

Prevalence and Antimicrobial Resistance Patterns of *Salmonella* from Different Raw Foods in Mexico

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MS 08-571: Received 17 November 2008/Accepted 14 January 2009

ABSTRACT

The presence of *Salmonella* was determined in 116 samples of poultry meat, 81 samples of pork, 73 samples of beef, 33 samples of cheese, 61 samples of fish, and 78 samples of vegetables collected from retail stores and supermarkets in Hidalgo State (Mexico). Ninety-three *Salmonella* strains isolated from raw foods were characterized, and MICs were determined for 10 antimicrobials. *Salmonella* was detected in 35.3% of poultry meat, 30.3% of cheese, 21.8% of vegetable, 17.3% of pork, and 15.1% of beef samples, but no *Salmonella* was detected in fish samples. Significantly higher counts were obtained in chicken meat ($P = 0.0001$), pork ($P = 0.0116$), cheese ($P = 0.0228$), and vegetables ($P = 0.0072$) obtained from retail stores compared with those samples obtained from supermarkets. *Salmonella* isolates had high levels of resistance to ampicillin (66.7% of isolates), tetracycline (61.3%), and chloramphenicol (64.5%) and low levels of resistance to cefotaxime (0%), gentamicin (3.2%), and kanamycin (4.3%). Higher levels of quinolone resistance were found in isolates from poultry meat and vegetables compared with that in other foods tested. High levels of multiresistant strains were found in all foods tested except fish, ranging from 100% of pork samples to 47.1% of vegetable samples. The present study revealed that *Salmonella* prevalence was higher in foods from retail stores than in foods from supermarkets. Resistance rates observed for *Salmonella* were largely comparable to those reported in other countries for most antimicrobials, although resistance to chloramphenicol tended to be higher.

Salmonella is one of the most important genera of pathogenic bacteria implicated in foodborne bacterial outbreaks and disease. *Salmonella* infections are a significant public health problem in many parts of the world. Authorities estimate that more than one million human illnesses can be attributed to this pathogen each year (10, 11).

In recent years, another worldwide health concern has been the occurrence of antibiotic-resistant strains of a number of pathogenic bacteria, including *Salmonella*, that can be acquired by humans through food (3). The use of antimicrobial agents is considered the most important factor for the emergence, selection, and dissemination of antimicrobial-resistant bacteria (33). Antimicrobials are used therapeutically in animals and humans for controlling bacterial infections and may be incorporated into livestock and poultry feed at subtherapeutic levels as growth promoters. This practice has been banned in some countries, including those of the European Union, but currently it is still permitted in most other countries.

The increase in antimicrobial resistance of pathogenic bacteria is a major concern, especially in developing countries (24). Contrary to the situation in developed nations, in which most antimicrobial prescriptions are in the hands of the medical community, in developing nations patients and farmers can purchase potent antibiotics directly from the pharmacist without a medical prescription (25). Con-

sequently, inadequate therapeutic protocols often are applied to humans and animals and may contribute to the emergence and spread of antibiotic-resistant bacterial strains (5, 26). The increases in both human and animal populations and the inadequate management of fecal waste in developing countries enables the environmental dissemination of resistant enteric bacteria that are selected during antimicrobial treatments (26).

Salmonella can be transmitted in several ways, but the majority of human infections are the result of consumption of contaminated foods. An estimated 95% of these infections are associated with food, especially food of animal origin (14, 20). Poultry and other types of meats and raw meat products are reportedly the most frequent vehicles in outbreaks of foodborne salmonellosis, especially when proper hygiene is neglected (2, 7).

In general, human nontyphoidal salmonellosis does not require antimicrobial treatment. However, when the pathogen causes a systemic infection, antimicrobial therapy is essential. Consequently, knowledge of the likelihood of resistance to antimicrobial agents may be life saving (13). For this reason, programs to monitor resistance are essential. Monitoring the use of antimicrobial agents and the emergence of resistant strains in animals destined for human consumption is a risk management option that can prevent the development and spread of antimicrobial resistance in microorganisms in food-producing animals (4, 25). Many countries, such as Canada, Denmark, Sweden, and the Unit-

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TABLE 1. Isolation of *Salmonella* from foods collected from retail stores and supermarkets in Hidalgo State (Mexico)

Sample type	No. of positive samples/no. of samples tested (% positive)			<i>P</i> ^a
	Supermarkets	Retail stores	Total	
Poultry	11/62 (17.7)	30/54 (55.6)	41/116 (35.3)	0.0001
Pork	2/38 (5.3)	12/43 (27.9)	14/81 (17.3)	0.0116
Beef	4/41 (9.8)	7/32 (21.9)	11/73 (15.1)	0.2685
Cheese	0/11 (0)	10/22 (45.5)	10/33 (30.3)	0.0228
Fish	0/34 (0)	0/27 (0)	0/61 (0)	
Vegetables	4/43 (9.3)	13/35 (37.1)	17/78 (21.8)	0.0072
Total	21/229 (9.2)	72/213 (33.8)	93/441 (21.1)	<0.0001

^a Significant differences ($P < 0.05$) between supermarkets and retail stores determined by χ^2 test with Yates's correction.

ed States, have established national surveillance programs to monitor the antibiotic susceptibility of enteric bacteria isolated from humans and animals (6, 9, 22, 30). Various bacterial species have been used for these monitoring programs. Commensal bacteria are often used, but the programs have focused especially on pathogenic zoonotic bacteria, such as *Salmonella*. However, very little information about the monitoring of antimicrobial resistance of zoonotic pathogenic bacteria isolated from food in developing nations (e.g., Mexico) is currently available.

The main goal of this study was to investigate the prevalence and antimicrobial susceptibility of *Salmonella* strains isolated from foods in Hidalgo State (Mexico). The implications of these results in terms of microbiological safety, especially concerning the development and spread of antimicrobial resistance to the food chain, also were evaluated.

MATERIALS AND METHODS

Collection of food samples. A total of 441 raw unprocessed food samples were collected from January 2007 to August 2008 from supermarkets and retail stores (including butcher and poultry shops). Samples included (i) drumsticks, breasts, wings, and necks of broiler chickens ($n = 116$), (ii) pork loin and minced pork ($n = 81$), (iii) beef fillets and chops and minced beef ($n = 73$), (iv) entire and filleted sea fish ($n = 61$), (v) vegetables generally consumed raw (lettuce, spinach, carrots, onions, tomatoes, and chilies) ($n = 78$), and (vi) Mexican-style cheese prepared from raw cow's milk ($n = 32$). No more than three samples of each group were obtained on different days from the same supermarket or retail store. Samples were placed in sterile bags and taken to the laboratory in an ice chest within 1 h for immediate processing. All supermarkets and butcher shops were located in Hidalgo State (east-central Mexico).

Microbiological analyses. Twenty-five-gram portions were obtained from each food sample, placed in a sterile bag (Seward, London, UK), diluted 1:9 (wt/vol) in sterile 0.1% buffered peptone water (bioMérieux, Hazelwood, MO), and stomached with a Seward stomacher. After stomaching, the buffered peptone water suspensions were incubated at 37°C for 24 h, and 0.1 ml of this broth culture was subcultured in 10 ml of Rappaport-Vassiliadis enrichment broth (Difco, Becton Dickinson, Sparks, MD) and incubated at 42°C for 24 h. Broth samples were streaked onto xylose lysine desoxycholate agar (Bioxon, Cuautitlan, Mexico) and brilliant green agar (Difco, Becton Dickinson). A maximum of three presumptive *Salmonella* colonies from each sample were picked, transferred onto Columbia agar containing 5% sheep blood (Difco, Becton Dickinson), and incubated at 37°C for 48 h to obtain pure

cultures. After characterization by Gram staining and oxidase and catalase activity tests, isolates were streaked onto triple sugar iron agar (TSI; Difco, Becton Dickinson) and lysine iron agar (LIA; Becton Dickinson). Isolates with typical color reactions on TSI and LIA were confirmed as *Salmonella* based on results of the latex agglutination test (Microbact, Ogdensburg, NY) and API 20E test strips (bioMérieux). Only one confirmed *Salmonella* isolate per food sample was included in the subsequent study.

Antimicrobial susceptibility testing of bacteria. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described in the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines (23). MICs for the following antibiotics against *Salmonella* isolates were determined: ampicillin, cephalotin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. All standard antimicrobials were obtained from Sigma Chemical Co. (St. Louis, MO), except ciprofloxacin (Bayer HealthCare AG, Leverkusen, Germany). Antimicrobials were generally tested in a twofold concentration series for the range of 0.125 to 128 mg/liter. Exceptions to this range were ciprofloxacin and gentamicin, which were tested at 0.03 to 32 mg/liter. Resistance was defined according to breakpoints published by the CLSI (8). Because there are no CLSI breakpoints published for streptomycin, the breakpoint for this antimicrobial was defined as ≥ 32 mg/liter (31). The MICs that inhibited 50% (MIC₅₀) and 90% (MIC₉₀) of total strains were calculated. *Escherichia coli* ATCC 25922 was used as a reference strain for this study. Isolates exhibiting resistance to at least three of the antimicrobial agents tested were considered multiresistant.

Statistical analysis. The χ^2 test with Yates's correction was used to analyze differences in isolation of *Salmonella*. The differences were considered to be significant at $P < 0.05$. Analyses were performed using Statgraphics version 5.0.1 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Salmonella was detected in 93 (21.1%) of the 441 25-g samples tested. Recovery of the organism was highest in poultry meat (35.3% of the samples) and raw cheese (30.3%) followed by crude vegetable foods (21.8%), pork (17.3%), and beef (15.1%). No *Salmonella* was detected in fish samples (Table 1). Within each food group, the highest prevalence of *Salmonella* in poultry samples was found for necks (54.2% of samples), and for both pork and beef the highest prevalence was found for minced meat (15.8 and 9.8%, respectively). For vegetables, the highest prevalence

TABLE 2. *In vitro* activity of diverse antimicrobial agents against 93 *Salmonella* isolates from foods in Hidalgo State (Mexico)

Antimicrobial	Measure ^a	Poultry (n = 41)	Pork (n = 14)	Beef (n = 11)	Cheese (n = 10)	Vegetables (n = 17)	Total (n = 93)
Ampicillin	MIC ₅₀	>128	>128	>128	4	2	>128
	MIC ₉₀	>128	>128	>128	64	>128	>128
	R (%)	34 (82.9)	12 (85.7)	8 (72.7)	3 (30)	5 (29.4)	62 (66.7)
Cephalotin	MIC ₅₀	4	16	2	2	4	4
	MIC ₉₀	64	>128	>128	64	64	64
	R (%)	16 (39)	6 (42.9)	4 (36.4)	3 (30)	5 (29.4)	34 (36.6)
Cefotaxime	MIC ₅₀	≤0.125	0.25	≤0.125	≤0.125	0.25	0.125
	MIC ₉₀	0.25	0.50	1	0.50	0.25	0.5
	R (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	MIC ₅₀	64	32	8	32	16	32
	MIC ₉₀	>128	>128	>128	>128	64	>128
	R (%)	33 (80.5)	9 (64.3)	5 (45.5)	7 (70)	6 (35.5)	60 (64.5)
Ciprofloxacin	MIC ₅₀	1	0.063	0.25	0.25	0.25	0.5
	MIC ₉₀	16	0.25	4	1	8	8
	R (%)	12 (29.3)	0 (0)	1 (9.1)	0 (0)	4 (23.5)	17 (18.3)
Gentamicin	MIC ₅₀	1	0.5	0.5	1	0.125	0.5
	MIC ₉₀	8	32	8	16	8	8
	R (%)	0 (0)	2 (14.3)	0 (0)	1 (10)	0 (0)	3 (3.2)
Nalidixic acid	MIC ₅₀	8	1	1	2	4	4
	MIC ₉₀	>128	32	32	4	>128	128
	R (%)	18 (43.9)	2 (14.3)	1 (9.1)	0 (0)	6 (35.3)	25 (26.9)
Kanamycin	MIC ₅₀	2	4	1	2	2	2
	MIC ₉₀	4	128	8	64	16	32
	R (%)	0 (0)	3 (21.4)	0 (0)	1 (10)	0 (0)	4 (4.3)
Streptomycin	MIC ₅₀	16	32	32	8	4	16
	MIC ₉₀	128	>128	64	32	128	128
	R (%)	19 (46.3)	9 (64.3)	7 (63.6)	4 (40)	7 (41.1)	46 (49.5)
Tetracycline	MIC ₅₀	32	16	4	16	8	16
	MIC ₉₀	>128	>128	64	>128	64	>128
	R (%)	32 (78)	9 (64.3)	4 (28.6)	6 (60)	6 (35.3)	57 (61.3)
Trimethoprim-sulfamethoxazole ^b	MIC ₅₀	0.5	16	1	<0.125	<0.125	0.5
	MIC ₉₀	64	>128	8	16	64	64
	R (%)	11 (26.8)	11 (68.8)	2 (27.3)	3 (30)	4 (23.5)	31 (33.3)

^a MIC₅₀, concentration that inhibited 50% of total isolates; MIC₉₀, concentration that inhibited 90% of total isolates; R (%), number (percentage) of resistant isolates.

^b MIC₅₀ and MIC₉₀ values refer to trimethoprim concentrations only.

was found for lettuce (33.3%) and carrots (27.3%). These prevalences of *Salmonella* are clearly higher than those reported for meats and live animals in other countries, for which the reported prevalence in products from various species was about 1 to 5% (4, 7, 14, 15, 18). However, the prevalences found in the present work are similar to or below those previously reported in Mexico by other authors. Prevalences of 46% in retail chicken, 63.3% in retail pork, and 60.5% in retail beef were found in Yucatan (35), and prevalences of 87.5% in raw pork (12) and 88% in chorizo (11) were reported in Guadalajara.

Although poultry meat and pork are historically considered at higher risk than other foods for *Salmonella* contamination (2, 11, 15), these results indicate that raw cheese and raw vegetable foods also can be important potential sources of salmonellosis.

The results obtained also indicate that for poultry meat ($P = 0.0001$), pork ($P = 0.0116$), cheese ($P = 0.0228$),

vegetables ($P = 0.0149$), and all foods together ($P < 0.0001$), samples obtained from retail stores (small shops and neighborhood bazaars) had a higher prevalence of *Salmonella* than did samples obtained from supermarkets. Similar results were obtained for chorizo in Mexico and for pork sausages in Ireland, where a higher prevalence of *Salmonella* was found in sausages from butcher shops than in prepacked sausages (1, 11, 16). These results indicate inferior sanitary conditions and poor food hygiene practices by retail food handlers and facilities in comparison to supermarkets, factors that play an important role in *Salmonella* contamination of foods.

Antimicrobial resistance in bacteria isolated from foods is a potential source of resistant bacterial strains in humans (4). When resistance is present among zoonotic organisms, such as *Salmonella*, it is then possible for resistant bacteria from foods to be transmitted to a consumer. Because most *Salmonella* infections are acquired from ingestion of con-

TABLE 3. Multiresistance patterns of *Salmonella* isolates from various foods in Hidalgo State (Mexico)

Sample type	No. (%) of isolates	Resistance profile ^a
Poultry	11 (26.9)	AmpCepChlTet
	8 (19.5)	AmpChlCipNalTet
	6 (14.6)	AmpChlStrNalTet
	6 (14.6)	AmpChlStrTetTsx
	2 (4.9)	AmpCepNalCipStrTsx
	2 (4.9)	ChlNalCipStrTsx
Pork	5 (35.7)	AmpCepChlStrTsx
	4 (28.6)	AmpChlStrTetTsx
	2 (14.3)	GenKanTetTsx
	2 (14.3)	AmpNalTet
	1 (7.1)	AmpCepKanTet
Beef	4 (36.4)	AmpCepChlStrTet
	2 (18.2)	AmpStrTsx
	1 (9.1)	AmpCipNal
Cheese	3 (30)	AmpCepChlTet
	2 (20)	ChlStrTetTsx
	1 (10)	GenKanTsx
Vegetables	3 (17.6)	AmpCepChlTetNalStr
	2 (11.8)	AmpCepNalCipTsx
	2 (11.8)	ChlStrTet
	1 (5.9)	CipNalStrTet

^a Amp, ampicillin; Cep, cephalotin; Chl, chloramphenicol; Tet, tetracycline; Cip, ciprofloxacin; Nal, nalidixic acid; Str, streptomycin; Tsx, trimethoprim-sulfamethoxazole; Gen, gentamicin; Kan, kanamycin.

taminated foods of animal origin, a likely cause of the increasing prevalence of antimicrobial-resistant *Salmonella* in recent years could be the use of antimicrobial agents in food animals (3, 13). Vegetables can become contaminated with resistant bacteria that have arisen in response to veterinary antimicrobial treatments and been carried to vegetable foods via wastewater and sewage (21, 25, 27).

Among the recent reports of antimicrobial resistance in *Salmonella* isolates from foods, results differ widely among compounds and countries (3, 4, 7, 18). For *Salmonella* isolates from clinical cases, differences were found among countries and geographic areas (3, 17, 29, 31) and among different studies involving clinical *Salmonella* strains from Mexico (13, 29, 35). The antimicrobial resistance rates obtained in our study (Table 2) were in most cases higher than those reported for clinical isolates for some antimicrobials such as chloramphenicol (64.5% of total isolates). However, clinical *Salmonella* samples obtained in Yucatan (Mexico) in another study (35) had higher resistance rates than those isolates obtained in the present study for some antimicrobials such as streptomycin (80.7%) and tetracycline (71.5%). In agreement with the findings reported by other authors, resistance of isolates was substantially higher for the compounds that have been in clinical use for the longest time, with the exceptions of gentamicin (3.2%) and kanamycin (4.3%) (4, 18, 19). These compounds have a long history but have been used less often in animals than have members of other antimicrobial families such as β -lactams or tetracyclines, and resistance of animal-origin *Salmonella*

isolates is consequently rare. Based on the data reported in all of the previous studies, third-generation cephalosporins (such as cefotaxime) remain extremely effective against *Salmonella* (0% of strains resistant). Depending on the origin of the samples, important differences were obtained in antimicrobial resistance. There is a remarkably high level of resistance to quinolones (nalidixic acid and ciprofloxacin) among *Salmonella* isolates of poultry and vegetable origin. Worldwide, the emergence of quinolone resistance and increasing susceptibility to *Salmonella* infection has been linked to the use of fluoroquinolones in food animals (17). The occurrence of higher levels of quinolone resistance in bacteria isolated from poultry was reported in recent years (32). Nevertheless, the presence of higher levels of resistance to both nalidixic acid and ciprofloxacin in *Salmonella* isolates from vegetables than in isolates from pork, beef, or cheese is surprising. This result seems to suggest that, as previously reported for *E. coli*, quinolone-resistant *Salmonella* could reach vegetable foods through human or poultry farm wastewater or sewage (27, 28).

The multidrug resistance (i.e., resistance to three or more antimicrobial agents) exhibited by *Salmonella* varied from 100% of isolates obtained from pork samples to 47.1% of isolates from vegetables. For all groups except vegetables, more than 60% of all isolates were multiresistant (Table 3). Multiresistant isolates could have originated as a result of coselection of resistance determinants. Exposure of a bacterial population to one antimicrobial agent may result in resistance to other agents without any prior exposure (28). The isolation of highly multiresistant *Salmonella* strains from animals in which antimicrobial use is more frequently employed (poultry and pigs) support this hypothesis, and such multiresistance could make it more difficult to obtain successful antimicrobial therapy for salmonellosis caused by strains of poultry or pork origin.

For decades, when antimicrobial treatment was necessary ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole were the drugs of choice (13). Because of increasing resistance to these antimicrobials, the use of fluoroquinolones (in adults) and extended spectrum cephalosporins (in children) has become common (7, 17). However, in some cases the presence of *Salmonella* isolates resistant to both fluoroquinolones and cephalosporins causes problems in salmonellosis therapy (34). In the present work, cephalotin resistance was found in *Salmonella* isolates from 29.4 to 42.9% of samples from all foods tested, whereas ciprofloxacin resistance depended on the food origin of isolates.

Salmonella was isolated from all foods tested except fish. Resistance to most antimicrobials tested was moderate except for cefotaxime, gentamicin, and kanamycin, and a high prevalence of multiresistant isolates was found. Some resistance profiles likely reflect the pattern of antimicrobial use on farms, as indicated by the higher quinolone resistance in poultry isolates than in those from other foods. This finding demonstrates the importance of epidemiological investigation of the origin of *Salmonella* isolates for selecting the appropriate antimicrobial treatment for food-borne salmonellosis. Surveillance studies should be con-

ducted in future years to study temporal trends and to determine whether the low level of resistance to newer antibiotics, such as third-generation cephalosporins, can be maintained.

ACKNOWLEDGMENTS

The authors are thankful for financial support from Dirección Xeral de Investigación, Desenvolvemento e Innovación (Xunta de Galicia, grant IN843A2007/42) and Consejo Nacional de Ciencia y Tecnología (project CB-2006-61310).

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