S. enterica* serotype agona was susceptible to all 17 antibiotics and was the only strain categorized as type B.

The 13 isolates of *S. enterica* serotype Istanbul were recovered from two brands of ground chicken and formed a single strain (type D) with three closely related patterns on pulsed-field gel electrophoresis (Fig. 1). Ten of these isolates had the same pattern (type D1). The 13 isolates were recovered from two stores on five sampling dates. Four isolates of *S. enterica* serotype Reading were isolated from turkey from two stores on two dates and had similar electrophoretic patterns.

The four isolates of *S. enterica* serotype typhimurium DT104 had identical patterns (all were type F1) that differed from the pattern of serotype typhimurium DT104b (type F2) by a single band, indicating close similarity. The two isolates of serotype typhimurium DT208 were from samples of ground chicken obtained from the same store on the same date and had closely related patterns (G1 and G2) and identical resistance phenotypes. The two isolates of *S. enterica* serotype orion were recovered from chicken and turkey from the same store and had identical patterns on pulsed-field gel electrophoresis (type H).

#### DISCUSSION

In this study, 20 percent of ground meat samples from supermarkets in the greater Washington, D.C., area were contaminated with 13 serotypes of salmonella. Of particular importance is the isolation of ceftriaxone-resistant salmonella as well as multidrug-resistant *S. enterica* serotype typhimurium definitive types DT208 and DT104. The latter can cause severe illness and is usually resistant to ampicillin, chloram-

**TABLE 1.** CHARACTERISTICS OF SALMONELLA ISOLATED FROM GROUND MEAT FROM THREE SUPERMARKETS IN THE GREATER WASHINGTON, D.C., AREA, JUNE TO AUGUST 1998.\*

ISOLATE NO.	S. ENTERICA SEROTYPE	ANTIBIOTIC-RESISTANCE PROFILE	SIZE (kb) OF INTEGRON AND IDENTITY OF RESISTANCE GENE†	bla <sub>CMY-2</sub> PRESENT‡	TYPE AND BRAND OF GROUND MEAT	STORE NO.	PFGE PATTERN§	SAMPLING DATE
1	Agona	Amo, Amp, Cef, Cet, Cep, Str, Sul, Tet, Tri	1.2, <i>aadA1</i>	Yes	Turkey, c	1	A3	6/8/98
2	Agona	Amo, Amp, Cef, Cet, Cep, Str, Sul, Tet, Tri	1.2, <i>aadA1</i>	Yes	Turkey, d	1	A2	6/15/98
3	Agona	Amo, Amp, Cef, Cet, Cep, Str, Sul, Tet, Tri	1.2, <i>aadA1</i>	Yes	Turkey, d	1	A2	6/15/98
4	Agona	Amo, Amp, Cef, Cet, Cep, Str, Sul, Tet, Tri	1.2, <i>aadA1</i>	Yes	Turkey, d	1	A1	6/15/98
5	Agona	Amo, Amp, Cef, Cet, Cep, Str, Sul, Tet, Tri	1.2, <i>aadA1</i>	Yes	Beef, f	1	A1	6/22/98
6	Agona	Str, Sul, Tet	1.0, <i>aadA1</i>	NT	Turkey, d	1	C	6/22/98
7	Agona	Str, Sul, Tet	1.0, <i>aadA1</i>	NT	Pork, e	1	C	6/22/98
8	Agona	Str, Sul, Tet	1.0, <i>aadA1</i>	NT	Turkey, d	2	C	6/30/98
9¶	Agona	Str, Tet	NT	NT	Chicken, a	2	UT	6/30/98
10	Agona		NT	NT	Turkey, c	2	B	8/1/98
11	Chomedey	Sul, Tri	2.0, <i>dfjXII</i> and <i>aadA2</i>	NT	Pork, e	1	NT	6/30/98
12	Djugu	Sul, Tri	2.0, <i>dfjXII</i> and <i>aadA2</i>	NT	Pork, e	3	NT	7/17/98
13**	Derby	Tet	NT	NT	Pork, e	3	NT	7/17/98
14	Heidelberg	Kan, Str, Sul, Tet	Absent	NT	Pork, e	3	NT	7/17/98

\*PFGE denotes pulsed-field gel electrophoresis, Amo amoxicillin–clavulanate, Amp ampicillin, Cef ceftiofur, Cet ceftriaxone, Cep cephalothin, Str streptomycin, Sul sulfamethoxazole, Tet tetracycline, Tri trimethoprim, NT not tested, UT untypable, Cml chloramphenicol, Ffc florfenicol, Gen gentamicin, and Kan kanamycin. The letters a through f indicate brands of meat.

†Sulfamethoxazole-resistant isolates were screened for class I integrons, and the following were identified: the gene for aminoglycoside adenyltransferase (*aadA*), the gene for dihydrofolate reductase (*dhfr*), the  $\beta$ -lactamase gene (*bla<sub>PSE-1</sub>*), and open reading frames (ORFs).

‡Isolates that were resistant to ceftiofur or ceftriaxone were screened for the extended-spectrum  $\beta$ -lactamase gene *bla<sub>CMY-2</sub>*.

§Patterns that differed by fewer than four bands were considered to represent subtypes within the main group (e.g., A1, A2, and A3). Patterns that differed from pattern A by four or more bands were considered to represent different strains.

¶Isolates 9 and 28 were from the same sample.

||Isolates 11 and 40 were from the same sample.

\*\*Isolates 13 and 42 were from the same sample.

††Isolates 19 and 20 were from the same sample.

phenicol, streptomycin, sulfonamides, and tetracycline.<sup>21</sup> The number of cases of infection with serotype typhimurium DT104 has increased in many countries.<sup>22-25</sup> A retrospective study of infections with *S. enterica* serotype typhimurium by the Centers for Disease Control and Prevention revealed that the percentage of isolates that were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline increased from 0.6 percent during the period from 1979 to 1980 to 34 percent in 1996.<sup>4</sup> Serotype typhimurium DT104 has been identified in numerous foods, including beef, pork, poultry, and unpasteurized dairy products, and its presence is thought to be due to the widespread clonal dissemination of multidrug-resistant isolates.<sup>4,26,27</sup> Foodborne transmission of this serotype has been well documented, and several outbreaks have involved the consumption of contaminated meat and dairy products or contact with cattle.<sup>22,28-31</sup> The isolation of *S. enterica* serotype typhimurium DT208 from

ground meats sold in supermarkets is also cause for concern, given the extensive pattern of resistance of this organism.

Our finding of identical isolates of *S. enterica* serotype Istanbul from two brands of ground chicken samples from two grocery stores over a seven-week sampling period demonstrates the potential for the contamination of food during handling and processing. However, the contamination of retail meats with resistant salmonella mainly reflects carriage of the organism by livestock; intervention strategies should therefore focus principally on reducing the number of pathogens present on farms and in slaughterhouses.

Although *S. enterica* serotype agona is less commonly associated with disease in humans than is *S. enterica* serotype typhimurium, it has been the cause of several foodborne outbreaks in recent years.<sup>32-34</sup> Our study identified five such isolates that were resistant to nine antibiotics, including ceftriaxone and ceftiofur. These isolates were recovered from differ-

## ISOLATION OF ANTIBIOTIC-RESISTANT SALMONELLA FROM RETAIL GROUND MEATS

TABLE 1. CONTINUED.

ISOLATE No.	<i>S. ENTERICA</i> SEROTYPE	ANTIBIOTIC-RESISTANCE PROFILE	SIZE (kb) OF INTEGRON AND IDENTITY OF RESISTANCE GENE†	<i>bla</i> <sub>CMY-2</sub> PRESENT‡	TYPE AND BRAND OF GROUND MEAT	STORE No.	PFGE PATTERN§	SAMPLING DATE
15	Istanbul	Str, Tet	NT	NT	Chicken, a	1	D1	7/29/98
16	Istanbul	Str, Tet	NT	NT	Chicken, a	1	D1	7/21/98
17	Istanbul	Str, Tet	NT	NT	Chicken, b	1	D1	7/21/98
18	Istanbul	Str, Tet	NT	NT	Chicken, b	1	D1	7/21/98
19††	Istanbul	Str, Tet	NT	NT	Chicken, a	1	D1	6/8/98
20††	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, a	1	D1	6/8/98
21	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, a	3	D1	7/15/98
22	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, a	3	D1	7/15/98
23	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, b	1	D1	7/29/98
24	Istanbul	Str, Tet	NT	NT	Chicken, b	1	D1	7/29/98
25	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, b	1	D2	7/29/98
26	Istanbul	Str, Tet	NT	NT	Chicken, a	1	D3	7/10/98
27	Istanbul	Str, Sul, Tet	Absent	NT	Chicken, a	3	D3	7/10/98
28¶	Kentucky	Str, Sul, Tet	Absent	NT	Chicken, a	2	NT	6/30/98
29	Meleagridis		NT	NT	Beef, f	3	NT	7/29/98
30	Orion	Str, Tet	NT	NT	Chicken, a	1	H	6/8/98
31	Orion	Str, Tet	NT	NT	Turkey, c	1	H	6/8/98
32	Reading		NT	NT	Turkey, d	3	E2	7/15/98
33	Reading		NT	NT	Turkey, d	3	E2	7/15/98
34	Reading		NT	NT	Turkey, c	1	E1	7/15/98
35	Reading		NT	NT	Turkey, c	1	E1	8/1/98
36	Seftenberg	Sul, Tet	Absent	NT	Pork, e	3	NT	8/6/98
37	Sinstorf		NT	NT	Beef, f	1	NT	7/27/98
38	Typhimurium DT104	Amp, Cml, Ffc, Str, Sul, Tet	1.0, <i>aadA2</i> ; 1.2, <i>bla</i> <sub>PSE-1</sub>	NT	Pork, e	2	F1	6/30/98
39	Typhimurium DT104	Amp, Cml, Ffc, Str, Sul, Tet	1.0, <i>aadA2</i> ; 1.2, <i>bla</i> <sub>PSE-1</sub>	NT	Pork, e	2	F1	6/30/98
40	Typhimurium DT104	Amp, Cml, Ffc, Str, Sul, Tet	1.0, <i>aadA2</i> ; 1.2, <i>bla</i> <sub>PSE-1</sub>	NT	Pork, e	1	F1	6/30/98
41	Typhimurium DT104	Amp, Cml, Ffc, Str, Sul, Tet	1.0, <i>aadA2</i> ; 1.2, <i>bla</i> <sub>PSE-1</sub>	NT	Chicken, a	1	F1	7/10/98
42**	Typhimurium DT104b	Amp, Cml, Ffc, Str, Sul, Tet	1.0, <i>aadA2</i> ; 1.2, <i>bla</i> <sub>PSE-1</sub>	NT	Pork, e	3	F2	7/7/98
43	Typhimurium DT208	Amo, Amp, Cef, Cet, Cep, Cml, Ffc, Gen, Kan, Str, Sul, Tet	2.7, <i>aadA</i> and 3 ORFs	Yes	Chicken, b	1	G1	7/25/98
44	Typhimurium DT208	Amo, Amp, Cef, Cet, Cep, Cml, Ffc, Gen, Kan, Str, Sul, Tet	2.7, <i>aadA</i> and 3 ORFs	Yes	Chicken, b	1	G2	7/25/98
45	Typhimurium	Sul, Tet, Tri	0.75, <i>dfrXIII</i>	NT	Chicken, a	1	NT	6/25/98

ent types of ground meat (turkey and beef) from the same store over a two-week period. These meats were ground at three different facilities, suggesting that the source of this serotype was the meat itself.

Ceftiofur is the only expanded-spectrum cephalosporin approved for use in food animals in the United States. Ceftriaxone is commonly used to treat children with salmonella infections, particularly invasive infections, because of its favorable pharmacokinetic properties and the low prevalence of resistance.<sup>10</sup> Previous reports have described salmonella strains with the same plasmid-mediated  $\beta$ -lactamase resistance gene (*bla*<sub>CMY-2</sub>), which confers resistance to both ceftiofur and ceftriaxone, that we found in our

salmonella isolates.<sup>9,10,19</sup> Thus, it has been argued that the use of ceftiofur in livestock accelerated the rate of the development of resistance to ceftriaxone in salmonella.<sup>10,19</sup> Though our data could not be used to attribute the presence of ceftriaxone-resistant phenotypes to the use of ceftiofur in livestock, it does support previous findings that foods of animal origin are potential sources of ceftriaxone-resistant salmonella infections in humans.<sup>9</sup> The dissemination of salmonella that is resistant to multiple drugs, including cephalosporins, through food has important public health implications.

The ability of bacteria to acquire antibiotic-resistance genes and subsequently spread them to many

**TABLE 2.** RESISTANCE PHENOTYPES OF SALMONELLA ISOLATED FROM GROUND MEAT.

ANTIMICROBIAL AGENT	MIC*	GROUND CHICKEN	GROUND TURKEY	GROUND PORK	GROUND BEEF	TOTAL (N=45)
		(N=20)	(N=12)	(N=10)	(N=3)	
	$\mu\text{g/ml}$	no. of resistant strains (%)				
Phenicol						
Florfenicol†	$\geq 8$	3	0	4	0	7 (16)
Chloramphenicol	$\geq 32$	3	0	4	0	7 (16)
Penicillins						
Ampicillin	$\geq 32$	3	4	4	1	12 (27)
Amoxicillin-clavulanate	$\geq 32$	2	4	0	1	7 (16)
Cephalosporins						
Cephalothin	$\geq 32$	2	4	0	1	7 (16)
Ceftiofur†	$\geq 8$	2	4	0	1	7 (16)
Ceftriaxone	$\geq 64$	2	4	0	1	7 (16)
Tetracycline	$\geq 16$	20	7	8	1	36 (80)
Aminoglycosides						
Amikacin	$\geq 64$	0	0	0	0	0
Apramycin†	$\geq 32$	0	0	0	0	0
Gentamicin	$\geq 16$	2	0	0	0	2 (4)
Kanamycin	$\geq 64$	2	0	1	0	3 (7)
Streptomycin	$\geq 64$	19	7	6	1	33 (73)
Sulfonamides and potentiated sulfonamides						
Sulfamethoxazole	$\geq 512$	11	6	9	1	27 (60)
Trimethoprim-sulfamethoxazole	$\geq 4$	1	4	2	1	8 (18)
Quinolones and fluoroquinolones						
Nalidixic acid	$\geq 32$	0	0	0	0	0
Ciprofloxacin	$\geq 4$	0	0	0	0	0

\*Minimal inhibitory concentrations (MICs) were determined by the broth-microdilution method, and the results were interpreted in accordance with the standards of the National Committee for Clinical Laboratory Standards.<sup>16,17</sup>

†This antimicrobial agent is used exclusively in animals.

different bacterial species is well known.<sup>35</sup> Integrons, one such mobile DNA element, have been associated with the transfer of resistance and often contain one or more linked antimicrobial-resistance genes.<sup>36</sup> Integrons are therefore particularly important, since a strong selection pressure exerted by antibiotics can potentially result in the mobilization and dissemination of linked multidrug-resistance phenotypes. The two integrons we identified in the isolates of *S. enterica* serotypes typhimurium DT104 and DT104b were identical to those characterized in strains of typhimurium DT104 found in many other outbreaks.<sup>18,21</sup>

We also identified integrons conferring resistance to streptomycin and trimethoprim in other serotypes of salmonella, suggesting that integrons play an important part in the transfer of resistance among these serotypes. This conclusion is supported by recent reports describing integrons in serotypes other than typhimurium.<sup>37,38</sup> However, integrons and their associated gene cassettes did not always account for the entire observed resistance phenotype, indicating that other mechanisms were also involved.

Our findings demonstrate that multidrug-resistant strains of salmonella, including ceftriaxone-resistant

isolates, are frequently present in retail ground meats in the greater Washington, D.C., area. The presence of these strains, particularly typhimurium DT104 and DT208, is of concern, considering their extremely resistant phenotypes and the association of DT104 with numerous foodborne outbreaks. Although we have no corresponding culture data from humans, our data provide support for the theory that the food supply is a major source of antimicrobial-resistant salmonella. The high prevalence of multidrug-resistant salmonella in retail ground meats reflects a reservoir of resistance in animals that can be transmitted to humans.

Efforts are needed to reduce the prevalence of resistant salmonella in food, including the adoption of guidelines for the prudent use of antimicrobial agents in animals used for food, the passage of new food-safety regulations, and a reduction in the number of pathogens present on farms and in slaughterhouses. In addition, a national surveillance program focusing on the identification and molecular subtyping of zoonotic foodborne bacterial pathogens that are present in retail foods should be established. Such measures will supplement ongoing surveillance programs such as the National Antimicrobial Resistance



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