

Occurrence, Serotype Diversity, and Antimicrobial Resistance of *Salmonella* in Ground Beef at Retail Stores in Jalisco State, Mexico

ELISA CABRERA-DIAZ,¹ CLAUDIA M. BARBOSA-CARDENAS,¹ JULIA A. PEREZ-MONTAÑO,^{1,2}
DELIA GONZALEZ-AGUILAR,¹ CARLOS PACHECO-GALLARDO,¹ AND JEANNETTE BARBA^{1*}

¹Departamento de Salud Pública, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km. 15.5 Carretera a Nogales, Zapopan, Jalisco, México 45110; and ²Departamento de Farmacobiología, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Marcelino García Barragán no. 1421, Guadalajara, Jalisco, México 44430

MS 13-109: Received 19 March 2013/Accepted 21 June 2013

ABSTRACT

The occurrence, serotype diversity, and antimicrobial resistance of *Salmonella* bacteria in commercial ground beef at retail establishments were investigated. *Salmonella* was isolated from 135 (56.7%) of 238 ground beef samples collected at the same number of butcher's shops located in three municipalities of Jalisco State, Mexico, during an 11-month period. The isolation frequency differed by municipality ($P < 0.05$) and was higher ($P < 0.05$) during the warm season (68.5%) than during the cold season (43.2%). Overall, 25 serotypes and 8 serogroups were identified among 135 *Salmonella* isolates; predominant were *Salmonella* group B (9.6%), *Salmonella* Anatum (8.9%), *Salmonella* Agona (6.7%), *Salmonella* Infantis (6.7%), and *Salmonella* Typhimurium (5.9%). All *Salmonella* isolates were tested for susceptibility to 11 antimicrobial drugs of human and veterinary use. Resistance to tetracycline was the most commonly observed (40.7%), followed by resistance to streptomycin (35.6%), trimethoprim-sulfamethoxazole (20.7%), and nalidixic acid (19.3%). Thirty-seven *Salmonella* isolates (27.4%) were multidrug resistant, and the majority corresponded to *Salmonella* Group B, *Salmonella* Anatum, and *Salmonella* Typhimurium. Three *Salmonella* isolates were resistant to seven different antimicrobials. The frequency of *Salmonella* in ground beef samples (56.7%) was higher than that observed in our previous investigation on beef carcasses (15.4%) at small abattoirs in the same region of Mexico. This may be a result of increasing contamination at these two points of the raw-beef production chain or may be an effect of the grinding process that facilitates a more-homogeneous pathogen distribution in the product. Poor hygiene, temperature abuse, and practices allowing cross-contamination during ground beef fabrication at these retail establishments increase the consumer's exposure to *Salmonella*.

Nontyphoidal *Salmonella* has been estimated to be responsible for 1 million foodborne illnesses each year in the United States and is the leading cause of hospitalizations and deaths due to foodborne illness (26). Foods of animal origin are the most-commonly identified vehicles, and ground beef has been implicated in several salmonellosis outbreaks (4, 5, 11, 19). The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture has estimated an overall *Salmonella* prevalence of 2.4% in ground beef after analyzing 264,402 samples collected at federally inspected establishments from 1998 to 2011 (31, 32, 34). Although the FSIS has reported a decrease in *Salmonella* prevalence in ground beef from 7.5% in 1993 to 1994 to 2.4% in 2011, outbreaks of human salmonellosis associated with ground beef continue to occur in the United States.

In Mexico, ground beef is commonly fabricated at retail establishments, such as supermarkets and butcher's shops,

where cross-contamination of the product from other raw meats or improperly sanitized equipment and utensils is common. In this country, Heredia et al. (14) reported the isolation of *Salmonella* from 11.4% of 88 ground beef samples collected at retail supermarkets and butchers' shops in Monterrey City, and López-Valadez et al. (16) found the pathogen in 36% of 25 ground beef samples collected from butcher's shops in Guadalajara City. Both studies were conducted on a small sample size, of 88 and 25 samples respectively, and therefore, inferences about *Salmonella* prevalence were very limited. Nevertheless, these reports suggest that ground beef may be an important vehicle of *Salmonella* in Mexico, where more than 120,000 cases of salmonellosis are reported each year to the National Center for Epidemiological Surveillance and Diseases Control (28).

An additional concern related to the presence of *Salmonella* in foods is the isolation of multidrug-resistant (MDR) strains. MDR *Salmonella* strains have been responsible for foodborne illnesses linked to the consumption of ground beef, and some of the isolates recovered from foodborne outbreaks were resistant to 9 or 11 antimicrobials

* Author for correspondence. Tel: +52(33)37771151, Ext. 33042; Fax: +52(33)36820574; E-mail: jeannbarba@gmail.com

(5, 11, 19). Human infections caused by MDR *Salmonella* were related to adverse outcomes or higher hospitalization rates among patients (13, 37).

The present study reports the occurrence, serotype diversity, and antimicrobial resistance profiles of *Salmonella* in ground beef at retail stores located in three municipalities in Jalisco State, Mexico.

MATERIALS AND METHODS

Sample collection. A total of 238 ground beef samples were collected from the same number of butcher's shops located in three municipalities in Jalisco State, Mexico. These municipalities corresponded to Guadalajara, Tlaquepaque, and Zapopan, and they were selected because they represent some of the most-populated areas of Jalisco and have the largest numbers of butcher's shops in the state. They will be referred to hereinafter as A, B, and C, respectively. The number of registered butcher's shops at municipality A, B, and C was 147, 333, and 835, respectively (15). The number of ground beef samples collected in each municipality was calculated with Win Episcope 2.0 software (10) according to the number of establishments per municipality and the expected prevalence of *Salmonella* ($\alpha = 0.05$). Samples were collected during an 11-month period from September 2009 to July 2010. Twelve butcher's shops were randomly selected and visited every week to collect one ground beef sample from each establishment. In order to obtain each sample, the butcher was asked to coarsely grind a beef trim and provide 100 g of the resulting ground beef, as this is the regular customer practice at this type of establishment. The sample was immediately placed into a sterile plastic bag. All samples were transported to the Food Safety Laboratory of the University of Guadalajara inside insulated containers with refrigerants and analyzed within 3 h after collection.

Salmonella isolation. An aliquot of 25 g from each ground beef sample was preenriched in 225 ml of buffered peptone water (BD, Franklin Lakes, NJ), homogenized for 1 min with a BagMixer (Interscience, Mourjou, France), and incubated at 35°C for 20 to 24 h. After incubation, the preenriched sample was homogenized for 1 min with a BagMixer, and then aliquots of 1.0 and 0.1 ml were transferred into 9 and 9.9 ml of tetrathionate broth (BD) and Rapaport-Vassiliadis-10 broth (BD), respectively, and incubated at 42°C for 20 to 24 h. After incubation, 0.5-ml aliquots of each broth were transferred to a sterile microtube and centrifuged at 9,200 $\times g$ for 3 min. The pellets were used for DNA extraction according to the instructions described by the manufacturer of the Wizard Genomic DNA Purification Kit (Wizard, Promega, Madison, WI). A multiplex PCR assay was performed to detect the presence of the *invA* (544 bp) and *fimA* (686 bp) genes using previously described primers and methods (23).

For those samples testing positive in the PCR test, aliquots of tetrathionate broth and Rapaport-Vassiliadis-10 broth were individually streaked onto brilliant green sulfa agar (BD) and xylose lysine Tergitol 4 agar (BD). All plates were incubated at 35°C for 24 to 48 h (33), and from each agar plate, three colonies showing characteristics typical for *Salmonella* were selected, if available, streaked onto triple sugar iron agar (BD) and lysine iron agar (BD), and incubated at 35°C for 24 h. *Salmonella* isolates were confirmed by slide agglutination using polyclonal serum A-Vi (BD) and by a PCR test. For the PCR test, DNA was obtained by cell lysis at 90°C for 5 min, and the conditions were the same as previously described (23). *Salmonella* isolates were stored in 15% glycerol tryptic soy broth (BD) at -20°C, and working cultures

were maintained on tryptic soy agar slants at 4°C. One isolate from each positive sample was randomly selected for serotyping and antimicrobial susceptibility testing.

Serotyping. A total of 135 *Salmonella* isolates were selected for serotyping. Each isolate was reactivated in tryptic soy broth at 35°C for 24 h and then individually streaked on brilliant green sulfa agar plates. One colony with characteristics typical for *Salmonella* was individually inoculated on tryptic soy agar slants, incubated at 35°C for 24 h, and then reconfirmed biochemically, serologically, and by PCR as previously described. Isolates were shipped to the National Laboratory for Diagnosis and Epidemiological Reference (Mexico City, Mexico) for serotype identification according to the White-Kauffmann scheme.

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined for 135 *Salmonella* isolates using the disk diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (8). Antimicrobial susceptibility test discs (BBL, BD, Sparks, MD) were used for the following antimicrobials of veterinary and human health importance: ampicillin (AMP, 10 µg), tetracycline (TET, 30 µg), nalidixic acid (NAL, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), ciprofloxacin (CIP, 30 µg), cephalothin (CEP, 30 µg), ceftriaxone (CRO, 30 µg), kanamycin (KAN, 30 µg), streptomycin (STR, 10 µg), gentamicin (GEN, 10 µg), and chloramphenicol (CHL, 30 µg). *Escherichia coli* ATCC 25922 was used as a quality-control microorganism. The diameters of the zones of complete inhibition were measured, and isolates were classified as resistant, intermediate, or susceptible according to the Clinical and Laboratory Standards Institute document M100-S18 (9). When resistance to three or more antimicrobials was observed in a single *Salmonella* isolate, it was considered MDR (20, 23).

Data analysis. The significant differences ($P < 0.05$) in *Salmonella* isolation frequency by municipality and season were assessed by the chi-square test using Statistical Package for Social Science, version 11.5.1 for Windows (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Overall, *Salmonella* was isolated from 135 (56.7%) of 238 ground beef samples collected from the same number of butcher's shops located at three different municipalities in Jalisco State, Mexico, during an 11-month period. The isolation frequencies of *Salmonella* were 59.3, 71.7, and 50.3% for samples collected at butcher's shops located in municipalities A, B, and C, respectively, and the frequency was significantly higher for samples collected at municipality B than at municipality C ($P < 0.05$) (Table 1). This indicates that the *Salmonella* occurrence in ground beef at retail establishments observed in this study is higher than previously reported for other areas in Mexico and for other countries. However, comparison among studies should be done cautiously due to differences in beef production and commercialization systems, as well as in sampling and isolation procedures. In the United States, the progress report for *Salmonella* testing in raw meats reported by the FSIS indicates an overall *Salmonella* prevalence of 2.4% after analyzing 241,985 samples of ground beef produced at federally inspected establishments from 1998 to 2009 (32). Another investigation conducted in the United States (3) reported the isolation of *Salmonella* in 4.2% of 4,136

TABLE 1. Frequency of *Salmonella* in ground beef at retail establishments in three municipalities of Jalisco State, Mexico

Municipality	Total no. ^a	No. of positive samples/no. of samples analyzed and % positive					
		Warm season ^b		Cold season ^c		Total	
		No.	%	No.	%	No.	%
A	27	7/11	63.6	9/16	56.3	16/27	59.3 AB ^d
B	60	29/39	74.4	14/21	66.7	43/60	71.7 B
C	151	51/77	66.2	25/74	33.8	76/151	50.3 A
Total	238	87/127	68.5 x ^e	48/111	43.2 y	135/238	56.7

^a Total number of ground beef samples analyzed per municipality and overall.

^b April to October (months of the year with an average temperature of >20°C).

^c November to March (months of the year with an average temperature of <20°C).

^d Values in the same column with the same letter (A, B, C) are not significantly different ($P > 0.05$).

^e Values in the same row with the same letter (x, y) are not significantly different ($P > 0.05$).

ground beef samples collected from 18 large producers who supply ground beef as patties or case-ready products to numerous restaurants and grocery chains across the country. Similar results were found in Seattle, Washington, where *Salmonella* was isolated from 3.8% of 1,750 ground beef samples collected at retail stores (25). In the United States, all federally inspected meat establishments have been required to implement the Hazard Analysis and Critical Control Point (HACCP) system (31), and the low *Salmonella* prevalence in ground beef produced at these types of establishments may be a consequence of pathogen control systems implemented by the beef production industry in this country. According to the FSIS, the *Salmonella* occurrence in ground beef has decreased from 7.5% to 2.4% with the initiation of the HACCP plans at large and small beef processors.

In Belgium, *Salmonella* was isolated from 3.5% of 1,384 ground beef samples collected from supermarkets and butcher's shops representing the entirety of Belgian establishments (12); in Canada, *Salmonella* was found in 1.3% of 1,002 ground beef samples purchased from retail outlets (29). In Turkey, *Salmonella* was isolated from 21.3% of 75 ground beef samples from retail stores (1), and a report from Senegal indicates that 87.4% of raw beef samples from retailers were positive for *Salmonella* (30). In Mexico, *Salmonella* occurrence was reported in 11.4% of 88 and 36% of 25 samples of ground beef collected at retail supermarkets and butcher's shops (14, 16).

Several factors may account for the high frequency of *Salmonella* isolation found in the present investigation. All butcher's shops included in this study obtain the beef trimmings for ground beef fabrication from small, local, nonfederally inspected abattoirs. Our research group conducted a previous investigation at this type of abattoir (23) and found that all of them failed to comply with good manufacturing practices and that none of them apply antimicrobial interventions on beef carcasses or have implemented a food safety system. Moreover, it was observed that 15.4% (6.4 to 28.6%) of beef carcasses produced at this type of harvesting establishment were positive for *Salmonella*, and *E. coli* was detected in 96% of beef carcass sponge samples, with Log CFU/cm² ranging

from -1.5 to 4.0. The presence of *E. coli* on 96% of the beef carcasses sampled clearly indicated that those abattoirs did not control fecal contamination. On the other hand, temperature abuse of beef trimmings used for ground beef fabrication at the butcher's shops visited was commonly observed, and cross-contamination, caused by grinding of beef and pork trimmings in the same grinder with no cleaning and sanitizing procedures in between, was also observed in most of the establishments during visits. These inappropriate handling practices and storage conditions facilitate the contamination of the product and allow for pathogen multiplication, increasing the consumer's exposure. A large outbreak of *Salmonella* Typhimurium infections was linked to ground beef consumption, and the epidemiological evidence suggested that incomplete sanitation of the meat grinder from the butcher's shop that supplied the product and the ingestion of raw ground beef were the main contributing factors in the outbreak (24).

The *Salmonella* isolation frequency was significantly higher ($P < 0.05$) during those months corresponding to the warm season (68.5%; Table 1) than during the cold season (43.2%). These results are consistent with other studies reporting a seasonal prevalence of *Salmonella* in ground beef (3) and beef carcasses (2), with higher isolation during the warmer months of the year.

Overall, 25 serotypes and 8 serogroups (partially identified serotypes) were identified among 135 *Salmonella* isolates (Table 2). *Salmonella* group B, recovered from 9.6% of the samples, followed by *Salmonella* Anatum (8.9%), *Salmonella* Agona (6.7%), *Salmonella* Infantis (6.7%), and *Salmonella* Typhimurium, *Salmonella* Havana, and *Salmonella* group E1 monophasic (all at 5.9%), were the predominant serotypes. The more-widely distributed serotypes were *Salmonella* group E1, *Salmonella* Anatum, and *Salmonella* Typhimurium, since they were found in samples from 26 different butcher's shops located in the three municipalities included in the study. *Salmonella* Infantis, *Salmonella* Braenderup, *Salmonella* Adelaide, and *Salmonella* groups G1 and G2 were isolated only from samples collected in municipality C, while *Salmonella* Worthington, *Salmonella* Azteca, *Salmonella* Cannstatt, *Salmonella* Montevideo, *Salmonella* Muenchen, *Salmonella*

TABLE 2. *Salmonella* serotypes isolated from ground beef at retail establishments in three municipalities of Jalisco State, Mexico

<i>Salmonella</i> serotype	No. (%) of isolates	Municipality
Anatum	12 (8.9)	A, B, C
Agona	9 (6.7)	B, C
Infantis	9 (6.7)	C
Havana	8 (5.9)	B, C
Typhimurium	8 (5.9)	A, B, C
Derby	7 (5.2)	A, B
Sinstorf	6 (4.4)	B, C
Panama	5 (3.7)	B, C
Brandenburg	4 (3.0)	A, C
Give	4 (3.0)	B, C
Rissen	4 (3.0)	A, C
Saintpaul	4 (3.0)	A, C
Albany	2 (1.5)	B, C
Braenderup	2 (1.5)	C
Bredeney	2 (1.5)	A, C
Kentucky	2 (1.5)	B, C
Lockleaze	2 (1.5)	A, C
Worthington	2 (1.5)	B
Adelaide	1 (0.7)	C
Azteca	1 (0.7)	B
Cannstatt	1 (0.7)	B
Montevideo	1 (0.7)	B
Muenchen	1 (0.7)	B
Muenster	1 (0.7)	B
Reading	1 (0.7)	A
Partially serotyped		
Group B	13 (9.6)	A, C
Group E1 monophasic	8 (5.9)	B, C
Group E1	6 (4.4)	A, B, C
Group C1	2 (1.5)	B
Group G2	2 (1.5)	B
Group G2 monophasic	1 (0.7)	C
Group G1	1 (0.7)	C
Group 18	1 (0.7)	B
Untypeable	2 (1.5)	C
Total	135 (100)	

Muenster, and *Salmonella* groups C1, G2, and 18 were exclusively identified in samples from establishments located in municipality B. The butcher's shops located in municipalities B and C purchase beef trimmings from different abattoirs, and this may account for the differences observed in serotype diversity.

Twelve serotypes (36.4% of the total identified) were similar to those previously isolated from beef carcasses at small abattoirs located in the same area of Mexico (23), demonstrating how they prevail throughout the beef production chain. The 21 serotypes not associated with beef carcass serotypes previously identified in this area may have been introduced into the ground beef at the butcher's shops where the product was fabricated. As previously explained, butcher's shops in Mexico commonly fabricate ground beef, ground pork, and sometimes ground chicken in the same grinder during the working day. This practice

facilitates the introduction of serotypes from different species into the ground meat. Furthermore, noncompliance with good manufacturing practices, poor equipment hygiene, and poor hygiene practices were commonly observed in all the butcher's shops visited, facilitating cross-contamination through surfaces, equipment, and tools that were improperly cleaned and sanitized.

Salmonella Anatum, *Salmonella* Agona, and *Salmonella* Typhimurium were some of the most-frequent serotypes found in this study and had been previously reported as frequent serotypes in retail beef samples and human clinical sources in other areas of Mexico (39, 40). *Salmonella* Anatum, *Salmonella* Typhimurium, *Salmonella* Derby, *Salmonella* Infantis, *Salmonella* Montevideo, and *Salmonella* Muenster found in the present investigation had been previously isolated from ground beef samples in other countries, including the United States (3, 41), Belgium (12), and Senegal (30). Seven serotypes identified in this study (28% of the total) are similar to those listed as the 20 most frequently found in human sources in the United States, as reported by the U.S. Centers for Disease Control and Prevention in 2009. Eight serotypes (32%), including *Salmonella* Agona, *Salmonella* Typhimurium, *Salmonella* Infantis, and *Salmonella* Montevideo, are similar to those most frequently isolated from nonhuman, nonclinical sources according to data provided by the National Veterinary Services Laboratories and reported by the U.S. Department of Health and Human Services (6). In addition, *Salmonella* Typhimurium, *Salmonella* Derby, *Salmonella* Agona, and *Salmonella* Infantis, frequently found in this study, are serotypes commonly isolated from porcine sources (6). Finding serotypes commonly related to pork may be a consequence of manufacturing practices at the butcher's shops, as previously explained.

A total of 135 *Salmonella* isolates were tested for susceptibility to eleven antimicrobial drugs of human and veterinary use by using the disk diffusion method. The results demonstrated that 54.1% (73 of 135) of *Salmonella* isolates were resistant to at least one antimicrobial drug. Resistance to TET was the most frequent, and it was found in 40.7% of the isolates (55 isolates), followed by resistance to STR in 35.6% (48 isolates), to SXT in 20.7% (28 isolates), and to NAL in 19.3% (26 isolates). Only 1 isolate (0.7%) was resistant to CIP, and no resistance to CRO or kanamycin was observed. Reduced susceptibility to NAL, CEP, and CHL was observed in 16.3, 14.8, and 8.1% of isolates (22, 20, and 11 isolates), and 5.9% of isolates (8 isolates) demonstrated reduced susceptibility to CIP or CRO. These findings are similar to those previously reported in other countries for raw meat at retail. In the United States, the National Antimicrobial Resistance Monitoring System conducts a surveillance program for retail meats to examine the prevalence of antimicrobial resistance among foodborne bacteria, including *Salmonella*. According to the National Antimicrobial Resistance Monitoring System, resistance to TET was the most frequent among *Salmonella* isolates from retail ground beef in 2006, followed by resistance to STR, AMP, and sulfisoxazole (22). In China, *Salmonella* isolates from retail raw beef were

TABLE 3. MDR phenotypes of *Salmonella* serotypes isolated from ground beef at retail establishments in Jalisco, Mexico

MDR profile ^a	No. (%) of isolates	<i>Salmonella</i> serotype(s) (no. of isolates) ^b	Municipality
AMP-TET-SXT-CEP-STR-GEN-CHL	3 (8.1)	Anatum (2), group B	B, C
AMP-TET-CEP-STR-GEN-CHL	1 (2.7)	Typhimurium	A
AMP-TET-SXT-STR-GEN-CHL	1 (2.7)	Panama	C
TET-NAL-SXT-STR-CHL	4 (10.8)	Rissen, Typhimurium (3)	A, B, C
AMP-TET-NAL-SXT-STR	4 (10.8)	Anatum, Derby, group B (2)	B, C
AMP-TET-SXT-STR-CHL	2 (5.4)	Group B, Sinstorf	C
AMP-TET-NAL-STR-CHL	1 (2.7)	Give	C
AMP-TET-SXT-STR	6 (16.2)	Agona, Brandenburg, Saintpaul, group B (3)	C
TET-SXT-STR-CHL	3 (8.1)	Anatum, Derby, Saintpaul	A, C
TET-NAL-STR-CHL	1 (2.7)	Anatum	B
AMP-TET-SXT-CEP	1 (2.7)	Group G2	B
AMP-TET-STR-CHL	1 (2.7)	Untypeable	C
AMP-TET-STR	3 (8.1)	Derby, group B, Typhimurium	A, C
TET-NAL-STR	4 (10.8)	Anatum, Derby, Saintpaul, Reading	A, B, C
TET-STR-CHL	1 (2.7)	Group B	A
TET-SXT-STR	1 (2.7)	Havana	B
Total	37 (100)		

^a AMP, ampicillin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; CEP, cephalothin; STR, streptomycin, GEN, gentamicin; CHL, chloramphenicol; NAL, nalidixic acid.

^b The numbers in parentheses represent the number of isolates of the particular serotype showing the MDR profile.

more commonly resistant to sulfamethoxazole, SXT, TET, and AMP (38). In Botswana, resistance to sulfafurazone (sulfisoxazole) was observed in 100% of *Salmonella* isolates from retail beef products, followed by resistance to TET and AMP (21). In Mexico, some reports indicate that resistance to STR, TET, AMP, and CHL was common among *Salmonella* isolates from raw beef at retail establishments (20, 39). Our previous research conducted in small abattoirs demonstrated that *Salmonella* isolates from beef carcasses were more frequently resistant to TET (46.2% of isolates), STR (42.3%), and CHL (23.2%). These antimicrobials have been used for a long time in animal production worldwide (18), and nowadays, TET is the drug marketed for food-producing animals with the largest sales and exports in the United States (35). The use of antimicrobials in animal production is affecting their intestinal microbiota and appears to be selecting for resistance in commensals and in zoonotic enteropathogens (17).

We found that 27.4% (37 of 135) of *Salmonella* isolates from ground beef were resistant to three or more antimicrobials and, thus, were considered MDR strains (Table 3). Three isolates were resistant to seven antimicrobials; these corresponded to *Salmonella* Anatum and *Salmonella* group B. The frequency of MDR *Salmonella* strains found in this study (27.4%) was lower than that observed in our previous investigation on beef carcasses (33.3%) at small abattoirs in the same region of Mexico (23). *Salmonella* group B was the most-common MDR serotype (9 of 37 isolates), followed by *Salmonella* Anatum (6 of 37 isolates) and *Salmonella* Typhimurium (5 of 37 isolates). Similarly, *Salmonella* group B and *Salmonella* Typhimurium were the most-frequent MDR serotypes we found on beef carcasses (23). MDR *Salmonella* Anatum, *Salmonella* Derby, and *Salmonella* Saintpaul have also been isolated from retail raw meats in Vietnam (36) and China (38).

The most-frequent MDR phenotype was AMP-TET-SXT-STR, as it was found in six isolates of *Salmonella* Agona, *Salmonella* Brandenburg, *Salmonella* Saintpaul, and *Salmonella* group B. This MDR profile was also observed in *Salmonella* Havana previously recovered from a beef carcass (23). Resistance to TET (37 of 37 isolates) was the most frequently observed among MDR *Salmonella* isolates, followed by STR (36 of 37 isolates), SXT (25 of 37 isolates), and AMP (23 of 37 isolates), and this is also similar to our previous findings on beef carcasses (23). Resistance to AMP was more frequent in MDR *Salmonella* isolates from ground beef (23 of 37 isolates, 62.2%) than in MDR isolates from beef carcasses (5 of 26 isolates, 19.2%). On the other hand, the MDR profile AMP-TET-CEP-STR-CHL, previously reported by Miranda et al. (20) as the most frequently and widely distributed among *Salmonella* isolates from retail beef (36.4%) in Mexico, was also found in the present investigation in one *Salmonella* Typhimurium isolate, which additionally showed resistance to GEN. This result suggests that *Salmonella* may be evolving, probably due to selective pressure (18).

A total of eight *Salmonella* Typhimurium isolates were recovered from 135 (5.9%) ground beef samples, and five of them were MDR strains (Table 3). In other reports from Mexico, *Salmonella* Typhimurium was the most-frequent serotype isolated from human clinical cases and showed high antimicrobial-drug resistance rates (40). Recently, the Centers for Disease Control and Prevention reported one multistate outbreak of MDR *Salmonella* Typhimurium infections linked to ground beef in the United States (7). These findings highlight the importance of this serotype as a cause of human illness. Although no single *Salmonella* Typhimurium isolate found in this study showed the typical pentaresistance (AMP, CHL, STR, sulfonamides, and TET

[ACSSuT]) found in the epidemic strain DT104, two isolates of *Salmonella* group B and *Salmonella* Sinstorf showed the ACSSuT phenotype. This is very important because *Salmonella* serotypes other than Typhimurium, such as MDR *Salmonella* Newport (27), have caused foodborne outbreaks linked to ground beef consumption in the United States.

Reduced susceptibility to seven antimicrobial drugs (CRO, CIP, NAL, CEP, SXT, kanamycin, and CHL) was observed among MDR *Salmonella* isolates, and for three of them, resistance to NAL was associated with a reduced susceptibility to CIP. Reduced susceptibility to a third-generation cephalosporin (CRO) and to a fluoroquinolone (CIP) among MDR *Salmonella* isolates of different serotypes recovered from ground beef is notable because these drugs are a potentially life-saving treatment for extraintestinal infections.

Although it was hypothesized that antimicrobial resistance might be different among isolates recovered from butcher's shops located at different municipalities because they receive the beef trimmings for ground beef production from different abattoirs, no differences were observed among municipalities in the frequencies of *Salmonella* isolates showing resistance to at least one antimicrobial or showing multidrug resistance ($P > 0.05$).

In conclusion, the occurrence of *Salmonella* in ground beef samples (56.7%) collected at butcher's shops was higher than that previously observed on beef carcasses (15.4%) at small abattoirs in the same region of Mexico. This may be a result of increasing contamination at these two points of the raw-beef production chain, or it may be an effect of the grinding process that facilitates a more-homogeneous pathogen distribution in the product. Meat retail establishments in Mexico are subject to enforcement to implement Good Manufacturing Practices; nevertheless, during the present investigation, it was confirmed that the majority of establishments visited did not comply with this enforcement. Poor hygiene and cross-contamination practices during ground beef fabrication at these retail establishments increases the risk of salmonellosis for consumers. An additional concern is the multidrug resistance observed in *Salmonella* isolates recovered during the present investigation, and we emphasize the importance of the prudent and responsible use of antimicrobial drugs in food animals.

ACKNOWLEDGMENTS

We are grateful to Ana Alicia Ávila Rosales and Anahí Esparza Becerra for their technical support. This work was funded by grants from Consejo Nacional de Ciencia y Tecnología de Jalisco and Universidad de Guadalajara (project numbers PS-2008-908 and PS-2009-524).

REFERENCES

- Arslan, S., and A. Eyi. 2010. Occurrence and antimicrobial resistance profiles of *Salmonella* species in retail meat products. *J. Food Prot.* 73:1613–1617.
- Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. W. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmarie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
- Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmarie. 2009. Prevalence and characterization of salmonellae in commercial ground beef in the United States. *Appl. Environ. Microbiol.* 75:1892–1900.
- Centers for Disease Control and Prevention. 2002. Outbreak of multidrug-resistant *Salmonella* Newport—United States, January–April 2002. *Morb. Mortal. Wkly. Rep.* 51:545–548.
- Centers for Disease Control and Prevention. 2006. Multistate outbreak of *Salmonella* Typhimurium infections associated with eating ground beef—United States, 2004. *Morb. Mortal. Wkly. Rep.* 55:180–182.
- Centers for Disease Control and Prevention. 2011. National *Salmonella* surveillance annual data summary, 2009. U.S. Department of Health and Human Services. Available at: http://www.cdc.gov/nationalsurveillance/salmonella_surveillance.html. Accessed 18 March 2013.
- Centers for Disease Control and Prevention. 2012. Multistate outbreak of *Salmonella* Typhimurium infections linked to ground beef. Available at: <http://www.cdc.gov/salmonella/typhimurium-groundbeef/index.html>. Accessed 16 March 2013.
- Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial disk susceptibility test. M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2008. Performance standards for susceptibility testing. 18th informational supplement. M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.
- De Blas, N., C. Ortega, K. Frankena, J. Noorduizen, and M. Thrusfield. 2000. Win Episcope 2.0. University of Utrecht and University of Edinburgh, Facultad de Veterinaria, Zaragoza and Agricultural University of Wageningen.
- Dechet, A. M., E. Scallan, K. Gensheimer, R. Hoekstra, J. Gunderman-King, J. Lockett, D. Wrigley, W. Chege, and J. Sobel. 2006. Outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium definitive type 104 infection linked to commercial ground beef, Northeastern United States, 2003–2004. *Clin. Infect. Dis.* 42:747–752.
- Ghafir, Y., B. China, N. Korsak, K. Dierick, J. M. Collard, C. Godard, L. De Zutter, and G. Daube. 2005. Belgian surveillance plans to assess changes in *Salmonella* prevalence in meat at different production stages. *J. Food Prot.* 68:2269–2277.
- 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.
- Heredia, N., S. Garcia, G. Rojas, and L. Salazar. 2001. Microbiological condition of ground meat retailed in Monterrey, Mexico. *J. Food Prot.* 64:1249–1251.
- Instituto Nacional de Estadística y Geografía. 2008. Directorio Estadístico Nacional de Unidades Económicas. Available at: <http://www3.inegi.org.mx/sistemas/mapa/denue/default.aspx>. Accessed 3 August 2013.
- López-Valadez, M. L., J. J. Varela-Hernández, S. L. Ruiz-Quezada, L. Navarro-Rubio, J. J. García-Reyna, V. Navarro-Hidalgo, and A. Villarruel-López. 2007. Deteción de *Salmonella* y *Listeria monocytogenes* por PCR y cultivo en carne molida de res obtenida de carnicerías, p. 47. Proceedings of the 9th International Food Safety Conference, Puerto Vallarta, Jalisco, Mexico.
- Mayrhofer, S., P. Paulsen, F. J. M. Smulders, and F. Hilbert. 2004. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int. J. Food Microbiol.* 97:23–29.
- McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* 34:S93–S106.
- McLaughlin, J. B., L. J. Castrodale, M. J. Gardner, R. Ahmed, and B. D. Gessner. 2006. Outbreak of multidrug-resistant *Salmonella* Typhimurium associated with ground beef served at a school potluck. *J. Food Prot.* 69:666–670.
- Miranda, J. M., A. C. Mondragon, B. Martinez, M. Guarddon, and J. A. Rodriguez. 2009. Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. *J. Food Prot.* 72:966–971.

21. Mrema, N., S. Mpuchane, and B. A. Gashe. 2006. Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. *Food Control* 17: 207–212.
22. National Antimicrobial Resistance Monitoring System. 2006. NARMS retail meat annual report. U.S. Food and Drug Administration, Centers for Disease Control and Prevention, and U.S. Department of Agriculture. Available at: <http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM073302.pdf>. Accessed 17 March 2013.
23. Perez-Montano, J. A., D. Gonzalez-Aguilar, J. Barba, C. Pacheco-Gallardo, C. A. Campos-Bravo, S. Garcia, N. L. Heredia, and E. Cabrera-Diaz. 2012. Frequency and antimicrobial resistance of *Salmonella* serotypes on beef carcasses at small abattoirs in Jalisco State, Mexico. *J. Food Prot.* 75:867–873.
24. Roels, T. H., P. A. Frazak, J. J. Kazmierczak, W. R. Mackenzie, M. E. Proctor, T. A. Kurzynski, and J. P. Davis. 1997. Incomplete sanitation of a meat grinder and ingestion of raw ground beef: contributing factors to a large outbreak of *Salmonella* Typhimurium infection. *Epidemiol. Infect.* 119:127–134.
25. Samadpour, M., M. W. Barbour, T. Nguyen, T. M. Cao, F. Buck, G. A. Depavia, E. Mazengia, P. Yang, D. Alfi, M. Lopes, and J. D. Stopforth. 2006. Incidence of enterohemorrhagic *Escherichia coli*, *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in retail fresh ground beef, sprouts, and mushrooms. *J. Food Prot.* 69: 441–443.
26. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
27. Schneider, J. L., P. L. White, J. Weiss, D. Norton, J. Lidgard, L. H. Gould, B. Yee, D. J. Vugia, and J. Mohle-Boetani. 2011. Multistate outbreak of multidrug-resistant *Salmonella* Newport infections associated with ground beef, October to December 2007. *J. Food Prot.* 74:1315–1319.
28. Secretaría de Salud. 2010. Anuario estadístico. Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades. Available at: <http://www.dgepi.salud.gob.mx/anuario/html/anuarios.html>. Accessed 15 March 2013.
29. Sorensen, O., J. Van Donkersgoed, M. McFall, K. Manninen, G. Gensler, and G. Ollis. 2002. *Salmonella* spp. shedding by Alberta beef cattle and the detection of *Salmonella* spp. in ground beef. *J. Food Prot.* 65:484–491.
30. Stevens, A., Y. Kabore, J. D. Perrier-Gros-Claude, Y. Millemann, A. Brisabois, M. Catteau, J. F. Cavin, and B. Dufour. 2006. Prevalence and antibiotic-resistance of *Salmonella* isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). *Int. J. Food Microbiol.* 110:178–186.
31. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Nationwide federal plant raw ground beef microbiological survey. Available at: <http://www.fsis.usda.gov/OHFS/baseline/rwrgbeef.pdf>. Accessed 10 March 2013.
32. U.S. Department of Agriculture, Food Safety and Inspection Service. 2004. Progress report on *Salmonella* testing of raw meat and poultry products, 1998–2003. Food Safety and Inspection Service. Available at: http://www.fsis.usda.gov/PDF/Salmonella_Progress_Report_1998-2003.pdf. Accessed 26 February 2013.
33. U.S. Department of Agriculture, Food Safety and Inspection Service. 2008. Isolation and identification of *Salmonella* from meat, poultry and eggs products, MLG 4.04. Food Safety and Inspection Service. Laboratory QA/QC Division, Athens, GA.
34. U.S. Department of Agriculture, Food Safety and Inspection Service. Progress report on *Salmonella* testing of raw meat and poultry products, 1998–2011. Executive summary. Food Safety and Inspection Service. Available at: http://www.fsis.usda.gov/PDF/Progress_Report_Salmonella_Testing_1998-2011.pdf. Accessed 10 March 2013.
35. U.S. Food and Drug Administration. 2011. 2011 Summary report on antimicrobials sold or distributed for use in food-producing animals. Available at: <http://www.fda.gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/UCM338170.pdf>. Accessed 11 March 2013.
36. Van, T. T. H., G. Moutafis, T. Istivan, L. T. Tran, and P. J. Coloe. 2007. Detection of *Salmonella* spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Appl. Environ. Microbiol.* 73:6885–6890.
37. Varma, J. K., K. D. Greene, J. Ovitt, T. J. Barrett, F. Medalla, and F. J. Angulo. 2005. Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984–2002. *Emerg. Infect. Dis.* 11:943–946.
38. Yang, B. W., D. Qu, X. L. Zhang, J. L. Shen, S. H. Cui, Y. Shi, M. L. Xi, M. Sheng, S. A. Zhi, and J. H. Meng. 2010. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *Int. J. Food Microbiol.* 141:63–72.
39. Zaidi, M. B., J. J. Calva, M. T. Estrada-Garcia, V. Leon, G. Vazquez, G. Figueroa, E. Lopez, J. Contreras, J. Abbott, S. Zhao, P. McDermott, and L. Tollefson. 2008. Integrated food chain surveillance system for *Salmonella* spp. in Mexico. *Emerg. Infect. Dis.* 14: 429–435.
40. Zaidi, M. B., P. F. McDermott, P. Fedorka-Cray, V. Leon, C. Canche, S. K. Hubert, J. Abbott, M. Leon, S. H. Zhao, M. Headrick, and L. Tollefson. 2006. Nontyphoidal *Salmonella* from human clinical cases, asymptomatic children, and raw retail meats in Yucatan, Mexico. *Clin. Infect. Dis.* 42:21–28.
41. Zhao, T., N. P. Doyle, P. J. Fedorka-Cray, P. Zhao, and S. Ladely. 2002. Occurrence of *Salmonella enterica* serotype Typhimurium DT104A in retail ground beef. *J. Food Prot.* 65:403–407.

Copyright of Journal of Food Protection is the property of Allen Press Publishing Services Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.