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Antimicrobial Susceptibility of *Salmonella* from Organic and Conventional Dairy Farms

K. A. Ray,* L. D. Warnick,*[1] R. M. Mitchell,* J. B. Kaneene,† P. L. Ruegg,‡ S. J. Wells,§ C. P. Fossler,§ L. W. Halbert,† and K. May†
*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853
†Population Medicine Center, College of Veterinary Medicine, Michigan State University, East Lansing 48824
‡Department of Dairy Science, University of Wisconsin, Madison 53706
§Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul 55108

ABSTRACT

The objective of this study was to compare antimicrobial susceptibility of *Salmonella* isolated from conventional and organic dairy farms in the Midwest and Northeast United States. Environmental and fecal samples were collected from organic (n = 26) and conventional (n = 69) farms in Michigan, Minnesota, New York, and Wisconsin every 2 mo from August 2000 to October 2001. *Salmonella* isolates (n = 1,243) were tested using a broth microdilution method for susceptibility to amoxicillin-clavulanic acid, ampicillin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. Herd-level logistic regression and logistic proportional hazards multivariable models were used to examine the association between farm management type and susceptibility to antimicrobial agents. For most antimicrobial agents tested, susceptibility of *Salmonella* isolates was similar on organic and conventional herds when controlling for herd size and state. Conventional farms were more likely to have at least one *Salmonella* isolate resistant to streptomycin using logistic regression (odds ratio = 7.5; 95% confidence interval = 1.7-55.4). Conventional farms were more likely to have *Salmonella* isolates with greater resistance to streptomycin (odds ratio = 5.4; 95% confidence interval = 1.5–19.0) and sulfamethoxazole (odds ratio = 4.2; 95% confidence interval = 1.2–14.1) using logistic proportional hazards models. Although not statistically significant, conventional farms tended to be more likely to have at least one *Salmonella* isolate resistant to 5 or more antimicrobial agents when compared with organic farms.
Key words: antibiotic, antimicrobial, *Salmonella*, organic

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[1]Corresponding author: ldw3@cornell.edu

INTRODUCTION

Salmonella is responsible for an estimated 1.4 million illnesses, 15,000 hospitalizations, and 500 to 600 deaths in the United States annually (Mead et al., 1999). Beef and dairy products are estimated to be responsible for 10% of all human *Salmonella* clinical cases from outbreaks in which the vehicle of transmission is known (Centers for Disease Control, 2000). Antimicrobial resistance among *Salmonella* isolates from food animals and the potential spread to humans has heightened public health concern and debate over antimicrobial agent use in food production systems (Seyfarth et al., 1997; Witte, 1998; Fey et al., 2000). Few studies to date have examined the effect of the discontinued use of antimicrobial agents on antimicrobial-resistant *Salmonella* in dairy cattle.

In the United States, certified organic dairy farms have restrictions on antimicrobial drug use (USDA, 1999). In a previous analysis of data from farms used for the current study, organic farms reported significantly less antimicrobial drug use than conventional farms (Zwald et al., 2004). There have been no studies examining the relationship between organic farming practices and isolation of antimicrobial drug-resistant *Salmonella* among dairy farms. The in vitro antimicrobial susceptibility of a microorganism is usually determined by broth dilution or disk diffusion methods. The MIC or inhibition zone diameter may be categorized as susceptible, intermediate, or resistant based on breakpoint standards established by the Clinical and Laboratory Standards Institute (**CLSI**; formerly National Committee for Clinical Laboratory Standards; NCCLS, 2000, 2002a,b). In the face of emerging antimicrobial resistance, it is important to examine reduced susceptibility of *Salmonella* isolates below resistant breakpoints. Methods for analyzing the MIC distribution of *Salmonella* isolates may also be useful in identifying risk factors for emerging resistance. Stegman et al. (2003) used survival analysis using logistic proportional hazards models to examine emerging antimicrobial resistance in *Enterococcus faecium* of poultry over time with

MIC included as the response variable. In this study, we used a similar method to examine the difference in antimicrobial MIC in *Salmonella* isolates from conventional and organic dairy farms. The objective of this study was to evaluate the association of farm management type (organic vs. conventional) with the presence of *Salmonella* with increased resistance to antimicrobial agents on dairy farms in the Midwest and northeastern United States.

MATERIALS AND METHODS

Study Herd Recruitment and Selection

One hundred thirty-one dairy farms from New York, Michigan, Minnesota, and Wisconsin were enrolled in this study. Herds were selected based on herd size (lactating and dry cows) and management type (organic or conventional). The target number of herds to enroll within each state, management, and herd size category (30 to 49, 50 to 99, 100 to 199, and >199 milk cows) was predetermined to provide adequate statistical power (0.8) to evaluate major risk factors. A list of dairy herds from each state was obtained from the respective State Departments of Agriculture. A letter describing the research project was sent to between 500 and 571 conventional herds per state (2,102 letters total) within 100 miles of participating universities (Cornell University, Ithaca, NY; Michigan State University, East Lansing; University of Wisconsin, Madison; and University of Minnesota, St. Paul). Farms interested in participating in the study were asked to respond by returning a prepaid postcard. Criteria for eligibility of dairy herds included having at least 30 milking cows, having at least 90% of cows of Holstein breed, raising their own replacement cattle, keeping a herd record system with unique identification for each cow, and shipping milk year round. The final list for conventional farm enrollment was obtained by randomly selecting 97 farms from a list of 295 conventional farms that responded with an interest in participation and that met the eligibility criteria. Each state identified organic dairy herds from independent organic certifying agencies, organic milk cooperatives, and personal contacts. All organic farms enrolled in this study were under organic management or were certified organic for at least 3 yr prior to enrollment. Although no national organic standards existed at the time of this study, rules among organic certifying agencies in this study were similar to current national organic standards (USDA, 1999). Herds that were not certified by an organic certifying agency, but were under organic management for at least 3 yr were enrolled as organic farms. The antibiotic and management practices of organic and conventional herds in this study were reported previously (Zwald et al., 2004). Thirty-two organic farms meeting eligibility criteria with an interest in participation were enrolled. Although 131 herds were selected for enrollment, biological samples were not collected from 2 of the farms enrolled. A more detailed description of study herd recruitment and selection criteria methods has been published previously (Fossler et al., 2004, 2005).

Sample Collection and Processing

Environmental and fecal samples were collected at approximately 2-mo intervals from August 2000 to October 2001 at 32 organic farms and 97 conventional farms in Michigan, Minnesota, New York, and Wisconsin. Fecal samples were taken from healthy cows and target cattle groups consisting of preweaned calves receiving milk or milk replacer, cows to be culled within 14 d, cows within 14 d of calving, and sick cows. Sick cows were defined as cows designated as sick by farm workers or a veterinarian within the previous week or cows having clinical signs of illness evident to farm or project workers on day of visit (except for localized reproductive tract or mammary infections). The number of fecal samples collected per herd and cattle group at each visit was based on herd size and calculated to provide similar herd-level sensitivity of *Salmonella* detection assuming the same prevalence for all herds (Warnick et al., 2003; Fossler et al., 2004, 2005). The target fecal sample size per visit was 30, 40, 50, and 55 for herds with 30 to 49, 50 to 99, 100 to 199, and greater than 199 milk cows, respectively. Individual cattle were sampled at each visit without regard to previous sampling status. A new glove was used to collect approximately 10 g of fecal material from the rectum of each selected animal. Fecal samples were placed in separate Whirl-Pak (Nasco, Fort Atkinson, WI) sterile sample collection bags for delivery or overnight shipment to Michigan State University.

At each visit, one sample from each of the following locations was collected by wiping areas with sterile gauze soaked in double-strength sterile skim milk: calving pen floor, sick pen floor, calf pen or hutch floor, feed bunk of lactating cows, lagoon or manure storage area, and bird droppings from cattle housing or feed storage areas. A swab was also taken from the flank of cows to be culled within 14 d. In addition, a 60-mL sample from the bulk milk tank, a milk line filter, and a 100-mL water sample from a lactating-cow water tank or pooled sample from 5 individual waterers were collected. If a particular source was not available for sampling, no sample was collected at that visit. Milk-line filters and environmental samples collected with sterile gauze pads were placed in separate Whirl-Pak (Nasco) plastic

bags and liquid samples were placed in plastic bottles for shipment to Michigan State University.

All fecal and environmental samples were stored on ice and taken to or shipped within 36 h to Michigan State University for *Salmonella* isolation and serogroup classification as previously described by Fossler et al. (2004). *Salmonella* cultures were stored at –80°C in a 50:50 solution of tryptic soy broth culture solution and 65% glycerol within 2 wk of delivery to the laboratory.

The MIC of *Salmonella* isolates from fecal and environmental samples was determined for 16 or 17 antimicrobial drugs using a broth microdilution method. Due to resource constraints, not all *Salmonella* isolates were recovered for MIC testing, but efforts were made to recover at least one isolate per positive sample. If more than one isolate was obtained from a single sample, the first isolate from a list of isolates within that sample was selected to be recovered for MIC testing. If there was no recovery from the frozen culture of the first isolate, attempt was made to recover at least one remaining isolate from that sample. An inoculating wire, sterilized by flaming was used to remove a small portion of the frozen *Salmonella* culture from storage. The frozen *Salmonella* culture was streaked for isolation onto xylose lysine desoxycholate-4 (XLT-4) selective agar (BD Diagnostic Systems, Sparks, MD). A single isolated colony was then streaked onto Mueller Hinton agar and incubated 24 h at 37°C. Antimicrobial MIC of *Salmonella* isolates were determined using the Sensititre semiautomated antimicrobial susceptibility testing system following the manufacturer's instructions (Trek Diagnostic Systems, Westlake, OH). For each antimicrobial agent, the minimum dilution that inhibited growth of the *Salmonella* isolate was recorded as the MIC. Quality control was performed every day antimicrobial susceptibility testing was conducted using *Escherichia coli* ATCC 25922. The CLSI ranges for quality control were used when available (NCCLS, 2002a,b). For drugs with no available CLSI quality control ranges, the MIC of the quality control organism was compared with a range of values generated from previous susceptibility tests of the same strain. Quality control results were always within expected ranges.

Two antimicrobial agent dilution panels (CMV4CNCD and CMV7CNCD, Trek Diagnostic Systems) were used to determine the MIC of *Salmonella* isolates. The antimicrobial concentrations were similar in both dilution panels. *Salmonella* isolates tested earlier in the study (approximately one-half of all isolates) were analyzed for the MIC of 17 antimicrobial agents using panel 1 (#CMV4CNCD). The remaining isolates tested later in the study were analyzed for the MIC of 16 antimicrobial agents using panel 2 (#CMV7CNCD). Only panel 1 contained apramycin and florfenicol, and only panel 2 contained cefoxitin. The dilution ranges for amikacin in the 2 panels only overlapped at one dilution (4 μg/mL) and all concentrations in both panels were below the CLSI resistant breakpoint. Amikacin, apramycin, florfenicol, and cefoxitin were not included in this analysis due to incomplete information related to these antimicrobial agents for all *Salmonella* isolates tested. The 14 antimicrobial agents included in this analysis were amoxicillin-clavulanic acid, ampicillin, ceftriaxone, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

Antimicrobial Resistance Classification

The CLSI interpretive criteria were used to classify *Salmonella* isolates as resistant or not resistant to individual antimicrobial agents based on MIC panel results (NCCLS, 2002a,b). The CLSI resistant breakpoints for all antimicrobial agents in this study were based on human data for Enterobacteriaceae. No interpretive criteria for Enterobacteriaceae were available for ceftiofur or streptomycin, so the resistant breakpoints presented in the National Antimicrobial Resistant Monitoring System report were used for these antimicrobial agents (USDA, 2000).

Most of the isolates were classified as susceptible or resistant based on MIC results with few isolates classified as having intermediate resistance. For analysis by logistic regression, isolates were classified as either resistant or not resistant. Isolates classified as resistant to more than 4 antimicrobial agents were also classified as exhibiting multiple drug resistance.

Statistical Analyses

Database and Statistical Software. All herd information and laboratory data were stored in a Microsoft Access (Microsoft Corporation, Redmond, WA) database and analyzed in SAS v.8.0 (SAS Institute, Inc., Cary, NC). Univariable descriptive statistics were obtained using the frequency procedure in SAS. Logistic regression was performed using the logistic procedure and the logistic proportional hazards model was performed using the PHREG procedure with TIES=DISCRETE in SAS.

Herd-Level Analysis. The susceptibility of 1,243 *Salmonella* isolates (from 95 herds) to 14 antimicrobial agents was determined and included in our analysis. The number of isolates per farm recoverable and tested for antimicrobial susceptibility varied between 1 and 153. All isolates with antimicrobial susceptibility re-

sults were included in this analysis. Due to the varied number of observations per farm and our interest in a herd-level factor, all regression analyses were performed at the herd level. Our main objective was to examine the association between farm type (organic vs. conventional) and *Salmonella* antimicrobial susceptibility. This was done in 2 ways for each antimicrobial agent; first by using logistic regression to analyze the effect of management type on the proportion of farms with at least one isolate classified as resistant, and second using logistic proportional hazards models to compare the distributions of the maximum farm-level MIC between management types. The maximum observed MIC found among all isolates per farm and corresponding antimicrobial susceptibility classification was used as the response variable in the logistic proportional hazards and logistic regression models, respectively.

Herd Size and State. Herd size and state were included in all models to control for possible confounding effects. Herd size (number of cows) was included as a continuous variable in the model because previous studies have reported a positive association between increasing herd size and *Salmonella* shedding (Kabagambe et al., 2000; Wells et al., 2001; Huston et al., 2002; Fossler et al., 2005). Among herds with susceptibility results, 51.0% of farms with less than 100 cows had at least one resistant *Salmonella* isolate, whereas 77.3% of the farms with 100 cows or more had at least one *Salmonella* isolate with reduced susceptibility ($P < 0.01$). Sixty percent of conventional herds had 100 milking cows or more compared with 26.9% of organic herds with 100 milking cows or more. For this analysis, only conventional farms within a comparable size range of organic farms were included, resulting in exclusion of 11 conventional herds with more than 400 cows. State and herd size distributions for the 95 farms included in this analysis are presented in Table 1. State was included in the model because an unequal number of organic and conventional farms were sampled from each state and antimicrobial susceptibility differed across states. State was also included in the model to control for possible sampling biases due to minor differences between states in the execution of the sample collection protocol.

Logistic Regression. Fourteen herd-level logistic regression models were used to examine the association between management type (organic vs. conventional) and resistance to individual antimicrobial agents, with resistance (positive or negative) to the individual antimicrobial agent as the response variable. A herd was classified as positive if at least one *Salmonella* isolate from that farm was classified as resistant to the antimicrobial agent of interest. A herd was classified as negative if none of the *Salmonella* isolates from a herd was classified as resistant to the antimicrobial agent of interest. Organic management was the reference level for management type in all logistic regression models. A separate model to examine the association between management type and resistance to any combination of at least 5 antimicrobial agents was also developed. In this model, a herd was considered positive if at least one *Salmonella* isolate from the herd exhibited resistance to at least 5 antimicrobial agents.

Table 1. Description of dairy herds studied to examine the association of management type with antimicrobial drug-resistant *Salmonella*

Farm location and type	No. of herds	Herd size (no. of cows)		
		Minimum	Median	Maximum
All states				
Organic	26	26	51	368
Conventional	69	30	109	391
Michigan				
Organic	2	73	221	36
Conventional	22	74	114	391
Minnesota				
Organic	7	26	42	70
Conventional	15	38	109	266
New York				
Organic	7	30	50	342
Conventional	15	30	99	357
Wisconsin				
Organic	10	36	59	363
Conventional	17	34	90	366

In the logistic regression model, the odds ratio function $[P/(1 - P)]$ for a vector of explanatory variables (x) is represented by the following equation:

$$\frac{P}{1 - P} = \exp\,(\beta_0 + \beta^T x),$$

where P is the probability that a herd with x covariates has at least one *Salmonella* isolate classified as resistant to the individual antimicrobial agent being tested. An analogous model was used with P equal to the probability that a herd with x covariates had at least one *Salmonella* isolate resistant to 4 or more antimicrobial agents. In these models, β_0 is equal to the intercept coefficient and β^T is a vector of regression coefficients for the set of x covariates (herd size, state, and farm management type). The parameter estimates (β^T) of the x covariates are equal to the log odds ratios.

Proportional Hazards Regression. The Cox proportional hazards (**PH**) model is commonly used in hazard regression when the hazard is equal to time to event (t), such as death, and is useful in the analysis of right-censored data, in which the event is not observed before the end of the observation period. This model can also be used for right-censored response variables other

than time (Therneau, 2000). In our model, we defined t as the within-farm maximum MIC of all *Salmonella* isolates found on the farm. Farms with at least one *Salmonella* isolate resistant to the highest concentration tested were included in the model as right-censored observations.

In the PH model, the conditional hazard function $[\lambda(t|x)]$ for a set of x covariates is represented by the following equation:

$$\lambda(t; x) = \lambda_0(t)\exp(\beta^T x),$$

where $\lambda_0(t)$ is a baseline hazard that is not specified and is estimated by nonparametric methods and β^T is a vector of regression coefficients for the set of x covariates. In our model, the x covariates were state, herd size, and management type (organic or conventional).

The PH model assumes that the maximum MIC from each herd can take on any concentration within the range of dilutions tested. This is obviously not the case given the standard method of using 2-fold dilutions for antimicrobial susceptibility testing. To account for the occurrence of a large number of ties at 2-fold dilution increments, time to event was transformed to the $\log_2$ of the maximum MIC observed per farm and treated as a discrete variable by transforming the PH model into a logistic model for hazards as described by Therneau (2000). It is reasonable to analyze MIC as a discrete variable because the 2-fold dilution method is commonly used in clinical and nonclinical diagnostic research and the susceptibility testing measurement is limited to these standard 2-fold dilution increments. The logistic PH model is constructed as follows:

$$\frac{\lambda(t; x)}{1 - \lambda(t; x)} = \frac{\lambda_0\,(t)}{1 - \lambda_0\,(t)} \exp\,(\beta^T x),$$

where the parameter estimates (β^T) of the x covariates from the logistic PH model are equal to the log odds ratios.

Fourteen herd-level logistic PH models were constructed to examine the association between farm management type (organic vs. conventional) and the maximum MIC exhibited to individual antimicrobial agents by *Salmonella* isolates from each farm. For some antimicrobial agents, the max $\log_2$(MIC) was less than or equal to zero. When this was observed, all maximum $\log_2$(MIC) values were transformed for the analysis by adding 10 to each maximum $\log_2$(MIC) of that antimicrobial agent. Some herds had *Salmonella* isolates resistant to the highest dilution of antimicrobial agent tested. If the most resistant isolate from a herd was not susceptible to the highest concentration tested, that herd was right-censored. If the highest observed MIC from a herd was below the joint concentration range of both panels, the observation was set equal to the lowest MIC detectable by both panels. In contrast to the logistic regression models, conventional management was used as the reference level for management type in all logistic PH models. This was done to make the odds ratios more easily comparable between models.

RESULTS

Descriptive Statistics

A total of 24,762 fecal and 5,056 environmental samples were cultured for *Salmonella* spp. from 129 farms (32 organic and 97 conventional) and *Salmonella* was isolated from samples from 120 farms. Serogroup and sample source frequencies of *Salmonella* isolated from study farms have been previously published (Fossler et al., 2004). *Salmonella* samples from 11 conventional herds with more than 400 cows were excluded from this analysis. *Salmonella* was isolated from 1,018 fecal and 228 environmental samples (1,246 total) from 109 herds (30 organic and 79 conventional) with less than 400 cows. More than one isolate was obtained from some samples resulting in 1,443 fecal and 335 environmental isolates (1,778 total) from these farms. We attempted to recover at least one isolate per sample for MIC testing. Some isolates could not be recovered due to difficulty in recovering a viable culture from storage. Therefore, MIC results were available for 1,243 isolates from 95 farms (26 organic, 69 conventional) for this analysis. The median number of cows on conventional farms was 109, whereas the median number of cows on organic farms was 51. Although efforts were made to enroll several organic farms from each state, only 2 organic farms from Michigan were enrolled in this study (Table 1).

The frequency of maximum MIC values of each antimicrobial agent recorded for each farm by management type is presented in Table 2. The frequency of resistance to individual antimicrobial agents by at least one *Salmonella* isolate from each farm is presented in Table 3.

The percentage of farms with at least one isolate resistant to individual antimicrobial agents ranged from 0% for ceftriaxone and ciprofloxacin to 37.9% for cephalothin (Table 3). None of the *Salmonella* isolated from conventional or organic farms exhibited resistance to ceftriaxone or ciprofloxacin. The number of farms with at least one *Salmonella* isolate exhibiting resistance to individual antimicrobial agents was greatest for tetracycline, cephalothin, amoxicillin-clavulanic acid, and ampicillin. There were 40.6% of conventional farms and 30.8% of organic farms with at least one *Salmonella* isolate resistant to tetracycline, 37.7% of conventional farms and 30.8% of organic farms with at

Table 2. Herd-level maximum MIC values of *Salmonella* isolates from organic and conventional dairy farms in the Northeast and Midwestern United States

		Conventional herds (n = 69)		Organic herds (n = 26)	
Antimicrobial agent	MIC (μg/mL)	No. of Herds	% of Herds	No. of Herds	% of Herds
Amoxicillin-clavulanic acid (RB[1] ≥ 32:16 μg/mL)	0.5:0.25[2]	0	0.0	1	3.8
	1:0.5	21	30.4	9	34.6
	2:1	4	5.8	0	0.0
	4:2	8	11.6	2	7.7
	8:4	6	8.7	3	11.5
	16:8	9	13.0	2	7.7
	32:16	5	7.2	1	3.8
	>32:16	16	23.2	8	30.8
Ampicillin (RB ≥ 32 μg/mL)	1[3]	0	0.0	0	0.0
	2	23	33.3	9	34.6
	4	15	21.7	6	23.1
	8	7	10.1	0	0.0
	16	2	2.9	0	0.0
	32	3	4.3	3	11.5
	>32	19	27.5	5	19.2
Ceftiofur (RB[4] ≥ 8 μg/mL)	0.125[3]	0	0.0	1	3.8
	0.25[3]	0	0.0	0	0.0
	0.5	42	60.9	18	69.2
	1	14	20.3	4	15.4
	2	0	0.0	0	0.0
	4	3	4.3	0	0.0
	8	2	2.9	0	0.0
	16[2]	5	7.2	2	7.7
	>16	3	4.3	1	3.8
Ceftriaxone (RB ≥ 64 μg/mL)	0.25	58	84.1	23	88.5
	0.5	1	1.4	0	0.0
	1	1	1.4	0	0.0
	2	0	0.0	0	0.0
	4	0	0.0	1	3.8
	8	2	2.9	1	3.8
	16	3	4.3	0	0.0
	32	4	5.8	1	3.8
	64	0	0.0	0	0.0
	>64	0	0.0	0	0.0
Cephalothin (RB ≥ 32 μg/mL)	1[2]	0	0.0	1	3.8
	2	10	14.5	5	19.2
	4	13	18.8	4	15.4
	8	7	10.1	2	7.7
	16	13	18.8	6	23.1
	32	12	17.4	3	11.5
	>32	14	20.3	5	19.2
Chloramphenicol (RB ≥ 32 μg/mL)	2[3]	0	0.0	0	0.0
	4	35	50.7	17	65.4
	8	18	26.1	6	32.1
	16	2	2.9	0	0.0
	32	2	2.9	2	7.7
	>32	12	17.4	1	3.8
Ciprofloxacin (RB ≥ 4 μg/mL)	0.015	32	46.4	14	53.8
	0.03	22	31.9	8	30.8
	0.06	13	18.8	2	7.7
	0.12	2	2.9	1	3.8
	0.25	0	0.0	1	3.8
	0.5	0	0.0	0	0.0
	1	0	0.0	0	0.0
	2	0	0.0	0	0.0
	4	0	0.0	0	0.0
	>4	0	0.0	0	0.0

Continued

Table 2 (Continued). Herd-level maximum MIC values of *Salmonella* isolates from organic and conventional dairy farms in the Northeast and Midwestern United States

		Conventional herds (n = 69)		Organic herds (n = 26)	
Antimicrobial agent	MIC (μg/mL)	No. of Herds	% of Herds	No. of Herds	% of Herds
Gentamicin	0.25	33	47.8	7	26.9
(RB ≥ 16 μg/mL)	0.5	13	18.8	10	38.5
	1	10	14.5	6	23.1
	2	2	2.9	2	7.7
	4	1	1.4	0	0.0
	8	3	4.3	0	0.0
	16	3	4.3	0	0.0
	>16	4	5.8	1	3.8
Kanamycin	8[3]	2	2.9	1	3.8
(RB ≥ 64 μg/mL)	16	50	72.5	20	76.9
	32	2	2.9	1	3.8
	64	2	2.9	2	7.7
	>64	13	18.8	2	7.7
Nalidixic acid	0.5[3]	0	0.0	0	0.0
(RB ≥ 32 μg/mL)	1[3]	0	0.0	0	0.0
	2[3]	0	0.0	0	0.0
	4	19	27.5	8	30.8
	8	42	60.9	11	42.3
	16	7	10.1	6	23.1
	32	1	1.4	0	0.0
	>32	0	0.0	0	0.0
	64[2]	0	0.0	0	0.0
	128[2]	0	0.0	1	3.8
	256[2]	0	0.0	0	0.0
	>256	0	0.0	0	0.0
Streptomycin	32	48	69.6	24	92.3
(RB[4] ≥ 64 μg/mL)	64	4	5.8	1	3.8
	>64	6	8.7	0	0.0
	128[2]	2	2.9	1	3.8
	256[2]	5	7.2	0	0.0
	>256	4	5.8	0	0.0
Sulfamethoxazole	16[3]	2	2.9	1	3.8
(RB ≥ 512 μg/mL)	32[3]	0	0.0	0	0.0
	64[3]	0	0.0	0	0.0
	128	47	68.1	21	80.8
	256	0	0.0	0	0.0
	512	3	4.3	3	11.5
	>512	17	24.6	1	3.8
Tetracycline	4	40	58.0	17	65.4
(RB ≥ 16 μg/mL)	8	0	0.0	1	3.8
	16	2	2.9	0	0.0
	32	6	8.7	4	15.4
	>32	21	30.4	4	15.4
Trimethoprim-sulfamethoxazole	0.12:2.38	47	68.1	19	73.1
(RB ≥ 4:76 μg/mL)	0.25:4.75	14	20.3	5	19.2
	0.5:9.5	7	10.1	0	0.0
	1:19	0	0.0	0	0.0
	2:38	0	0.0	1	3.8
	4:76	0	0.0	0	0.0
	>4:76	1	1.4	1	3.8

[1]RB = Resistant breakpoint.

[2]Only Panel 1 (CMV4CNCD) contained this concentration of the antimicrobial agent.

[3]Only Panel 2 (CMV7CNCD) contained this concentration of the antimicrobial agent.

[4]RB for ceftiofur and streptomycin obtained from National Antimicrobial Resistance Monitoring System 2000 Annual Report (USDA, 2000); RB for all other antimicrobial agents obtained from CLSI (NCCLS, 2002a,b).

Table 3. Frequency of farms with at least one resistant *Salmonella* isolate to individual antimicrobial agents by farm management type

Antimicrobial agent	Conventional (n = 69)	Organic (n = 26)	Total (n = 95)
	No. of farms (%)	No. of farms (%)	No. of farms (%)
Amoxicillin-clavulanic acid	21 (30.4)	9 (34.6)	30 (31.6)
Ampicillin	22 (31.9)	8 (30.8)	30 (31.6)
Ceftiofur	10 (14.5)	3 (11.5)	13 (13.7)
Ceftriaxone	0 (0)	0 (0)	0 (0)
Cephalothin	26 (37.7)	8 (30.8)	34 (35.8)
Chloramphenicol	14 (20.3)	3 (11.5)	17 (17.9)
Ciprofloxacin	0 (0)	0 (0)	0 (0)
Gentamicin	7 (10.1)	1 (3.8)	8 (8.4)
Kanamycin	15 (21.7)	4 (15.4)	19 (20.0)
Nalidixic acid	1 (1.5)	1 (3.8)	2 (2.1)
Streptomycin	21 (30.4)	2 (7.7)	23 (24.2)
Sulfamethoxazole	20 (29.0)	4 (15.4)	24 (25.3)
Tetracycline	28 (40.6)	8 (30.8)	36 (37.9)
Trimethoprim-sulfamethoxazole	1 (1.5)	1 (3.8)	2 (2.1)
≥5 Antimicrobial agents	17 (24.6)	3 (11.5)	20 (21.1)

least one *Salmonella* isolate resistant to cephalothin, 30.4% of conventional farms and 34.6% of organic farms with at least one *Salmonella* isolate resistant to amoxicillin-clavulanic acid, and 31.9% of conventional farms and 30.8% of organic farms with at least one *Salmonella* isolate resistant to ampicillin. The number of farms with at least one *Salmonella* isolate exhibiting resistance to individual antimicrobial agents was lowest for ceftriaxone, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole (Table 3). *Salmonella* isolates resistant to 5 or more antimicrobial agents were present on both types of farms, occurring on 24.6% of conventional farms and 11.5% of organic farms.

Multivariable Analysis of Individual Antimicrobial Agents

Logistic regression was used to examine the relationship between antimicrobial resistance and farm management type for individual antimicrobial agents. Herd size and state were included in each model to control for confounding effects. No significant 2-way interaction effects were observed. Streptomycin was the only antimicrobial agent with a significant association between farm type and proportion of farms with resistance. Conventional farms were more likely to have at least one streptomycin resistant *Salmonella* isolate [odds ratio (**OR**) = 7.5; 95% confidence interval (**CI**) = 1.7–55.4]. Increasing herd size was also associated with a significant increase in odds for the presence of at least one *Salmonella* isolate resistant to streptomycin. Increasing herd size was associated with increased risk of a farm having at least one isolate resistant to one or more of the following antimicrobial agents: amoxicillin-clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, gentamicin, streptomycin, sulfamethoxazole, or tetracycline.

Proportional hazards analysis was used to examine the relationship between farm management type and antimicrobial drug resistance using logistic PH models. This method is useful for detecting differences in MIC when most observations are below the resistant breakpoint used for logistic regression. Few observations were above the resistant breakpoint for nalidixic acid, ciprofloxacin, ceftriaxone, trimethoprim-sulfamethoxazole, and gentamicin. No significant association was found between farm management type and these antimicrobial agents with the logistic PH model. Streptomycin and sulfamethoxazole exhibited a significant association ($P < 0.05$) between MIC and farm management type with the logistic PH model. Isolates from conventional farms were associated with higher streptomycin MIC than isolates from organic farms (OR = 5.4). A similar association was observed for sulfamethoxazole, with isolates from conventional farms exhibiting higher MIC than isolates from organic farms (OR = 4.2). Logistic proportional hazard analysis and logistic regression results for all antimicrobial agents are summarized in Table 4.

Multivariable Analysis of Resistance to At Least 5 Antimicrobial Agents

In the multivariable analysis with state and farm type as categorical variables and herd size as a continuous variable, conventional farms tended to be more likely than organic farms to have at least one *Salmonella* isolate resistant to 5 or more antimicrobial drugs. ($P = 0.12$; OR = 3.1; 95% CI = 0.83–15.3; Table 5). Two-way interaction effects were nonsignificant ($P > 0.15$).

Table 4. Association between farm management type (conventional vs. organic) and presence of at least one *Salmonella* isolate with increased resistance to individual antimicrobial agents controlling for state and herd size

	Logistic regression model:odds ratio[1]			Logistic PH model:odds ratio[2]		
		95% Confidence interval			95% Confidence interval	
Antimicrobial agent	Point estimate	Lower bound	Upper bound	Point estimate	Lower bound	Upper bound
Amoxicillin-clavulanic acid	0.81	0.29	2.3	0.74	0.40	1.4
Ampicillin	1.2	0.41	3.5	1.1	0.55	2.1
Ceftiofur	1.3	0.32	6.8	1.5	0.69	3.4
Ceftriaxone	N/A[3]	N/A	N/A	1.6	0.51	5.1
Cephalothin	1.1	0.39	3.3	1.0	0.55	1.9
Chloramphenicol	2.4	0.60	12.6	1.7	0.77	3.9
Ciprofloxacin	N/A	N/A	N/A	0.87	0.40	1.9
Gentamicin	3.8	0.55	78.7	1.1	0.58	2.2
Kanamycin	2.3	0.6	10.1	2.0	0.73	5.7
Nalidixic acid	0.38	0.01	11.0	0.47†	0.20	1.1
Streptomycin	7.5*	1.7	55.4	5.4**	1.50	19.0
Sulfamethoxazole	2.8	0.78	12.9	4.2*	1.20	14.1
Tetracycline	1.8	0.63	5.5	1.8	0.80	4.1
Trimethoprim-sulfamethoxazole	0.54	0.02	15.2	1.2	0.50	3.1

[1]Organic management is the reference level.

[2]Conventional management is the reference level; PH = proportional hazards.

[3]N/A = Distribution of data not appropriate for analytical method.

†$P \leq 0.10$; *$P < 0.05$; **$P < 0.01$.

DISCUSSION

Antimicrobial use in animal production systems has been scrutinized as the primary cause of the emergence and dissemination of antimicrobial resistant *Salmonella* (Cohen and Tauxe, 1986; Fey et al., 2000) and other enteric bacteria. Few studies have examined the change in antimicrobial resistance among enterobacteria in food animals after the discontinued use of antimicrobial drugs. In the United States, the largest source of information available to examine emerging resistance comes from human and veterinary clinical isolates submitted to the Centers for Disease Control and the National Antimicrobial Resistance Monitoring System (CDC, 2005). Much of the data published on antimicrobial susceptibility of *Salmonella* isolates from dairy cattle has been from clinically ill cattle. *Salmonella* serotypes and resistance patterns frequently observed in clinical isolates may not be representative of isolates from healthy cattle. A study conducted by Wells et al. (2001) reported that approximately 10% of *Salmonella* isolates from healthy dairy cattle were resistant to at least 1 of 17 antimicrobial agents tested. *Salmonella* isolates from healthy and ill dairy cattle and the environment from 26 organic and 69 conventional dairy farms from 4 states were available for our analysis, providing a more representative sample for characterizing antimicrobial susceptibility among conventional and organic dairy farms than analysis of clinical isolates alone.

Table 5. Multivariable logistic regression model to examine dairy farm management type (organic vs. conventional) as a risk factor for the presence of at least one *Salmonella* isolate resistant to at least 5 antimicrobial agents

			95% Confidence interval[1]		
Variable	Level	Odds ratio[1]	Lower bound	Upper bound	*P*-value
Herd size (no. of cows)	Continuous	1.005	1.000	1.011	0.05
State	MI	0.21	0.04	0.87	0.16
	MN	0.30	0.06	1.25	
	NY	0.39	0.09	1.54	
	WI	1.00			
Farm type	Conventional	3.1	0.83	15.3	0.12
	Organic	1.00			

[1]Maximum likelihood approximation.

Salmonella with resistance to at least one of amoxicillin-clavulanic acid, ampicillin, cephalothin, kanamycin, streptomycin, sulfamethoxazole, or tetracycline was observed among the highest percentages of farms in our study. These findings are in agreement with other reports of antimicrobial resistance among *Salmonella* isolated from dairy cows. Results from a longitudinal study conducted on 6 dairies in New Mexico and Texas found *Salmonella* isolates to be most frequently resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfamethoxazole, and tetracycline (Edrington et al., 2004). Wells et al. (2001) also found resistance to ampicillin, streptomycin, and tetracycline to account for the highest percentages of resistance among *Salmonella* isolates from dairy cows across 19 states. Describing *Salmonella* isolates as resistant in our study and other similar investigations are based on laboratory measurements of susceptibility. Although useful for comparison among farms and for monitoring changes in susceptibility over time, we recognize that classifying *Salmonella* isolates as resistant based on CLSI breakpoints or other commonly used interpretive criteria is not necessarily related to clinical efficacy.

A survey of antimicrobial use was administered to all dairy farms enrolled in this study and a summary of reported usage can be found in Zwald et al. (2004). Over 90% of organic farms reported no antimicrobial administration to milking cows. The majority of conventional dairy owners reported antibiotic use for the treatment of various gastrointestinal, respiratory, and mammary infections in the herd. In addition, 49% of conventional farms in this survey reported use of medicated milk replacer whereas only one organic farm (3%) reported the use of medicated milk replacer. The most commonly reported antimicrobial agents used within the previous 60 d on conventional dairy farms were penicillins, cephalosporins, and tetracyclines (Zwald et al., 2004). Although resistance to these antimicrobial agents was observed among a high percentage of dairy herds, it is interesting to note that no significant difference in resistance to these individual antimicrobial agents was observed between organic and conventional dairy farms in our study.

The analysis of antimicrobial susceptibility data is largely constrained by susceptibility breakpoints and dilution panel ranges. The CLSI interpretive criteria for classification of susceptible, intermediate, and resistant isolates based on MIC results are determined based on pharmacological properties of the antimicrobial agent, microbiological characteristics of the pathogen, and clinical efficacy data (NCCLS, 2002a). Although these breakpoints create natural cut-offs for dichotomizing antimicrobial susceptibility when analyzing MIC data, this results in the loss of some information with regard to differences in incremental increases in resistance. In addition, it can be difficult to detect differences in antimicrobial resistances among associated measures when observations are largely distributed above or below susceptibility breakpoints, as was the case for ceftriaxone, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole in our study. In practice, classification of isolates as susceptible or resistant is often the main objective and it is difficult to obtain exact MIC values for several antimicrobial agents when testing numerous microbial isolates. In this study, we attempted to account for these measurement constraints by employing 2 analytical methods, logistic regression and proportional hazards regression, to examine the relationship between increased resistance and farm management type.

Both models produced very similar OR for all antimicrobial agents, but the logistic PH model always produced narrower CI. Conventional farms were significantly associated with increased resistance to streptomycin in both models ($P < 0.05$), whereas the strength of association between conventional farms and increased resistance to sulfamethoxazole was statistically significant in the logistic PH model only ($P < 0.05$). In this case, right-censoring and taking into account uncertainty of true MIC values beyond the range of dilutions tested provided enough information to show a significant difference between increased resistance and farm management type with the PH model. Sulfonamide use was reported within the previous 60 d on 23.7% of conventional study farms compared with 0% of organic study farms. This might be a reason for the observed difference in increased resistance to sulfamethoxazole between *Salmonella* isolates from organic and conventional farms. Farm management type was not significantly associated with increased resistance to the other antimicrobial agents by logistic regression or logistic PH analysis; however, statistical power may not have been adequate for detecting a significant difference for some antimicrobial agents given our sample size of 95 herds.

Streptomycin was the first aminoglycoside discovered, and is still used in animal production systems. Streptomycin/penicillin is approved for the treatment and prevention of mastitis in nonlactating dairy cows. Streptomycin use was not reported on many conventional farms enrolled in this study, but information was not collected on individual antimicrobial agents used for dry-cow therapy. Longitudinal and other studies examining antimicrobial resistance among *Salmonella* isolates on US dairies have found high percentages of isolates resistant to streptomycin (Wells et al., 2001; Edrington et al., 2004). Given the low numbers of conventional farms reporting aminoglycoside use, our

findings suggest that current selection pressure through streptomycin use may not have been the only factor contributing to the increased presence of streptomycin resistant *Salmonella* on conventional dairy farms. Before 1990, streptomycin was widely used to treat a variety of animal diseases. Streptomycin resistance could be due to an established resistance mechanism genetically linked to other beneficial genes on an integron or selected for by other antimicrobial agents utilizing the same resistance mechanism.

Most MIC observations for nalidixic acid were below the resistant breakpoint, and in fact, susceptible to the lowest concentration of nalidixic acid tested. Logistic PH analysis allowed us to examine the relationship between farm management type and resistance to dilutions below the resistant breakpoint. It is interesting to note that *Salmonella* isolates from organic dairy farms tended to be less susceptible to nalidixic acid ($P = 0.07$) when the maximum observed MIC values of *Salmonella* isolates from organic and conventional dairy farms were compared with the logistic PH model. Nalidixic acid is an antimicrobial agent from which the fluoroquinolones were derived. Nalidixic acid is not used to treat animal diseases but is used to detect resistance to fluoroquinolone. Resistance to nalidixic acid is rare in *Salmonella* from cattle, but more common in *Salmonella* from poultry. A study conducted in England and Wales comparing antimicrobial susceptibility of *Salmonella* isolates from food producing animals and humans reported 2% and 11% of *Salmonella* spp. resistant to nalidixic acid from cattle and poultry, respectively (Threlfall et al., 2003). Our finding that *Salmonella* isolates from organic farms tended to be more resistant to increasing concentrations of nalidixic acid underscores the importance of considering factors other than antimicrobial use on individual farms when examining the emergence and dissemination of antimicrobial-resistant *Salmonella*.

If recent antimicrobial drug use on individual farms were the sole factor associated with antimicrobial resistant *Salmonella*, we would expect to see greater differences between increased resistance and farm management type than what was observed. Our knowledge of antimicrobial use among the farms in our study is limited to herd-level, farmer-reported antimicrobial drug use so we were unable to examine the direct association between the amount of antimicrobial drug use and the antimicrobial resistance of *Salmonella* from these herds. Organic farms from this study had been under organic management for varying lengths of time before the study began. Previous antimicrobial use before these herds transitioned to organic management could have influenced our results. Nevertheless, organic herds in our study were under organic management for at least 3 yr before enrollment. In addition, cross-resistance to antimicrobial agents has been demonstrated in *Salmonella* with adaptive resistance to the disinfectants triclosan and chlorhexidine (Braoudaki and Hilton, 2004; Randall et al., 2004). Information on biocide use among the organic and conventional farms in our study was not available, but biocide use may play a role in selecting for *Salmonella* with increased resistance to antimicrobial agents. Spatial and temporal clustering of *Salmonella* isolates has been observed (Threlfall et al., 1994; Sato et al., 2001), and movement of animals, transport vehicles, wildlife, and personnel between herds may have facilitated the dispersion of antimicrobial resistant *Salmonella* among the dairy farms in our study. Our findings highlight the importance of examining factors other than antimicrobial use on individual farms, such as the spread of antimicrobial-resistant *Salmonella* between herds, when monitoring antimicrobial-resistant *Salmonella* on dairy farms.

The emergence of *Salmonella* strains such as *S.* Typhimurium and *Salmonella* Newport, which are often resistant to multiple antimicrobial agents, has heightened public health awareness and concern about antimicrobial resistant *Salmonella* found in food production systems. *Salmonella* Typhimurium DT104 is commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Multidrug-resistant *S.* Newport is commonly resistant to multiple antimicrobial agents including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, amoxicillin-clavulanic acid, cephalothin, cefoxitin, and ceftiofur (USDA, 2003). At least one *Salmonella* isolate was found on most of the dairy farms originally enrolled in this longitudinal study and organic farm management type was not associated with *Salmonella* shedding (Fossler et al., 2004). However, our herd-level analysis examining the association between the presence of *Salmonella* resistant to 5 or more antimicrobial agents and management type found that conventional farms tended to have one or more isolates resistant to at least 5 antimicrobial agents ($P = 0.12$). The cut-off of resistance to at least 5 antimicrobial agents for classification as multiple resistant was selected because penta-resistance arising from plasmid-mediated transposons has been implicated in the emergence and dissemination of multidrug-resistant *Salmonella* (Liebert et al., 1999).

Our primary objective was to examine the association between resistance to antimicrobial agents and farm management type, but we also examined the association with herd size. We did find a strong association between increasing herd size and a herd having at least one *Salmonella* isolate resistant to at least 5 antimicro-

bial agents. In addition, herd size was significantly associated with increased odds of having at least one *Salmonella* isolate resistant to one or more of the following individual antimicrobial agents: amoxicillin-clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, gentamicin, streptomycin, sulfamethoxazole, or tetracycline. For this analysis, we only included herd sizes of up to 400 milking cows because no organic herds larger than 400 milking cows participated. According to 2001 USDA data, only 5.4% of all dairy operations within Michigan, Minnesota, New York, and Wisconsin had more than 200 milking cows (USDA, 2001).

CONCLUSIONS

Of the 14 antimicrobial agents tested, a significant association between increased resistance of *Salmonella* isolates from a dairy herd and farm management type was found only for streptomycin and sulfamethoxazole, with conventional farms harboring *Salmonella* isolates with more resistance. In some cases, analysis of MIC data by logistic proportional hazards models provided a more sensitive test for detecting incremental differences in antimicrobial drug susceptibility for MIC data distributed below resistant breakpoints than did logistic regression. Most MIC observations for nalidixic acid were below the resistant breakpoint and an association between farm management type and nalidixic acid susceptibility was more significant in the logistic proportional hazards model ($P = 0.07$) than in the logistic regression model ($P = 0.53$). Proportional hazards analysis could be a useful tool for analyzing risk factors of emerging resistance in *Salmonella* and other bacteria. *Salmonella* resistant to 5 or more antimicrobial agents tended to be associated with conventional farms in this study. Much emphasis has been placed on the local selection of antimicrobial-resistant enteric bacteria through antimicrobial agent use in food production systems. Findings from this study highlight the importance of examining the spread of antimicrobial-resistant *Salmonella* in addition to the local selection through antimicrobial drug use on individual farms.

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