

Research Paper

Microbiological Profile of Three Commercial Poultry Processing Plants in Colombia

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ABSTRACT

Poultry meat production in Colombia has significant growth potential to fulfill national demands and to become an important global exporter. Entering export markets requires compliance with international food safety standards and the support of a rigorous national inspection system. To support the development of national standards, information about the microbiological profiles of poultry operations is needed, and no official microbiological baseline is currently available. A total of 480 chicken carcass rinses and 64 fecal samples were collected at different process sites from three commercial poultry processing establishments located in different regions of Colombia. Samples were analyzed to determine the prevalence of *Salmonella* and the levels of *Escherichia coli* in chicken rinse. Six steps were selected for sampling in the slaughter, evisceration, and chilling processes. The overall *Salmonella* prevalence after water immersion chilling at the three establishments was 12.5% (73 of 584 samples). *E. coli* levels were 1.2 to 2.2 log CFU/mL (mean, 1.65 log CFU/mL) after the chilling process. Significant differences ($P < 0.05$) were found for *E. coli* levels among the processing sites at the three establishments; however, there were no significant differences in the distribution of *Salmonella*-positive samples through the sites at each plant. These results can be used as reference data for microorganisms in chicken meat facilities in Colombia and will help the poultry industry and regulators in the design of new prevention programs and food safety management systems.

Key words: Colombia; *Escherichia coli*; Microbial profile; Poultry; *Salmonella*

The Colombian poultry industry has grown considerably in the past decade as a result of increased consumer demand for higher quality, more varied, and safer protein sources. This steady growth has opened opportunities for Colombian poultry meat to enter international markets as long as sanitary measures are in compliance with global and country-specific standards.

To support this process, the National Institute for Food and Drug Surveillance in Colombia (Instituto Nacional de Vigilancia de Medicamentos y Alimentos) has been working on a regulatory framework that will mirror some of the components of the food safety control system created by the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS). Colombian decree 1500, published in May 2007 (15), includes a series of prerequisite sanitary conditions for poultry processing operations, antemortem and postmortem inspection components, and a requirement to implement sanitation standard operating procedures and hazard analysis and critical control point (HACCP) food safety management systems with verifiable voluntary microbial standards (5). Facilities were required to comply with these measures by August 2016 and therefore have gone through a process of capacity building and major

infrastructure modifications in recent years. The Colombian inspection service has also undergone a significant process of modernization and training for its personnel to enable verification of the implementation of the regulation by the proposed compliance date (5). However, microbial performance standards for compliance have not been included in the regulation, leaving to the processing facilities the responsibility for demonstrating the level of control of their food safety systems. An official microbial baseline data source to be utilized as a reference for poultry processors has not been published, despite several efforts aimed at completing it. Some estimates of pathogen prevalence and indicator levels in Colombian poultry at retail have been made, but no comprehensive national baseline or in-plant reference data are currently available for poultry during processing. Therefore, information on microbial levels throughout the poultry processing chain from representative geographical locations in Colombia is needed so processors can measure their performance and compare their data to national and international reference sources.

Poultry operations in Colombia are conventional, with a high degree of vertical integration that has allowed major operations to reduce production costs and compete internationally for export markets. Most natural microflora related to poultry production are not pathogenic to humans (6); however, as in other countries, pathogenic organisms such

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as *Salmonella* and *Campylobacter* spp. are the key target organisms for control in these operations. During production and processing, the risk of contamination by any of these pathogens is significant, because any item that contacts a single bird might cause contamination, and any item that touches more than one bird might create cross-contamination (16).

Food safety management programs along the poultry processing chain are required to support the implementation of recent regulatory requirements in Colombia. However, no official microbial baseline or peer-reviewed reports are available on the prevalence of major poultry-associated pathogens and the levels of indicator organisms in chicken carcasses and parts at various stages of processing representative of the local conditions and processing practices. The main objective of this study was to collect samples at various stages of production from three commercial chicken processing facilities located in representative geographical regions of Colombia to establish reference microbial profiles for *Salmonella* prevalence and *Escherichia coli* levels during commercial processing of chickens.

MATERIALS AND METHODS

Characteristics of poultry processing facilities. Three processing establishments were selected for this study. Plant A is located in the central region of Colombia at an elevation of 2,207.10 m and an average temperature of 15°C. This plant processes 42,000 birds per day and runs two shifts of 8 h/day. Plant B is located in the southern region, at 995.79 m and an average of 24°C, processes 183,000 birds per day, and runs two shifts of 8 h/day. Plant C is located in the northern region, at 33.82 m and an average of 28°C, processes 55,000 birds per day, and runs two shifts of 8 h/day. Each establishment has unique characteristics in terms of elevation, temperature range throughout the year, and production capacity. A summary of these variables is provided in Figure 1. All establishments utilize 10 and 50 ppm of sodium hypochlorite (NaOCl) as a chemical intervention in the immersion chiller tank but no major chemical interventions in other processing steps.

The full process line includes reception, hanging, stunning, slaughter, bleeding, scalding, defeathering, rehang, automatic evisceration, inspection, carcass rinse, inside-outside bird washer, prechilling (15 min at 12°C with recycled water from the chilling stage), and the chilling (45 to 60 min at 0°C, with chlorine intervention). Final products can be packaged and sold as fresh or frozen whole carcasses, and some of the carcasses are cut for sale as chicken parts.

Experimental design. Three commercial chicken processing facilities from geographically distinct regions of Colombia known for high levels of poultry production were selected for this study (Fig. 1). Plants A, B, and C represented the central, southern, and northern regions of Colombia, respectively. A cross-sectional study was carried out in 2015 between April and May for plant C and between October and November for plants A and B. The prevalence of *Salmonella* and the levels of *E. coli* in chicken rinse samples collected at various processing sites were evaluated. A total of 480 chicken carcasses and 64 fresh chicken fecal samples (24 samples from six sites in plant A, 40 samples from six sites in plant B, and 40 samples from five sites in plant C) were collected at various times during the two consecutive months of poultry production operations on two processing days per week. Sampling sites throughout the processing line were selected based on major operations with the

potential to affect microbial loads. Samples were collected after scalding, after defeathering, after evisceration, after prechilling and after chilling. Additional variables such as weather effects, regional differences, and intraflock, interflock, and interfarm variability were not controlled for in the sampling design.

Chicken rinse sample collection at various processing steps. Chicken carcass rinse samples were collected at sites in all three establishments participating in this study according to FSIS method MLG 4.08 (24). At specific processing steps, chicken carcasses selected at random were removed with sterile gloves from the processing line and placed in individual sterile poultry stomacher bags (Nasco, Fort Atkinson, WI). Four hundred milliliters of buffered peptone water (BPW; BD, Detroit, MI) was added to each bag and carefully distributed by shaking vigorously for 1 min. Approximately 100 mL of the rinse solution was aseptically transferred into a sterile screw-top container and shipped to a contract laboratory by an overnight delivery service. The temperature of received samples was recorded, and only samples at 2.5 to 5°C were accepted for microbiological analysis. In plant B, which processes more than 100,000 birds per day, samples were collected randomly between plant work shifts 1 and 2 to better account for the distribution of carcasses between shifts.

Fecal sample collection. Fecal samples were pooled by aseptically collecting approximately 100 g of fresh feces from the cages used to transport the broiler chickens to the slaughter plants. A 100-mL specimen container ($n = 64$) was used to collect each sample as soon as the chickens were removed from the cages. Samples were cooled and shipped under refrigeration to a contract laboratory for microbial analysis.

Chicken rinse sample collection at various times at each processing step in plant B. To estimate the cumulative effect of bacterial organisms during a complete work shift, additional whole chicken carcass samples were collected and analyzed for *E. coli* levels at five processing steps (after scalding, after defeathering, after evisceration, after prechilling, and after chilling) at five time periods (0, 2.5, 5, 8, and 11 h) after the initiation of the slaughter process in plant B. Samples were collected only at plant B for this component of the study.

Microbiological analysis. Fecal samples, diluted 1:9 (w/v) with BPW, were placed in a stomacher and homogenized for 1 min at 230 rpm. Samples (100 mL) of each carcass rinse and of the fecal fluids were collected into sealed containers, further serially diluted (1:10) in BPW, and used to determine *E. coli* levels. Samples were processed in duplicate by transferring 1 mL of the corresponding dilution to *E. coli*-coliform Petrifilm plates (3M, St. Paul, MN), which were incubated at 35°C for 24 h following method AOAC 998.08 (22).

Salmonella prevalence was evaluated using a molecular detection system (MSD100, 3M) with method AOAC 2013.09 (2) following the manufacturer's instructions. The BPW ISO enrichment medium (3M) was prewarmed to $37 \pm 1^\circ\text{C}$ and then aseptically combined with each carcass rinse or fecal sample at 1:10 dilution. Samples were homogenized thoroughly for 2 min and incubated at $37 \pm 1^\circ\text{C}$ for 24 h. Enriched samples were transferred to lysis tubes and heated at $100 \pm 1^\circ\text{C}$ for 15 min. Lysates from each sample were transferred to a reagent tube, loaded into a molecular detection speed loader tray (3M), and then analyzed using molecular detection software (3M). Samples positive for *Salmonella* were then cultured using conventional method NTC 4574 (12). Enriched samples were grown in xylose lysine desoxycholate agar



Plant	Region	Elevation (m)	Average temperature (°C)	Average precipitation (mm)*	No. of employees	birds/day processed	NaOCl concentration chiller
A	Central	2207.10	15	28	400	42,000	10 ppm
B	South	995.79	24	58	1,100	183,000	10 ppm
C	North	33.82	28	105	600	55,000	35 ppm

*Data estimated of precipitation of two consecutive months when samples were collected (IDEAM, 2015)

FIGURE 1. Geographical location and profile of the three poultry processing plants evaluated in this study. IDEAM, Instituto de Hidrología, Meteorología y Estudios Ambientales, Bogotá, Colombia.

(Hardy Diagnostics, Santa Maria, CA) and in brilliant green sulfa agar (Difco, BD) and incubated at $37 \pm 1^\circ\text{C}$ for 24 h. Isolates with typical *Salmonella* morphology were confirmed by agglutination using a Poly-O (A and Vi) antiserum test (Difco, BD).

Statistical analysis. A one-way analysis of variance (ANOVA) followed by Sidak's multiple comparison test ($P < 0.05$) were used to determine the significance of differences between the samples collected at various processing sites for each establishment and between establishments. A two-way ANOVA was used to determine the main effect and the interaction of *E. coli*

levels and the time of sampling, followed by Tukey's multiple comparison test. *Salmonella* results were reported as prevalence, and significant differences were identified with a chi-square test. The statistical analyses were carried out using Prism 7.01 statistical software (GraphPad Software, San Diego, CA).

RESULTS

***Salmonella* prevalence.** *Salmonella* was recovered from chicken rinse and fecal samples at various processing sites (Table 1). Chicken rinse samples from plant A had no

TABLE 1. Prevalence of *Salmonella* recovered from chicken samples collected at various locations in processing plants

Process location	No. of samples positive/no. tested (% positive) ^a			
	Plant A (n = 144)	Plant B (n = 240)	Plant C (n = 200)	Mean (n = 584)
Fecal material (before slaughter)	0/24	8/40 (20)	NA	8/64 (12.5)
Chicken carcass rinse				
After scalding	0/24	8/40 (20)	7/40 (17.5)	15/104 (14.4)
After defeathering	0/24	8/40 (20)	2/40 (5)	10/104 (9.6)
After evisceration	0/24	11/40 (27.5)	2/40 (5)	13/104 (12.5)
After prechilling	0/24	9/40 (22.5)	6/40 (15)	15/104 (14.42)
After chilling	0/24	7/40 (17.5)	5/40 (12.5)	12/104 (11.5)

^a Results represent the data collected during two consecutive months on two sampling days per week. Plant A, $n = 3$ per day; plant B, $n = 5$ per day; plant C, $n = 5$ per day. In plant A, no samples were positive for *Salmonella* during this study. NA, not applicable because sampling at this process location was not included. Limit of detection was $<1\%$.

TABLE 2. *Escherichia coli* recovered from chicken carcass rinses at various sampling locations in each processing plant

Process location	<i>E. coli</i> (log CFU/mL) ^a			
	Plant A (n = 144)	Plant B (n = 240)	Plant C (n = 200)	Mean (n = 584)
Fecal material (before slaughter)	5.72 A	TNTC	NA	5.72
Chicken carcass rinse				
After scalding	5.05 A X	2.68 A Y	1.52 A Z	3.08
After defeathering	5.32 A X	3.16 B Y	1.08 A Z	3.19
After evisceration	3.79 B X	2.84 AB Y	1.17 A Z	2.60
After prechilling	3.05 BC X	1.60 C Y	1.28 A Y	1.98
After chilling	2.21 CD X	1.58 C Y	1.16 A Z	1.65

^a Results represent the data collected during two consecutive months on two sampling days per week. Plant A, $n = 3$ per day; plant B, $n = 5$ per day; plant C, $n = 5$ per day. TNTC, too numerous to count (15 to 150 total colonies in 20-cm² Petrifilm plate area). NA, not applicable because sampling at this process location was not included. Within a column (comparison between processing sites), means followed by different letters A, B, C, or D are significantly different according to an ANOVA and Sidak's multiple comparison tests at $P < 0.05$. Within a row (comparison between plants) means followed by different letters x, y, or z are significantly different.

detectable *Salmonella*. However, in plant B rinse samples *Salmonella* prevalence increased after prechilling and was 27.5% of tested samples (confidence interval [CI], 15.14 to 44.13%) after evisceration (Table 1). In plants B and C, *Salmonella* prevalence after chilling was 12.5% (CI, 4.7 to 27.6%) and 17.5% (CI, 7.9 to 33.4%), respectively. For plants A, B, and C, *Salmonella* prevalence was 0% (0 of 144 samples), 21.2% (51 of 240 samples), and 11% (22 of 200 samples), respectively, during the 2 months of this study. The overall *Salmonella* prevalence for all chicken samples at the three slaughtering plants was 12.5% (73 of 584 samples; CI, 9.98 to 15.52%).

***E. coli* levels.** *E. coli* levels at the six processing sites for each establishment are presented in Table 2. Levels at plants A and B were significantly different from those at plant C after the scalding and defeathering steps, and levels at all plants were significantly different from each other ($P < 0.05$) at the last sampling location (after chilling). Plant C had the lowest levels at all processing sites compared with the other plants (Table 2). Of the 584 total samples tested, 69 (11.8%) had *E. coli* levels below the limit of detection of 10 CFU/mL. Of the remaining 478 samples, 196 (41%) had an *E. coli* levels of 10^3 to 10^4 CFU/mL of rinse (Table 3).

Cumulative evaluation of *E. coli* levels during a full work shift, plant B. Additional data were collected in plant B, which had the highest poultry production volume of the three plants included in this study. Samples for *E. coli* analysis were collected at each of five processing sites at five times during a single work shift: 0, 2.5, 5, 8, and 11 h after the initiation of the production process. Results obtained for the first process site (after scalding) indicated significant differences ($P < 0.05$) between the samples at the initial sampling times at 0 and 2.5 h and those at the later times, 8 and 11 h, after continuous processing (Table 4). In general, no significant differences ($P > 0.05$) in *E. coli* levels were found at the other sampling times and the subsequent processing sites.

DISCUSSION

The results of this study provide reference data for *Salmonella* prevalence and *E. coli* levels at various chicken processing steps in plants in three representative regions of Colombia. Results differed between and within each participating poultry processing plant, possibly because each plant was unique based on such variables as location, weather, altitude, production levels, infrastructure, processing step variables, utilization of antimicrobial interventions, flocks processed, and farm infrastructure. Hence, these results must be carefully considered before they are utilized as a representative microbial profile reference source to support food safety management programs. Each plant can use the information to identify potentially important processing steps for controlling foodborne pathogens and hygiene indicators during operations.

The overall prevalence of *Salmonella* in the whole carcass rinses samples obtained after the chilling process was 12.5%. This prevalence is comparable to that in similar studies conducted in Costa Rica (10%) (19), Brazil (10%) (3), and Canada (16.9%) (4) but higher than the prevalence in the United States (3.7%) (23), the United Kingdom (3.6%) (11), and Denmark (0%) (11). However, the variability between facilities and regions is significant even in countries with high *Salmonella* prevalence. These high prevalence levels probably resulted from intestinal tearing during evisceration and cross-contamination during scalding, defeathering, and chilling, and any single point of contact can be enough to spread bacteria to chicken carcasses and the plant environment (10). No *Salmonella* was found in samples from plant A. Because various factors can affect *Salmonella* detection, these results cannot be solely attributed to the elevation of the facility. Despite the fact that recent studies have indicated an effect of geo-location, average temperature, and annual precipitation on the prevalence of pathogenic microorganisms in poultry (13), this experiment was not designed to elucidate these relationships.

The facilities evaluated in this study rely on chlorine to control bacterial contamination on carcasses and in processing water because of its low cost, safety, and ease of use in

TABLE 3. Distribution of *E. coli* in processing plants

Level (log CFU/mL of rinse) at process location	No. of positive samples				% of total (<i>n</i> = 584)	Total no. positive (<i>n</i> = 584)	Total % positive (<i>n</i> = 584)
	Plant A (<i>n</i> = 144)	Plant B (<i>n</i> = 240)	Plant C (<i>n</i> = 200)	Mean (<i>n</i> = 584)			
Before slaughter (fecal material)							
<10	0	0	NA ^a	0	0.0	0	0.0
10–100	0	3		3	0.5	3	0.5
100–1,000	0	0		0	0.0	3	0.5
1,000–10,000	0	0		0	0.0	3	0.5
10,000–100,000	6	0		6	1.0	9	1.5
100,000–1,000,000	6	0		6	1.0	15	2.6
>1,000,000	12	0		12	2.1	27	4.6
TNTC ^b	0	37		37	6.3	64	11.0
After scalding							
<10	0	0	23	23	3.9	87	14.9
10–100	0	8	13	21	3.6	108	18.5
100–1,000	0	15	4	19	3.3	127	21.7
1,000–10,000	14	17	0	31	5.3	158	27.1
10,000–100,000	8	0	0	8	1.4	166	28.4
100,000–1,000,000	2	0	0	2	0.3	168	28.8
>1,000,000	0	0	0	0	0.0	168	28.8
TNTC	0	0	0	0	0.0	168	28.8
After defeathering							
<10	0	0	28	28	4.8	196	33.6
10–100	0	3	9	12	2.1	208	35.6
100–1,000	0	7	3	10	1.7	218	37.3
1,000–10,000	2	30	0	32	5.5	250	42.8
10,000–100,000	7	0	0	7	1.2	257	44.0
100,000–1,000,000	9	0	0	9	1.5	266	45.5
>1,000,000	6	0	0	6	1.0	272	46.6
TNTC	0	0	0	0	0.0	272	46.6
After evisceration							
<10	0	0	26	26	4.5	298	51.0
10–100	0	2	12	14	2.4	312	53.4
100–1,000	3	24	2	29	5.0	341	58.4
1,000–10,000	13	14	0	27	4.6	368	63.0
10,000–100,000	7	0	0	7	1.2	375	64.2
100,000–1,000,000	1	0	0	1	0.2	376	64.4
>1,000,000	0	0	0	0	0.0	376	64.4
TNTC	0	0	0	0	0.0	376	64.4
After prechilling							
<10	0	0	22	22	3.8	398	68.2
10–100	3	30	18	51	8.7	449	76.9
100–1,000	12	10	0	22	3.8	471	80.7
1,000–10,000	4	0	0	4	0.7	475	81.3
10,000–100,000	5	0	0	5	0.9	480	82.2
100,000–1,000,000	0	0	0	0	0.0	480	82.2
>1,000,000	0	0	0	0	0.0	480	82.2
TNTC	0	0	0	0	0.0	480	82.2
After chilling							
<10	0	0	21	21	3.6	501	85.8
10–100	9	31	16	56	9.6	557	95.4
100–1,000	12	8	3	23	3.9	580	99.3
1,000–10,000	3	1	0	4	0.7	584	100
10,000–100,000	0	0	0	0	0.0	584	100
100,000–1,000,000	0	0	0	0	0.0	584	100
>1,000,000	0	0	0	0	0.0	584	100
TNTC	0	0	0	0	0.0	584	100

^a NA, not applicable because sampling at this process location was not included.^b TNTC, too numerous to count.

TABLE 4. *E. coli* populations recovered in plant B from chicken carcass rinses at various sampling locations at five sampling times during a processing shift^a

Process location	<i>E. coli</i> (log CFU/mL) at sampling time:									
	0:00		2:30		5:00		8:00		11:00	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
After scalding	2.76 AB	2.1–3.4	2.39 A	2.0–2.8	2.31 AB	1.7–3.0	2.67 A	2.2–3.2	3.28 B	3.0–3.6
After defeathering	3.06 A	2.7–3.4	3.31 A	3.0–3.6	3.00 A	2.2–3.8	3.19 A	2.9–3.5	3.25 A	3.1–3.4
After evisceration	2.70 A	2.2–3.1	2.85 A	2.5–3.3	2.88 A	2.6–3.2	2.73 A	2.2–3.1	3.11 A	2.7–3.5
After prechilling	1.42 A	0.9–2.0	1.72 A	1.3–2.1	1.75 A	1.3–2.2	1.59 A	1.1–2.0	1.52 A	1.2–1.9
After chilling	1.74 A	1.4–2.1	1.61 A	1.1–2.1	1.55 A	1.0–2.0	1.75 A	1.1–2.5	1.27 A	1.0–1.5

^a Within a row (comparison between sampling times at individual location), means followed by different letters are significantly different. Results represent the data collected during two consecutive months on two sampling days per week, $n = 40$ for each process location.

the processing plant. Nevertheless, chlorine pH and concentration and the quality of the incoming water can affect the antimicrobial efficacy of chlorine on chicken carcasses (18) and therefore could explain the variable results obtained in these processing plants. Proper use of chlorine in immersion chilling tanks or as a rinsing step is effective for reducing *Salmonella* prevalence (9, 17).

The chilling process is one of the most critical steps for microbial control during poultry processing. The main objective of chilling is to inhibit pathogen growth by lowering the temperature of the carcass to reduce the overall risk of foodborne disease (21). Antimicrobial interventions can be applied directly to the surface of whole carcasses and parts by showers, sprays, and dipping solutions; however, extensive bird-to-bird contact can spread pathogens in the chiller by cross-contamination (14). Based on the results obtained from this study, the application of chlorine (>10 ppm) in the chilling process as performed at plants A and B may have had an effect on *E. coli* levels. However, in plant C no significant reductions in these levels were found after the application of the same antimicrobial intervention at the same processing step.

In this study, an additional objective to evaluate the change in *E. coli* levels during a processing shift. Samples were collected at various processing steps at various times during the full work shift at plant B. The variability in the data indicates an overall trend for increasing *E. coli* levels, but the differences were not significant when comparing early and late sampling times for the same processing step. The continuous overflow of water and the introduction of clean and fresh water plus the other stress conditions such heat and acid during processing appeared to prevent accumulation of bacteria at the various processing steps (1, 20).

Colombia's economy is the third largest in Central and South America. Poultry is one of the economic activities that grown steadily in the past 50 years (7). Colombia also is one of the fastest growing markets, with a growth of 82.14% between 2000 and 2010 in total U.S.-Colombia agricultural trade (exports and imports) (8). The Trade Promotion Agreement between these two countries went into effect in May 2012 (25). This agreement includes the opening of the Colombian market to U.S. poultry exports with a 27,040 ton³ duty-free access to Colombia of fresh, chilled, frozen,

and processed chicken leg quarters with a 4% annual growth over 18 years. The National Federation of Poultry Farmers in Colombia had incentivized the implementation of HACCP programs in slaughter poultry establishments as a voluntary measure to improve food safety around the country and as a way to assist in securing the equivalency of inspection approval to reciprocate the exchange of poultry products between these countries. The development and implementation of food safety management programs in the Colombian poultry industry require the availability of comparison data that could help processors in benchmarking their operations to identify intervention needs and improve the safety of chicken meat. Although this study does not provide complete baseline data for the whole industry in Colombia, these data do provide reference information for comparative purposes and can be used for the continuous improvement of food safety efforts in the Colombian poultry industry.

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