Prevalence of *Salmonella* enterica on poultry processing equipment after completion of sanitization procedures

Tomi Obe,* Rama Nannapaneni,[†] Wes Schilling,[†] Li Zhang,* Chris McDaniel,* and Aaron Kiess[*,1]

Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA; and [†]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS 39762, USA

ABSTRACT   *Salmonella* is a poultry-borne pathogen that causes illness throughout the world. Consequently, it is critical to control *Salmonella* during the process of converting broilers to poultry meat. Sanitization of a poultry processing facility, including processing equipment, is a crucial control measure that is utilized by poultry integrators. However, prevalence of *Salmonella* on equipment after sanitization and its potential risk to food safety has not been evaluated thoroughly. Therefore, the objective of this study was to evaluate the persistence of *Salmonella* on poultry processing equipment before and following cleaning and sanitization procedure. A total of 15 locations within 6 commercial processing plants were sampled at 3 time points: (A) after processing; (B) after cleaning; and (C) after sanitization, on 3 separate visits for a total of 135 samples per plant. *Salmonella*-positive isolates were recovered from samples using the United States Department of Agriculture MLG 4.09 conventional method. Presumptive *Salmonella* colonies were subjected to biochemical tests for confirmation. *Salmonella* isolates recovered after sanitization were serotyped and tested for the presence of specific virulence genes. A completely randomized design with a $6 \times 3 \times 15$ factorial arrangement was utilized to analyze the results for *Salmonella* prevalence between processing plants. Means were separated using Fishers protected least significant difference when $P \leq 0.05$. For *Salmonella* prevalence between processing plants, differences ($P < 0.0001$) were observed in the 6 plants tested where the maximum and minimum prevalence was 29.6 and 7.4%, respectively. As expected, there was a difference ($P < 0.0001$) in the recovery of *Salmonella* because of sampling time. *Salmonella* prevalence at time A (36%) was significantly higher, whereas there was no difference between time B (12%) and C (9%). There was a location effect ($P < 0.0001$) for the prevalence of *Salmonella* with the head puller, picker, cropper, and scalder having a significantly higher prevalence when compared with several other locations. At sampling time C, a trend toward a difference ($P = 0.0899$) was observed for *Salmonella* prevalence between the 6 plants, whereas significant differences were observed because of location ($P = 0.0031$). Five prominent *Salmonella* enterica serovars were identified, including Kentucky, Schwarzengrund, Enteritidis, Liverpool, and Typhimurium with *S.* Kentucky being the most prevalent. PCR analysis of 8 *Salmonella* virulence genes showed that the *invA*, *sipB*, *spiA*, *sseC*, and *fimA* were detected in all isolates, whereas genes carried on plasmids and/or fimbriae varied remarkably among all isolates. This study established *Salmonella* prevalence and persistence in poultry processing facilities after antimicrobial application through sanitization procedures which could result in contamination of poultry carcasses and food safety risks because of poultry meat.

Key words: *Salmonella*, prevalence, sanitization, poultry processing, virulence

INTRODUCTION

Poultry integrators in the United States are continually working toward producing safe poultry meat

(NCC, 2020). To achieve this goal, it is critical for integrators to follow regulatory programs and procedures that are directed at controlling foodborne pathogens during broiler processing (Simmons et al., 2003; McKee, 2012; NCC, 2020). Foodborne infections remain a public health challenge in the United States. In 2018, the Foodborne Diseases Active Surveillance Network (FoodNet) of the CDC identified over 25,000 infections and approximately 6,000 hospitalizations because of foodborne pathogens (CDC, 2018). *Salmonella* infections was reported as the second most common

foodborne infection accounting for 9,084 infections at a rate of 18.3 cases per 100,000 people (Tack et al., 2019). Additionally, *Salmonella* infections caused the most hospitalizations and deaths in the same year with the top 3 *Salmonella* serotypes causing salmonellosis being Enteritidis, Newport, and Typhimurium. The Food Safety and Inspection Service (**FSIS**) of the United States Department of Agriculture (**USDA**) also identified *Salmonella* in 3.7% of all the chicken carcasses sampled under the Hazard Analysis Critical Control Point verification program in 2014, and the top 2 serotypes identified were Kentucky and Enteritidis (USDA-FSIS, 2016). *Salmonella* control during the processing of poultry meat is crucial. Among all food commodities, chicken causes the most *Salmonella* outbreak-associated illnesses, hospitalizations, and deaths than any other food (CDC, 2016). Moreover, per capita consumption of broiler meat has continued to increase for the past 2 decades and is expected to continue to increase in the future (NCC, 2019).

Efficient cleaning and sanitization procedures are part of the plant operating procedures that are intended to control foodborne pathogens, including *Salmonella* and *Campylobacter* in poultry processing facilities (Olsen et al., 2003; Potter et al., 2012; Lebron, 2013). Poultry processing facilities clean and sanitize their plant and equipment after processing meat to produce safe, wholesome products and to eliminate any pathogenic microorganisms that may be present. However, some microorganisms are able to adhere to food processing equipment surfaces and remain active after cleaning and sanitization (Chmielewski and Frank, 2003; Carpentier, 2011; Fagerlund et al., 2017). The persistence of such pathogenic microorganisms on the equipment surface could result in cross-contamination of a pathogen-free flock during processing (Rasschaert et al., 2008). Antimicrobial application or sanitization is a step conducted after the cleaning process. Sanitizers such as sodium hypochlorite, quaternary ammonium compounds, and hydrogen peroxides are among the antimicrobial agents approved by the USDA for the disinfection of poultry carcasses as well as for the sanitization of poultry processing equipment and inside the facility (USDA-FSIS, 2017a). The potency of these sanitizing agents is essential for microbial inactivation during the cleaning and sanitization procedure. The efficacy of different antimicrobial agents at inactivating foodborne pathogens like *Salmonella* during poultry processing and on retail poultry meat has been established (Firildak et al., 2015; Kim et al., 2017; Li et al., 2017; Moore et al., 2017; Zhang et al., 2019). Others have reported the ability of sanitizers like chlorine to reduce foodborne pathogens on different food-contact surfaces encountered in food processing plants (Shen et al., 2012; Schlisselberg and Yaron, 2013; Smith et al., 2015).

Recovery of *Salmonella* from poultry transport equipment, the slaughterhouse, and the processing environment has been documented, but minimal information is available about its recovery from poultry processing equipment after antimicrobial application (Simmons

et al., 2003; Reiter et al., 2007; Lestari et al., 2009; Mezal et al., 2014; Khan et al., 2018). A study conducted in France recovered *Campylobacter jejuni* from the picker and eviscerator before cleaning and disinfection, and this pathogen persisted on the equipment and was recovered again after the disinfection procedure (Peyrat et al., 2008). Similarly, several *Salmonella* serotypes were recovered from processing equipment of Malaysian wet markets and a small-scale processing plant (Nidaullah et al., 2017). The persistence and prevalence of *Salmonella* on poultry processing equipment highlighted in Figure 1, not only after poultry meat processing but also after cleaning and sanitizing the processing equipment has not been well documented in the United States. The equipment could be indirectly contaminated with pathogens that reside in the gut of live birds and cross-contaminate poultry meat if improperly cleaned. Hence, it is imperative to understand the extent to which *Salmonella* persist in the poultry processing environment, as this knowledge could improve the effectiveness of current *Salmonella* control measures. In the current study, the prevalence of *Salmonella* in the poultry processing environment and its persistence on processing equipment after the cleaning and sanitization procedures within different poultry processing plants is reported. The recovered isolates were further identified by serotyping and characterized based on virulence genes.

MATERIALS AND METHODS

Experimental Design

Six different poultry processing plants (designated 1, 2, 3, 4, 5, and 6) belonging to 3 poultry integrators in the southern region of the United States were visited from January 2018 to January 2019. Each plant has a shift dedicated to thorough cleaning and sanitization, and each were visited for sample collection on 3 different days. Samples were collected from 15 different pieces of equipment (locations) at 3 different time periods described as A–after processing, B–after cleaning, and
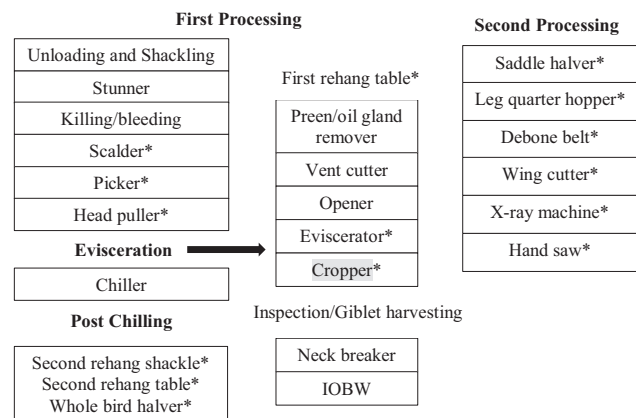
Figure 1. Poultry processing equipment layout with *sampling points. Abbreviation: IOBW, inside/outside bird washer.

C–after sanitization. A total of 135 samples were collected at each plant, making 45 samples per time period. The processing equipment sampled are highlighted in Figure 1.

Sample Collection

Swab samples of poultry processing equipment were collected using 3M sponge-stick with 10 mL buffered peptone water or neutralizing broth (SSL10NB, 3M Co. St. Paul, MN). The surface of each piece of equipment from Figure 1 was sampled 3 times. Each piece of equipment was swabbed horizontally by covering a surface area of about 13 cm^2 for 30 s, and the same spot was swabbed 3 times as described above. Each sample was immediately placed in a cooler with ice packs. All samples were immediately transported in a cooler containing ice and analyzed immediately upon reaching the lab at Mississippi State University.

Identification of Isolates

Media was purchased from Fisher Scientific, Hampton, NH, unless otherwise specified. Detection and isolation of *Salmonella* from samples was carried out following the USDA MLG 4.09 conventional method (USDA-FSIS, 2017b). Each sample was pre-enriched in 50 mL of buffered peptone water (BPW, BD218105) and incubated at 37°C for 20 to 24 h. After pre-enrichment, an aliquot of 100 µL and 500 µL was transferred into 10 mL of modified rappaport vassiliadis (mRV, CM0910 B) and tetrathionate broth (TT Hajna, BD249120) respectively and incubated at 42°C for 22 to 24 h. One loop full of culture in mRV and TT was subsequently streaked, in duplicate, onto brilliant green sulfa (BGS, BD271710) and xylose lysine tergitol 4 (XLT4, BD223420) agar. The agar plates were incubated at 37°C for 18 to 24 h. Afterward, presumptive *Salmonella* colonies were selected for biochemical testing by inoculating into triple sugar iron (BD226540) and lysine iron agar (BD211363) slants in tandem. The positive *Salmonella* isolates that were recovered from the slants at time period C (post sanitization) were sent to the National Veterinary Service Laboratory (NVSL, Ames, IA) for serotyping and used for all subsequent analysis.

Isolate Collection and Preservation

Following identification of the isolates recovered after sanitization, a loop of culture from *Salmonella*-positive triple sugar iron slant was streaked onto XLT4 plates and incubated at 37°C for 24 h. A distinct *Salmonella* colony was picked from the XLT4 plate and streaked onto tryptic soy agar (BD236950) and incubated. Afterward, cells were harvested and preserved in cryotubes containing a 20% glycerol solution (G33500, Fisher Scientific) and stored at −80°C.

DNA Extraction

For extraction of *Salmonella* DNA, isolates recovered after sanitization were streaked from the cryotubes onto tryptic soy agar plates. Following incubation, colonies from each plate were inoculated into 10 mL tryptic soy broth (BD211825) and incubated at 37°C for 24 h. After incubation, 2 mL of the culture was transferred to a microcentrifuge tube and centrifuged at 5,000 × g for 3 min followed by removing the supernatant from the pellet. This process was continued until all 10 mL of the culture was centrifuged. The pellets were resuspended twice in 2 mL phosphate buffered saline (J75889AE). The DNA was extracted from the pellet using the QIAamp DNA mini kit (51,304, Qiagen, Hilden, Germany) and analyzed for quantity and purity using a Nanodrop one UV-VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA) with a 260/280 nm ratio of 1.8 to 2.0 purity standard.

PCR Detection of Salmonella Virulence-Related Genes

Eight genes attributed to virulence in *Salmonella* were selected. Four of the genes (*invA*, *sipA*, *spiA*, and *sseC*) are located within the *Salmonella* pathogenicity islands (**SPI**) 1 and 2, 3 targets (*spvB*, *spvC*, and *pefA*) are found on the *Salmonella* virulence plasmid (pSLT), and 1 gene (*fimA*) encodes Type 1 fimbriae of *Salmonella* (Fàbrega and Vila, 2013; Suez et al., 2013).

After DNA extraction from each isolate, the presence of the virulence genes was determined using a PCR technique previously described by Oliveira et al. (2002). DNA from reference strains of *S. Typhimurium* ATCC 14028, *S. Enteritidis* ATCC 4931, and *S. Heidelberg* ATCC 8326 were included. All primers that were used to detect virulence genes are listed in Table 1. For each virulence gene, amplification took place in a 25 µL reaction containing 12.5 µL 2× Promega GoTaq master mix (M7122, Promega, Madison, WI), 0.5 µL each (10 µmol) F/R primers, 10.5 µL nuclease-free water, and 1 µL (80–120 ng) of DNA template. PCR was carried out in a Eppendorf EP *gradient* master cycler (Eppendorf Biotech Company, Hamburg, Germany) using the following cycling conditions: Initial denaturation at 98°C for 3 min, followed by 40 cycles of denaturation at 98°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. Gel electrophoresis of PCR products was carried out on 2% agarose gel (A201100, GoldBio, St. Louis, MO) containing SYBR safe DNA gel stain (Invitrogen S33102, Fisher Scientific) for visualization and a 100 bp DNA ladder (D001500, GoldBio) as a marker.

Statistical Analysis

Differences in *Salmonella* prevalence on equipment amongst plants over time were determined by analysis of variance in the General Linear Model using SAS

Table 1. List of primers used in this study.

Target gene	Primers	Sequence	Product size	Reference
invA	invA-F	GTGAAATTATCGCCACGTTCGGGCAA	284	Rahn et al., 1992
	invA-R	TCATCGCACCGTCAAAGGAACC		
sipB	sipB-F	GGACGCCGCCCGGGAAAAACTCTC	875	Skyberg et al., 2006
	sipB-R	ACACTCCCGTCGCCGCCTTCACAA		
spiA	spiA-F	CCAGGGGTCGTTAGTGTATTGCGTGAGATG	550	Skyberg et al., 2006
	spiA-R	CGCGTAACAAAGAACCCGTAGTGATGGATT		
sseC	sseC-F	ATGAATCGAATTCACAGTAA	1,455	Bhowmick et al., 2011
	sseC-R	TTAAGCGCGATAGCCAGCTA		
spvB	spvB-F	CTATCAGCCCCGCACGGAGAGCAGTTTTTA	717	Tarabees et al., 2017
	spvB-R	GGAGGAGGCGGTGGCGGTGGCATCATA		
spvC	spvC-F	ACTCCTTGCACAACCAAATGCGGA	571	Chaudhary et al., 2015
	spvC-R	TGTCTTCTGCATTTCGCCACCATCA		
fimA	fimA-F	CCTTTCTCCATCGTCCTGAA	85	Naravaneni and Jamil, 2005
	fimA-R	TGGTGTTATCTGCCTGACCA		
pefA	pefA-F	GCGCCGCTCAGCCGAACCAG	157	Tarabees et al., 2017
	pefA-R	GCAGCAGAAGCCCAGGAAACAGTG		

software v 9.4 (SAS Institute, 2013; Steel and Torrie, 1980). A 6 (plants) × 3 (time period) × 15 (locations) factorial arrangement of treatments in a completely randomized design was used to determine the effect of plant, time, and location on *Salmonella* prevalence. When significant differences ($P \leq 0.05$) were observed, Fisher's protected Least Significant Difference Test ($P \leq 0.05$) was used to separate the means.

RESULTS

Prevalence of Salmonella *on Poultry Processing Equipment Because of Plants*

Prevalence of *Salmonella* on poultry processing equipment among the 6 poultry processing plants based on overall sampling times was compared (Table 2). Overall, plants 1, 2, and 4 from integrators 1 and 2 had higher prevalence ($P < 0.001$) of *Salmonella* on their processing equipment when compared with plants 5 and 6 from integrator 3. When looking at prevalence within an integrator, there was no significant difference ($P = 0.22$) in *Salmonella* prevalence on equipment between plants 1 and 2 within integrator 1. Similarly, no differences were observed ($P = 0.76$) in prevalence between plants 5 and 6 within integrator 3. In contrast, *Salmonella* prevalence differed ($P = 0.03$) on the processing equipment of plants 3 and 4 within integrator 2.

Prevalence of Salmonella *on Poultry Processing Equipment Because of Time*

Salmonella prevalence was higher ($P < 0.001$) on the equipment at the end of the day's processing (time period A, 36%) compared with after the cleaning procedure, which included scrubbing and washing with detergent (time period B, 12%). In contrast, there were no differences ($P = 0.39$) in prevalence between time periods B and C (9%), which was after sanitization (Table 3).

Prevalence of Salmonella *on Poultry Processing Equipment Because of Location*

Salmonella prevalence differed ($P < 0.0001$) among the equipment (locations) that was sampled in this study (Table 4). *Salmonella* was observed to persist more on some of the first processing equipment on the kill line, including the head puller, picker, and scalder when compared with others on the evisceration line, like the eviscerator in all the processing plants. Prevalence was similar ($P > 0.05$) on most of the second processing equipment among all plants.

Persistence of Salmonella *on Processing Equipment After Sanitization Because of Plant and Location*

After antimicrobial application (sanitization), there was a trend ($P = 0.09$) where less *Salmonella* could be recovered from processing equipment (Table 5). For persistence of *Salmonella* on the equipment because of plants, there were no significant differences in prevalence between plants 1 and 2 ($P = 0.76$), which are managed by integrator 1, plants 3 and 4 ($P = 0.76$) managed by integrator 2, and plant 5 and 6 ($P = 0.76$) managed by integrator 3. However, plants 3 and 4 had the highest percent prevalence compared to the other plants. Integrator 3 had the lowest percent prevalence when compared with integrators 1 and 2. Moreover, *Salmonella* was recovered from all the processing plants after

Table 2. Overall prevalence of *Salmonella* on poultry processing equipment of different processing plants.

Integrator	Plant	Prevalence (%)
1	1	29.6[a]
	2	23.7[a,b]
2	3	17.8[b,c]
	4	28.2[a]
3	5	7.4[d]
	6	8.9[c,d]

[a-d]Each plant was visited 3 times. Means with different superscripts indicate significant differences.

N = 135, SEM = 3.41, $P < 0.001$.

Table 3. Prevalence of *Salmonella* on poultry processing equipment of different processing plants over 3 time periods.

Time	Prevalence (%)
A—Postprocessing	36.3[a]
B—Postcleaning	12.2[b]
C—Postsanitization	9.3[b]

[a,b]Means with different superscripts indicate significant differences. N = 270, SEM = 2.41, $P < 0.001$.

Table 5. Recovery of *Salmonella* from poultry processing equipment of different processing plants after sanitization.

Integrator	Plant	Prevalence (%)
1	1	8.9
	2	11.1
2	3	15.6
	4	17.8
3	5	2.2
	6	0.0

N = 45, SEM = 5.02, $P = 0.09$.

sanitization except plant 6 where there was no *Salmonella* found on the equipment. When looking at the persistence of *Salmonella* on the equipment (location) after sanitization, recovery of *Salmonella* was observed to be significantly higher ($P = 0.0002$) on first processing equipment, including the cropper, scalder, picker, and head puller when compared with several other types of equipment (Table 6). There were no differences ($P = 0.62$) in prevalence between the cropper and scalder, picker, and head puller. However, significant differences ($P = 0.05$) were observed when the cropper was compared with the debone belt and leg quarter hopper, which belong to the second processing equipment and were the only second processing equipment where *Salmonella* persisted.

Serotypes of Isolates Recovered From Processing Equipment After Sanitization

A total of 25 *Salmonella* isolates were recovered from various pieces of equipment from different processing plants after the sanitization (time period C) procedure. The various *Salmonella* serovars isolated are listed in Table 7. The recovered isolates belonged to 5 distinct *Salmonella* serovars. The most prevalent serovar isolated was *Salmonella* Kentucky (n = 12, 48%) followed by *Salmonella* Schwarzengrund (n = 5, 20%). Four isolates were identified as *Salmonella* Enteritidis (16%), 3

were Liverpool (12%), and only 1 (4%) was *Salmonella* Typhimurium.

Virulence of Salmonella *Isolates Recovered From Processing Equipment After Sanitization*

All the isolates that were recovered after sanitization were further characterized by examining 8 virulence genes in *Salmonella*. At least 4 virulence genes (*invA*, *sipB*, *spiA*, and *sseC*) found within SPI 1 and 2, and 1 related to fimbriae (*fimA*) were detected in all 25 *Salmonella* isolates (Table 8). Detection of the genes located on *Salmonella* virulence plasmid (pSLT) varied among the isolates. Among the genes carried on the plasmid, *spvB* (23/25) and *pefA* (24/25) were detected more frequently from the isolates *spvC* (9/25) was detected less frequently (Table 8).

DISCUSSION

Overall Prevalence of Salmonella *by Plants, Time Periods, and Location*

For several years, non-typhoidal *Salmonella* has remained a pathogen of importance to public health because it causes gastroenteritis in both developed and

Table 4. Prevalence of *Salmonella* on poultry processing equipment of different processing plants.

Processing step	Location[1]	Prevalence (%)
First processing (kill line)	Head puller	40.7[a]
	Picker	53.7[a]
	Scalder	42.6[a]
First rehang	First rehang	20.4[b,c]
First processing (evis line)	Cropper	44.4[a]
	Eviscerator	24.1[b]
Second rehang	Second rehang shackle	7.4[c,d]
	Second rehang table	0[d]
Second processing	Debone belt	13.0[b,c,d]
	Hand saw	3.7[d]
	Halver	9.3[b,c,d]
	Leg quarter hopper	9.3[b,c,d]
	Saddle halver	5.6[c,d]
	Wing cutter	9.3[b,c,d]
	X-ray belt	5.6[c,d]

[a-d]Superscripts indicate significant differences. N = 54, SEM = 5.39, $P = 0.001$.
[1]Equipment within each processing plants sampled. Means with different.

Table 6. Recovery of *Salmonella* from poultry processing equipment of different processing plants after sanitization.

Processing step	Location[1]	Prevalence (%)
First processing (kill line)	Head puller	27.8[a]
	Picker	27.8[a]
	Scalder	27.8[a]
First rehang	First rehang	0.0[b]
First processing (evis line)	Cropper	33.3[a]
	Eviscerator	0.0[b]
Second rehang	Second rehang shackle	0.0[b]
	Second rehang table	0.0[b]
Second processing	Debone belt	11.1[a,b]
	Hand saw	0.0[b]
	Halver	0.0[b]
	Leg quarter hopper	11.1[a,b]
	Saddle halver	0.0[b]
	Wing cutter	0.0[b]
	X-ray belt	0.0[b]

[a,b]Superscripts indicate significant differences. N = 18, SEM = 7.93, $P = 0.0002$.
[1]Equipment within each processing plants sampled. Means with different.

Table 7. *Salmonella* serovars recovered after sanitization of different processing plants.

Salmonella enterica	No. of isolates	Prevalence (%)
Salmonella Enteritidis	4	16
Salmonella Typhimurium	1	4
Salmonella Schwarzengrund	5	20
Salmonella Kentucky	12	48
Salmonella Liverpool	3	12
Total	25	

developing countries. Majowicz et al. (2010) estimated the global burden of *Salmonella* infection, gastroenteritis that were foodborne to be 80.3 million cases each year. According to the CDC, consumption of contaminated chicken meat is still a significant source of *Salmonella* infection in the United States (CDC, 2016). In other parts of the world where chicken meat is readily available, *Salmonella* contamination has been reported to cause illnesses and deaths (Barua et al., 2014; Nidaullah et al., 2017). Contamination can occur during different stages of poultry processing, including through the improper cleaning of processing equipment (Olsen et al., 2003; Lestari et al., 2009). Although *Salmonella* prevalence in raw chicken meat has been reported, this study presents the persistence of *Salmonella* on processing equipment after chickens have been processed through the cleaning and sanitization process.

In the present study, the presence of *Salmonella* on processing equipment of different poultry processing plants was tracked, and *Salmonella* was recovered from all the processing plants sampled. Many factors may have contributed to the recovery of *Salmonella* from the plants that were sampled. These include the antimicrobial that was used, the cleaning procedure, and the attachment of *Salmonella* to the equipment as biofilms. It has been previously suggested that *Salmonella* could persist in an environment by attaching firmly to abiotic surfaces, thus rendering antimicrobial applications ineffective (Gram et al., 2007). Although prevalence was lower in some plants compared with others, the pathogen was recovered from all the plants immediately after processing. It is expected that there would be a high prevalence of *Salmonella* on the equipment after processing chickens (time period A) because *Salmonella* is a commensal pathogen of the gut for many animals including poultry (Cosby et al., 2015). There was a reduction in the recovery of *Salmonella* from time periods A to B (after cleaning), but no significant reduction

was observed in prevalence between time periods B and C (after sanitization). The process of cleaning with detergent, some of which may have low levels of antimicrobial activity, could explain the reduction in bacterial contamination on the equipment surfaces. The cleaning process in all the plants visited required both physical and chemical activity. This process involved physical scrubbing of the equipment with a sponge, brush, and chemical detergent. Moreover, the application of sanitizers like chlorine or a quaternary ammonium compound on the equipment is presumed to further reduce bacterial contamination. It is possible that *Salmonella* have persisted on the equipment surface over time and thus are able to tolerate the antimicrobials that are used for sanitization. Previous studies have reported that *Salmonella* could acquire tolerance to antimicrobials when exposed to subinhibitory concentrations over time (Condell et al., 2012; Obe et al., 2018). Furthermore, high prevalence of *Salmonella* was observed for some of the first processing equipment, whereas there was no change in prevalence for several pieces of second processing equipment. The first processing of birds including evisceration, where the eviscerator removes the internal content of the carcass, which can cause contamination of both the carcass and the equipment with *Salmonella* (Russell and Walker, 1997). However, most processing plants use equipment spray with antimicrobial agents to reduce contamination during poultry processing (Bourassa, 2018).

Prevalence of Salmonella *After Sanitization (Time Period C) by Plants and Location*

To determine whether *Salmonella* persists on the equipment after sanitization, prevalence at time period C was only analyzed. *Salmonella* prevalence was considerably higher in plants managed by integrators 1 and 2 compared with integrator 3, which has at least 1 plant where there was no *Salmonella* recovered from its equipment after sanitization. Similar to overall prevalence, plants with higher overall prevalence had higher prevalence after sanitization, which suggests that the antimicrobials that were used may be ineffective, or the cleaning procedure employed by the plant may not be adequate to reduce microbial contamination on the equipment surfaces. Another possible explanation is that the plants with persistent *Salmonella* on the equipment surface had a higher initial bacterial load as seen in

Table 8. *Salmonella* virulence genes detected in the isolates.

Virulence genes	Location	No. of isolates positive (%)	No of isolates negative (%)
invA	SPI-1	25 (100%)	0
sipB		25 (100%)	0
spiA	SPI-2	25 (100%)	0
sseC		25 (100%)	0
spvB	pSLT plasmid	23 (92%)	2 (8%)
spvC		9 (36%)	16 (64%)
pefA		24 (96%)	1 (4%)
fimA	fimbriae	25 (100%)	0

overall prevalence at sampling time period A (after processing). Furthermore, *Salmonella* was found to persist more on first processing equipment than several other pieces of equipment. Some of the equipment are located on the kill side of the processing plant and could easily be contaminated with *Salmonella* from a positive flock and therefore require more rigorous cleaning. Studies have reported prevalence of *Salmonella* and *Campylobacter* on equipment like scalders, pickers, and eviscerators at the end of processing. However, similar to our findings, *Campylobacter* has been recovered from the picker, eviscerator, and conveyor belt before and after antimicrobial application, whereaas *Salmonella* was found to be prevalent on the picker after sanitization (Trampel, 2000; Olsen et al., 2003; Peyrat et al., 2008). Other equipment, especially those used in the second processing area, had significantly lower prevalence after processing, and no bacteria was recovered from the equipment after sanitization. This may be due to a lower initial bacterial load coupled with different interventions aimed at reducing *Salmonella* contamination during poultry processing. Antimicrobial intervention in the chiller is 1 of the critical control measures against *Salmonella* during poultry processing, but recovery has been reported from carcasses at the exit chiller (Parveen et al., 2007; Wideman et al., 2016). This could cause contamination of second processing equipment that have been thoroughly cleaned and sanitized. Also, of all the equipment sampled, the head puller was positive for *Salmonella* at all the 6 plants. Similarly, debone belt and leg quarter hopper were positive after sanitization. A possible explanation is that the equipment may be hard to reach for thorough cleaning and may therefore require more attention during the cleaning procedure.

Serotypes of Salmonella *Isolates Recovered After Sanitization*

There were 5 distinct *Salmonella* serovars identified in this study including Kentucky, Schwarzengrund, Enteritidis, Liverpool, and Typhimurium. *S.* Enteritidis and *S.* Typhimurium are 2 of the top 3 serovars identified in salmonellosis. According to CDC data, the number of infections caused by *S.* Enteritidis has increased from 2008 to 2018 and that the source of the infections could be traced to poultry and eggs (CDC, 2018). While *S.* Enteritidis was the most common serotype identified by USDA-FSIS from poultry establishments over a 3-year period, recovery of *S.* Typhimurium was reduced in the same establishments over the same 3-year period (CDC, 2018). *Salmonella* Kentucky is the most prevalent serovar identified in this study, and it has emerged as the top serovar identified in live poultry, turkey, and chicken meat (Lestari et al., 2009; Foley et al., 2011). Furthermore, the USDA data showed *S.* Kentucky was the most prevalent serovar from routine testing of chicken samples as part of the Hazard Analysis Critical Control Point verification program (USDA-FSIS, 2016). *Salmonella* Schwarzengrund was the second

most prevalent serovar that was isolated from processing equipment. This serovar is not among the top 10 serovars commonly identified in poultry meat by CDC or USDA but has been implicated in multistate foodborne outbreaks that resulted in illnesses and hospitalizations (CDC, 2007). In addition, *S.* Kentucky and *S.* Schwarzengrund that have been previously recovered from poultry products have expressed resistance to multiple antibiotics of clinical importance (Aarestrup et al., 2007; Lestari et al., 2009). The reduction in the recovery of Typhimurium from poultry and infection in humans has been linked to vaccination and better production practices (Dórea et al., 2010). Vaccinating commercial poultry against Typhimurium and Enteritidis could help reduce the incidence of salmonellosis that is caused by these serovars, but also increase infections caused by other serovars like Kentucky and Schwarzengrund to which vaccines have not been developed (Foley et al., 2011). Therefore, vaccinating poultry against emerging strains of *Salmonella* implicated in salmonellosis may help to further control *Salmonella* contamination in poultry meat.

Virulence of Salmonella *Isolates Recovered After Sanitization*

The ability of *Salmonella* to cause infection in humans has been extensively studied using *S.* Typhimurium. In this study, to determine the extent to which the recovered isolates could cause infection, the presence of virulence genes implicated in colonization by *S.* Typhimurium were examined. These genes are located within the *Salmonella* pathogenicity islands (SPI 1 & 2), virulence plasmid (pSLT), and the fimbrial subunit. Their functions include host recognition and invasion, survival, and replication within the epithelial cells, inhibition of inflammatory response and actin polymerization, and adhesion to specific epithelial cells (Fàbrega and Vila, 2013; Mezal et al., 2014). Many of the genes tested with the exception of the 1 found in the plasmid were detected in the recovered isolates. This observation is in agreement with other studies, where similar genes associated with multiple *Salmonella* strains that were isolated from poultry houses, chicken samples, and clinical samples were compared, with similarities found in their virulence. In fact, the poultry and clinical isolates shared virulence genotypes, which suggests that the poultry isolates can cause infection in humans (Diarra et al., 2014; Mezal et al., 2014; Yang et al., 2016; Rauch et al., 2018). Similarly, the findings in this study suggest that if the recovered isolates were to contaminate chicken meat and safe food handling practices were not followed, salmonellosis could occur. Notably, all the recovered *S.* Kentucky carried at least one virulence plasmid gene, even though *S.* Kentucky is not the most reported serovar in *Salmonella* infection. *Salmonella* virulence plasmids have been suggested to play a pivotal role in *Salmonella* infection (Guiney et al., 1995; Yang et al., 2016). Barua et al. (2014) found

similarities in the PFGE profile of *S.* Kentucky from poultry and human sources. Likewise, Rauch et al. (2018) observed the same *Salmonella* Kentucky sequence types in isolates from chicken meat and clinical samples. Additionally, studies have found that *Salmonella* isolates carrying the virulence plasmid possess resistance to multiple antibiotics, which could make treatment with clinically important antibiotics challenging (Barua et al., 2014; Yang et al., 2016).

CONCLUSION

In conclusion, contaminated processing equipment could serve as a potential source of cross-contamination of poultry carcasses during poultry processing because pathogens are able to survive the cleaning and sanitization procedure, thus causing food safety risks (Peyrat et al., 2008; Perez-Arnedo and Gonzalez-Fandos, 2019). It is critical to mention that all the processing plants that were visited in this study dedicated substantial time to the cleaning and sanitization procedure of the equipment and facility between the end of the shift and the next processing, but more effort may be required to address *Salmonella* contamination during poultry processing. The prevalence of *S.* Kentucky observed in this study is worth further exploration because virulence genes previously identified in *S.* Typhimurium and *S.* Enteritidis infection were detected. Also, further examination of antibiotic resistance profiles of the recovered isolates would be noteworthy.

A drawback observed in this study is the lack of data on the prevalence of *Salmonella* in the flocks processed at each of the plant sampled. This information could help to link the serovars of *Salmonella* recovered at each plant to the flock processed and reveal whether the serovars have been persisting on the equipment from a previous flock or processing day. The conclusions in this study could also be better supported by tracking prevalence of *Salmonella* on the equipment to the chickens processed by the plant at retail level. Regardless, this study fills some gaps in knowledge regarding the efficiency of the cleaning and sanitization procedure to reduce *Salmonella* contamination. This information could further be utilized to determine the mechanism by which the recovered isolates persist in the processing environment.

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