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**Prevalence of *Salmonella* and *Campylobacter* from retail poultry in the
Southeastern United States**

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Abstract

Chicken meat the most consumed poultry products in the USA and one the most associated with foodborne illnesses. The objective of this study was to analyse the prevalence of two common pathogens in poultry products, *Salmonella* and *Campylobacter*, in retail poultry at the Southeastern United States. A total of 74 samples were collected from four different grocery stores. Sample types were selected based on availability, divided into breasts, wings, thighs, and tenders and categorically collected from conventional, antibiotic-free, organic and air-chilled productions. All samples originated from 14 different processing plants based on the different codes marked in each package. Conventional detection was employed using bacterial culturing on selective media, XLT4 and Campy Cefex Agar. Also, molecular methods Gene-Up for *Salmonella* (SLM2) (AOAC Official Method of Analysis 2020.02) and the 3MTM Molecular Detection Assay 2 (MDS) for *Salmonella* and *Campylobacter* were applied to ensure accurate results. A prevalence of 13.5% for *Salmonella* ($p = <0.0001$) and 6.8% for *Campylobacter* ($p = <0.0001$), respectively, was determined. No differences were determined, but breasts and wings showed higher prevalences for both pathogens. The highest contamination was for conventional and antibiotic-free (ABF) productions for *Salmonella* while *Campylobacter* showed the opposite. In processing plants, the highest *Salmonella* prevalence was 42% and the lowest 0%, similar to *Campylobacter* with 40% and 0%. Results revealed that the presence of skin, location in the body, and potentially certain practices employed in chicken production or processing may have influenced contamination levels. Reinforced sanitation protocols is important to reduce contamination to the lowest possible level.

Keywords: cross contamination, microbial resistance, prevalence

Resumen

La carne de pollo es la más consumida en EE. UU. y una de las más asociadas con enfermedades transmitidas por alimentos. El objetivo de este estudio fue analizar la prevalencia de dos patógenos comunes en dicho producto, *Salmonella* y *Campylobacter*, en aves minoristas en el sureste de Estados Unidos. Se recogieron 74 muestras de cuatro supermercados, seleccionadas según la disponibilidad. Se dividieron en pechugas, alas, muslos y filetes y se recolectaron categóricamente de producciones convencionales, libres de antibióticos, orgánicas y refrigeradas por aire. Procedían de 14 plantas diferentes según los códigos de cada empaque. Convencionalmente, se empleó un cultivo bacteriano en medios selectivos, XLT4 y Campy Cefex Agar. Además, se aplicaron los métodos moleculares Gene-Up para *Salmonella* (SLM2) (AOAC 2020.02) y el ensayo de detección molecular 2 (EDM) 3MTM para ambas bacterias. Se determinó una prevalencia de 13,5% para *Salmonella* ($p = <0,0001$) y 6,8% para *Campylobacter* ($p = <0,0001$), respectivamente. No resultaron diferencias, pero las pechugas y alas mostraron prevalencias mayores para ambos patógenos. La mayor contaminación resultó en producciones convencionales y libres de antibióticos para *Salmonella*, mientras que *Campylobacter* mostró lo contrario. En las plantas procesadoras, la mayor prevalencia de *Salmonella* fue de 42% y la menor de 0%, similar a *Campylobacter* con 40% y 0%. Los resultados revelaron que la presencia de piel, la ubicación en el cuerpo y las diferentes prácticas empleadas en cada producción influyeron en los niveles de contaminación. Es importante reforzar los protocolos de saneamiento, para reducir la contaminación al menor nivel posible.

Palabras clave: contaminación cruzada, prevalencia, resistencia microbiana

Introduction

Nowadays, chicken meat is the most consumed meat in the United States and is one of the largest industries in Alabama, responsible for around 86,000 jobs and a production of one billion birds annually (Alabama Poultry and Egg Association [APEA], 2024) . It is a flexible livestock sector with a big demand, no religious taboos, is the fastest growing and is considered affordable and easily accessible. It has an important role in the economy since rural poultry represents about 80% of poultry stocks in low resource countries, making this sector fundamental for the sustenance of resource-poor farmers (Food and Agriculture Organization of the United Nations [FAO], 2013). Also, chicken meat provides benefits to our health since it contains essential macronutrients and micronutrients which make it healthier than other types of meat. Chicken meat includes high-quality complete proteins, nine essential amino acids, minerals, vitamins, low salt levels, and does not naturally contain carbohydrates (Connolly & Campbell, 2023).

All these qualities make poultry production one of the most controlled sectors to protect human health. In the study carried out by Bhisare et al. in 2014, it is mentioned that poultry meat is often contaminated with different pathogenic microorganisms, for instance, *Salmonella*, *Campylobacter*, *S. aureus*, *E. coli*, and *Listeria*. Among these pathogens, two of the most prevalent ones associated with foodborne illness worldwide are *Salmonella* and *Campylobacter*, causing significant morbidity and mortality annually. The poultry industry, especially, has been identified as a primary reservoir for these pathogens, posing substantial public health risks. *Salmonella* and *Campylobacter* infections are a big concern. Annually, *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the USA (Centers for Disease Control and Prevention [CDC], 2020b). On the other hand, *Campylobacter* cases in the USA cause approximately 1.5 million illnesses annually, usually caused due to cross contamination during food preparation and people who eat raw or undercooked poultry (CDC, 2020a).

Salmonella, first discovered and isolated from the intestines of pigs with a common illness called swine fever, is known as one of the most frequently isolated foodborne pathogens worldwide. The most common illnesses caused by this bacteria are gastroenteritis, bacteremia, and enteric fever (Aras et al., 2015). *Salmonella* is a rod-shaped, facultative anaerobe, gram-negative bacteria, which belongs to the family Enterobacteriaceae (Barlow & Hall, 2002). Based on the World Health Organization (2018), there are two species: *Salmonella bongori* and *Salmonella enterica*. Also, over 2500 different serotypes or serovars have been identified. Related to the sources and transmission, *Salmonella* is widely distributed in domestic and wild animals, and can pass through the food chain from animal feed to primary production, ending in establishments and institutions (World Health Organization [WHO], 2018).

Campylobacter. This bacterium is a gram-negative, microaerophilic genus, belongs to the family *Campylobacteriaceae*, and there are more than 20 species, but there are just a few that can cause human illness, for instance, *Campylobacter coli* and *Campylobacter jejuni* (CDC, 2023). Known as a fragile bacterium, it grows better at 37 °C to 42 °C and is well adapted to birds since its body temperature is adequate. This bacterium is common in the intestinal microbiota of birds and some mammal species. Based on the Center for Disease Control and Prevention (CDC), 2023, humans can be infected by *Campylobacter* commonly through consumption of contaminated meat products, essentially poultry. The infections are characterized by abdominal pain, diarrhea, nausea, fever and in some cases, vomiting.

In favor of controlling this problem, understanding the behavior of *Salmonella* and *Campylobacter* in retail poultry is fundamental to carry out effective control measures to mitigate the spread of the pathogens and reduce the burden of foodborne diseases. The objective of this study was to analyze the prevalence of *Salmonella* and *Campylobacter* from retail poultry in the Southeastern United States, determine if there is dependence between sample types, productions,

and processing location with contamination levels, as well as discuss possible causes related to the persistence of these pathogens.

Methodology

Study Location

Located in Auburn, Alabama. The study was carried out at the Poultry Science Department of Auburn University. Microbiological studies were conducted at the microbiology laboratory of Dr. Dianna Bourassa. All the supplies were provided by the entity. The samples were obtained from four local supermarkets.

Concept of Prevalence and Formula

Prevalence is known as a frequent epidemiological measure of how commonly a specific condition is present in a population. To estimate it, all samples were collected randomly ensuring an adequate quantity (a random selection increase chances to have representative results). Based on a general formula established and commonly used, the prevalence was calculated by dividing the number of positive samples by the total of samples analyzed and multiplying the result by 100 to obtain a percentage (Roe & Doll, 1995).

$$\text{Prevalence (\%)} = \frac{\text{Total of positives samples}}{\text{Total of samples}} \times 100 \quad [1]$$

Microbiological Analysis

A microbiological study to define the prevalence and characterization of *Salmonella* and *Campylobacter* was conducted. From March 2024 to April 2024, samples were procured from four different establishments located at Auburn, Alabama, and transported in refrigeration conditions to the laboratory to go through prevalence analysis. After each collection, each package was sprayed with ethanol (70%) and identified with a number, plant code, treatment, and type of sample.

Both conventional and molecular methods were used. Both conventional and molecular methods were used. The conventional method consisted of initially adding 150 mL of non-selective

culture media Buffered Peptone Water (BPW) to 454 g (1 lb) of each sample. After this, all samples with BPW were plated in petri dishes with selective culture media XLT4 and Cefex to see if it was possible to get direct counts without incubating sample bags. The same day, all samples were subjected to Gene Up analysis to enumerate *Salmonella* if present. Samples were incubated overnight to be subjected to MDS the next day. Those that were positive, according to MDS molecular method, were selectively enriched in Rappaport Vassiliadis (RV) and Tetrathionate Broth base (TT) to isolate colonies that could be saved for further analyses. For the detection of *Salmonella*, the molecular methods Gene-Up *Salmonella* (SLM2) (AOAC Official Method of Analysis 2020.02) and the 3MTM Molecular Detection Assay 2 (MDS) were applied. The detection of *Campylobacter* was limited only with the use of conventional method with selective media with enrichment and the 3MTM Molecular Detection Assay 2 (MDS).

Gene Up Method (AOAC Official Method of Analysis 2020.02)

The Gene Up method is a real-time PCR pathogen detection system, which is simple, efficient, and it has an accurate method for diagnosing pathogens. It utilizes Fluorescence Resonance Energy Transfer (FRET) hybridization probes for the rapid detection of *Salmonella* species in different foods. It combines the detection of real-time amplification curves and melt peaks to find out positive or negative results (Johnson et al., 2021). This method has a range of detection from >10 CFU/mL to report a positive result. For each analysis, a Gene-Up SLM kit was used with the Gene-Up thermocycler. This kit contains all the components for PCR such as sample-specific primers and probes and an internal amplification control. The software automatically interpreted the results in a range of time of 4 – 8 hours. In some cases, it requires 12 to 26 hours depending on the matrix.

3MTM Molecular Detection Assay 2 (MDS) for the Detection of Salmonella and Campylobacter

The Molecular Detection Assay (MDS) is a molecular method used for the rapid and precise detection of *Salmonella* and *Campylobacter* in enrichments of food, animal feed, and food process environmental samples. “The 3M Molecular Detection Assay use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification” (Rosauer et al., 2022). This equipment is exclusive for laboratory use only and is not oriented for pharmaceutical, veterinary, cosmetic, or clinical analysis.

Sample Types and Collection

The type of samples was selected based on the availability within each establishment. Among these types were purchased breast, wings, tenders, and thighs. Between the samples, four different treatment categories were evaluated, such as conventional, organic, antibiotic free (ABF), and air chilled. The number of samples was defined in a range between 22 and 27 chicken packages per collection day. After the collection, the samples were transported the same day keeping them in refrigeration condition to the microbiology laboratory to start the microbiological analysis.

Samples Collected and Repetitions

A total of three repetitions were done to obtain all study results. One repetition consisted of four days of analysis, starting with collection of samples in four different supermarkets on Day 1. For each collection day, the same supermarkets were visited. Table 1, 2 and 3 shows how each collection for week 1, 2 and 3 respectively.

Table 1*First sample collection*

Sample	Sample Type	Plant Code	Production Type
1	Thighs	A	ABF
2	Breast	B	Conventional
3	Wings	C	Organic
4	Breast	D	ABF
5	Thighs	E	Conventional
6	Breast	F	Conventional
7	Wings	G	Conventional
8	Tenders	B	ABF
9	Wings	B	ABF
10	Breast	H	ABF
11	Wings	I	Conventional
12	Tenders	I	Conventional
13	Thighs	I	Conventional
14	Breast	J	Air Chilled
15	Thighs	K	ABF
16	Thighs	E	Conventional
17	Wings	L	ABF
18	Wings	L	ABF
19	Thighs	J	Air Chilled
20	Thighs	M	Conventional
21	Breast	M	Conventional
22	Wings	M	Conventional

Note. Production types: Conventional: standard production without specific restrictions on antibiotic use. Antibiotic-Free (ABF): production where no antibiotics are used at any stage of growth. Organic: production which follows organic standards including pesticides-free feed, chickens have access to outdoors, and no antibiotic use. Air Chilled: production where the chicken is chilled using air rather than water to preserve its flavor and natural texture.

Table 2*Second sample collection*

Sample	Sample Type	Plant code	Production Type
1	Tenders	F	Conventional
2	Tenders	F	Conventional
3	Breast	K	ABF
4	Breast	L	ABF
5	Breast	F	Conventional
6	Wings	F	Conventional
7	Breast	F	Conventional
8	Tenders	I	Conventional
9	Wings	I	Conventional
10	Breast	I	Conventional
11	Breast	H	ABF

Sample	Sample Type	Plant code	Production Type
12	Breast	E	ABF
13	Tenders	E	ABF
14	Breast	L	ABF
15	Wings	A	ABF
16	Wings	M	Conventional
17	Tenders	H	ABF
18	Breast	J	Air Chilled
19	Breast	H	ABF
20	Wings	H	Conventional
21	Wings	B	ABF
22	Breast	B	Conventional
23	Breast	N	ABF
24	Tenders	L	ABF
25	Tenders	B	Conventional

Note. Production types: Conventional: standard production without specific restrictions on antibiotic use. Antibiotic-Free (ABF): production where no antibiotics are used at any stage of growth. Organic: production which follows organic standards including pesticides-free feed, chickens have access to outdoors, and no antibiotic use. Air Chilled: production where the chicken is chilled using air rather than water to preserve its flavor and natural texture.

Table 3

Third sample collection

Sample	Sample Type	Plant code	Production Type
1	Breast	A	ABF
2	Breast	J	Organic Air chilled
3	Breast	E	ABF
4	Breast	A	ABF
5	Wings	L	ABF
6	Breast	J	Organic
7	Tenders	A	ABF
8	Breast	E	ABF
9	Wings	M	Conventional
10	Breast	L	ABF
11	Breast	M	Conventional
12	Tenders	M	Conventional
13	Breast	F	Conventional
14	Breast	F	Conventional
15	Breast	K	ABF
16	Wings	F	Conventional
17	Breast	L	ABF
18	Breast	A	ABF
19	Wings	A	Conventional
20	Tenders	L	ABF
21	Breast	E	Conventional
22	Tenders	B	ABF
23	Breast	F	Conventional

Sample	Sample Type	Plant code	Production Type
24	Tenders	I	Conventional
25	Breast	I	Conventional
26	Wings	I	Conventional
27	Breast	A	ABF

Note. Production types: Conventional: standard production without specific restrictions on antibiotic use. Antibiotic-Free (ABF): production where no antibiotics are used at any stage of growth. Organic: production which follows organic standards including pesticides-free feed, chickens have access to outdoors, and no antibiotic use. Air Chilled: production where the chicken is chilled using air rather than water to preserve its flavor and natural texture.

Protocol of Analysis per Repetition Week

Day 1

Step 1.

Sample collection and transportation.

Samples were collected from four different establishments and transported to the laboratory in coolers with ice packs. For transportation a conventional car was used.

Step 2.

Sample reception, identification, and weighting.

Meat packages were received at refrigeration temperature then sprayed with 70% ethanol and labeled with information including date collected, part type, and plant code. After labeling, packages were opened to weigh 1 lb (454 g) and transferred to large whirl pack bags. Equipment: Ethanol (70%), markers, large whirl pack bags.

Step 3.

Addition of Buffered Peptone Water (BPW)

150 mL of Buffered Peptone Water (BPW) was added for each bag with 1 lb (454 g) of sample and was hand massaged for 2 minutes. Equipment: Non-selective culture media Buffered Peptone Water (BPW), sample bottles.

Step 4.

Plating samples in selective culture media (Conventional method).

After the addition of BPW in each sample, two plates of Xylose lysine tergitol 4 (XLT4) and two plates of Campy Cefex agar base were plated with 100 uL of each sample. XLT4 plates were incubated at 37 °C for 24 hours and Cefex plates at 42 °C for 48 hours inside bags with *Campylobacter* gas (5% O₂, 10% CO₂, balance N₂).

Step 5.

Samples incubation for Salmonella MDS and tubes preparation for Gene Up and Campylobacter MDS

After the plating in selective culture media, 40 mL from sample bags were transferred to 50 mL tubes to start Gene Up method the same day of collection (day 1). Another 30 mL from sample bag were transferred to 30 mL of *Campylobacter* Enrichment Broth in 50 mL tubes and were incubated at 42 °C for 24 hours to do *Campylobacter* MDS the next day (day 2). After this, all sample bags were incubated at 37 °C for 24 hours to do *Salmonella* MDS also the next day (day 2).

Step 6

Gene Up method (day 1)

Using the 50 mL tubes with 40 mL from sample bags the Gene-Up method was conducted. DNA was extracted from samples using specialized extraction kits. Using the Gene Up system the DNA extracted was subjected to real time PCR amplification to get results the next day.

Day 2

Step 7.

Gene Up results lecture

After around eight hours approximately of data running, the results were collected and downloaded from the computer to a USB.

Step 8.

XLT4 plates counting and streak Campylobacter enrichment broth with sample rinsate

After 24 incubation hours at 37 °C each XLT4 plate was checked to see results for *Salmonella*. After 24 incubation hours at 42 °C each 50 mL tube with *Campylobacter* enrichment broth and sample

rinsate was streaked to other Cefex plates and incubated for 48 hours. These tubes were also used for MDS.

Step 9.

MDS method and results

Campylobacter enrichment broth with sample rinsate was used for *Campylobacter* Molecular Detection Assay. The sample bags incubated for 24 hours at 37 °C were used for *Salmonella* MDS. Results were ready in one hour.

Step 10.

Salmonella enrichments elaboration.

Positive samples for *Salmonella* were used for enrichment. 0.1 mL from incubated sample bags of each positive sample was transferred to 10 mL of Tetrathionate broth base (TT) and incubated for 24 hours. For Rappaport-Vassiliadis (RV) were transferred 0.1 mL to 10 mL. Also, media controls (10 mL of TT broth and 10 mL RV broth without adding samples) were prepared and incubated.

Day 3

Step 11.

Salmonella enrichments plating and first Cefex plates results checking

All RV and TT enrichments tubes were plated in XLT4 plates after 24 hours of incubation. Each plate was labeled with sample number and enrichment media. Enrichments plated were incubated for 24 hours. After 42 incubation hours Cefex plates with samples were checked to see positive or negative results for *Campylobacter*.

Day 4

Step 12.

Last plates results checking and isolation for future studies

Cefex plates streaked on Day 2 were checked to see positives or negatives. Data from Molecular Detection Assay (MDS) was used to verify these results.

XLT4 plates with enrichments RV and TT were checked to see results. To verify, data from Gene Up was used. Stocks were made for all isolates for future serotype determination and plates with positive results for both bacteria were saved for future studies.

Experimental Design

Under the conditions of this study, one of the most important parts was to understand which factors affected prevalence and resulted in bacterias survival leading to all positive results obtained. Chi-Square (X^2) analysis was employed to confirm if there was dependence between variables such as sample type, production, and location with the prevalence of these bacterias in chicken meat. The software used was SAS OnDemand for academics and the significance level was 0.05.

Results and Discussion

During this study, a total of 74 samples were analyzed from four different establishments using one conventional method and two molecular. Among the samples, there were 35 breasts, 18 wings, 7 thighs, and 4 tenderloins. In Table 3, the total prevalence value is shown for both bacterias. This percentage includes all positive samples from all sample types and productions. For *Salmonella* was determined a 13.5% (n=10) final prevalence and for *Campylobacter* a value of 6.8% (n=5). Significant differences were determined for both bacterias with a p value of <.0001, lower than 0.05, suggesting there is a higher tendency of a negative result and that a positive result is related to other factors and not a random result (Table 4).

Table 4

General prevalence of Salmonella and Campylobacter in chicken meat

Bacteria	Total of samples	Positives	Prevalence (%)	X ² p value (Negative and Positive)
<i>Salmonella</i>	74	10	13.5%	<.0001
<i>Campylobacter</i>		5	6.8%	<.0001
X ² P value				0.1732

Note. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square test.

Prevalence of *Salmonella*

In the results of the analysis for *Salmonella*, the number of positives detected was 10 samples among the 74 samples analyzed. In this study, in the case of Gene Up method, the colony forming units (CFU) range of detection employed was above 10 CFU/mL, which matches the limit established by the FSIS regulations. This regulation says that all samples with more than 10 CFU/mL exceed the acceptable limit and should not be available at stores. Overall prevalence and levels of contamination by sample type, production, and plant code, the total prevalence determined was 13.5% (n=10 > 10 CFU/mL) of this bacterium on retail poultry products. A similar result was reported in an investigation conducted in the United States when a prevalence of 8.6% was determined in a total of 72 breast

samples obtained from seven different establishments in a specific city (not mentioned) in the USA, employing a conventional method with selective culture media as XLT4 and selective enrichments like Tetrathionate broth base (TT) and Rappaport-Vassiliadis (RV) (Mujahid et al., 2023). Another study conducted from March 2019 to February 2020 in Maryland, USA, presented a prevalence of *Salmonella* of 49.3% detected in a total of 480 samples of chicken carcasses obtained from a retail store in the Eastern Shore of Maryland (Jeewantha et al., 2023). Both results indicate that a prevalence of 13.5% is in the usual range detected in retail poultry.

Abundant investigations associate the prevalence of *Salmonella* and its development of “antimicrobial resistance”, referring to its capacity of adaptation according of each different environment, which means that it has developed resistance to routine practices of elimination and sanitation using chemical treatments and antimicrobial drugs (Tomicic et al., 2019). Documented evidence confirms *Salmonella* increased its resistance to antibiotics such as tetracycline, Sulfonamides (Santos et al., 2021) nalidixic, ampicillin (Schroniltgen et al., 2020), amoxicillin, cefazolin (Bythwood et al., 2019), and so on. The antimicrobial resistance factor is strongly correlated with the different serotypes present in chicken meat since some serotypes present stronger resistance to specific antibiotics than others. The presence of this pathogen is an indicator of a possible lack in good sanitation practices during chicken processing, but not necessarily. The lack of adequate following of SSOP, monitoring of critical limits in processes like scalded (62 – 63 °C), eviscerate, cooling (2.2 – 4 °C), and so on, increase the risk of contamination at any point of the chicken production chain (Barrientos, 2019). However, with perfect processing and sanitation, a highly contaminated flock at the farm will result in contaminated carcasses.

Prevalence of *Campylobacter*

For *Campylobacter* the prevalence detected was 6.8% (n=5) which is a value significantly lower in comparison to recent investigation. In 2019, at the University of Georgia, a prevalence of 15.8% and 52.8% was detected for conventional and alternative chicken meat samples, associating it to cross contamination through contaminated feces and soils, rodents, insects, and poor hygiene practices by workers during processing (Golden & Mishra, 2020). *Campylobacter* is also a bacterium which has developed resistance to antibiotics in recent years. As the pathogen obtained resistance to fluoroquinolones, antibiotics like erythromycin became ineffective against the bacteria.

The best method for the detection of *Campylobacter* was the conventional method using selective culture media Cefex and *Campylobacter* broth base. The most common serotypes of bacteria isolated with this enrichment are *Campylobacter jejuni* and *Campylobacter coli*, both associated with 50 – 70% of *Campylobacteriosis* cases that cause 190 deaths annually and which number of cases is increasing each year (Jo et al., 2017) On the other hand, the 3MTM Molecular Detection Assay 2 (MDS) was not able to detect all positives. Possibly, there was an inhibition effect during testing, which is a common situation with this type of molecular method. *Campylobacter* is also a very sensitive pathogen to some environmental conditions.

Evident differences between studies indicate that the results are not directly comparable as each study has differing sample types, number of samples, methodologies, location, and repetitions. However, a prevalence of 13.5% for *Salmonella* and 6.8% for *Campylobacter* is in the range usually detected for related investigations. This range can be considered as a low prevalence level in comparison to the values presented by USDA FSIS in the microbiological baseline data of past years since they determined a prevalence in raw chicken parts of 26.3% for *Salmonella* and 21.4% for *Campylobacter* obtained from different processing plants in 2012 (U. S. Department of Agriculture [USDA], 2012). However, is fundamental to ensure that prevalence must be at the lowest level possible.

Prevalence of Salmonella and Campylobacter per Sample Type, Plant Code, and Production Type

Prevalence per Sample Type for Salmonella

Levels of contamination of *Salmonella* in chicken meat by sample: As shown in Table 5, prevalence was analyzed for four different sample types: breasts, wings, thighs, and tenders. When considering the chi-square test across all sample types to find differences between them, a value of 0.8078 was calculated, which indicated they are not different from each other. Significant differences were found in the individual analysis of breasts, wings, and tenders. The prevalence in breast samples was 11.4%, with 4 positives out of a total of 35. The chi-square analysis resulted in a value of <.0001, which means there were significant differences between positives and negative results of *Salmonella* in breast samples. Wings presented a prevalence of 11.1%, 2 positives of 18 samples, being the chi square 0.001 that also suggests that the distribution of the results is not random, and the positives are dependent of other factors. Prevalence in tenders was the highest with 21.4%, 3 positives of 14 samples, also presenting a p value = 0.0325 < 0.05. In contrast with these samples, thighs showed a prevalence of 14.3% (1 positive of 7) and a chi square of 0.0588 > 0.05, which is closer to the p value, but still not reaches it, meaning there is just a tendency to have significant differences.

Table 5

Prevalence of Salmonella determined in chicken meat by sample type

Sample type	Total samples	Positives for <i>Salmonella</i>	Negatives for <i>Salmonella</i>	Prevalence (%)	X ² p value per sample
Breast	35	4	31	11.4%	<.0001
Wings	18	2	16	11.1%	0.001
Thighs	7	1	6	14.3%	0.0588
Tenders	14	3	11	21.4%	0.0325
X ² P value					0.8078

Note. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square.

As a first observation of these results, the highest levels of contamination were found in tenders, breast and wings with 21.4%, 11.4% and 11.1% respectively. Official documents have confirmed that these parts are the most common associated with *Salmonella* presence, sharing that

chicken necks are more likely to be contaminated with *Salmonella* (55%) than any other part, including breast, wings (20 – 44%), and legs (USDA, 2013). It is important to note that chicken tenders are extracted from breasts. As a recommendation, the FSIS guideline to control *Salmonella* in raw poultry (2013) suggests that establishments should not use chicken necks in comminuted poultry products, unless these products are intended for lethality treatment.

Other studies have also associated the presence of *Salmonella* with qualities in chicken meat and its different parts. A similar study conducted at Atlanta, Georgia, reported no significant differences in *Salmonella* prevalence on chicken parts with skin-on (wings) and skin off (breast, thighs, and tenders). However, revealed a higher prevalence in parts with skin-on (42.2%) than skin-off parts (17.6%), being the prevalence in skin-on chicken breasts (44.7%) higher than in breasts meat with no skin and so on for skin-off thighs (Guran et al., 2017). This suggests that chicken skin acts as a greater source of *Salmonella* transmission since it can contain the bacteria in the skin surface exposed during processes such as grinding and spread throughout, which might be related in this study with the prevalence found in wings (11.1%). Another study explains that chicken's body has ideal qualities for the survival and growing of *Salmonella*, which makes it a good reservoir since this bacterium can survive in a range of 5 – 46 °C (optimum 37 – 38 °C), which is a temperature present in their bodies naturally (for chicks 39 °C and grownups chickens from 40 – 41 °C). Also, *Salmonella* survive in a water activity of 0.94 - 0.99 and a pH value of 3.8 - 9.5, similar to chicken meat qualities (Wessels et al., 2021).

Prevalence per Sample Type for Campylobacter

Table 6 shows the prevalence of *Campylobacter* for each chicken meat sample. No significant differences were founded across all samples (P value = 0.5229 > 0.05). Breast presented a prevalence of 6% (2 positives and 33 negatives), and a probability of <.0001, indicating there are other factors relate to positive results. Wings had the highest prevalence, with 2 positives and 16 negatives (11%) and a chi square of 0.001 showing significant differences. In the case of thighs samples, prevalence

was 14% (1 positive and 6 negatives), but with a probability of 0.0588, so no significant differences were determined for this type. On the other hand, no positives for *Campylobacter* were detected in 14 samples of tenders, as a result, chi square was not calculated.

Table 6

Prevalence of Campylobacter determined in chicken meat by sample type

Sample type	Total samples	Positives for <i>Campylobacter</i>	Negatives for <i>Campylobacter</i>	Prevalence (%)	X ² p value
Breast	35	2	33	6%	<.0001
Wings	18	2	16	11%	0.001
Thighs	7	1	6	14%	0.0588
Tenders	14	0	14	0%	-
X ² P value					0.5229

Note. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square test.

Many factors are associated with the survival of *Campylobacter* from production to the processing chain. *Campylobacter* survives inside the digestive system by colonizing the mucus of the epithelial cells in the intestines and the ceca, but can also be recovered from other parts of the spleen and liver. Some studies reveal that the introduction of this pathogen in flocks comes from different risk factors like rodents, insects, wild birds, drinking water system, age and number of poultry houses, farm equipment, partial depopulation, and insufficient biosecurity (Arsenault et al., 2007). This primary production level is the focus of contamination, leading to a quick transmission due to the coprophagic behavior of chickens, and to the present prevalence in chicken meat during the processing chain.

Chicken bodies also have qualities that benefits the growth of the bacteria before processing , such as a high body temperature (41- 42 °C), the anaerobic environment and slightly alkaline pH (6.5 – 7.5) inside chickens' intestines, and a diverse microbiome present in the digestive system (Oakley et al., 2013). Skin-on parts, such as wings, count with a rough and porous surface with folds and feather follicles that can trap the bacteria, bringing protection environment where *Campylobacter* can adhere and persist during processing, protecting it from disinfectants and heat treatments (Corry & Atabay,

2001). Both sample types, breast and wings, are vulnerable to cross contamination during evisceration since the digestive system is the colonization focus of the bacteria, from which intestines can rupture, spreading the bacteria to other chicken parts and supplies used in processing. Another study conducted in 2024 revealed that serotypes like *Campylobacter jejuni* have developed tolerance to low temperatures (4°C), commonly using cold shock proteins (CSPs), giving it the capability to survive in poultry meat in the cold chain, making it a serious food safety concern (Hur et al., 2024).

Prevalence per Production Type for Salmonella

In this study, four different types of chicken meat production were analyzed to compare prevalences: conventional, antibiotic free (ABF), air chilled, and organic. The number of samples were collected depended on availability in each of the four stores visited. Among 74 chicken samples, 35 were conventional, 33 were ABF, 4 were Air Chilled, and 2 were organic. Using the chi-square analysis a p value = <.0001 was calculated when all productions were compared, indicating significant differences in the prevalence of *Salmonella* across the production systems. However, it is important to note that the sample amount for organic and air chilled productions can be considered as low in comparison to conventional and antibiotic free (ABF). While no positives results were found in these productions, the low number of samples limits the generalizability of results. Research with larger sample sizes is recommended.

As shown in Table 7, no p value was calculated for air chilled and organic production since no positives for *Salmonella* were detected. Only conventional and antibiotic free productions showed significant differences with p value of <.0001 for both respectively. Antibiotic free and conventional had the higher prevalences with a 15.2% and 14.3% of positive percentages respectively. These productions are comparable due to the 35 and 33 samples analyzed. In this case, no significant differences were reported ($p = 0.9197 > 0.05$) between conventional and antibiotic free samples. Under the conditions of this study, this result shows both practices present similar contamination risks

of *Salmonella*, which might be related to its similarities in production practices despite the difference of antibiotic absence for antibiotic free (ABF) poultry products.

Table 7

Salmonella prevalence per production type

Production type	Total samples	Positives for <i>Salmonella</i>	Negatives for <i>Salmonella</i>	Prevalence (%)	X ² P value
Conventional	35	5	30	14.3%	<.0001
ABF	33	5	28	15.2%	<.0001
Air Chilled	4	0	4	0.0%	-
Organic	2	0	2	0.0%	-
X ² P value					<.0001

Note. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square test.

Prevalence per Production Type for Campylobacter

Table 8 presents the prevalence of *Campylobacter* for all productions analyzed. Based on the chi square analysis conducted with SAS software, there are differences between productions ($p = 0.0139 < 0.05$). Each one has different practices during chicken raising or processing, which may explain the different risk levels of contamination.

The levels of contamination for this bacterium were lower for conventional production in contrast with *Salmonella*, where only 3 positives were detected (prevalence of 8.6%) and a p value of <.0001 showed significant differences between negatives and positives results. For antibiotic free, among 33 samples there were no positives, as a result, SAS software did not calculate chi square and probability. Of 4 air chilled samples, only one presented a positive result and so was for organic, where 1 of 2 samples was positive for *Campylobacter*. However, both productions had higher p values (0.3179 and 1 respectively) that indicates no differences can be established and that the results are not related to its production conditions. A higher number of samples is required to ensure more accurate results and comparisons. Comparing conventional and antibiotic free (ABF) no differences were determined ($p \text{ value} = 0.0854$), which means no production was better than the other when contamination risk is considered.

Table 8*Campylobacter* prevalence per production type

Production type	Total samples	Positives for <i>Campylobacter</i>	Negatives for <i>Campylobacter</i>	Prevalence (%)	X ² P value
Conventional	35	3	32	8.6	<.0001
ABF	33	0	33	0.0	-
Air Chilled	4	1	3	25.0	0.3179
Organic	2	1	1	50.0	1
X ² P value					0.0139

Note. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square test.

The results of this study demonstrate significant differences in the prevalence of *Salmonella* and *Campylobacter* across various poultry production methods. Specifically, *Salmonella* presented a higher level of prevalence in conventional and antibiotic free (ABF) production systems compared to organic and air chilled, where there were no positives. Similarly, *Campylobacter* was found in 8.6% of conventional samples, but no presence of this pathogen was detected for antibiotic free (ABF). This value is lower than *Salmonella* prevalence, but suggests that similar causes lead to its presence. Despite the limited sample number, Air chilled prevalence was higher for *Campylobacter* than *Salmonella* with a 25% positive, and the same results were found in organic, where there was one positive (50%) of *Campylobacter* and no presence of *Salmonella*.

Some literature affirms that different conditions of production systems can significantly impact the microbial safety of poultry products. For instance, conventional production typically involves high stocking densities and the use of antibiotics. Antibiotics are used to reduce the risk of disease and increase feed efficiency. The administration is done before slaughtered, and goes through monitoring to verify levels of residues are not above the tolerance level established (USDA, 2019). Moreover, the use of antibiotics, although intended to prevent disease, can also contribute to the development of antibiotic-resistant strains of *Salmonella* and *Campylobacter* (as mentioned before) complicating control efforts and increasing the probability of obtaining positive results. In the case of antibiotic free (ABF) samples, the preference of consumers on buying ABF products is related to their

own perception rather than scientific investigation. Consumers associate the use of antibiotics in poultry products as the primary driver of antibiotic-resistant infections in humans despite the lack of scientific evidence to support this affirmation. Some studies affirm that not using antibiotics increases the risk of having contaminated products with numerous microorganisms involved with enteric and systemic diseases, leading to use a variety of strategies to minimize the effects on flock development of not using antibiotics (Cervantes, 2015) It is important to mention that poor biosecurity measures benefits bacteria survival as well as stocking density, which also increase the risk of contamination between chickens during raising for both conventional and ABF since the space is reduce and infected chickens might have contact with not affected ones.

Air chilled production showed low contamination levels. Based on some studies, air chilled production is a practice which popularity has increased in the last years, as a result of its efficacy minimizing cross contamination between carcasses and reducing prevalence of *Salmonella* and *Campylobacter* by using cool air (from -1 °C to 2 °C) to reduce body temperature individually, unlike water chilling, where carcasses are chilled in the same water (Duarte et al., 2019). For organic production, there is information that affirm this practices tend to have a lower prevalence of *Salmonella* than non-organic ones (no *Salmonella* was found in organic production in this study), which may be attributed to better management practices, lower stocking density, and no application of antibiotics (Jeewantha et al., 2024). The prevalence of *Campylobacter* in organic chicken has been shown to be more variable. Higher prevalences have been observed in organic than conventional chicken, probably associated with the capacity of *Campylobacter* to adapt well to environments with access to open air, allowing it to persist and transmit across stocks (Luangtongkum et al., 2006)

These results and information indicate that poultry production methods significantly influence in the level of prevalence of *Salmonella* and *Campylobacter*. Conventional and ABF systems are associated with the higher prevalences rates and organic and air-chilled systems exhibited lower

prevalence but only for *Salmonella*. Future studies should aim to further investigate these relationships, particularly with larger samples sizes and across different geographical regions.

Prevalence per Plant Code for Salmonella

A total of 14 plant codes were registered among the 74 samples collected. 50% of processing plants presented positive results for *Salmonella*, each with unique contamination profiles. Plant E had the highest prevalence for *Salmonella* with 42.9% (3 positives from 7 samples) despite not reaching significant differences ($p = 0.7055$). Plant I showed a prevalence of 22.2% (2 out of 9 samples), but no significant differences ($p = 0.0956$), while plant H had a lower contamination level with a prevalence of 20% (1 out of 5 samples) but also did not reach statistical significance ($p = 0.1797$). Plants B, A, and L had the lowest prevalences with values of 14.3% (1 out of 7), 12.5% (1 out of 8), and 11.1% (1 out of 9) respectively, but only plant L presented p value of 0.0196 indicating significance. Similarly, another study realized by Obe (2020) *Salmonella* prevalence was evaluated in 6 different processing plants in different times, where differences were observed ($p < .0001$) between them and the maximum and minimum percentages of contamination were 29.6% and 7.4% respectively. The plant with the highest prevalence determined (plant E) in this study exceeded this percentage with 42.9%, but other plants like I, H, B, A and L showed lower levels and between this range. As both investigations presented similar percentages, these results should not be considered as unusual.

Prevalence per Plant Code for Campylobacter

Campylobacter behavior in processing plants was different from *Salmonella*. Positive results were detected only in 3 plants among 14: F, J, and M. The highest prevalence was present in plant J with a 40% contamination (2 out of 5 samples) with a p value of 0.6547 indicating no statistical significance despite the prevalence level. M and F had lower prevalences (14.3% and 20%) and high p values (0.0588 and 0.0578). These results of the statistical analysis did not show significant differences, but the existence of contamination, even in low levels, can also be considered as a good indicator of a lack of good sanitation practices and its necessity to evaluate control measures during

process flow. The survival of *Campylobacter* from poultry farms to final products evidence the several adaptative responses or environmental niches throughout all poultry production chain despite all the sanitation procedures implemented.

Table 9 summarized the obtained results for the 14 plants. Most of the positive samples came from plants of conventional and antibiotic-free (ABF) productions, which were the production types with the highest prevalences.

Table 9*Prevalence of Salmonella and Campylobacter per plan code*

Plant code	Total samples	Positives for <i>Salmonella</i>	Negatives for <i>Salmonella</i>	Prevalence (%)	X ² P value	Plant code	Positives for <i>Campylobacter</i>	Negatives for <i>Campylobacter</i>	Prevalence (%)	X ² P value
A	8	1	7	12.5	0.0339	A	0	8	0.0	0
B	7	1	6	14.3	0.588	B	0	7	0.0	-
C	1	0	1	0.0	-	C	0	1	0.0	-
D	1	0	1	0.0	-	D	0	1	0.0	-
E	7	3	4	42.9	0.7055	E	0	7	0.0	-
F	10	1	9	10.0	0.0114	F	2	8	20.0	0.0578
G	1	0	1	0.0	-	G	0	1	0.0	-
H	5	1	4	20.0	0.1797	H	0	5	0.0	-
I	9	2	7	22.2	0.0956	I	0	9	0.0	-
J	5	0	5	0.0	-	J	2	3	40.0	0.6547
K	3	0	3	0.0	-	K	0	3	0.0	-
L	9	1	8	11.1	0.0196	L	0	9	0.0	-
M	7	0	7	0.0	-	M	1	6	14.3	0.0588
N	1	0	1	0.0	-	N	0	1	0.0	-
X ² P value					0.7691					
						0.2517				

Notes. A significance level of 0.05 was used for statistical analysis. A p-value less than 0.05 indicates statistically significant differences using the chi square test.

There are multiple steps or critical control points (CCP) during chicken meat processing involved with cross contamination, as well as leading to bacteria persistence. Many studies mention processes such as scalding, defeathering, evisceration, neck removal, inside and outside washing as important cross-contamination points in one way or another. Scalding is a process where carcasses go through immersion in warm water (51 – 64 °C) for 1 or 2 minutes to facilitates defeathering. There is data that affirms that *Campylobacter*, in comparison to other pathogens, for instance, *E. coli*, total aerobic microbes, and coliforms, presents higher levels of recovery during defeathering y increasing from 1.8 to 3.7 CFU/mL (Hakeem & Lu, 2020). Evisceration is another critical step of cross-contamination as *Campylobacter* and *Salmonella* colonizes gastrointestinal tract of poultry birds in large numbers. During evisceration is possible to have intestines rupture, so both bacterias can spread rapidly to other parts of the body, especially to the lower half of carcasses (breast and thighs) as the birds are hung upside-down by the feet. These points of possible cross-contamination require monitoring procedures as the one approved by the United States Department of Agriculture (USDA) that says that to reduce these risks is necessary to do visual examination for fecal material examining the inside and outside surfaces of 10 random carcasses per hour.

All poultry processing plants implement cleaning and sanitation procedures to ensure disinfected equipment and the lowest contamination levels possible. Physical and chemical interventions are implemented to reduce bacteria growing. Irradiation and ultrasound are some examples of physical treatments that reduce bacterial load effectively. On the other hand, air chilled is an example of a treatment that controls bacterial growing and reduce cross-contamination by the elimination of shared water contact and maintaining low temperatures to limit bacterial proliferation. For chemical treatments, organic acids, chlorine-based treatments, acetic and lactic acid, acidified sodium chloride, and quaternary ammonium are used (Loretz et al., 2010). An efficient and correct execution of these sanitation protocols helps to control foodborne pathogens. However, now is a fact that pathogens (including *Salmonella* and *Campylobacter*) are able to adhere to food processing

equipment surfaces as well as resist physical and chemical interventions to stay active even after cleaning and sanitation (Obe et al., 2020).

Estimating the prevalence of *Salmonella* and *Campylobacter* in dependence of the origin place of processing is fundamental to ensure that good biosecurity and sanitation protocols are followed correctly so new strategies might be implemented to achieve a lower level of contamination, as well as improve the traditional practices employed. For this study, all plants with the higher prevalences are directly related to possible inefficient execution of sanitation practices. There is no key treatment or procedure that ensures a prevalence of 0% in final products, but the adequate application of them can help to reduce these risks as much as possible. Is important to establish achievable goals focused on keeping *Salmonella* and *Campylobacter* levels at an undetectable level.

Conclusions

The study revealed that the prevalence of *Salmonella* (13.5%) and *Campylobacter* (6.8%) in chicken parts found is lower compared to similar research.

It was determined that breast and wings samples, which come from conventional and antibiotic-free (ABF) productions are more likely to be good reservoirs of these bacterias, suggesting a significant relationship between the prevalence levels of *Salmonella* and *Campylobacter* and the variables studied.

The persistence of these pathogens is associated with microbial resistance and cross contamination which indicates the importance of improving sanitation procedures and establishing goals to achieve the lowest prevalence possible.

Recommendations

Carry on serotype determination to define which types of *Salmonella* and *Campylobacter* are commonly present in retail poultry at the Southeastern United States.

Increase sample amounts for limited categories to ensure more precise results.

Conduct longitudinal studies that monitor the prevalence of *Salmonella* and *Campylobacter* throughout different seasons of the year.

Continue this study visiting different production farms and processing plants to make a deeper analysis of the mechanisms of cross-contamination, prevalence for both bacterias, and its cause

References

- Alabama Poultry and Egg Association (2024). About The Alabama Poultry & Egg Association. *Alabama Poultry and Egg Association*. <https://alabamapoultry.org/about-apea/#:~:text=The%20poultry%20industry%20is%20one,make%20up%20Alabama's%20poultry%20industry>.
- Aras, Z., Sanioglu, G., & Onur, T. (2015). Salmonella detection in different types os packed raw poultry meat by culture Elisa and PCR methods. *ResearchGate*, 31, Article 1, 1531–1536. https://www.vivanttechnologies.com/images/Resources/publication/journal_607.pdf
- Arsenault, J., Letellier, A., Quessy, S., Normand, V., & Boulianne, M. (2007). Prevalence and risk factors for Salmonella spp. and Campylobacter spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *ScienceDirect*, 81(4), 250–264. <https://www.sciencedirect.com/science/article/abs/pii/S0167587707001006?via%3Dihub>
- Barlow, M., & Hall, B. G. (2002). Origin and Evolution of the AmpC B-Lactamases of *Citrobacter freundii*. *American Society for Microbiology*, 5, Article 46. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC127158/>
- Barrientos, L. (2019). *Diseño de un Plan de Análisis de Peligros y Puntos de Control Crítico (HACCP) en una empresa de faenamiento de pollo, Arequipa, 2018* [Thesis]. Universidad Continental, Perú. <https://core.ac.uk/download/pdf/327087532.pdf>
- Bythwood, T., Soni, V., Lyons, K., Hurley, A., Lee, M., Hofacre, C., Sanchez, S., & Maurer, J. (2019). Antimicrobial Resistant Salmonella enterica Typhimurium Colonizing Chickens: The Impact of Plasmids, Genotype, Bacterial Communities, and Antibiotic Administration on Resistance. *Frontiers*, 3. <https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2019.00020/full>
- Centers for Disease Control and Prevention. (2020a). *Campylobacter (Campylobacteriosis)*. <https://www.cdc.gov/campylobacter/index.html#:~:text=Campylobacter%20causes%20an%20estimated%201.5,year%20in%20the%20United%20States>
- Centers for Disease Control and Prevention. (2020b). *Salmonella*. <https://www.cdc.gov/salmonella/index.html#:~:text=CDC%20estimates%20Salmonella%20bacteria%20cause,the%20United%20States%20every%20year>
- Centers for Disease Control and Prevention. (2023). *Campylobacter (Campylobacteriosis)*. <https://www.cdc.gov/campylobacter/technical.html#:~:text=Campylobacter%20is%20a%20gram%2Dnegative,by%20one%20species%2C%20Campylobacter%20jejuni>.
- Cervantes, H. (2015). Antibiotic-free poultry production: Is it sustainable? *ResearchGate*, 91–97. https://www.researchgate.net/publication/276884454_Antibiotic-free_poultry_production_Is_it_sustainable
- Connolly, G., & Campbell, W. W. (2023). Poultry Consumption and Human Cardiometabolic Health-Related Outcomes: A Narrative Review. *MDPI*, 15, Article 16. <https://www.mdpi.com/2072-6643/15/16/3550>
- Corry, J., & Atabay, H. (2001). Poultry as a source of Campylobacter and related organisms. *Oxford Academic*, 90(6), 96–114. <https://academic.oup.com/jambio/article-abstract/90/S6/96S/6721394?redirectedFrom=fulltext>

- Duarte, T., Belk, A., Martin, J., & Belk, K. (2019). A Comparison of Water Chilling and Air Chilling on Poultry Shelf Life. *ResearchGate*, 3, Article 2, 66. https://www.researchgate.net/publication/340213464_A_Comparison_of_Water_Chilling_and_Air_Chilling_on_Poultry_Shelf_Life
- Food and Agriculture Organization of the United Nations. (2013). *Poultry Development Review*. <https://www.fao.org/3/i3531e/i3531e.pdf>
- Golden, C., & Mishra, A. (2020). Prevalence of Salmonella and Campylobacter spp. in Alternative and Conventionally Produced Chicken in the United States: A Systematic Review and Meta-Analysis. *ScienceDirect*, 83. <https://www.sciencedirect.com/science/article/pii/S0362028X22103418>
- Guran, H., Mann, D., & Alali, W. (2017). Salmonella prevalence associated with chicken parts with and without skin from retail establishments in Atlanta metropolitan area, Georgia. *ScienceDirect*, 73. <https://www.sciencedirect.com/science/article/abs/pii/S0956713516304777>
- Hakeem, M., & Lu, X. (2020). Survival and Control of Campylobacter in Poultry Production Environment. *Frontiers in Cellular and Infection Microbiology*, 10, Article 615049. <https://doi.org/10.3389/fcimb.2020.615049>
- Hur, J., Kim, J., Kang, M., Jung, H., Ryu, S., & Jeon, B. (2024). Cold tolerance in Campylobacter jejuni and its impact on food safety. *ScienceDirect*, 175. <https://www.sciencedirect.com/science/article/abs/pii/S0963996923012310#:~:text=Despite%20the%20microaerophilic%20nature%20of,survival%20at%204%20%C2%BC>.
- Jeewantha, A., Schwarz, J., Diria, A., Bowers, J., & Parveen, S. (2023). Prevalence and antibiotic resistance of Salmonella in organic and non-organic chickens on the Eastern Shore of Maryland, USA. *Frontiers*, 14. <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1272892/full>
- Jeewantha, A., Schwarz, J., Diria, A., Bowers, J., & Parveen, S. (2024). Prevalence and antibiotic resistance of Salmonella in organic and non-organic chickens on the Eastern Shore of Maryland, USA. *Frontiers*, 14. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10794514/>
- Jo, Y., Oh, H., Yoon, Y., Lee, S., Ha, J., Kim, W., Hwang, K., Sanghyun, H., & Se-Rim, K. (2017). Enrichment Broth for the Detection of Campylobacter jejuni and Campylobacter coli in Fresh Produce and Poultry. *ScienceDirect*, 80. <https://www.sciencedirect.com/science/article/pii/S0362028X22095680?via%3Dihub>
- Johnson, R., Mills, J., Pittete, J., & Rannou, M. (2021). Evaluation of the GENE-UP® EHEC Detection Method for the Detection of Enterohemorrhagic E. coli in Select Foods: Collaborative Study: First Action Method 2020.06. *ResearchGate*, 104, Article 4. https://www.researchgate.net/publication/351006960_Evaluation_of_the_GENE-UPR_EHEC_Detection_Method_for_the_Detection_of_Enterohemorrhagic_E_coli_in_Select_Foods_Collaborative_Study_First_Action_Method_202006
- Loretz, M., Stephan, R., & Zweifel, C. (2010). Antimicrobial activity of decontamination treatments for poultry carcasses: A literature survey. *ScienceDirect*, 21(6), 791–804. <https://www.sciencedirect.com/science/article/abs/pii/S0956713509003156>

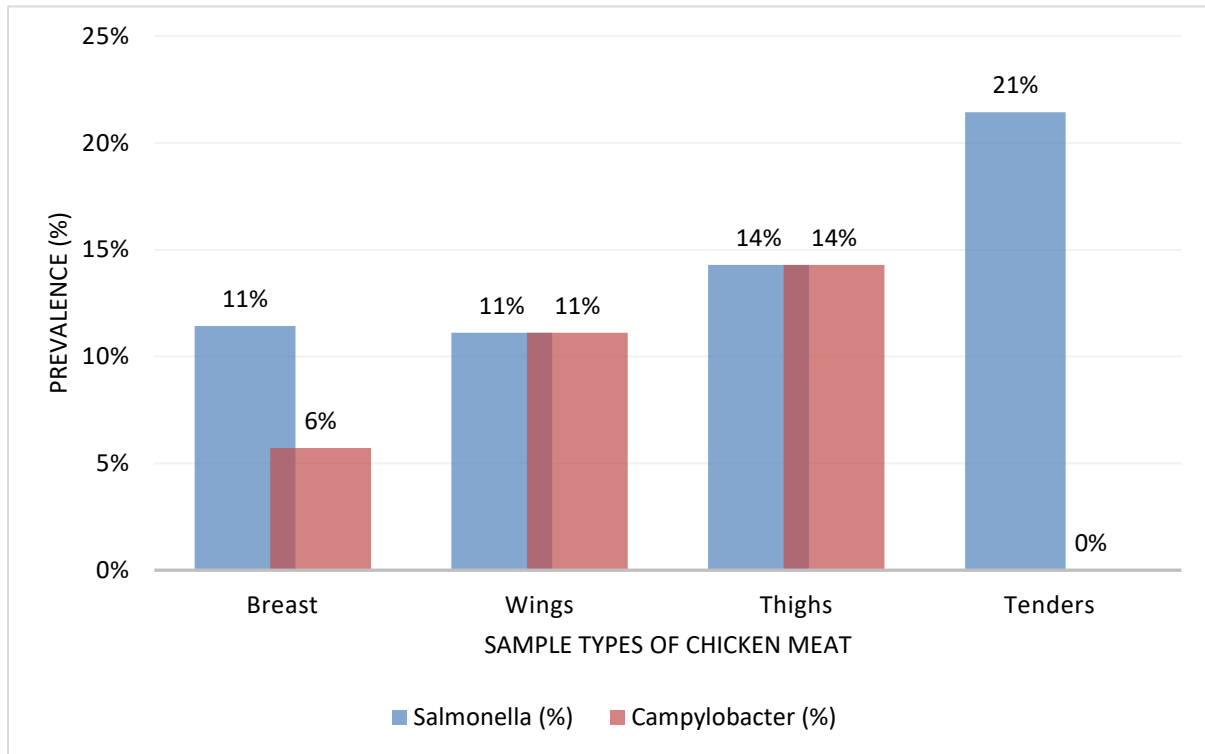
- Luangtongkum, T., Morishita, T., Ison, A., Huang, S., McDermott, P., & Zhang, Q. (2006). Effect of Conventional and Organic Production Practices on the Prevalence and Antimicrobial Resistance of *Campylobacter* spp. in Poultry. *National Library of Medicine*, 72, Article 5. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1472326/>
- Mujahid, S., Hansen, M., Miranda, R., Newsom, K., & Rogers, J. (2023). Prevalence and Antibiotic Resistance of *Salmonella* and *Campylobacter* Isolates from Raw Chicken Breasts in Retail Markets in the United States and Comparison to Data from the Plant Level. *MDPI*, 13, Article 3. <https://www.mdpi.com/2075-1729/13/3/642>
- Oakley, B., Morales, C., Line, J., Berrang, M., Meinersmann, R., Tillman, G., Wise, M., Siragusa, G., Hiett, K., & Seal, B. (2013). The Poultry-Associated Microbiome: Network Analysis and Farm-to-Fork Characterizations. *Plos One*, 8, Article 2. <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057190>
- Obe, T., Nannapaneni, R., Schilling, W., Zhang, L., McDaniel, C., & Kiess, A. (2020). Prevalence of *Salmonella enterica* on poultry processing equipment after completion of sanitization procedures. *National Library of Medicine*, 99, Article 9, 4539–4548. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7598133/>
- Roe, B., & Doll, H. (1995). Health and numbers: basic biostatistical methods.: Prevalence and incidence. *Journal of Clinical Nursing*, 178–188. https://www.blackwellpublishing.com/specialarticles/jcn_9_188.pdf
- Rosauer, M., Lopez, G., Silbernagel, K., & Horine, L. (2022). 3M Molecular Detection Assay 2 - *Salmonella* for Detection of *Salmonella* in Dried Cannabis Flower and Dried Hemp Flower: Targeted Matrix Extension AOAC Performance Tested MethodsSM 091501. *Journal of AOAC International*, 105, Article 6. https://www.researchgate.net/publication/361502464_3M_Molecular_Detection_Assay_2_-_Salmonella_for_Detection_of_Salmonella_in_Dried_Cannabis_Flower_and_Dried_Hemp_Flower_Targeted_Matrix_Extension_AOACR_Performance_Tested_MethodsSM_091501
- Santos, S., Almeida, A., Magalhaes, A., Souza, C., Castilho, D., & Rodrigues, I. (2021). Presence of Tetracycline and Sulfonamide Resistance Genes in *Salmonella* spp.: Literature Review. *National Library of Medicine*, 10, Article 11. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8615168/>
- Schroniltgen, I., Catro, R., Herreras, M., & Rodriguez, R. (2020). Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. *National Library of Medicine*, 13, Article 10. <https://doi.org/10.14202/vetworld.2020.2070-2084>
- Tomicic, Z., Cabarkapa, I., Colovic, R., & Djuragic, O. (2019). SALMONELLA IN THE FEED INDUSTRY: PROBLEMS AND POTENTIAL SOLUTIONS. *ResearchGate*, 2, Article 1, 130–137. https://www.researchgate.net/publication/331564179_SALMONELLA_IN_THE_FEED_INDUS TRY_PROBLEMS_AND_POTENTIAL_SOLUTIONS
- U. S. Department of Agriculture. (2012). *Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey*. U. S. Department of Agriculture. <https://www.fsis.usda.gov/node/1970>
- U. S. Department of Agriculture. (2013). *FSIS Guideline for Controlling Salmonella in Raw Poultry*. U. S. Department of Agriculture (USDA). <https://www.fsis.usda.gov/guidelines/2021-0005>

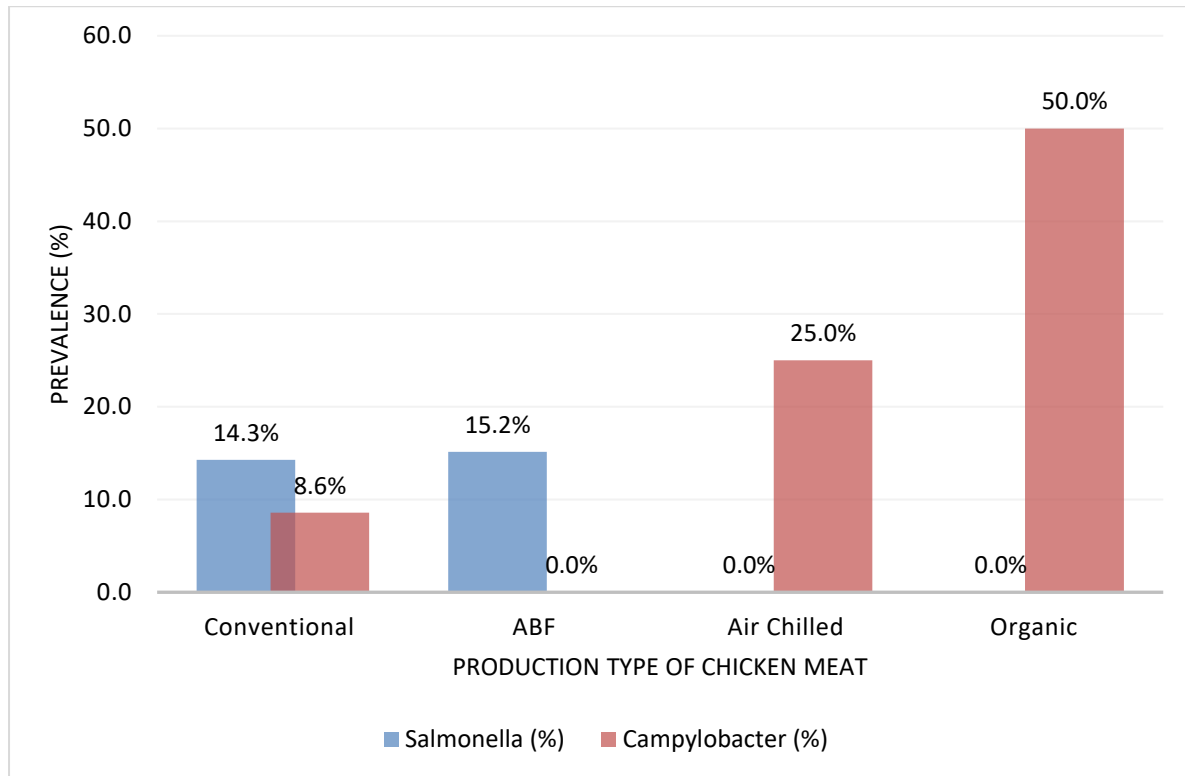
- U. S. Department of Agriculture. (2019). *Chicken from Farm to Table: Hormones & Antibiotics*. U. S. Department of Agriculture (USDA). <https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/poultry/chicken-farm-table#6>
- Wessels, K., Rip, D., & Gouws, P. (2021). Salmonella in Chicken Meat: Consumption, Outbreaks, Characteristics, Current Control Methods and the Potential of Bacteriophage Use. *ResearchGate*, 10, Article 8. <https://doi.org/10.3390/foods10081742>
- World Health Organization. (2018). *Salmonella (non-typhoidal)*. World Health Organization (WHO). [https://www.who.int/news-room/fact-sheets/detail/salmonella-\(non-typhoidal\)#:~:text=Salmonella%20is%20of%20the,have%20been%20identified%20to%20date.](https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)#:~:text=Salmonella%20is%20of%20the,have%20been%20identified%20to%20date.)

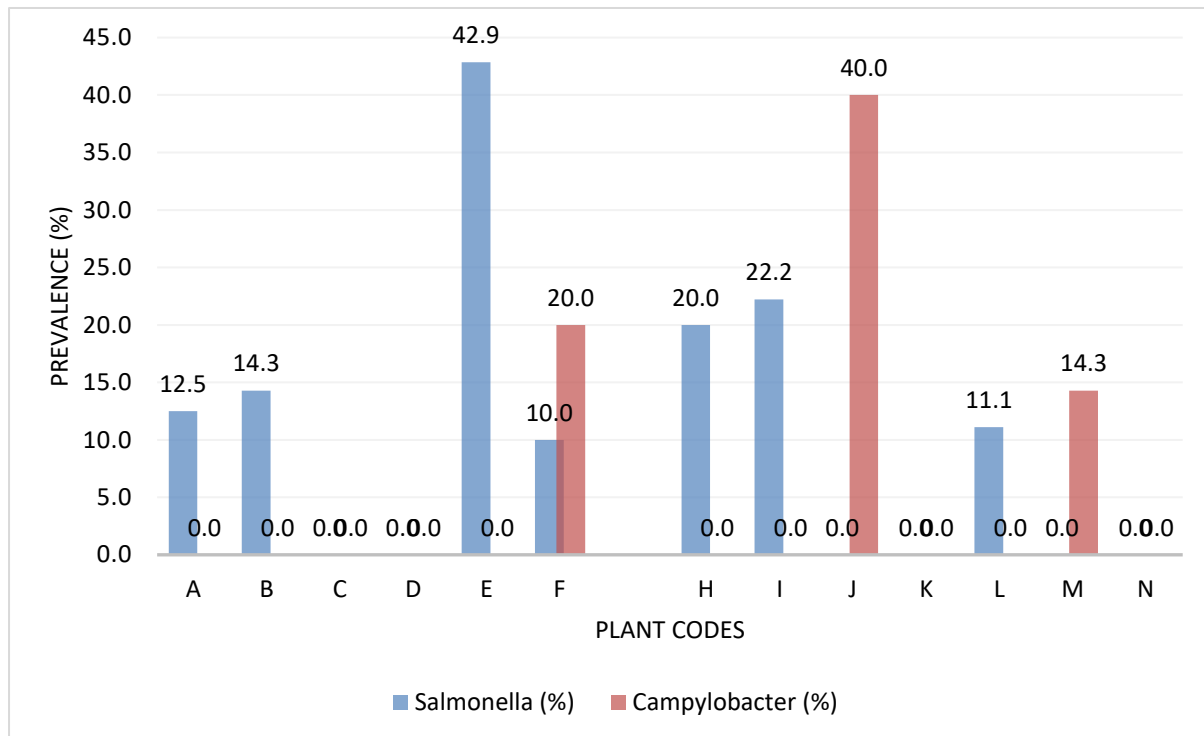
Appendices

Appendix A

Prevalence of Salmonella and Campylobacter per Sample Type



Appendix B*Prevalence of Salmonella and Campylobacter per Production Type*

Appendix C*Prevalence of Salmonella and Campylobacter per Plant Code*

Appendix D

First Data Compilation

Retail 1 (March 11, 2024)												
Sample	Sample Type	Plant code	Production Type	Gene Up +/- (<i>Salmonella</i>)		MDS +/-		<i>Campylobacter</i> post enrichment	<i>Salmonella</i> enrichment		Stocks	
				' +/-	CFU/ml	<i>Salmonella</i>	<i>Campylobacter</i>		Rappaport Vassiliadis (RV)	Tetrahionate Broth Base (TT)	RV	TT
1	Thighs	A	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
2	Breast	B	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
3	Wings	C	Organic	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
4	Breast	D	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
5	Thighs	E	Conventional	Positive	97.72	Negative	Negative	Negative	Positive	Positive		1
6	Breast	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Positive	-	-		
7	Wings	G	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
8	Tenders	B	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
9	Wings	B	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
10	Breast	H	ABF	Uncertain	33.88	Negative	Negative	Negative	Negative	Negative		0
11	Wings	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
12	Tenders	I	Conventional	Positive	19.05	Negative	Negative	Negative	Negative	Negative		1
13	Thighs	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
14	Breasts	J	Air Chilled	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
15	Thighs	K	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
16	Thighs	E	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
17	Wings	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
18	Wings	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-		
19	Thighs	J	Air Chilled	Negative	<10CFU/ml	Negative	Valid	Positive	-	-		
20	Thighs	M	Conventional	Negative	<10CFU/ml	Negative	Valid	Negative	-	-		
21	Breast	M	Conventional	Negative	<10CFU/ml	Negative	Valid	Negative	-	-		
22	Wings	M	Conventional	Negative	<10CFU/ml	Negative	Invalid	Negative	-	-		

Appendix E

Second Data Compilation

Sample	Sample Type	Plant code	Production Type	Gene Up +/- (<i>Salmonella</i>)		MDS +/-		<i>Campylobacter</i> post enrichment	<i>Salmonella</i> enrichment	
				' +/-	CFU/ml	<i>Salmonella</i>	<i>Campylobacter</i>		Rappaport Vassiliadis (RV)	Tetrahionate Broth Base (TT)
1	Tenderloins	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
2	Tenderloins	F	Conventional	Positive	173.78	Inspect	Negative	Negative	Negative	Negative
3	Breasts	K	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
4	Breasts	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
5	Breasts	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
6	Wings	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
7	Breasts	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
8	Tenderloins	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
9	Wings	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
10	Breasts	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
11	Breasts	H	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
12	Breasts	E	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
13	Tenderloins	E	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
14	Breasts	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
15	Wings	A	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
16	Wings	M	Conventional	Negative	<10CFU/ml	Error	Negative	Negative	-	-
17	Tenderloins	H	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
18	Breasts	J	Air Chilled	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
19	Breasts	H	ABF	Positive	208.93	Negative	Negative	Negative	Negative	Negative
20	Wings	H	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
21	Wings	B	ABF	Positive	114.82	Negative	Negative	Negative	Negative	Positive
22	Breasts	B	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
23	Breasts	N	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
24	Tenderloins	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	-	-
25	Tenderloins	B	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	-	-

Appendix F

Third Data Compilation

Sample	Sample Type	Plant code	Production Type	Gene Up +/- (<i>Salmonella</i>)		MDS +/-		<i>Campylobacter</i> post enrichment	<i>Salmonella</i> enrichment	
				' +/-	CFU/ml	<i>Salmonella</i>	<i>Campylobacter</i>		Rappaport Vassiliadis (RV)	Tetraionate Broth Base (TT)
1	Breasts	A	ABF	Uncertain	53.7	Negative	Negative	Negative	Negative	Negative
2	Breasts	J	Organic Air chilled	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
3	Breasts	E	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
4	Breasts	A	ABF	Uncertain	40.74	Negative	Negative	Negative	Negative	Positive
5	Wings	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Positive	Negative
6	Breasts	J	Organic	Negative	<10CFU/ml	Negative	Positive	Positive	Positive	Negative
7	Tenders	A	ABF	Positive	2511.89	Negative	Negative	Negative	Negative	Positive
8	Breasts	E	ABF	Positive	2041.74	Negative	Negative	Negative	Negative	Positive
9	Wings	M	Conventional	Negative	<10CFU/ml	Negative	Negative	Positive	Positive	Negative
10	Breasts	L	ABF	Positive	2041.74	Negative	Negative	Negative	Positive	Positive
11	Breasts	M	Conventional	Uncertain	2398.83	Negative	Negative	Negative	Negative	Negative
12	Tenders	M	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
13	Breasts	F	Conventional	Uncertain	2187.76	Negative	Negative	Negative	Negative	Negative
14	Breasts	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
15	Breasts	K	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
16	Wings	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Positive	Negative
17	Breasts	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
18	Breasts	A	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
19	Wings	A	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
20	Tenders	L	ABF	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
21	Breast	E	Conventional	Positive	3311.31	Negative	Negative	Negative	Negative	Negative
22	Tenders	B	ABF	Uncertain	2691.53	Negative	Negative	Negative	Positive	Positive
23	Breasts	F	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
24	Tenders	I	Conventional	Negative	<10CFU/ml	Negative	Negative	Negative	Negative	Negative
25	Breast	I	Conventional	Uncertain	1995.26	Negative	Negative	Negative	Negative	Negative
26	Wings	I	Conventional	Positive	47.86	Negative	Negative	Negative	Negative	Negative
27	Breasts	A	ABF	Uncertain	1513.56	Negative	Negative	Negative	Positive	Positive