FOODBORNE PATHOGENS AND DISEASE
Volume 18, Number 3, 2021

DOI: 10.1089/fpd.2020.2853

Prevalence and Antimicrobial Susceptibility of *Salmonella* Serovars Isolated from U.S. Retail Ground Pork

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Abstract

One objective of this study was to determine overall prevalence of *Salmonella* in ground pork from U.S. retail stores over three seasons including both case-ready and store-ground packages. Package types collected included: overwrap, chub, modified atmosphere packaging, and other (plastic or wax paper wrapped). Because package type represents different production systems and are subject to varied microbiological government regulation and testing methodologies, both USDA-FSIS and FDA *Salmonella* isolation protocols were performed. Another objective of the study was to determine serotypes and antimicrobial susceptibility profiles of the isolates obtained from the ground pork samples. Ground pork aliquots were subjected to real-time PCR. Recovered isolates were serotyped and minimum inhibitory concentration analysis to 15 antimicrobials was determined using microbroth dilution. Overall prevalence of *Salmonella* in ground pork from the 865 samples collected was 1.39%. Prevalence was not affected by package type ($p=0.29$) nor grind location (case-ready vs. store-ground; $p=0.17$). Season affected *Salmonella* prevalence ($p=0.05$) with most isolates found during fall, and there was a tendency for geographic region to affect prevalence ($p=0.07$). The USDA *Salmonella* isolation method was more effective at recovering isolates ($p=0.01$) compared with the FDA methodology and yielded a kappa statistic of 0.26 as a measure of agreement. The serotypes isolated included: Infantis, 4,5,12:i:-, Brandenburg, Typhimurium var 5-, Seftenberg, and Johannesburg with only two packages containing multiple serotypes. No isolates were resistant to antibiotics commonly used to treat human *Salmonella* infections including extended spectrum cephalosporins or fluoroquinolones. Although the recovery of *Salmonella* from retail ground pork samples was rare, *Salmonella* Typhimurium (and its monophasic variant 4,5,12:i:-), which are among the most common serovars recovered from human infections, were recovered. Therefore, more effective strategies to further reduce or eliminate these pathogens from retail pork products are warranted.

Keywords: pork, *Salmonella*, antimicrobial resistance, ground pork

Introduction

Salmonella is a commonly reported foodborne pathogen in the United States and throughout the world and causes gastroenteritis (Botteldoorn *et al.*, 2003). Salmonellosis in humans can result from consumption of *Salmonella*-contaminated foods such as beef, pork, and eggs (ICMSF, 1996). *Salmonella* was the most commonly reported foodborne bacterial infection in 2011 (16.42 cases/100,000 people); thus the 2010 national health objective to reduce the incidence of foodborne *Salmonella* illness to 6.8 cases/100,000 people was not met. Scallan *et al.* (2011) reported 11% of foodborne illnesses in the United States were attributable to *Salmonella*, whereas 35% of hospitalizations and 28% of deaths from foodborne pathogens involved *Salmonella*. Tack *et al.* (2020) reported *Salmonella* as the second most common laboratory diagnosed bacterial infection behind *Campylobacter*. Although *Salmonella* infections may occur through other modes of fecal–oral transmission, the majority of documented *Salmonella* illnesses in the United States are attributed to foodborne contamination. Scallan *et al.* (2011) reported *Salmonella* as a common causative agent for U.S. foodborne illnesses and suggested that one million foodborne illnesses were attributable to nontyphoidal *Salmonella*, with similar reports in Europe (Parlement Europeen Et Conseil De L'Union, 2001).

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Salmonella have been reported as a bacterial contaminant of raw pork that have also been associated with foodborne infections leading to human illness (Escartin *et al.*, 2000). Studies suggest that among foodborne illness in the United States, 9–15% of all *Salmonella* infections, and 7.5% of *Salmonella* Enteritidis and Typhimurium infections, are associated with consuming pork or pork products (Hald *et al.*, 2004; Pires and Hald, 2010). Although the risk of *Salmonella* infection from pork consumption is low compared with the consumption of other fresh retail meat products [e.g., poultry; M'Ikanatha *et al.* (2010)], the commonality in strains isolated from pigs and human infections combined with *Salmonella's* ubiquitous nature in the swine production settings makes it a pathogen of concern for the pork industry. In addition, *Salmonella*, in fresh retail pork have been reported to be resistant to clinically important antibiotics (White *et al.*, 2001; Bottemiller, 2012). Therefore, the two main objectives for this study were (1) to determine the prevalence of *Salmonella* in retail ground pork, evaluate factors that may be associated with *Salmonella* prevalence, and distinguish between *Salmonella* prevalence in case-ready packages versus store-ground pork using USDA and FDA *Salmonella* isolation protocols; and (2) to determine the serotypes of the *Salmonella* isolates and their antimicrobial susceptibility profiles.

Materials and Methods

Sample collection

A convenience sample of packages of ground pork ($n = 865$) was obtained from retailers across the United States over a 12-month period. Pork samples were obtained from ≥12 cities within each of the 4 geographical regions within the United States (West, South, Central/Midwest, and East) evenly dispersed across three seasons (fall: September–November, winter/spring: January–March, summer: June–August; Table 1). Twenty-four packages were obtained from each city. Approximately 1/2 of the packages collected at each location were case-ready ground pork produced off-site, and the remaining packages were ground/produced on-site at the retail store. Packages were obtained from grocery stores, supercenters, and other markets that sold fresh meats. Before refrigerated shipping, each package was placed in a sealed plastic bag to prevent cross-contamination between packages in the shipping container. After package information was recorded, all identifiers were removed, and packages were assigned a three-digit code before analysis. Of the 865 packages of ground pork collected, ~55% were overwrap packages, 6% were chub packages, 20% were modified atmosphere packages (MAP), and 19% were wrapped in butcher paper with or without a plastic liner (Other; Supplementary Table S1).

Five, 2.5 cm cores of ground pork were aseptically removed from each package and placed in a Whirl Pack® bag. The cores were mixed thoroughly, and 25-g aliquots were removed for FDA and USDA *Salmonella* isolation analyses. In addition, a 10-g aliquot from six different packages representing a single location and package type was homogenized to create a composite. A 25-g aliquot of the composite was analyzed by real-time PCR (RT-PCR) utilizing both internal and external positive and negative controls (RT-PCR; BAX, Dupont, Wilmington, DE; 100201; AOAC) similar to that conducted by Chaves *et al.* (2015b) and Chaves *et al.* (2015a). If a pooled composite sample was positive for *Salmonella*, individual packages represented in the composite were tested using the same procedure.

USDA isolation

Individual ground pork samples from a positive composite sample were diluted 10-fold with 225 mL BPW and stomached at 230 rpm for 2 min (Seward Stomacher 400, Davie, FL). Samples were incubated at 37°C for ~24 h before analysis. Postenrichment, samples were subjected to RT-PCR analysis to determine *Salmonella* presence. If positive, samples were subjected to USDA *Salmonella* isolation techniques

Table 1. Prevalence of *Salmonella* Isolated from Retail Ground Pork Based on Region, Season, Package Type, and Grind Location

	Model-adjusted prevalence (%)	*No. of positive/total*	p	*95% Confidence interval*
Region			0.07	
West	0.00	0/228		0.00–100.00
Central/Midwest	1.04	2/192		0.39–2.74
South	0.00	0/216		0.00–100.00
East	4.37	10/229		2.84–6.67
Season			0.05	
Fall	3.47	10/288		2.11–5.65
Winter/spring	0.76	2/263		0.25–2.30
Summer	0.00	0/314		0.00–100.00
Package type			0.29	
Chub	3.85	2/52		1.35–16.81
MAP	2.29	4/175		0.91–5.64
Overwrap	1.26	6/476		0.58–2.60
Other[a]	0.00	0/162		0.00–100.00
Grind location			0.17	
Case-ready	1.90	9/473		0.89–4.46
Store grind	0.77	3/392		0.18–2.91

[a]Other = Butcher-wrapped paper package with or without a plastic liner.
MAP, modified atmosphere packages.

as described by USDA-FSIS Microbiological Laboratory Guidelines (US Department of Agriculture and Food Safety and Inspection Service, 2019). The enrichment was placed in tetrathionate (TT) and Rappaport-Vassiladis (RV) media and incubated at 37°C and 42°C, respectively, for 18–24 h. After incubation, samples were streaked onto brilliant green sulfa and xylose lysine tergitol-4 agar. Phenotypic colonies were streak plated on new selective media before biochemical confirmation as described hereunder.

FDA isolation

After a sample was deemed positive by RT-PCR analysis, a 25-g aliquot of ground pork was placed into a sterile container with 225 mL of lactose broth for enrichment. Samples were stomached at 230 rpm for 2 min and incubated at 25°C for 1 h. Samples were then enriched at 35°C for ~24 h. Procedures were followed for *Salmonella* isolation as described in the FDA Bacteriological Analytical Manual (Andrews *et al.*, 2007). After incubation, 0.1 mL was transferred to 10 mL RV medium, and an additional 1 mL of enrichment was transferred to TT broth. After vortexing, the RV media was incubated ~24 h at 42°C, and the TT media was incubated for ~24 h at 35°C. After incubation, one loopful (10 μL) from RV media was streaked onto bismuth sulfite agar, xylose lysine desoxycholate agar, and Hektoen enteric agar. The same process was repeated for TT media. The plates were incubated for ~24 h at 35°C and were subsequently examined for the presence of phenotypic colonies.

Confirmation

Colonies exhibiting phenotypic *Salmonella* characteristics were re-streaked onto the same selective media from which they were recovered and were subjected to further biochemical confirmation. Specifically, isolates were subjected to latex agglutination (Oxoid, Thermo Scientific, United Kingdom), and were streaked onto triple sugar iron slants and MacConkey agar. Multiple isolates from each selective media were incubated and frozen in 10% glycerol for storage at –80°C. Isolates were also stored on tryptic soy agar slants and maintained for further antimicrobial resistance and serotyping analysis. Finally, to confirm the effectiveness of RT-PCR (BAX) in the detection of *Salmonella* in the packages, 144 packages determined to be negative after RT-PCR were subjected to USDA and FDA *Salmonella* isolation techniques.

Antimicrobial resistance profiling

Antimicrobial resistance of individual isolates was evaluated by determining their minimum inhibitory concentration (MIC) to a standard panel of 15 antibiotics (amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole) using microbroth dilution. Isolates were first inoculated in sterile water and were analyzed on a spectrophotometer and adjusted to a 0.5 McFarland standard. A 10 μL aliquot was then inoculated into 10 mL of Mueller–Hinton broth. Then, 96-well plates containing predetermined concentrations of antibiotics (NARMS CMV3AGNF MIC panel; TREK Diagnostic Systems, Cleveland, OH) were inoculated and incubated for 18–24 h at 35°C. Growth was visually determined (Trek Diagnostics Systems, Inc., Cleveland, OH) with susceptibility and resistance of isolates based on established clinical or surveillance breakpoints (Clinical Laboratory Standards Institute [CLSI]; National Antimicrobial Resistance Monitoring System [NARMS]).

Isolate characterization and serotyping

To examine the genetic similarity of individual *Salmonella* isolates, pulse-field gel electrophoresis 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 restriction enzyme (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 with clustering settings of 1.00% optimization and 1.00% band position tolerance using Bionumerics software (Applied Maths, Kortrijik, Belgium). *Salmonella* isolates were submitted to the National Veterinary Services Laboratory in Ames, IA for serotyping using polyvalent and single factor antisera to determine the O and H antigens.

Statistical analysis

Crude prevalence estimates were calculated as the proportion of all packages from which a *Salmonella* isolate was recovered. Crude prevalence was summarized for all packages and by region, season, package type, and grind location. The ability of the FDA and USDA culture methodologies to recover *Salmonella* from the same packages of ground pork was compared using McNemar's chi-square test to detect disagreement and the kappa statistic to estimate agreement beyond that expected by chance.

Adjusted prevalence estimates were obtained using logistic regression mixed models to estimate the adjusted probability that an individual package of ground pork would be contaminated with *Salmonella*. The store in which the package was purchased was included in each model as a residual random effect. Variables representing the season, geographical region, package type, and grind location were tested for inclusion in the model as categorical fixed effects using a forward stepwise model building procedure. All data were analyzed using SAS v.9.3 (SAS Institute, Cary, SC) with the Tukey option to separate mean differences at $p \leq 0.05$ for all comparisons.

Results and Discussion

Prevalence

Salmonella was recovered from 12 packages (1.39%; Tables 1 and 2), and a total of 61 isolates were collected. Of the packages from which *Salmonella* was recovered, 75% (9 of 12) were case-ready packages packaged off-site from the retail location. In addition, 83.3% of the positive packages (10 of 12) were collected in the fall season (September–November). Eight of the positive packages were collected in September, and two of the positive packages were collected

Table 2. Serotypes of *Salmonella*-Positive Ground Pork Procured from U.S. Retail Locations

State	*Package*	*Package type*	*Season*	*Method*	*Serotype(s)*
CO	Case-ready	Chub	Fall	USDA	Infantis
CO	Case-ready	Chub	Fall	USDA	Infantis
OH	Store-ground	Overwrap	Winter	USDA; FDA	4,5,12:i:-
OH	Case-ready	MAP	Winter	USDA; FDA	Brandenburg
PA	Case-ready	Overwrap	Fall	USDA	Typhimurium var 5-
PA	Case-ready	Overwrap	Fall	USDA	Typhimurium var 5-
PA	Case-ready	Overwrap	Fall	USDA	Typhimurium var 5-; Seftenberg
PA	Store-ground	Overwrap	Fall	USDA	Typhimurium var 5-
PA	Store-ground	Overwrap	Fall	USDA	Typhimurium var 5-
PA	Case-ready	MAP	Fall	FDA	Johannesburg
PA	Case-ready	MAP	Fall	USDA	Seftenberg; Johannesburg
PA	Case-ready	MAP	Fall	USDA	Johannesburg

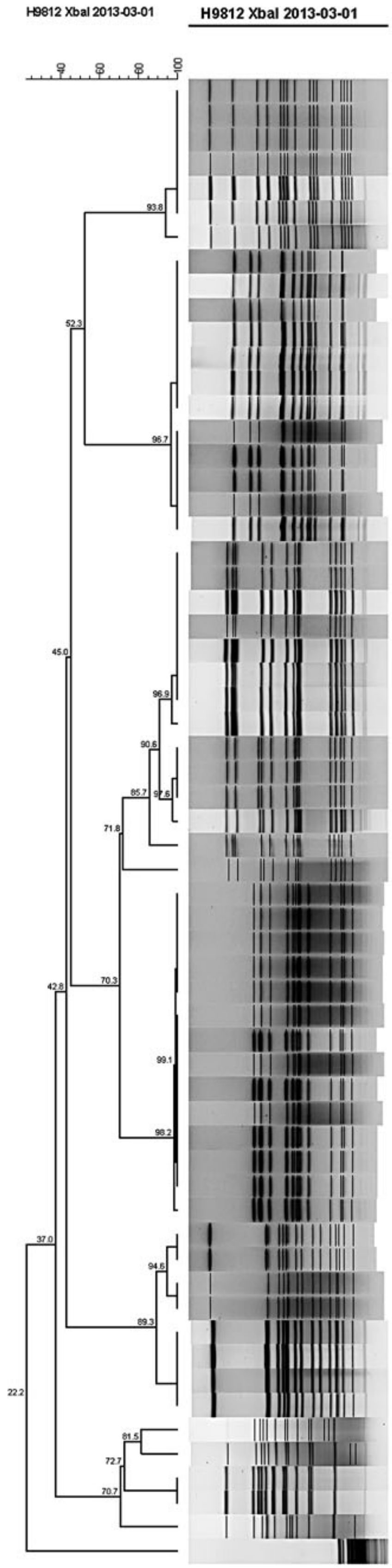
in November. The remaining two packages were collected in February. Overwrap packaging comprised 50% of *Salmonella*-positive packages (6 of 12), whereas MAP and chub packaging represented 33.3% and 16.7%, respectively. Most positive packages were collected from the East sampling region (83.3%; 10 of 12), with the other two packages collected in the Central/Midwest sampling region. The overall prevalence of *Salmonella* in retail ground pork was very low, indicating that *Salmonella* in retail ground pork may not be a large contributor to human foodborne illness in the United States. After PCR evaluation, 144 samples that tested negative were subjected to FDA and USDA culture isolation methodologies. *Salmonella* was not recovered from any of the packages after these analyses.

Although limited research has been conducted on *Salmonella* prevalence in ground pork in the United States, similar prevalence results have been reported in other countries. *Salmonella* prevalence in Irish pork was reported between 2.0% and 2.9% (Boughton *et al.*, 2004; Prendergast *et al.*, 2009). *Salmonella* surveillance in Belgium indicated prevalence between 0.3 and 4.3% (Delhalle *et al.*, 2009). Two studies conducted in one metropolitan region in the United States reported 2% and 3.3% of retail pork products were contaminated with *Salmonella* (Zhao *et al.*, 2006). In another U.S. study, Duffy *et al.* (2001) reported *Salmonella* contamination in 8.3% and 10.4% of whole muscle and enhanced whole muscle pork cuts, respectively. In Vietnam, Van *et al.* (2007) reported 62% of retail pork products contained *Salmonella*. The EFSA (2018) reported that pig meat and their products were responsible for 4.5% foodborne outbreaks associated with *Salmonella*, whereas only 1.85–2.15% of pork carcasses tested positive for *Salmonella*. *Salmonella* surveillance in other meat products has also been investigated in the United States. Mollenkopf *et al.* (2011) reported 6.3% of pork chops and 5% of pork ribs were contaminated with *Salmonella* in two U.S. regions. A Romanian study reported 9.1% of 146 retail pork products were contaminated with *Salmonella* over a 2-year sampling period (Tirziu *et al.*, 2020).

Multiple factors such as region, season, package type, and grind location were analyzed to determine if each played a role in the *Salmonella* prevalence of retail ground pork. *Salmonella* contamination tended to be different among regions ($p = 0.07$; Table 1) and differed across seasons ($p = 0.05$; Table 1). More *Salmonella* was isolated from packages obtained in the fall season than the winter/spring season ($p = 0.04$). The fall season yielded the greatest *Salmonella* recovery at 3.47% compared with 0.76% for the winter/spring season. Of interest, no *Salmonella* was isolated in the summer season. Greater *Salmonella* prevalence in the fall season and the East region may be the result of two stores in the same town that yielded 50% (6 of 12; case-ready packages) of the positive isolates in this study, indicating store may play a larger role than season or region when estimating ground pork *Salmonella* prevalence at retail. Naumova *et al.* (2007) reported increased foodborne illness through *Salmonella* during summer. In addition, Barkocy-Gallagher *et al.* (2003) reported less *Salmonella* on the hides and feces of cattle during winter and spring. Kouppari *et al.* (2003) reported human clinical infections of *Salmonella* Enteridis may be season dependent. Although there was a difference between *Salmonella* prevalence across seasons ($p = 0.05$), seasonality may not greatly affect prevalence in ground pork owing to the thermoneutral environment in which pigs are produced. Schmidt *et al.* (2012) sampled pork-processing facilities, and although there were differences between seasons, a strong relationship between *Salmonella* contamination and season was not observed. However, Sorensen *et al.* (2005) reported seasonal variance on *Salmonella* prevalence at pork abattoirs in Denmark.

Salmonella isolation was unaffected by package type ($p = 0.29$); however, a greater number of chub type packages (3.85%) were contaminated with *Salmonella* compared with other package types (Table 1). A total of 1.26% of overwrap packages collected contained *Salmonella*, whereas chub and MAP contained 3.85 and 2.29%, respectively (Table 1). Most of the packages collected were overwrap (55%), and the majority of retail ground pork available for sale in the stores sampled were packaged in overwrapped trays. This is the packaging technology primarily used by stores who grind their own pork, whereas butcher paper wrapping is the second most common packaging used at the store. No *Salmonella* was isolated from butcher-wrapped (other) packages throughout the study (Table 1). Vipham *et al.* (2012) reported package type did not affect *Salmonella* recovery in ground and whole muscle beef cuts collected in the United States. This study appears to be the first to examine ground pork package type and its relation to *Salmonella* contamination in retail settings.

There was no difference in the *Salmonella* prevalence of retail store-ground pork compared with case-ready packages ($p = 0.17$; Table 1). A total of nine case-ready packages were contaminated with *Salmonella* (1.90%), whereas only three



Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	RV XLT-4
Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	TT BGS
Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	TT BGS
Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	TT XLT-4
Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	TT XLT-4
Senftenberg	PA	Case-ready	MAP	Fall	USDA	644	TT XLT-4
Senftenberg	PA	Case-ready	Overwrap	Fall	USDA	631	TT XLT-4
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT HE
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLT-4
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT HE
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLD
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLD
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLT-4
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLT-4
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	RV XLT-4
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT BGS
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT BGS
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT HE
Brandenburg	OH	Case-ready	MAP	Winter	USDA; FDA	209	TT XLD
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	629	TT XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	629	TT XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	631	RV XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	631	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	632	TT BGS
Typhimurium var 5-	PA	Store-ground	Overwrap	Fall	USDA	633	TT BGS
Typhimurium var 5-	PA	Store-ground	Overwrap	Fall	USDA	633	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	632	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	630	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	630	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	630	TT XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	630	TT XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	631	TT BGS
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	630	TT XLT-4
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV XLD
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT XLD
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV XLT-4
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT BGS
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT HE
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV HE
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV HE
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV XLD
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV XLT-4
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT BGS
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT HE
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT HE
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	TT XLD
4,5,12:i:-	OH	Store-ground	Overwrap	Winter	USDA; FDA	207	RV XLD
Infantis	CO	Case-ready	Chub	Fall	USDA	17	BGS
Infantis	CO	Case-ready	Chub	Fall	USDA	17	XLT-4
Infantis	CO	Case-ready	Chub	Fall	USDA	16	XLT-4
Infantis	CO	Case-ready	Chub	Fall	USDA	17	XLT-4
Infantis	CO	Case-ready	Chub	Fall	USDA	16	XLT-4
Infantis	CO	Case-ready	Chub	Fall	USDA	16	XLT-4
Infantis	CO	Case-ready	Chub	Fall	USDA	17	BGS
Infantis	CO	Case-ready	Chub	Fall	USDA	17	XLT-4
Johannesburg	PA	Case-ready	MAP	Fall	USDA	648	TT XLT-4
Johannesburg	PA	Case-ready	MAP	Fall	USDA	648	TT XLT-4
Johannesburg	PA	Case-ready	MAP	Fall	FDA	642	TT BS
Johannesburg	PA	Case-ready	MAP	Fall	FDA	642	TT BS
Johannesburg	PA	Case-ready	MAP	Fall	USDA	644	TT XLT-4
Typhimurium var 5-	PA	Case-ready	Overwrap	Fall	USDA	632	TT XLD

FIG. 1. Dendrogram showing *Xba*1 pulsed-field electrophoresis profiles for *Salmonella* spp. isolates recovered from retail packages of ground pork purchased from grocery stores throughout the United States. Text columns indicate the Salmonella serotype, the U.S. state in which the ground pork was purchased, grinding of the product, packaging of the product, season the product was purchased, culture protocol, package ID, and enrichment and differentiation media used.

TABLE 3. MINIMUM INHIBITORY CONCENTRATIONS OF ANTIBIOTICS NEEDED TO INHIBIT GROWTH OF *SALMONELLA* ISOLATES FROM PACKAGES OF RETAIL GROUND PORK*

ug/ml	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*
Amoxicillin/ Clavulanic Acid							21 0.35		2 0.0333	14 0.2333	14 0.2333	8 0.1333	1 0.0166				
Ampicillin							20 0.3333	1 0.0166	1 0.0166	1 0.0166	1 0.0166	1 0.0166	35 0.5833				
Azithromycin								3 0.05	36 0..6	13 0.2167	2 0.333	6 0.1					
Ceftoxitin							17 0.2833	32 0.5333	8 0.1333	1 0.0166	0 0.00		2 0.0333				
Ceftiofur				1 0.0166	1 0.0166	15 0.25	40 0.666	2 0.0333	1 0.0166								
Ceftriaxone					56 0.9333	4 0.0667											
Chloramphenicol									45 0.75	7 0.117	2 0.0333	0 0.0	6 0.1				
Ciprofloxacin	57 0.95	7 0.1167	1 0.0166														
Gentamicin						44 0.7333	9 0.15	1 0.0166	1 0.0166		1 0.0166	4 0.0667					
Naladixic Acid							0 0.0	26 0.4333	32 0.5333	2 0.0333							
Streptomycin									3 0.05	17 0.2833	9 0.15	2 0.0333	3 0.05	26 0.4333			
Sulfisoxazole													4 0.0667	15 0.25	1 0.0166	40 0.667	
Tetracycline									25 0.4167	3 0.05	1 0.0166	4 0.0667	29 0.4833				
Trimethoprim/ Sulfamethoxazole				53 0.8833	3 0.05	1 0.0166			1 0.0166	1 0.0166							

*Black line on the left denote the line in which everything to the left of the line is considered susceptible to the antibiotic at that concentration.
*Black lines on the right denote the line in which everything to the right of the line is considered susceptible to the antibiotic at that concentration.
*Spaces between the lines indicate intermediate resistance to the antibiotic at that concentration.

Table 4. Frequency of Antibiotic Susceptibility and Resistance by Serotype for Isolates from Retail Ground Pork Samples in the United States[1]

		Antibiotics[a]										
Serotype	*Isolates (n)*	*Sus*	*Amp*	*Aug2*	*Chl*	*Fis*	*Fox*	*Gen*	*Str*	*Sxt*	*Tet*	*Xnl*
Brandenburg	12	0.58	0.17	0.17		0.08	.	.	0.25	.	0.25	0.08
Infantis	4	0.50	.	.	.	.	.	.	.	0.25	0.25	.
Johannesburg	5	0.60	0.20	.	.	0.20	.	0.20	0.20	0.20	0.40	.
Seftenberg	7	.	0.57	0.29	.	1.00	.	0.14	0.29	.	0.86	.
Typhimurium var 5-	16	.	0.94	0.88	0.44	0.88	.	0.13	0.44	.	0.56	.
4,5,12:i:-	16	0.06	0.94	0.31	.	0.94	0.13	.	0.94	.	0.94	.

Frequency = number of isolates resistant to each antibiotic/total number of isolates of each serotype. The isolate was determined to be resistant by minimum inhibitory concentration analysis utilizing a NARMS pork antibiotic panel.

[a]Antiobiotics: Sus, susceptible to all antibiotics; Amp, ampicillin; Aug2, amoxicillin/clavulanic acid 2:1 ratio; Chl, chloramphenicol; Fis, sulfisoxazole; Fox, cefoxitin; Gen, gentamicin; Str, streptomycin; Sxt, trimethoprim/sulfamethoxazole; Tet, tetracycline; Xnl, ceftiofur.

store-ground packages contained *Salmonella* (0.77%; Table 1). Case-ready ground pork is subject to USDA microbiological testing, whereas store-ground pork is subject to FDA pathogen tests. In this study, both FDA and USDA *Salmonella* isolation protocols were conducted simultaneously on a single package to compare the effectiveness of each testing methodology. The USDA-FSIS method was the most effective method to isolate *Salmonella* compared with FDA isolation ($p = 0.01$). All but one isolate was recovered utilizing the USDA-FSIS *Salmonella* isolation protocol, whereas the FDA *Salmonella* isolation protocol recovered only 3 of 12 isolates. These data suggest the two *Salmonella* isolation methodologies produced varied results. A kappa statistic of 0.26 (95% confidence interval: –0.04 to 0.57) was calculated by comparing the agreement of the methodologies, suggesting there was 26% agreement between methods, beyond chance, in recovery of *Salmonella* from ground pork samples in this study, although no information was discovered in the literature directly comparing these methods for *Salmonella* isolation.

A total of 6 serotypes were recovered from the 12 packages and 61 isolates obtained (Table 2). The serotypes isolated included the following: Infantis ($n = 2$), 4,5,12:i- ($n = 1$), Brandenburg ($n = 1$), Typhimurium var 5- ($n = 5$), Seftenberg ($n = 2$), and Johannesburg ($n = 3$). Within serotype, strains were generally highly similar (Fig. 1). Only two packages contained multiple serotypes. All other isolates from packages containing *Salmonella* were found to be the same serotype. One of the packages containing multiple serotypes contained Typhimurium and Seftenberg, and the other contained Seftenberg and Johannesburg. Korsak *et al.* (2003) reported the presence of *Salmonella* Seftenberg, Typhimurium, Infantis, and Brandenburg in the feed of swine, the large intestine, and the surface of the carcass after harvest. In the Netherlands, Swanenburg *et al.* (2001) also discovered Typhimurium, Brandenburg, and Infantis on pork carcasses. Schlosser *et al.* (2000) reported the presence of Johannesburg, Typhimurium, and Infantis on pork carcasses and isolated Typhimurium and Infantis, along with other species, in raw ground pork. More recently Tack *et al.* (2020) reported the most common *Salmonella* serotypes were Enteritidis, Newport, Typhimurium, Javiana, I 4,[5], 12:i:-, and Infantis in 2019. In Romania, Tirziu *et al.* (2020) reported the isolation of *Salmonella* Infantis, Typhimurium, Derby, Enteritidis, Brandenburg, and others in ground pork with the majority of the isolates being Typhimurium.

Antimicrobial resistance

Antimicrobial resistance was determined by MIC analysis utilizing a NARMS surveillance panel of antibiotics (Table 3). Sixty isolates were analyzed to determine antimicrobial resistance profiles utilizing breakpoints from the CLSI. Forty isolates exhibited resistance to sulfisoxazole, 36 isolates were classified as resistant to ampicillin, and 37 isolates were classified as resistant to tetracycline (Table 4). Only one isolate exhibited resistance to trimethoprim/sulfamethoxazole. None of the isolates were determined to be resistant to ciprofloxacin, ceftiofur, ceftriaxone, or nalidixic acid. Few isolates exhibited resistance to antibiotics commonly used to treat *Salmonella* infections in humans. Of more importance than frequency of resistance may be the lack of resistant isolates to important antimicrobials used in human medicine. The CDC reports that severe *Salmonella* infections in humans may be treated with fluoroquinolones, extended-spectrum cephalosporins, and ampicillin (for susceptible infections), of which none of the *Salmonella* isolates from this study were resistant (Hohmann, 2001; CDC, 2019).

Antibiotic-resistant *Salmonella* is a public health and veterinary medical concern because conventional treatments may not be effective in mitigating human or animal illness, and many of the resistance mechanisms are transferrable to other bacteria (Van *et al.*, 2007). Sinwat *et al.* (2015) reported that all isolates from pork, chicken, and humans were resistant to at least one antibiotic, and >95% of the isolates were classified as multidrug resistant. A report from Romania classified 92% of isolates as resistant to at least one antibiotic (Tirziu *et al.*, 2020).

Conclusion

The overall prevalence of *Salmonella* in ground pork in this study was low, and likely the result of control measures implemented by the Hazard Analysis Critical Control Point system as well as harvest and processing procedures applied to pork products before consumption. Current testing procedures have become more efficient in identifying contaminated products; however, the methodologies used to isolate *Salmonella* in this study produced varied results. Further research is warranted to optimize the effectiveness of these testing methodologies. Our results indicate multiple *Salmonella* serotypes may contaminate ground pork. In addition, *Salmonella* isolated from ground pork at retail did not contain bacteria resistant to medically important antibiotics commonly used for treating human salmonellosis. Although the industry should continue to utilize technologies and practices to reduce pathogens in pork products, the risk to U.S. public health associated with *Salmonella* in ground pork appears to be low. This surveillance study may also provide public health authorities with additional information to utilize when managing decisions related to human *Salmonella* infections.

Disclosure Statement

No competing financial interests exist.

Funding Information

This research was funded, in part, by the National Pork Board through the Pork Checkoff, NPB # 12-170.

Supplementary Material

Supplementary Table S1

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