

Prevalence and Antimicrobial Resistance of *Campylobacter* spp. and *Salmonella* Serovars in Organic Chickens from Maryland Retail Stores

Shenghui Cui, Beilei Ge, Jie Zheng, and Jianghong Meng*

Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742

Received 7 October 2004/Accepted 17 January 2005

Retail organic ($n = 198$) and conventional ($n = 61$) chickens were analyzed. Most organic (76%) and conventional (74%) chickens were contaminated with campylobacters. Salmonellae were recovered from 61% of organic and 44% of conventional chickens. All *Salmonella enterica* serovar Typhimurium isolates from conventional chickens were resistant to five or more antimicrobials, whereas most *S. enterica* serovar Typhimurium isolates (79%) from organic chickens were susceptible to 17 antimicrobials tested.

Campylobacters and salmonellae are common bacterial pathogens associated with human gastroenteritis (14). Contaminated raw or undercooked poultry is particularly important in transmitting campylobacters and salmonellae (8, 11). Consumers and farmers have been increasingly interested in organic food products. The number of organic livestock increased sharply during the last decade, and the market for organic meat products is expected to grow considerably (9). Although the growth of the organic food market has been fueled by consumers' perception of organic products as healthier and safer (19), organic livestock production is not designed to reduce pathogen loads in food animals (3, 20). Organic meat production involves potentially higher microbiological safety risks due to raising animals outdoors, the use of slow-growing breeds, the prohibition of antimicrobial use, and the use of very small slaughtering facilities (3, 19). However, little is known about the microbiological status of organic animal products. In a Danish study, campylobacters were found in all of 22 organic broiler flocks compared to one-third of 79 conventional flocks, and most *Campylobacter* isolates (>90%) from the organic and conventional flocks were susceptible to antimicrobials (10). Sato et al. (18) reported no significant difference in the campylobacter prevalence and antimicrobial resistance in organic and conventional dairy herds in Wisconsin. However, two isolates from the conventional herds were resistant to ciprofloxacin.

The objectives of this study were to determine the prevalence of campylobacters and salmonellae in retail organic chickens in order to evaluate the microbiological safety of organic chicken relative to meat derived from conventionally raised chickens and to characterize the bacteria and their antimicrobial susceptibility.

Organic (198 samples) and conventional (61 samples) chicken carcasses were randomly collected from three organic and three conventional retail stores, respectively, in Maryland between September 2002 and August 2003. Four organic and six conventional chicken brands were included in the sampling.

Campylobacters and salmonellae were isolated from chickens using previously described methods (23, 24). Three presumptive *Campylobacter* or *Salmonella* isolates from each positive sample were selected and confirmed by morphological and

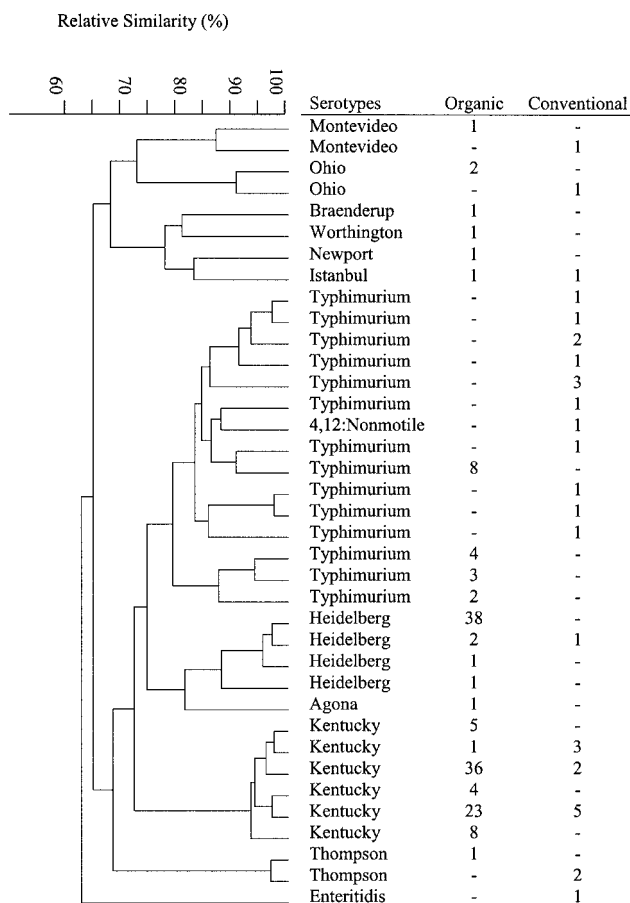


FIG. 1. Dendrogram of PFGE of *Salmonella* isolates recovered from organically and conventionally produced chicken samples. The tree of relative genetic similarity was constructed based on the neighbor-joining method; scale at 100 means identical. The numbers under columns organic and conventional are the numbers of chicken samples from which each serotype was recovered.

* Corresponding author. Mailing address: Department of Nutrition and Food Science, 0112 Skinner Building, University of Maryland, College Park, MD 20742. Phone: (301) 405-1399. Fax: (301) 314-3313. E-mail: jmeng@umd.edu.

TABLE 1. Contamination with *Salmonella* and *Campylobacter* bacteria in organically and conventionally produced chicken samples

Bacteria	No. (%) of contaminated samples ^a	
	Organic (n = 198)	Conventional (n = 61)
Campylobacters	150 (76)	45 (74)
<i>C. jejuni</i>	68 (45)	28 (62)
<i>C. coli</i>	71 (47)	18 (40)
Other campylobacters	31 (20)	7 (16)
Salmonellae	121 (61)	27 (44)
Serovar Kentucky	72 (59)	10 (37)
Serovar Heidelberg	40 (33)	1 (4)
Serovar Typhimurium	20 (17)	12 (44)
Other salmonellae	11 (9)	6 (2)

^a Twenty-seven samples contained two or more *Campylobacter* species, and 21 samples had two or more *Salmonella* serotypes.

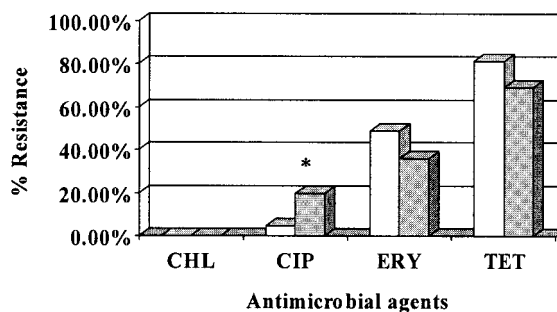
microscope examination and PCR assays (12, 13, 17). *Salmonella* serotypes were determined at the U.S. Department of Agriculture National Veterinary Service Laboratories in Ames, Iowa. Approximately 76% of the organic and 74% of the conventional chicken samples were positive for campylobacters (Table 1). Similar numbers of the organic chicken samples were contaminated with *Campylobacter coli* (47%) and *Campylobacter jejuni* (45%). Conventional chickens were more commonly contaminated with *C. jejuni* (62%) than *C. coli* (40%). A number of the samples (10% organic and 13% conventional chickens) were contaminated with two or more

TABLE 2. Antimicrobials, dilution ranges, and resistance breakpoints used in antimicrobial susceptibility tests for campylobacters and salmonellae^a

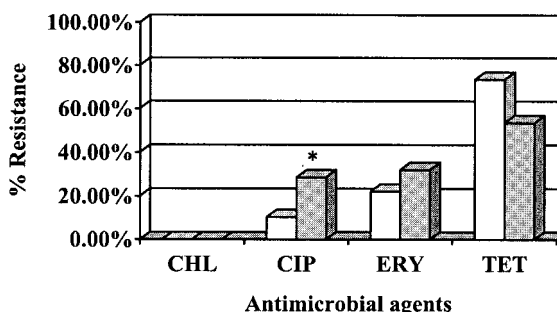
Antimicrobial	Dilution range tested (µg/ml)	Resistance breakpoint (µg/ml)
Campylobacters		
Chloramphenicol	0.25–128	≥32
Ciprofloxacin	0.03–16	≥4
Erythromycin	0.125–64	≥8
Tetracycline	0.125–64	≥16
Salmonellae		
Amikacin	4–32	≥64
Amoxicillin-clavulanic acid	0.5–32	≥32
Ampicillin	2–32	≥32
Apramycin	2–32	≥32
Ceftiofur	0.5–16	≥8
Ceftriaxone	0.25–64	≥32
Cephalothin	1–32	≥32
Chloramphenicol	4–32	≥32
Ciprofloxacin	0.015–4	≥4
Florfenicol	2–16	≥16
Gentamicin	0.25–16	≥16
Kanamycin	16–64	≥64
Nalidixic acid	4–256	≥32
Streptomycin	32–256	≥64
Sulfamethoxazole	128–512	≥512
Tetracycline	4–32	≥16
Trimethoprim-sulfamethoxazole	0.12–2/4–76	≥4/76

^a Antimicrobial susceptibility testing was performed according to NCCLS guidelines (16). *C. jejuni* ATCC 33560 was used as the quality control organism for campylobacters, whereas *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used for salmonellae.

A. *Campylobacter* species (n=195)



B. *C. jejuni* (n=96)



C. *C. coli* (n=89)

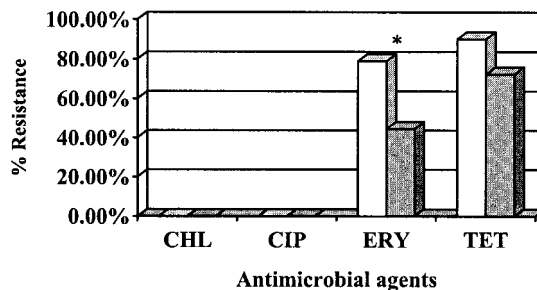


FIG. 2. Antimicrobial resistance of campylobacters isolated from 150 organic (cross-hatch bars) and 45 conventional (solid bars) chickens. Abbreviations: CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline. An asterisk indicates a significant difference.

Campylobacter species. Salmonellae were isolated from 61% of the organic and 44% of the conventional chickens (Table 1). Eleven serotypes were identified among the salmonellae from the organic chickens; Kentucky, Heidelberg, and Typhimurium were the top three serotypes (Fig. 1). Typhimurium and Kentucky were the predominant serotypes (78%) among eight serotypes of salmonellae from the organic chickens (Fig. 1).

Pulsed-field gel electrophoresis (PFGE) with XbaI digestion was performed to determine genomic DNA fingerprinting profiles of *Salmonella* isolates (355 from organic and 74 from conventional chickens) using the procedures described previously (23). Thirty-seven distinct PFGE patterns were identified (Fig. 1); 14 were found only in conventional chicken samples, 18 were only in organic chicken samples, and 5 were in both

TABLE 3. Antimicrobial resistance of *S. enterica* serovars Kentucky and Typhimurium isolated from organic and conventional chicken samples

Antimicrobial resistance profile ^a	Serovar Kentucky		Serovar Typhimurium	
	Organic (n = 72)	Conventional (n = 10)	Organic (n = 19)	Conventional (n = 12)
AUG-AMP-CEP-FOX-STR-TET-TIO	2 (3) ^b			2 (17)
AUG-AMP-CEP-FOX-TET-TIO		1 (10)		2 (17)
AUG-AMP-CEP-FOX-TIO	1 (1)	1 (10)		8 (67)
GEN-SMX-STR-TET	5 (7)		1 (5)	
KAN-STR-TET	1 (1)			
STR-TET	40 (56)	6 (60)	1 (5)	
KAN-NAL			1 (5)	
TET	1 (1)			
NAL			1 (5)	
Susceptible to all tested antimicrobials	23 (32)	2 (20)	15 (79)	0 (0)

^a AUG, amoxicillin-clavulanic acid; AMP, ampicillin; CEP, cephalothin; TIO, ceftiofur; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; SMX, sulfamethoxazole; STR, streptomycin; TET, tetracycline; NAL, nalidixic acid.

^b The number (percentage) of isolates is shown.

conventional and organic chickens. No identical pattern was shared among *Salmonella enterica* serovar Typhimurium isolates from organic versus conventional chickens.

Antimicrobial resistance of bacterial pathogens has become a significant concern for public health. Antimicrobial resistance in campylobacters and salmonellae isolated from both food and clinical sources appears to be increasing in many countries. Nachamkin et al. (15) reported a dramatic increase (from 8.3% in 1996 to 40.5% in 2001) in fluoroquinolone-resistant *C. jejuni* in Pennsylvania. Our previous findings revealed that a common resistance among campylobacters from retail chicken and turkey was to tetracycline (82%), followed by erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%) (6). The rise of antimicrobial-resistant salmonellae is attributed in part to the clonal spread of multidrug-resistant varieties, including *S. enterica* serovar Typhimurium DT104 (21). In Great Britain, the reported rates of antimicrobial-resistant *S. enterica* serovar Typhimurium more than doubled between 1981 and 1989 (22). In the United States, resistance to tetracycline has increased from 9% in 1980 to 24% in 1990 and ampicillin resistance increased from 10% to 14% (2, 7). Resistance to fluoroquinolones increased from 0.4% in 1996 to 1% in 2001, and resistance to ceftriaxone increased over 20-fold, from 0.1% in 1996 to 2% in 2001. We reported that many salmonellae from ground meats were resistant to tetracycline (80%), streptomycin (73%), and sulfamethoxazole (69%) (23). Ceftiofur- and ceftriaxone-resistant salmonellae were also isolated from ground turkey, chicken, and beef. These data have significant public health implications since fluoroquinolones and ceftriaxone are the most commonly used antimicrobial agents for the treatment of invasive *Salmonella* infections in adults and children, respectively (1, 4).

In the present study, we used agar dilution and the Sensititre system as described previously (5, 23) to determine antimicrobial susceptibility of campylobacters and salmonellae, respectively. The antimicrobials and resistance breakpoints used in the study are presented in Table 2. All campylobacters were susceptible to chloramphenicol (Fig. 2). Resistance to tetracycline was most common (78%), followed by resistance to erythromycin (46%) and ciprofloxacin (8%). More isolates from the conventional chickens (20%) were resistant to ciprofloxacin

than those from the organic chickens (5%). Rates of resistance to erythromycin and tetracycline were higher in organic chicken isolates (49% and 81%, respectively) than conventional chicken isolates (36% and 69%, respectively).

The antimicrobial resistance profiles of the *Salmonella* isolates differed among the serotypes and also depended upon the sources from which they were recovered. *S. enterica* serovars Typhimurium and Kentucky were more resistant to antimicrobials than *S. enterica* serovar Heidelberg. *S. enterica* serovar Typhimurium isolates recovered from 79% of the organic chicken samples were susceptible to all 17 antimicrobials tested (Table 3). In contrast, all 12 *S. enterica* serovar Typhimurium isolates from conventional chicken samples were resistant to five to seven different antimicrobials (Table 3). However, one serovar Heidelberg and one serovar Newport isolate from the organic chickens were resistant to nine antimicrobials, including amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. Regardless of the source, the isolates of serovars Ohio, Agona, Braenderup, Thompson, Worthington, and Montevideo were susceptible to all antimicrobials tested.

Our results revealed that the prevalence and antimicrobial susceptibility of campylobacters and salmonellae varied among different animal production systems. While organic chickens were more frequently contaminated with campylobacters and salmonellae, the pathogens from organic animal production were more susceptible to certain antimicrobials. However, findings based on the data from one study may be inconclusive. Baseline data on the microbiological status of organic products are needed to ensure the safety of food supplies as sales of organic foods are expected to increase.

We are indebted to Patrick McDermott from the Division of Animal and Food Microbiology, Center for Veterinary Medicine, Food and Drug Administration, for assistance with and comments on the preparation of this article.

REFERENCES

- Angulo, F. J., K. R. Johnson, R. V. Tauxe, and M. L. Cohen. 2000. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. *Microb. Drug Resist.* 6:77-83.
- Besser, T. E., C. C. Gay, J. M. Gay, D. D. Hancock, D. Rice, L. C. Pritchett,

- and E. D. Erickson. 1997. Salmonellosis associated with *S typhimurium* DT104 in the USA. *Vet. Rec.* **140**:75.
3. Engvall, A. 2001. May organically farmed animals pose a risk for *Campylobacter* infections in humans? *Acta Vet. Scand. Suppl.* **95**:85–87.
 4. Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs. 2000. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *N. Engl. J. Med.* **342**:1242–1249.
 5. Ge, B., S. Bodeis, R. D. Walker, D. G. White, S. Zhao, P. F. McDermott, and J. Meng. 2002. Comparison of the Etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. *J. Antimicrob. Chemother.* **50**:487–494.
 6. Ge, B., D. G. White, P. F. McDermott, W. Girard, S. Zhao, S. Hubert, and J. Meng. 2003. Antimicrobial-resistant *Campylobacter* species from retail raw meats. *Appl. Environ. Microbiol.* **69**:3005–3007.
 7. Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo. 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N. Engl. J. Med.* **338**:1333–1338.
 8. Gomez, T. M., Y. Motarjemi, S. Miyagawa, F. K. Kaferstein, and K. Stohr. 1997. Foodborne salmonellosis. *World Health Stat. Q.* **50**:81–89.
 9. Greene, C. R. 2001. U.S. organic farming emerges in the 1990s: adoption of certified systems. U.S. Department of Agriculture, Washington, D.C.
 10. Heuer, O. E., K. Pedersen, J. S. Andersen, and M. Madsen. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Lett. Appl. Microbiol.* **33**:269–274.
 11. Jacobs-Reitsma, W. 2000. *Campylobacter* in the food supply, p. 467–482. In I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, D.C.
 12. Linton, D., A. J. Lawson, R. J. Owen, and J. Stanley. 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J. Clin. Microbiol.* **35**:2568–2572.
 13. Malorny, B., J. Hoorfar, C. Bunge, and R. Helmuth. 2003. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. *Appl. Environ. Microbiol.* **69**:290–296.
 14. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5**:607–625.
 15. Nachamkin, I., H. Ung, and M. Li. 2002. Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA, 1982–2001. *Emerg. Infect. Dis.* **8**:1501–1503.
 16. National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals—approved standards M31-A, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
 17. Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss III, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol. Cell. Probes* **6**:271–279.
 18. Sato, K., P. C. Bartlett, J. B. Kaneene, and F. P. Downes. 2004. Comparison of prevalence and antimicrobial susceptibilities of *Campylobacter* sp. isolates from organic and conventional dairy herds in Wisconsin. *Appl. Environ. Microbiol.* **70**:1442–1447.
 19. Sundrum, A. 2001. Organic livestock farming. A critical review. *Livest. Prod. Sci.* **67**:207–215.
 20. Thamsborg, S. M. 2001. Organic farming in the Nordic countries—animal health and production. *Acta Vet. Scand. Suppl.* **95**:7–15.
 21. Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe. 1996. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. *Lancet* **347**:1053–1054.
 22. Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int. J. Food Microbiol.* **62**:1–5.
 23. White, D. G., S. Zhao, R. Sudler, S. Ayers, S. Friedman, S. Chen, P. F. McDermott, S. McDermott, D. D. Wagner, and J. Meng. 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N. Engl. J. Med.* **345**:1147–1154.
 24. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. *Appl. Environ. Microbiol.* **67**:5431–5436.