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Post Market Assessment of the Microbial Quality of Chicken

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Abstract: Foodborne pathogens, most significantly *Salmonella* species and *Campylobacter* species, have been commonly associated with raw poultry, and are leading causes of foodborne illness in the United States. Thus, the microbial quality of poultry is an on-going concern. Also, during the last two years, Foster Farms, a major poultry producer, had serious sanitation problems and *Salmonella* contamination. For these reasons, determining the microbial state of raw chicken and how Foster Farms brand chicken compared with other brands was of interest. Raw chicken parts were obtained from retail grocery stores in the Pomona, CA area. The total number of bacteria, total number of gram negative bacteria, and presence of coliforms, *E.coli*, *Pseudomonas*, *Salmonella* and *Shigella* for the raw chicken were analyzed. The methods of analysis used were selective media and the RapID ONE bacterial identification test. Both Foster Farms brand chicken and other brands were tested. The results of this study showed aerobic bacteria and gram-negative bacteria were detected in 100% of the chicken. Coliforms were found in 71% of the chicken, showing a high level of fecal contamination and possible presence of pathogens. Possible *Salmonella* contamination was detected in 14% of the chicken. *Shigella* was found in 29% of the chicken, indicating a new emerging foodborne pathogen concern. The microbial quality of the chicken from the Pomona, CA area was similar to that of chicken from throughout the country, and the results of the Foster Farms brand chicken were similar to other brands. These results show that issues with microbial quality of chicken are not unique to Foster Farms brand chicken, but a continuous concern forchicken production in general.

Key words: foodborne pathogens, coliforms, *Salmonella*, *Shigella*, microbiology, chicken

1. Introduction

Foodborne pathogens have been commonly associated with raw poultry. *Salmonella* species and *Campylobacter* species are the most significant pathogens associated with raw poultry [1], and the leading causes of bacterial foodborne disease in the United States and worldwide, each responsible for an estimated 1.4 to 2.5 million cases in the U. S. each year [1-4]. Thus, the microbial quality of poultry is a continuous concern that directly affects the public health. In order to reduce the number of pathogenic microorganisms on meat and the incidence of foodborne illness from meat consumption in 1996, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) began requiring microbial testing by slaughter plants. In the case of poultry, the purpose of the testing was to verify removal of fecal contamination and associated bacteria and to routinely test for presence of *Salmonella* [5-7]. Since 1998, the FSIS has been reporting the percent of samples positive for *Salmonella* in meat products [8]. *Campylobacter* has also become recognized as a cause of human disease and a prevalent foodborne pathogen on raw chicken [2], and since 2011, the FSIS has required the testing and reporting of *Campylobacter* levels in poultry [8].

During the last two years (2013 and 2014), Foster Farms, a major producer of poultry, had both serious sanitation problems and *Salmonella* contamination, leading to recalls of meat products, outbreak of illness

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and plant closures [9-12]. These problems raised concern over the microbial quality of raw chicken currently in the market place, especially Foster Farms brand chicken. For these reason, this study examined the microbial quality of raw chicken from the market place and how Foster Farms brand compared with other brands. Raw chickens were obtained from the local grocery stores and standard media-based microbiology methods were used to determine the microbial quality. Both Foster Farms brand chicken and other brands of chicken were tested for the presence of general types of bacteria, indicator organisms and pathogens. In particular, total number of bacteria, total number of gram negative bacteria, and specific types of bacteria, including coliforms, which are indicator organisms for fecal contamination and presence of potential pathogens [13-17], and the pathogens, *Salmonella* and *Shigella*, were determined.

2. Materials and Methods

2.1 Preparation of the Chicken

Chicken was purchased from major grocery stores in the Pomona, California area between September 2014 and May 2015. The brand of the chicken was recorded. Chicken were identified as either Foster Farms brand chicken or non-Foster Farms brand chicken. Chicken was either cut into parts or obtained as parts. Wings, thighs, drumsticks and breasts were used. Two pieces of chicken were kept for direct contact plating. Chicken was removed from the bone and cut into small pieces, weighed and the weight was recorded. Chicken was placed in a blender and autoclaved distilled or doubly-distilled water was added to make a 1/5 or 1/10 dilution (1 gram chicken to 4 ml water for the 1/5 dilution and 1 gram chicken to 9 ml water for the 1/10 dilution). The chicken was blended until a slurry was formed. This chicken/water slurry was used with autoclaved distilled water to make 1/10 serial dilutions: 1/10, 1/100, 1/1000, and 1/10,000. Seven chickens were tested in total. Three chickens from Foster Farms brand and four chickens from other brands were used.

2.2 Bacterial Cultures

Bacterial stock cultures were used to provide positive and negative controls. The species used are Salmonella enterica subspecies entericabiovar Typhimurium (Salmonella typhimurium), Salmonella enterica subspeciesenterica (Salmonella enterica), Shigellaflexneri, Escherichia coli, Enterococcus faecalis, Enterobacter aerogenes, Pseudomonas aeruginosa, and Micrococcus species. These bacteria were obtained from the general Cal Poly-Pomona stock cultures maintained by the Microbiology Technician for the Department of Biological Sciences. The bacterial cultures are maintained as frozen -70°C glycerol stocks and when needed streaked out on nutrient agar slants and incubated until sufficient growth had occurred.

2.3 Growth on Media

2.3.1 Nutrient Agar Plates and Nutrient Broth

For direct contact method, the chicken pieces were directly rubbed on the surface of the nutrient agar (Difco Brand, Becton, Dickinson and Company, Franklin Lakes, NJ). With the blended chicken slurry mixture and the subsequent serial dilutions, 0.1 ml was spread evenly onto nutrient agar plates. The nutrient agar plates were incubated at 37°C for 24 to 48 hours, under aerobic conditions. The number of colonies on the nutrient agar plates was counted, and the total colony forming units (CFU) per gram of chicken was determined. The following equation was used to calculate the CFU/gram of chicken.

$$\text{Total Colonies}\left(\frac{\text{cfu}}{\text{gram}}\right) = \frac{(\#\text{ colonies on the plate})}{\text{dilution factor x volume plated}}$$

An example of an equation used in this study is shown.

$$\text{Total colonies}\left(\frac{\text{cfu}}{\text{ml}}\right) = \frac{114\text{ colonies}}{\frac{1}{5}\text{ x }\frac{1}{10}\text{ x }\frac{1}{10}\text{ x 0.1 mL}}$$

$$= 5.70 \text{ x } 10^5 \text{ cfu/mL}$$

The dilution factor was determined from the initial dilution to make the chicken slurry mixture and all additional serial dilutions. For some of the blended chicken slurry mixture or subsequent dilutions, nutrient broth was used as an enrichment culture for the bacteria from the chicken samples. In this case, 0.1 ml of the blended chicken slurry mixture or subsequent dilutions was added to nutrient broth and incubated at 37°C for 24 to 48 hours.

2.3.2 Culture Maintenance

Nutrient agar and Triple Sugar Iron (TSI) agar slants were used to maintain cultures.

2.3.3 Salmonella Shigella (SS) Agar Plates

For direct contact method, one piece of the chicken part was directly rubbed on the surface of the *Salmonella Shigella* (SS) agar (Acumedia Brand, Neogen, Lansing, MI). For the blended chicken slurry mixture and serial dilutions, 0.1 ml of dilution was added to the SS agar plates and spread evenly. The SS agar plates were incubated at 37°C for 24 to 48 hours. After incubation, the colonies on the agar plates are examined for colony color and appearance. Pink to rosy red are *E. coli*. Cream to pink are *Enterobacter* species or *Klebsiella* species. Colorless colonies with black centers are *Salmonella* species. *Proteus* species will also produce colorless colonies with black centers, but most are inhibited from growing on the SS agar. Colorless colonies are *Shigella* species, but may be non-H_2S producing *Salmonella* species or other bacteria, such as *Enterobacter* species [18-20]. The colonies per plate were also counted, and the CFU/gram of chicken was determined.

The SS agar was also used with the enriched culturing from the chicken samples. In this case, 0.1ml from the incubated nutrient broth culture was added to SS agar and spread evenly. The SS agar plates were incubate at 37°C for 24 to 48 hours. The colonies were observed for the color and appearance. The colonies per plate were also counted, and the CFU/gram of chicken was determined.

2.3.4 Triple Sugar Iron Agar Deep Slants

Colonies from the SS agar plates were inoculated onto TSI agar deep slants (Difco or BBL^{TM} TSI Agar Slant, Becton, Dickinson Company, Sparks, MD) with an inoculating needle. The butt of the slant was stabbed, and then the needle was streaked up the slant. The TSI slants were incubate at 37°C for 24 to 48 hours. The TSI slant was examined for growth, color of slant and butt, presence of gas or H_2S. If sugars are not fermented, the media remains alkaline and red in color. If sugars are fermented, the media becomes acidic and yellow in color. Whether the butt or slant is yellow is dependent on which sugars are fermented by the bacteria. A black precipitant in the butt indicates H_2S production [21-23]. From the results, the possible type of bacteria was determined. The possible types of bacteria were designated based on the TSI result shown in Table 1.

2.4 Gram Stain and Microscopy

A gram stain was performed for the bacterial cultures with positive growth on the TSI slants. Bacterial cultures were obtained from the TSI slants with positive growth, and microscopy smears were made with these cultures on glass slides. The smear was heat fixed, then gram stained by standard procedures and viewed under a light microscope at 1000x magnification using a 100x oil objective.

Table 1 Possible types of bacteria from TSI results.

Microorganism	**Slant**	**Butt**	**Gas**	**H_2S**
*Escherichia coli or other coliforms**	A	A	+	-
Proteus mirabilis	K	A	-	+
*Pseudomonas aeruginosa or other aerobic environmental gram -, rod shaped bacteria***	K	K	-	-
Salmonella typhimurium, Citrobacterfreundi, Proteus sp.	K	A	+/-	+
Shigellaflexneri, Serratia, Citrobactersp.	K	A	-	-

Note: A: acid; K: alkaline; +: positive; -:negative; +/-: usually negative or positive. *: common examples, *Klebsiella, Enterobacter, Citrobacter or Serratia*, **: common examples *Alcaligenesfaecalis* and *Acinectobacter*.

2.5 Oxidase Test

An oxidase test was performed for five bacterial cultures with positive growth on the TSI slants. These colonies were from the last three batches of chicken and were the colonies also tested by the RapID ONE bacterial identification test. Bacterial culture sample was placed on oxidase test strips (Key Scientific Products, Stamford, Texas) according to the test strip procedure and then examined for presence or absence of a dark blue color within 15 seconds.

2.6 RapID ONE Bacterial Identification Test

Five colonies from the last three batches of chicken were maintained by re-streaking on TSI slants, and then, tested for bacterial identification by the RapID ONE test (Thermo Scientific, Waltham, MA). The protocol provided was followed to perform the RapID ONE test. An inoculating loop was used to pick the bacterial culture from the TSI slant and suspend the bacterial culture in the RapID inoculation fluid. The turbidity of this bacterial suspension was compared with the McFarland #2 Turbidity Standards. Inoculating fluid was added as necessary to achieve the appropriate turbidity. The suspension was vortexed to mix it thoroughly. The lid was peeled off of the RapID ONE panel. Transfer fluid and inoculating fluid was pipetted into the upper right-hand corner panel, and the lid was resealed. The panel was tilted away from the reaction cavities, and then the panel was gently moved from left to right to evenly distribute the suspension in the wells. Then the suspension was slowly tilted towards the reaction cavities, so that the suspension flowed from the well to the reaction cavities. Air bubbles were removed by gently tappin the suspension. The panel was checked to make sure the reaction cavities were evenly filled with suspension, and then the panel was incubated at 37°C for 4 hours. After the incubation, the lid was peeled off and 2 drops of the RapID ONE reagent were added to cavities 15 (PRO) through 17 (PYR). The results from cavity 1 (URE) through 18 (ADON) were read and recorded as positive or negative. Two drops of RapID Spot Indole Reagent were added to cavity 18 (ADON/IND) and allowed to sit for 10 seconds to 2 minutes, and then the result was read and recorded. Results were entered into the ERIC database in order to obtain a bacterial identification.

3. Results

3.1 Quantitation of Bacteria on the Raw Chicken

One aspect of microbial quality in food is the total number of microorganisms present on the food. All batches and parts of chicken examined showed the presence of colonies on the nutrient agar plates. Thus, 100% of the raw chickens had bacteria on them. Where possible the number of colonies on each plate was counted. The plates with the most accurate number of colonies were used for calculating the colony forming units (CFU) per gram of chicken in the original sample. Quantitative assessment for total aerobic bacteria in the chicken samples (Foster Farms brand or other brands) are shown in Table 2.

Chicken pieces were also directly applied to the nutrient agar in the direct contact method to also assess the microbial quality of the chicken. Bacteria were seen on all of these plates and in all except one case at levels too numerous to count.

Quantitation of microorganisms was also performed with the SS agar plates. Quantitation from SS agar plates provides an assessment of the amount of gram-negative bacteria on the chicken. All batches and parts of chicken examined showed the presence of colonies on the SS agar plates. Thus, 100% of the raw chickens had gram-negative bacteria present on them. Where possible the number of colonies on each plate were counted. The plates with the most accurate number of colonies was used to calculate the colony forming units (CFU) per gram of chicken in the original sample. This approach provided a quantitative assessment of the total gram-negative bacteria in the chicken samples. The results from all chicken samples, Foster Farms brand or other brands are shown in Table 2.

Table 2 Quantitation of bacteria in raw chicken.

Chicken Samples	Total aerobic plate counts		Total gram negative bacteria	
	Range	Average	Range	Average
Total	3.45×10^4 to 1.37×10^6 CFU/g	6.60×10^5 CFU/g	1.5×10^3 to 1.49×10^6 CFU/g	3.86×10^5 CFU/g
Foster Farms	3.45×10^4 to 1.37×10^6 CFU/g	5.45×10^5 CFU/g	1.5×10^3 to 1.49×10^6 CFU/g	5.76×10^5 CFU/g
Other Brands	5.95×10^5 to9.25×10^5 CFU/g	7.75×10^5 CFU/g	1.8×10^3 to 1.22×10^6 CFU/g	2.43×10^5 CFU/g

Direct contact method was also performed. All plates had bacterial colonies on them. Most had numbers too numerous to count. For those that could be counted, the range of gram-negative bacteria/contact was 3.90×10^2 to 3.62×10^3 CFU/contact.

3.2 Determination of Types of Bacteria on the Raw Chicken

In addition to being a selective media, SS agar can be used to distinguish between different enteric bacteria based on ability to ferment lactose and produce H_2S [18-20]. Colonies from the SS agar were examined for color and appearance, which was recorded. Colonies of each type seen on SS agar and from every batch of chicken were picked and transferred to a TSI deep slant. After incubation, the appearance of the TSI slants were observed for growth, color of the buttand the slant, presence of H_2S or gas. The results are shown in Table 3. These results are based on presence or absence of any bacterial colony detected in the entire batch of chicken with the corresponding slant/butt/gas/H_2S result. In some cases, only one colony of the designated type was observed. In other cases, multiple colonies were observed.

Using appearance on the TSI and Table 1, a bacterial type designation was made and shown in Table 3. *Pseudomonas aeruginosa* or similar aerobic environmental bacteria were very pervasive in the raw chicken. Not only did every batch of chicken tested have colonies with a TSI result of K/K/-gas/+H_2S, but multiple colonies were detected from individual batches and colonies were detected from direct contact, the slurry mix and from different parts of the chicken. No colonies with a TSI result of K/A/-gas/+H_2S, which is suggestive of *Proteus mirabilis*, were identified in any batch of chicken tested. Five of seven batches of chicken showed a TSI result of A/A/+gas/-H_2S, indicative of the presence of coliforms. Two of three batches for Foster Farms brand showed presence of coliforms and three of four from non-Foster Farms brands. Two of seven batches showed presence of potential *Salmonella* species with a TSI result of K/A/+gas/+H_2S, both were from the Foster Farms brand of chicken. Three of seven batches of chicken showed presence of potential *Shigella* species with a TSI result of K/A/-gas/-H_2S, two from the Foster Farms brand and one from the non-Foster Farms brand.

The RapID ONE bacterial identification test (Thermo Scientific, Waltham, MA) was performed on colonies from the last three batches of chicken. Five colonies from TSI slants were maintained by re-streaking on TSI slants. These colonies were shown to be gram negative rods and oxidase negative, and were tested by the RapID ONE bacterial identification method. Two colonies from a non-Foster Farms brand chicken with TSI results of A/A/+gas/-H_2S and thus, suggestive of *E.coli*or other coliforms, were identified

Table 3 TSI Results from raw chicken.

Number of chicken with result	Slant	Butt	Gas	H_2S	Possible microorganism
5 of 7 (2 of 3 FF) (3 of 4 non-FF)	A	A	+	-	*E. coli or other coliforms*
none	K	A	-	+	*Proteus mirabilis*
all	K	K	-	-	*Ps. aeruginosa, or other aerobic bacteria*
2 of 7 (Both positive FF)	K	A	+/-	+	*Salmonella typhimurium, Citrobacterfreundi , Proteus*
3 of 7 (2 of 3 FF) (1 of 4 non-FF)	K	A	-	-	*Shigellaflexneri,Serratia, Citrobacterspecies*

Note: FF: Foster Farms; A: acid; K: alkaline; +: positive; -: negative; +/-: usually negative or positive.

by the RapID ONE method as *Citrobacterfreundii* and *Serratiaodorifera* 1 & 2. Two colonies from a non-Foster Farms brand with TSI results of K/A/-gas/-H_2S and thus, suggestive of *Shigellaflexneri*, were identified as *Yersinia kristensenii* and *Shigella* species by the RapID ONE method. One colony of a Foster Farms brand chicken with TSI results of K/A/+gas/+H_2S and thus, suggestive of *Salmonella typhimurium* was identified as *Shigella* species by the RapID ONE method.

Gram stain was performed with bacterial colonies from the TSI slants. All bacteria examined were gram-negative and rod shaped with moderate length. This result is consistent with *SS agar* inhibiting gram-positive bacteria and thereby, selecting for gram-negative bacteria. This result is consistent with the types of bacteria indicated by the TSI results and RapID ONE method. All specific bacteria types mentioned above are gram-negative and rod shaped with moderate length.

Overall these results show that aerobic environmental gram-negative bacteria were present and pervasive on all chicken. *Proteus mirabilis* was not present on any of the chicken. Coliforms were present on most (71%) of the chicken. This result indicates that most of the chicken tested was contaminated with fecal material. *Shigella* species were confirmed by the RapID ONE test to be present on two batches (29%) of the chicken. One additional batch of chicken showed potential *Shigella* by the TSI slant and one batch of chicken showed potential *Salmonella* species, but the colonies were not further tested to confirm the bacterial identification. Although not definitive, these results indicate an additional potential for presence of pathogens on the chicken.

3.3 Comparison of Results for Chicken from Foster Farms Brand to Other Brands

Since Foster Farms plants were having problems with sanitation and *Salmonella* contamination, a comparison of the microbial quality of the Foster Farms brand chicken with other brands was made. The total bacteria and the total gram-negative bacteria on the chicken was similar for the Foster Farms brand and the other brands. Both Foster Farms and the other brands have aerobic environmental gram-negative bacteria, which are general non-pathogenic bacteria, present on all batches of chicken in a pervasive and high level. Both Foster Farms brand and other brands had coliforms present on the chicken and at similar levels with coliforms isolated from about 70% of the batches of chickens. Thus, the majority of chicken tested had fecal contamination and this contamination was true for the Foster Farms brand, but also for chicken from other brands. Both Foster Farms brand and the other brands showed one batch of chicken (25%-33%) with a confirmed positive test for *Shigella.* The Foster Farms brand also showed additional potential *Shigella* and *Salmonella* by the TSI slants. One batch of chicken for each potential bacteria. Colonies were not further tested to confirm the bacterial identification. These results show that both Foster Farms and other brands have issues with human pathogens and specifically *Shigella* on the chicken, as seen with the confirmed positive tests for *Shigella*. In addition, Foster Farms level of pathogens may be higher than other brands, since their chicken showed potential presence of pathogens, *Shigella* and *Salmonella*, by the TSI slants.

3.4 Comparison of Pomona, California Area Results to Nationwide Results for Raw Chicken

A comparison of the microbial results for the raw chicken obtained post-market from the Pomona, California area to nationwide results for raw chicken was made. For the nationwide results, a baseline report for chicken parts during 2012 produced by the FSIS was used, since the chicken used in this study was in the form of chicken parts as opposed to whole chicken. This report revealed that 97% of the chicken tested showed bacteria present by aerobic plate counts (APC), thus aerobic bacteria, with a mean value of 5.6×10^8

CFU/ml. The chicken parts were tested for *Enterobacteriaceae*, which are bacteria from the intestines of animals, gram-negative and moderately shaped rods [13, 24]. *Enterobacteriaceae* were present in 97% of the chicken parts, and the mean amount on the chicken was 1.7×10^4 CFU/ml. Total coliforms were present in 89% of the chicken parts with a mean value of 2.5×10^3 CFU/ml. Sixty-two percent of the chicken had generic *E.coli* present with a mean value of 7.0×10^2 CFU/ml. The percent of the chicken that had *Salmonella* present was 26% and *Campylobacter* was 21%.

A comparison of the microbial quality of the chicken tested between the 2012 nationwide report and the results from this study are shown in Table 4. The results from this study show similar percentage of chicken with total viable aerobic bacteria as the national results; however, number of bacteria detected is substantially different with a 1000 fold greater number in the national study. Overall both studies show that almost every chicken had the presence of general aerobic bacteria and in high numbers. As for gram-negative bacteria, the study here looked at growth on SS agar plates, which detects most, but not all gram-negative bacteria. The national study looked at presence of *Enterobacteriaceae*, which is a large family of gram-negative bacteria. Similar results were obtained for gram-negative bacteria or *Enterobacteriaceae*. As might be expected the numbers for the *Enterobacteriaceae* were slightly lower than the gram-negative bacteria. Overall both studies showed that almost every chicken had gram-negative bacteria or *Enterobacteriaceae* present and in high numbers.

Both studies show a high prevalence of coliforms on the chicken. The level is a little lower for the results from this study than the national level, but still both indicate high presence of coliforms with almost 3 of every 4 chickens showing presence of coliforms. Both the nationwide results and this study show a high incidence (~25%) of pathogenic bacteria on the chicken. The nationwide results show a higher prevalence of *Salmonella* than this study. The nationwide study shows high prevalence of *Campylobacter*, which was not tested in this study. This study showed a high incidence for the presence of *Shigella*, the nationwide study did not report findings for *Shigella*. Together these results show high incidence of pathogenic bacteria on the chicken, although the specific pathogens appear to be different.

Table 4 Comparison of microbial quality of chicken with national results.

Type of Bacteria	National		Pomona, CA	
Total aerobic bacteria	Count	%	Count	%
	5.6×10^8 CFU/ml	97%	6.60×10^5 CFU/gram	100%
Total gram-neg. bacteria (or *Enterobacteriaceae*)	1.7×10^4 CFU/ml	96%	3.86×10^5 CFU/gram	100%
Total coliforms	2.5×10^3 CFU/ml	89%	-----	71%
Salmonella	-----	26%	-----	14%
Campylobacter	-----	21%	-----	-----
Shigella	-----	-----	-----	29%

4. Discussion

In the U.S. and throughout the world, foodborne pathogens have been associated with raw chicken in a continuous manner for long periods of time [1-4]. Also raw chicken produced for the general marketplace have contained high levels of general bacteria and bacteria of fecal origin [6, 25-29]. While some decreases in pathogens have been observed, their presence on the chicken still remains significant [8]. Thus, there has been a continuous need to examine the microbial quality of raw chicken. Recent issues with sanitation and *Salmonella* outbreaks [9-12] have also revealed the need for examination of the microbial quality of raw chicken.

The levels of total viable aerobic bacteria in this study were similar to those in the 2012 nationwide baseline study [28] with almost all chicken tested showing presence of bacteria (100% and 97%). In both cases, the amounts of bacteria were high in numbers, but higher for the nationwide study. The mean amounts

were 6.6×10^5CFU/g for this study and 5.6×10^8 CFU/ml nationwide study. The universal presence of total viable aerobic bacteria on raw chicken at levels of 10^3or higher is consistent with other studies and results worldwide [6, 25, 27]. This study and the 2012 nationwide baseline study [28] showed almost all raw chicken had gram-negative bacteria or *Enterobacteriaceae* (100% and 97%) and relatively similar mean values of 3.86×10^5 CFU/gram for this study and 1.7×10^4 CFU/ml for the 2012 nationwide study, indicating that bacteria from the intestines of the chicken does end up spread on the raw chicken. A study from Sweden also shows presence of *Enterobacteriaceae* on raw chicken although at about 100 fold lower numbers [25]. Overall, this study is consistent with previous studies, and these studies shows that environmental, gram-negative and intestinal bacteria are universally present on raw chicken in the U. S. and other countries.

This study showed coliforms present on 71% of the raw chickens, which is a high prevalence. The 2012 nationwide baseline study also showed a high prevalence at 89% [28]. In this study, the majority of chickens showed presence of coliforms, which means the majority of raw chicken has fecal contamination and potential for the presence of pathogens [13-17]. The same is true for the nationwide study examined here. Taken together, these results show a consistent nature of coliform contamination on the majority of raw chickens, and thus, throughout the U. S., raw chicken are being produced with fecal contamination and potential for pathogens. These results also show that the FSIS efforts to have chicken produced without fecal contamination has not materialized. This study did show coliforms at a slightly reduced level compared to nationwide levels.

It may be possible that the preparation of the chicken in this study is somewhat improved for microbial quality, but it also possible is that detection methods were more limited. The SS agar is a selective agar and does inhibit some coliforms. So some coliforms may not be detected by this approach. Other studies from raw chickens throughout the world, Canada, Australia and Kenya, show levels between 59.6% and 99.7% [26, 27, 29]. These studies show that fecal contamination of raw chicken is not only a U.S. problem, but also a worldwide problem.

Salmonella has consistently been associated with poultry [1, 8, 30]. In the U. S., the levels have declined from 10-16% in the early to mid 2000s to approximately 4% now in whole young chickens [8]. However the rates in chicken parts, 26% in 2012 [28], and ground chicken, 18% in 2014 [11], show that approximately ¼ of the raw chicken produced as parts or ground for human consumption in the U.S. contains *Salmonella*, which is a significant level. This level of *Salmonella* present in raw chicken is not unique to the U.S., but such levels are seen worldwide. Studies show 36% to 48% of retail chicken samples with *Salmonella* in Australia [26], 39% of raw chicken legs [30] and 38% from carcass rinses with *Salmonella* in Canada [27], and 6.8% of carcasses with *Salmonella* in Ghana [31]. Similar levels are seen in the gut contents or feces of chicken, 7.2% of live birds gut contents in Ghana [31] and 23.7% from a European Union-wide baseline study [32].

This study here shows fewer batches of chicken parts had *Salmonella* than the 2012 nationwide study, 14% versus 26%, and the 14% are only possible *Salmonella*, since a confirmatory test was not performed, so the true level could be lower. Thus for the selected chicken tested here *Salmonella* was reduced from the levels normally observed in the U. S. Since the *Salmonella* are harbored within the intestine of the birds, the level in raw poultry is tied to the level in the live birds [31, 32]. However, slaughtering, butchering and preparation process of the raw poultry can spread the *Salmonella* to more of the poultry than was harbored in the gut of the live birds [33]. The levels of raw chicken in *Salmonella* in this study are lower than other U. S. studies. This lower level could be due to decreased level in the live chickens and/or less spreading of the

Salmonella during the slaughtering, butchering and preparation.

This study shows 29% of the raw chicken tested were confirmed for the presence of *Shigella* species. *Shigella* species are pathogens to humans, causing bacterial dysentery, also known as shigellosis [16, 34-36]. Illness from *Shigella* species is seen in both developing and developed countries. Very low numbers of individual *Shigella* are needed to cause infection, thus its mere presence brings a level of concern. While contaminated water and food play a role in the spread of shigellosis, the pathogen is usually spread from person to person [36, 37]. When spread through food often humans have been the carrier of the *Shigella* to the food [37]. The natural hosts of *Shigella*have been humans and primates, and not animals raised for traditional food consumption, such as cattle, pig or poultry [38]. In the U. S., raw chicken is not routinely tested for *Shigella* as is the case for *Salmonella* and *Campylobacter* [1, 8]. The detection of *Shigella* species on the chicken in this study is very significant because it reveals the presence of a serious pathogen in a new food environment. Recently, there has been evidence that *Shigella* is being harbored in new hosts [38-40], including chickens [38, 41]. A study of the poultry-associated microbiome showed closely related genera to *Shigella* as part of the core microbiome of chickens, indicating that *Shigella* may need to be added to the list of poultry-associated pathogens [42]. These results along with this study show that *Shigella* has now becoming an emerging concern in chickens.

Although Foster Farms brand chickens had issues with sanitation and *Salmonella* in the last two years, the results of this study show similar levels of microorganisms between the Foster Farms brand chicken and other brands. Levels of total bacteria, gram-negative bacteria and coliforms was high for both Foster Farms brand chickens and the other brands. Both showed presence of *Shigella*, a human pathogen. The only difference observed was in the level of *Salmonella*. Foster Farms showed 14% possible *Salmonella*, but these bacteria were not further tested to confirm the result. No *Salmonella* was observed in the other brands. For both Foster Farms brand and other brands the level of *Salmonella* is lower than the amounts seen nationwide. The similarity between Foster Farms brand chicken and other chickens observed in this study may be due to changes made in the Foster Farms production of chicken, since the chicken tested in this study were obtained after the sanitation and *Salmonella* issues had been raised. However, both types of chicken tested show high levels of coliforms, an indicator bacteria, and presence of pathogenic bacteria. So while Foster Farms brand microbial quality was similar to other brands, the raw chicken from Foster Farms brand and non-Foster Farms brands had issues with microbial contamination. This study shows that all chickens have issues with microbial quality, and that the problem is not unique to the Foster Farms brand.

5. Conclusions

This study showed that the raw chicken obtained in the Pomona, CA area had high levels of total bacteria and gram-negative bacteria. All chicken tested had general bacteria on them. The chicken also had a high level of coliforms which shows fecal contamination on the chicken and possible presence of pathogens. The chicken showed presence of pathogenic bacteria at levels similar to those of other studies. Overall, these results are similar to the microbial quality of raw chicken observed throughout the U.S. and also worldwide. Foster Farms brand chicken had similar microbial quality to the other brands tested. Although Foster Farms had problems with production of chicken, issues of microbial quality are not unique to the Foster Farms brand. All chicken show high levels of general bacteria, presence of fecal indicator organisms and pathogens. These results from this study and the other studies examined here show that efforts to reduce fecal contamination have not materialized and while levels

of pathogens have decreased, they are still present in some raw chicken. All raw chicken should still be considered to potentially contain human pathogens and therefore, be cooked thoroughly. This study also showed an important difference in the type of pathogen detected. In this study, *Salmonella* levels were lower than normally observed, but *Shigella* was detected. While *Salmonella* is decreasing, shown overall by FSIS and results of this study, *Shigella* is emerging as a concern in chicken. Both recent studies and this study are showing this new trend of *Shigella* association with chickens. This presence of *Shigella* on chicken reveals an emerging foodborne pathogen concern.

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