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Organic or Antibiotic-Free Labeling Does Not Impact the Recovery of Enteric Pathogens and Antimicrobial-Resistant *Escherichia coli* from Fresh Retail Chicken

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Abstract

We investigated the implied health benefits of retail chicken breast labeled as ''organic'' or ''antibiotic-free'' when compared to conventional products based on frequency of contamination by *Salmonella* spp., *Campylobacter* spp., and coliform bacteria resistant to fluoroquinolones, extended-spectrum cephalosporins, or carbapenems. We purchased 231 prepackaged chicken breasts from 99 grocery stores representing 17 retail chains in Ohio, Michigan, and Pennsylvania from June to September 2012. Ninety-six (41.5%) packages were labeled ''antibiotic free'' and 40 (17.3%) were labeled ''organic,'' with the remaining 95 (41.1%) making neither label claim. *Salmonella* were recovered from 56 (24.2%) packages, and the recovery rate was not different between product types. Five percent of packages contained *Salmonella* carrying the extended-spectrum cephalosporin resistance gene $bla_{\text{CMY-2}}$, representing 21.4% of *Salmonella* isolates. *Campylobacter* spp. were recovered from 10.8% of packages, with observed recovery rates similar for the three product types. Using selective media, we recovered *Escherichia coli* harboring $bla_{\text{CMY-2}}$ from over half (53.7%) of packages, with similar recovery rates for all product types. In addition, we recovered *E. coli* carrying $bla_{\text{CTX-M}}$ from 6.9% of packages, and *E. coli* with QRDR mutations from 8.2% of packages. Fluoroquinolone-resistant *E. coli* recovered using selective media were more common ($p < 0.05$) in conventional (18.9%) compared to organic (0) and antibiotic-free (2.1%) packages. Our results indicate that, regardless of product type, fresh retail chicken breast is commonly contaminated with enteric pathogens associated with foodborne illness and commensal bacteria harboring genes conferring resistance to critically important antimicrobial drugs.

Introduction

Fresh chicken meat is an important vehicle for the zoonotic foodborne transmission of enteric pathogens (Batz *et al*., 2012). Retail chicken products are frequently contaminated with both *Salmonella* and *Campylobacter*, the bacterial pathogens most commonly associated with foodborne disease in the United States (Mead *et al*., 1999; Scallan *et al*., 2011). These organisms innocuously colonize the gastrointestinal tract of broiler chickens raised in population-dense production environments (Arsenault *et al*., 2007) and can contaminate carcasses during processing (Bryan and Doyle, 1995). The US Food and Drug Administration (FDA) 2011 report of the National Antimicrobial Resistance Monitoring System (NARMS) found that 45.7% of fresh chicken breasts were contaminated with *Campylobacter* spp. and 12% with *Salmonella* spp. (FDA, 2011). Foodborne zoonotic transmission subsequently occurs as a result of inadequate cooking or poor kitchen hygiene resulting in cross contamination (Gorman *et al*., 2002).

While most cases of salmonellosis and campylobacteriosis are self-limiting local infections that do not require medical attention in otherwise healthy individuals (Kumar *et al*., 1982), a proportion will progress to systemic infections that require antimicrobial therapy in order to prevent patient death (Stoycheva and Murdjeva, 2006). Populations at increased risk of adverse outcomes include children, the elderly, and immunocompromised individuals (Acheson and Hohmann, 2001). *Salmonella* and *Campylobacter* present in fresh retail chicken products may also be resistant to human antimicrobial drugs important for therapeutic treatment of invasive Gram-negative bacterial infections. The 2011 NARMS reported that

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22.4% of *C. jejuni* recovered from chicken breasts were resistant to ciprofloxacin, and that 33.5% of *Salmonella* spp. recovered from chicken breasts were resistant to ceftriaxone (FDA, 2011). Foodborne outbreaks of ciprofloxacin-resistant *Campylobacter* (Engberg *et al*., 2004) and ceftriaxone-resistant *Salmonella* (Dutil *et al*., 2010) associated with consumption of fresh retail chicken have been reported. Resistant infections may increase the likelihood of treatment failure, resulting in increased healthcare costs (Holmberg *et al*., 1987) and a greater risk of patient death (Helms *et al*., 2002).

Some retail chicken products are marketed as ''organic'' or ''antibiotic free'' with the implication that they are healthier than similar products without these label claims. As a result, consumers may reasonably expect that they are less likely to contain pathogens or antimicrobial-resistant organisms such as *Escherichia coli*. Any product labeled as organic must meet specific criteria for production practices established by the US Department of Agriculture (USDA) including no use of antimicrobial drugs (USDA, 2011). However, there are no requirements for production practices that can be expected when the term ''antibiotic free'' is present on food product labels. The available scientific literature suggests that fresh retail meat products labeled as organic or antibiotic free are microbiologically similar to products without those label claims (Smith-Spangler *et al*., 2012), although this has not yet been fully established. Our objective was therefore to determine the differences in contamination with pathogens and antimicrobial-resistant organisms between retail boneless chicken breasts labeled as organic or antibiotic free when compared to conventional products without those label claims.

Materials and Methods

Sampling

Prepackaged fresh boneless chicken breast with label claims of ''organic'' or ''antibiotic-free'' and conventional packages without either label claim were purchased from supermarkets in three US states between June and September 2012. A convenience sample of retail grocery stores was selected based on product availability, and only stores with boneless chicken breasts available representing at least two of these three label claims were sampled. A single package representing each of the available product types at each store was selected from available product in retail cases, purchased, and transported or shipped overnight to our laboratory at The Ohio State University.

Bacterial culture

For bacterial culture, one breast half per package was mixed in 275 mL buffered peptone water (BPW) and incubated overnight at 37°C. The following day, the chicken rinsate was aseptically inoculated to MacConkey agar and streaked for isolation. After overnight incubation at 37°C, a single *E. coli* isolate from each package was confirmed indole positive and stored for further characterization.

For the recovery of *E. coli* resistant to fluoroquinolones, 4 mL of incubated chicken rinsate was inoculated to 36 mL of nutrient broth containing 16 μg/mL nalidixic acid and incubated for 18–24 h at 37°C. This broth was then streaked onto MacConkey agar containing 2 μg/mL ciprofloxacin and incubated overnight. A single lactose-positive, indole-positive *E. coli* isolate from each positive package was reserved for further characterization.

E. coli resistant to extended-spectrum cephalosporins and carbapenems were cultured by adding 4 mL of incubated chicken rinsate to 36 mL nutrient broth containing 2 μg/mL cefotaxime (Mollenkopf *et al*., 2012). After overnight incubation, this broth was streaked onto MacConkey agar supplemented with 4 μg/mL cefepime to identify isolates with a $bla_{\text{CTX-M}}$ phenotype, onto MacConkey agar containing 8 μg/mL cefoxitin to identify isolates with a $bla_{\text{CMY-2}}$ phenotype, and to MacConkey agar with 2 μg/mL meropenem to identify phenotypic carbapenem resistance.

For isolation of *Salmonella* spp. we transferred a 100-μL chicken breast/BPW rinsate aliquot to Rappaport-Vassiliadis R10 broth, incubated overnight at 42°C and differentially selected on xylose-lysine-Tergitol 4 agar (XLT-4) with overnight incubation (Heider *et al*., 2009). From each sample, bacteria from a single black colony on XLT-4 were isolated on MacConkey agar and confirmed as *Salmonella* using standard biochemical reactions including triple sugar iron agar, urea broth, and polyvalent antisera. *Salmonella* spp. isolates were screened for extended-spectrum cephalosporin, carbapenem, and fluoroquinolone resistance by inoculation onto selective agar (Mollenkopf *et al*., 2012).

For isolation of *Campylobacter* spp., 50 mL of the chicken breast/BPW rinsate was transferred before incubation to Whirl-pak bags with 50 mL of double-strength Bolton broth and incubated at 42°C for 48 h under microaerophilic conditions. We inoculated this broth to Campy-Cefex agar and incubated under microaerophilic conditions at 42°C for 48 h. Suspect *Campylobacter* isolates were confirmed and speciated using multiplex polymerase chain reaction (PCR) with 16S rRNA primers specific for *Campylobacter* genus, as well as for *C. jejuni* (*mapA*) and *C. coli* (*ceuE*) as previously described (Linton *et al*., 1997; Denis *et al*., 1999).

Isolate characterization and antimicrobial susceptibility testing

To examine the genetic similarity of *Salmonella* and *Campylobacter* isolates, pulsed-field gel electrophoresis (PFGE) genotyping (CHEF-DRIII; Bio-Rad Laboratories, Hercules, CA) was performed on total genomic DNA. Agarose plugs prepared with the *Salmonella* isolates were digested using *Xba*I (Promega, Madison, WI) following previously reported protocols (Ribot *et al*., 2006). After electrophoresis, banding patterns were compared and levels of similarity assigned using generally accepted criteria (Tenover *et al*., 1995). *Salmonella* isolates were compiled into pulsotypic groups by using the Dice coefficient similarity index and the unweighted pair-group method with arithmetic averages (UPGMA) with clustering settings of 1.00% optimization and 1.00% band position tolerance via Bionumerics software (Applied Maths, Kortrijik, Belgium). *Salmonella* isolates that appeared clonal on *Xba*I PFGE were compared by PFGE following digestion using *Spe*I to confirm their relatedness. Agarose plugs prepared with *Campylobacter* isolates were digested using *Sma*I (Promega, Madison, WI) and imaged as described (Ribot *et al*., 2001; Sanad *et al*., 2011). Similarity and clustering analysis were accomplished using the Dice coefficient and UPGMA with 1.00% optimization and 1.50% band position tolerance.

The resistance phenotype of a single *Salmonella*, *Campylobacter*, and *E. coli* recovered from nonsupplemented MacConkey media to represent each positive package was characterized by minimum inhibitory concentrations (MICs). *Salmonella* and *E. coli* MICs to a standard panel of 15 antimicrobial drugs were generated using a semiautomated broth microdilution system (NARMS CMV2AGNF, TREK Diagnostic Systems, Cleveland, OH) following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009). *Campylobacter* MICs were generated to a standard panel of nine antimicrobial drugs (NARMS CAMPY, TREK Diagnostic Systems) as described (Sanad *et al*., 2011).

Discrimination of resistance genes

The quinolone-resistance determining regions (QRDR) corresponding to the *gyrA* and *parC* subunits of the fluoroquinolone-resistant *E. coli* isolates were amplified by PCR and bidirectionally sequenced to assess DNA-binding surface amino acid substitutions (Genewiz, South Plainfield, NJ) (Kato *et al*., 1990; Everett *et al*., 1996; Heisig, 1996). Additionally, *E. coli* isolates expressing fluoroquinolone resistance were tested by PCR for the carriage of plasmid-mediated *qnr* using a global *qnr* primer pair (Chen *et al*., 2012).

The presence of $bla_{\text{CTX-M}}$ and $bla_{\text{CMY-2}}$ in extended-spectrum cephalosporin-resistant *E. coli* and *Salmonella* isolates was confirmed by PCR utilizing previously reported primer sets (Mollenkopf *et al*., 2012). $bla_{\text{CTX-M}}$ genes were bidirectionally sequenced using the corresponding PCR amplification primers and analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/).

Plasmid characterization

The plasmid content of each $bla_{\text{CTX-M}}$ *E. coli* isolate was visualized by electrophoresis using a standard procedure (Kado and Liu, 1981). $bla_{\text{CTX-M}}$-harboring plasmids were codified according to a PCR-based replicon typing procedure that detects 18 replicon types based on incompatibility group loci (Carattoli *et al*., 2005, 2006; Johnson *et al*., 2007).

Data analysis

Prevalence of each outcome of interest was expressed on a per-package basis. Differences in the likelihood of recovering specific pathogens or antimicrobial-resistant organisms between the three package types were estimated using logistic regression mixed models with SAS Proc GLIMMIX (SAS v. 9.3, SAS Institute, Cary, NC). Variables representing the state in which the package was purchased, vacuum packaging, weight of the package, and date of purchase were assessed in the models as fixed effects, while variables representing the individual grocery store, the retail grocery store chain, and the processing plant were assessed in the models as random effects in order to investigate and control for potential confounding. Multiple pairwise comparisons of least-square means were accomplished using the Tukey-Kramer method (Hayter, 1984).

Results

We purchased 231 packages of fresh retail boneless chicken breast from 99 grocery stores belonging to 17 store chains located in 3 US states between June and September 2012 (Table 1). Most packages (61%) were purchased from stores located throughout Ohio. Another 36% of packages were purchased from stores located throughout southern Michigan, and the 8 packages purchased in Pennsylvania were from stores in the Pittsburgh metropolitan area. The packages originated from 27 chicken processing plants, although the processing plant was not identifiable on the label of 9 (4%) packages. Ninety-six (41.5%) of the packages were labeled ''antibiotic free'' and 40 (17.3%) of the packages were labeled ''organic'' (Table 1). Most (83.1%) packages were foam trays covered with clear plastic stretch wrap and the remaining (16.9%) were in vacuum packaging.

We recovered *Salmonella* spp. from 56 (24.2%) of the packages of boneless chicken breasts (Table 1). Over 5% of the packages contained *Salmonella* carrying the extended-spectrum cephalosporin resistance gene $bla_{\text{CMY-2}}$, representing over 21% of all *Salmonella* isolates. We also recovered *Campylobacter* spp. from 10.8% of the packages (Table 1), most of which (72%) were *C. jejuni* while the remainder (28%) were *C. coli*. We observed little variability in pathogen recovery rates between states, but there were considerable differences between grocery store chains and between processing plants, with *Salmonella* recovery rates as high as 41% for one large regional chain of grocery stores. We could not detect differences in recovery rates of *Salmonella*, *Salmonella* carrying $bla_{\text{CMY-2}}$, or *Campylobacter* spp. among conventional, organic, or antibiotic-free chicken breasts.

We recovered *E. coli* harboring $bla_{\text{CMY-2}}$ from over half (53.7%) of the packages using selective media (Table 1). In addition, we recovered *E. coli* carrying $bla_{\text{CTX-M-1}}$ from 6.9% of packages, all of which were located on IncI1 plasmids. We also recovered *E. coli* with QRDR mutations conferring fluoroquinolone resistance from 8.2% of packages using selective media (Table 1). We did not recover carbapenem-resistant isolates. We could not detect differences among product types in the probability of recovering *E. coli* harboring either $bla_{\text{CMY-2}}$ or $bla_{\text{CTX-M}}$ using selective media. Using Fisher's exact test, we were able to detect differences among the product types in the probability of detecting *E. coli* with QRDR mutations conferring fluoroquinolone resistance. However, because no organic packages contained *E. coli* with these QRDR mutations, multivariable models utilizing this outcome would not converge. As a result, we were unable to control for potential confounders and so cannot draw strong conclusions regarding this observed difference.

Distributions of MICs of *Salmonella* spp., *Campylobacter* spp., and commensal *E. coli* recovered without antimicrobial selection pressure are summarized in Figure 1. We could not detect differences in the proportion of isolates with reduced susceptibility or in the median MIC to each of the antimicrobial drugs tested between conventional, organic, or antibiotic-free packages of chicken breasts.

Analysis of the relatedness of the *Salmonella* isolates (Fig. 2) revealed multiple clonal or highly similar strains recovered from packages purchased in different stores located in different states, originating from different processing plants, and recovered at different times throughout the summer. In addition, multiple clonal or highly similar *Salmonella* strains were recovered from at least two of the three different product types. Three highly similar

Table 1. Recovery of Potential Foodborne Pathogens and *Escherichia coli* Harboring Specific Bacterial Resistance Genotypes from 231 Retail Packages of Fresh Boneless Chicken Breasts Purchased from Grocery Stores in 3 U.S. States

	n	Salmonella *spp.*	Salmonella *spp. w/ CMY*	Campylobacter *spp.*	*Reduced susceptibility* E. coli		
					CTX-M	*CMY-2*	*QRDR mutations*
Overall	231	56 (0.24)	12 (0.05)	25 (0.11)	16 (0.07)	124 (0.54)	19 (0.08)
Production system							
Conventional	95	24 (0.25)	8 (0.08)	12 (0.13)	5 (0.05)	49 (0.52)	17 (0.18)
Antibiotic free	96	25 (0.26)	2 (0.02)	11 (0.12)	9 (0.09)	54 (0.56)	2 (0.02)
Organic	40	7 (0.18)	2 (0.05)	2 (0.05)	2 (0.05)	21 (0.53)	0
State							
Ohio	140	36 (0.26)	9 (0.06)	7 (0.05)	13 (0.09)	84 (0.60)	16 (0.12)
Michigan	83	18 (0.22)	3 (0.04)	17 (0.20)	2 (0.02)	34 (0.41)	3 (0.05)
Pennsylvania	8	2 (0.25)	0	1 (0.13)	1 (0.13)	6 (0.75)	0
Retail chain							
A	77	16 (0.21)	2 (0.03)	10 (0.13)	0	36 (0.47)	4 (0.05)
B	39	9 (0.23)	2 (0.05)	2 (0.05)	2 (0.05)	17 (0.44)	1 (0.03)
C	34	14 (0.41)	5 (0.15)	2 (0.06)	6 (0.18)	26 (0.76)	10 (0.29)
D	25	6 (0.24)	1 (0.04)	5 (0.20)	1 (0.04)	14 (0.56)	1 (0.04)
E	21	4 (0.19)	0	0	3 (0.14)	10 (0.48)	2 (0.10)
F	8	3 (0.38)	2 (0.25)	1 (0.13)	1 (0.13)	7 (0.88)	0
G	6	1 (0.17)	0	0	0	4 (0.67)	0
All others[a]	21	3 (0.14)	0	5 (0.24)	3 (0.14)	10 (0.48)	1 (0.05)
Processing plant							
1	46	9 (0.20)	1 (0.02)	5 (0.11)	0	21 (0.46)	0
2	31	4 (0.13)	0	0	2 (0.06)	12 (0.39)	2 (0.06)
3	16	5 (0.31)	1 (0.06)	3 (0.19)	1 (0.06)	10 (0.63)	1 (0.06)
4	16	10 (0.63)	5 (0.31)	1 (0.06)	2 (0.13)	13 (0.81)	9 (0.56)
5	15	2 (0.13)	0	2 (0.13)	0	8 (0.53)	0
6	15	4 (0.27)	1 (0.07)	1 (0.07)	1 (0.07)	12 (0.80)	0
7	14	1 (0.07)	0	2 (0.14)	0	7 (0.50)	1 (0.07)
8	11	1 (0.09)	0	0	1 (0.09)	4 (0.36)	0
9	11	5 (0.45)	1 (0.09)	2 (0.18)	0	4 (0.36)	2 (0.18)
10	10	4 (0.40)	1 (0.10)	3 (0.30)	3 (0.30)	9 (0.90)	0
11	8	1 (0.13)	0	0	2 (0.25)	4 (0.50)	1 (0.13)
12	5	3 (0.60)	1 (0.20)	0	1 (0.20)	3 (0.60)	1 (0.20)
13	4	1 (0.25)	0	0	1 (0.25)	3 (0.75)	0
All others[b]	29	6 (0.21)	1 (0.03)	6 (0.21)	2 (0.07)	14 (0.67)	2 (0.07)
Vacuum packaged							
Yes	39	12 (0.31)	3 (0.08)	4 (0.10)	4 (0.10)	23 (0.59)	3 (0.08)
No	192	44 (0.23)	9 (0.05)	21 (0.11)	12 (0.06)	101 (0.53)	16 (0.08)

Data are presented as frequency (proportion) of packages.
[a]Includes chicken breast packages from 10 retail grocery store chains.
[b]Includes chicken breast packages from 14 processing plants including 9 packages for which the processing plant could not be determined.
QRDR, quinolone-resistance determining regions.

(>97%) *Salmonella* isolates were each recovered from different product types purchased from different store chains located in two states and originated from different processing plants. In two situations, clonal or highly similar *Salmonella* strains differed in their carriage of plasmid-borne bla_{CMY-2}.

Analysis of the relatedness of the *C. jejuni* (Fig. 3) and *C. coli* (Fig. 4) isolates also revealed highly similar strains recovered from different product types, different store chains, and originating from different processing plants. Two highly similar (>90%) *C. jejuni* isolates were recovered from conventional and antibiotic-free package types in different store chains located in Ohio and Michigan that originated from different processing plants. Highly similar (>95%) *C. coli* isolates were also recovered from different product types purchased from different store chains, and originating from different processing plants.

Discussion

We found little difference in the microbiological quality of fresh boneless chicken packages labeled as organic or antibiotic free compared to conventional packages without those label claims. *Salmonella* spp., including multidrug-resistant strains, frequently contaminated all three product types. Commensal *E. coli* carrying bla_{CMY-2} and bla_{CTX-M} on transferable plasmids were also frequently recovered for all three package types. A recent review of the scientific literature pertaining to the safety of organic foods also concluded that bacterial contamination of retail chicken is common but unrelated to organic

	μg/ml	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amoxicillin/ Clavulanic Acid	*Salmonella*							33	3	1	1	1	4	13			
	E. coli							1	9	51	17	15	16	12			
Ampicillin	*Salmonella*							32	3	2	2	2		15			
	E. coli							6	29	39	3	3	2	39			
Azithromycin	*Salmonella*								10	45			1				
	E. coli						2	9	19	70	13	4	4				
	Campylobacter					3	2	2	3	2	8	3		2			
Ceftoxitin	*Salmonella*							6	23	9	2	2	9	5			
	E. coli						1	2	44	30	12	4	8	20			
Ceftiofur	*Salmonella*					1	22	13	2	3	4	11					
	E. coli				1	25	58	10	9	6	4	8					
Ceftriaxone	*Salmonella*					36	2	2	1	1	2	8	1	3			
	E. coli					102	1	2	2		6	5	2	1			
Chloramphenicol	*Salmonella*									28	28						
	E. coli								18	39	53	3	1	7			
Ciprofloxacin	*Salmonella*	47	2				3	1		2	1						
	E. coli	94	9	11	3	1		2	1								
	Campylobacter	11	10	3	1												
Clindamycin	*Campylobacter*					1			2	1	9	12					
Erythromycin	*Campylobacter*								1	6	1	2	12	3			
Florfenicol	*Campylobacter*							8	13	4							
Gentamicin	*Salmonella*					11	30	7	4			3	1				
	E. coli					4	33	61	7	1	2	4	9				
	Campylobacter						10	7	3	3				1			
Kanamycin	*Salmonella*										54		1		1		
	E. coli										102	8	3		8		
Naladixic Acid	*Salmonella*								36	17			1	2			
	E. coli						9	9	78	21		1		3			
	Campylobacter									9	6	9		1			
Streptomycin	*Salmonella*												34	14	8		
	E. coli												96	7	18		
Sulfisoxazole	*Salmonella*											1	6	21	4	1	23
	E. coli											33	15	29	11	1	32
Telithromycin	*Campylobacter*							3	1	2	19						
Tetracycline	*Salmonella*									17				39			
	E. coli									80	4	4	7	26			
	Campylobacter											3	3	19			
Trimethoprim/ Sulfamethoxazole	*Salmonella*				52	2	2										
	E. coli				92	20	8		1								

FIG. 1. Distribution of minimum inhibitory concentrations (MICs) among *Salmonella* spp., *Campylobacter* spp., and commensal *Escherichia coli* recovered without antimicrobial selection pressure from 231 retail packages of fresh boneless chicken breasts purchased from grocery stores in 3 US states. (numbers of isolates are shown in the body of the table). Broken lines represent susceptible breakpoints and solid lines represent resistant breakpoints where available. Corresponding to the concentration listed at the top of each column (μg/mL), the included range of each antimicrobial is shown in gray. For amoxicillin/clavulanic acid, the indicated range refers to amoxicillin concentration; clavulanic acid was included in wells at half the amoxicillin concentration. For trimethoprim/sulfamethoxazole, the indicated range refers to trimethoprim; sulfamethoxazole was included in wells at 19 times the concentration of trimethoprim.

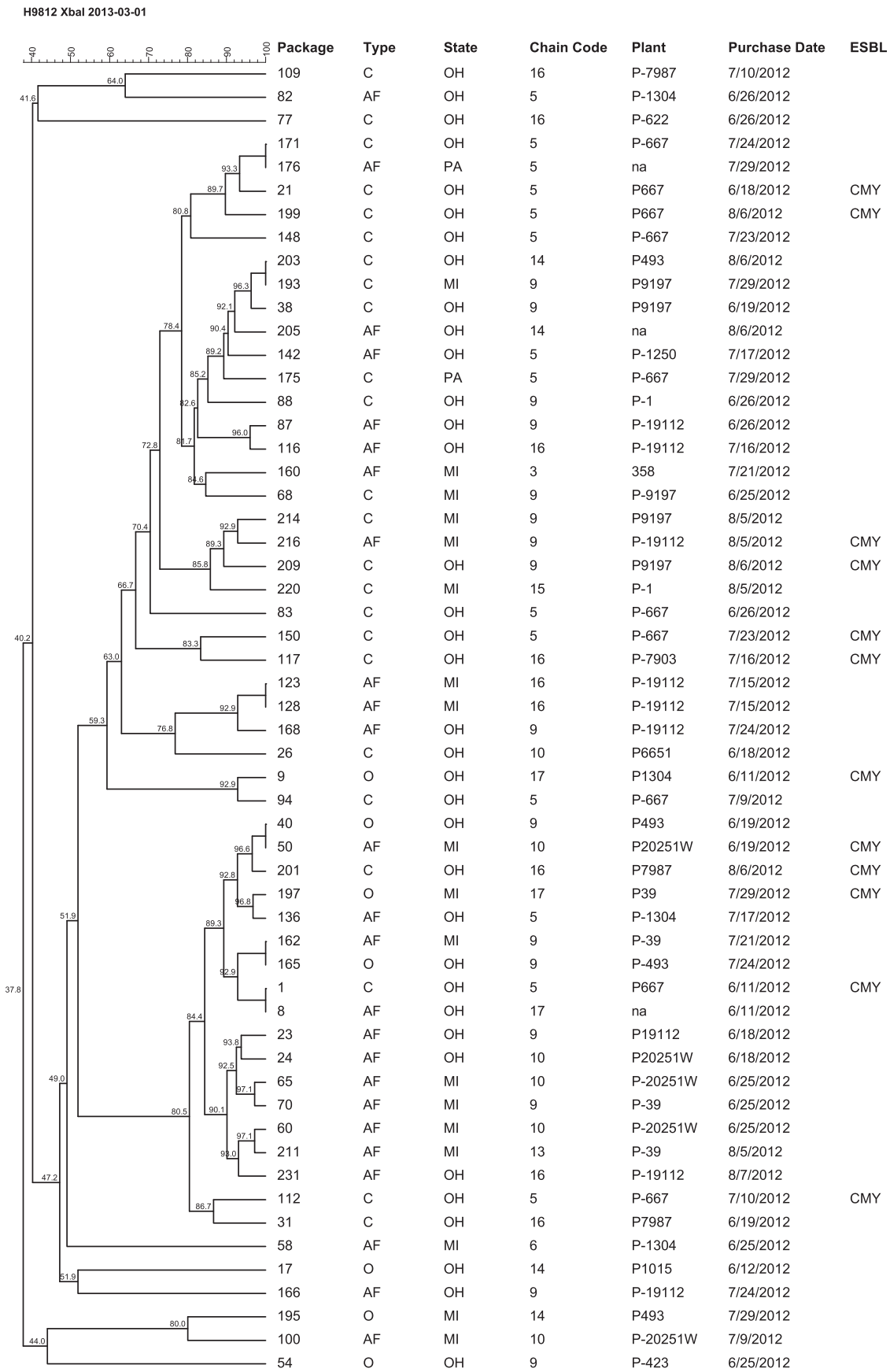
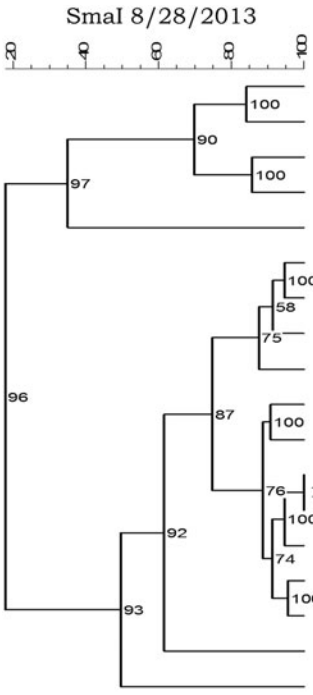


FIG. 2. Dendrogram showing *Xba*I pulsed-field gel electrophoresis profiles for *Salmonella* spp. recovered from retail packages of fresh boneless chicken breasts purchased from grocery stores in 3 US states. For product type, C = conventional, O = organic, AF = antibiotic-free. ESBL, extended-spectrum β-lactamase.



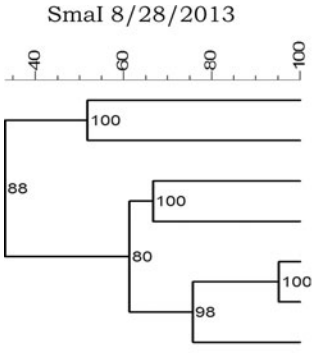
Package	Type	State	Chain Code	Plant	Purchase date
182	O	OH	17	P-1304	7/30/2012
188	C	OH	5	P-667	7/30/2012
149	AF	OH	5	P-1304	7/23/2012
69	C	MI	9	P-325	6/25/2012
174	C	PA	11	P-40183	7/29/2012
105	AF	MI	16	P-19112	7/09/2012
88	C	OH	9	P-1	6/26/12012
99	AF	MI	7	P-20604	7/09/2012
87	AF	OH	9	P-19112	6/26/2012
66	C	MI	10	P-6651	6/25/2012
68	C	MI	9	P-9197	6/25/2012
60	AF	MI	10	P-20251W	6/25/2012
75	AF	MI	16	P-19112	6/25/2012
67	AF	MI	9	P-19112	6/25/2012
59	C	MI	10	P-6651	6/25/2012
61	O	MI	10	P-20251W	6/25/2012
49	C	MI	9	P-1	6/19/2012
138	C	OH	9	P-177	7/17/2012

FIG. 3. Dendrogram showing *Sma*I pulsed-field gel electrophoresis profiles for 18 *Campylobacter jejuni* recovered from retail packages of fresh boneless chicken breasts purchased from grocery stores in 3 US states. For product type, C = conventional, O = organic, AF = antibiotic-free.

status (Smith-Spangler *et al*., 2012). However, they did find evidence that the risk of isolating bacteria resistant to three or more antibiotics was higher in conventional than in organic chicken, although they did not consider specific resistance genotypes (Smith-Spangler *et al*., 2012). In addition, they did not include antibiotic-free products in their review. Our results provide little evidence of differences in the microbiological quality of fresh retail chicken labeled organic, antibiotic free, or conventional without either label claim.

We recovered *Campylobacter* spp. from 11% of the packages, and we could not detect a difference in recovery between product types. Our *Campylobacter* recovery rate was lower than expected based on previous reports from fresh retail chicken products (Williams and Oyarzabal, 2012; Trimble *et al*., 2013). We believe that our recovery of *Campylobacter* spp. may have been reduced by our practice of transporting the fresh packages of chicken breasts at ambient temperature from the stores to the laboratory during summer months. Temperature has been shown to impact the survival of *Campylobacter* in fresh meat products (Park, 2002). This type of nondifferential misclassification of *Campylobacter* contamination status is generally expected to introduce bias toward the null hypothesis of no difference between product types (Chen *et al*., 2013). However, it has been previously reported that recovery of *Campylobacter* is similar between conventional and antibiotic-free chicken products (Price *et al*., 2005).

The AmpC $bla_{\text{CMY-2}}$ was commonly present in *E. coli* and *Salmonella* recovered from boneless chicken breast packages. $bla_{\text{CMY-2}}$ has been previously reported in fresh meat products including beef, pork, and chicken (Mollenkopf *et al*., 2011; White *et al*., 2001; Dutil *et al*., 2010; Chen *et al*., 2004; Folster *et al*., 2012). In addition, $bla_{\text{CMY-2}}$ has been reported to be widely disseminated in food animal populations (Tragesser *et al*., 2006; Heider *et al*., 2009; Mollenkopf *et al*., 2012). The extended-spectrum β-lactamase $bla_{\text{CTX-M}}$ has only recently been detected in *E. coli* and *Salmonella* from livestock in the United States (Wittum *et al*., 2010, 2012; Mollenkopf *et al*., 2012). In addition, an *E. coli* isolate carrying $bla_{\text{CTX-M}}$ was recovered from retail chicken in Pennsylvania that was identical to a human clinical isolate (Doi *et al*., 2010). Extended-spectrum cephalosporins are not approved for use in broiler production in the United States, although ceftiofur sodium is approved for use in day-old chicks for the control of early



Package	Type	State	Chain Code	Plant	Purchase date
164	C	OH	9	P-177	7/24/2012
63	C	MI	9	P-9197	6/25/2012
127	AF	MI	8	P-39	7/15/2012
50	AF	MI	10	P-20251W	6/19/2012
57	C	MI	6	P-18557	6/25/2012
62	AF	MI	9	P-19112	6/25/2012
58	AF	MI	6	P-1304	6/25/2012

FIG. 4. Dendrogram showing *Sma*I pulsed-field gel electrophoresis profiles for seven *Campylobacter coli* recovered from retail packages of fresh boneless chicken breasts purchased from grocery stores in 3 US states. For product type, C = conventional, AF = antibiotic-free.

mortality associated with *E. coli*. However, the frequency of ceftiofur use in chickens has not been reported.

We recovered clonal or highly similar *Salmonella* strains from packages representing different grocery store chains and different processing plants. Because broiler chickens are raised in grower/finisher barns using all in/all out production systems, our result suggests that these common *Salmonella* strains must originate from a common source earlier in the production system such as a common hatchery or common parent stock. Hatcheries have previously been associated with the widespread dissemination of *Salmonella* in chicks (Gaffga *et al*., 2012; Wilkins *et al*., 2002). However, the role of broiler chicken parent stock in the maintenance and dissemination of *Salmonella* strains has not been reported. We also recovered highly similar *C. jejuni* and *C. coli* from diverse sources, suggesting a common source prior to broiler barns. Field research to identify common sources for *Salmonella* and *Campylobacter* spp. dissemination and to develop effective interventions might result in reduced zoonotic foodborne transmission of these pathogens.

We observed considerable variability in the contamination rates of retail meat products associated with the specific processing plant from which the product originated, which resulted in highly variable contamination rates among individual stores and between store chains. The large observed differences between processing plants may be due to differences in live bird infection rates, or in management and process control factors in place at individual plants resulting in different contamination rates. This result suggests that retail stores can directly influence the contamination rate of fresh chicken products by modifying their purchasing practices to take differences in plant contamination rates into account. Unfortunately, this information may not be readily available to individuals making purchasing decisions for retail grocery stores. All poultry processing plants in the United States must monitor *Salmonella* contamination rates of their fresh meat products as part of required Hazard Analysis Critical Control Point surveillance programs (FDA, 2014). However, this information is not available in a format that is accessible to consumers or that can be used by retailers to inform their purchasing decisions.

Conclusions

Retail meat products labeled ''organic'' or ''antibiotic free'' are marketed with an implied health benefit, and consumers of these products may reasonably expect that they be less likely to contain pathogens or antimicrobial-resistant organisms. Our results indicate that, regardless of product type, fresh retail chicken breast is commonly contaminated with enteric pathogens associated with foodborne illness and commensal bacteria harboring genes conferring resistance to critically important antimicrobial drugs. Consumers, regardless of product type, will need to practice appropriate kitchen hygiene in order to reduce their risk of foodborne illness.

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Disclosure Statement

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