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# *Salmonella* spp. detection in chicken meat and cross-contamination in an industrial kitchen

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**Chicken meat is a widely consumed food. However, broilers are implicated in contamination by *Salmonella* spp., since poultry is considered asymptomatic carrier of the pathogen. The objective of this study was to detect the presence of *Salmonella* spp. in chicken *in natura* and ready for consumption, as well as in the hands of employees, personal protection equipment and utensils in an industrial kitchen. In total, 18 *in natura* chicken samples, 18 cooked chicken samples and 30 surfaces were analyzed. Research was conducted in two stages: before and after the presentation of bacteriological analysis and the observational research for managers and employees of food preparation, for the discussion and changes in the procedures to handle and prepare chicken. *Salmonella* spp. was detected in 55.5% of *in natura* chicken in stage 1 and in 44.5% in stage 2. In cooked chicken, positive results were observed in 33.4 and 11.2% in stages 1 and 2. Concerning surfaces, the microorganism was detected in 40% (stage 1) and 53.3% (stage 2) of tested samples. The results show the occurrence of problems in the chicken processing chain, with evident cross-contamination, posing risks to the health of the end consumer.**

**Key words:** Poultry, food safety, food microbiology, food contamination.

## INTRODUCTION

The constant expansion of chicken meat trade at global level is due to the fact that this meat product has excellent nutritional value and relatively low production and processing costs. Chicken meat is rich in important nutrients such as proteins, lipids, vitamins and minerals. It enjoys considerable acceptance by consumers, and may be consumed by humans of all ages (Núcleo de Estudos e Pesquisas em Alimentação, 2011).

However, chicken meat is involved in the transmission of several pathogens that cause food-borne diseases that are important in public health. This high prevalence is due to the fact that these pathogens are distributed

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across the whole production chain, from birth of chickens to the end product. This contamination may be worsened by temperature and humidity conditions in farms. Among the most important pathogen present on chicken body surfaces is *Staphylococcus aureus*, while *Salmonella*, *Campylobacter* and *Escherichia* are the genera known to colonize the intestinal tract of broilers (Foley et al., 2008; Mendes, 2012).

The contamination of chicken meat by *Salmonella* spp. is the object of constant research and control in several countries due to the high prevalence, health risks to consumers and economic costs. *Salmonella* spp. is present in the environment, and around 2,300 different serotypes of this microorganism may contaminate the intestines of animals, water and foods in general. The list of the most susceptible foods to contamination by *Salmonella* spp. is long, and includes meats in general, eggs, milk and dairy products, fish, some kinds of sweets, and others (CDC, 2013a; Foley et al., 2008; Fortuna et al., 2012).

The National Program of Pathogen Reduction, Microbiological Monitoring and *Salmonella* spp. Control in Chicken and Turkey Carcasses was published in Ordinance 70, 2003, to establish standards and quality control measures for poultry products, and developed an information system on the pathogen, guaranteeing food safety for domestic and export markets (Brasil, 2003). The Brazilian legislation established the threshold of zero bacterial count of *Salmonella* spp. in 25 g of a bird meat sample (Brasil, 2001).

Contamination by *Salmonella* spp. is stressed in food poisoning outbreaks. In the USA, approximately 9.4 million cases of food-borne diseases are reported a year. Laboratory analyses confirmed that 19,531 infections were associated with food-borne pathogens in the country in 2012, and *Salmonella* spp. were implicated in 16.42% of the confirmed food poisoning cases (CDC, 2013b; CDC 2013c).

In Brazil, between 2000 and 2011, of the 3,927 food poisoning cases reported, 1,660 were due to *Salmonella* spp. Concerning the origin of infection, 51.84% of these cases were acquired at home, and 17.93% in restaurants and similar places. The foods most commonly implicated in these poisoning cases were prepared with a mixture of ingredients, with eggs, as well as sweets and desserts, water, *in natura* beef, processed meat and offal (Secretaria de Vigilância em Saúde, 2011).

Contamination of foods by *Salmonella* spp. is due to inappropriate handling and hygiene conditions, among other reasons. The person preparing foods plays a crucial role in contamination, since the hand is one of the main vehicles of microorganism transmission (CDC, 2013d; Fortuna et al., 2012). In a study on the microbiological quality of hands of 44 workers in 13 state schools in Brazil, 2.3% were positive for *Salmonella* spp. (Souza and Santos, 2009). After the analysis of food, eggs, mayonnaise and chicken implicated in 10 salmonellosis outbreaks the researchers suggested that the possible causes of contamination were mistakes in handling, which promoted cross-contamination. A study on a salmonellosis outbreak in a restaurant chain revealed that raw chicken, seasoning and the chopping board used to slice cooked chicken were responsible for contamination of foods by *Salmonella* serotype Montevideo (Patel et al., 2010).

The clinical characteristics of salmonellosis are diarrhea, nausea, abdominal pain, mild fever, shivers, and occasional vomiting, headaches and weakness. In some cases, people affected may present more severe signs. During the disease, the patient releases the bacterium via feces, which may be spread in the environment (CDC, 2013d).

The processing of foods in restaurants has to obey safety standards. Chan and Chan (2008) discovered that restaurants were the most common sources of food-borne etiological agents between 1996 and 2005. In a study that investigated the presence of *Salmonella* spp. in feces of workers who prepared food in two university canteens, four (10%) were positive for the bacterium, which represents a serious public health problem (Sandrea Toledo et al., 2011). Also, in another study, the presence of *Salmonella* spp. was detected in salads in over 50% of commercial restaurants surveyed in the city of Rio de Janeiro, Brazil. Problems concerning cross-contamination, poor personal hygiene of restaurant workers and in the control in food storage were considered critical (Antonio and Ghisi, 2011). Several studies carried out in kitchens and restaurants point to problems in constructive, physical, functional, hygienic and sanitary aspects both in preparation of foods and in physical installations and utensils, in the training of workers and personal hygiene, which are critical points in the production of safe foods (Bramorski et al., 2008; Colombo et al., 2009; Faheina Jr et al., 2008).

The objective of the study was to detect the presence of *Salmonella* spp. in chicken meat *in natura* and ready for consumption, in the hands of employees, in personal protective equipment and in utensils used and in the food preparation process, considering food safety in accordance with official regulations. This information may be useful in the development of more efficacious food preparation procedures.

## MATERIALS AND METHODS

### Epidemiological study

An exploratory-descriptive and quantitative study, including data survey and laboratory investigation was carried out. Data were collected by systematic observation using an instrument to record the work process throughout the production chain of foods, from the moment when raw foods are received to the distribution of ready food in the restaurant. In this survey, the facts and phenomena studied were known and examined (Lakatos and Marconi, 2011).

The laboratory investigation included the bacteriological analyses

to detect *Salmonella* spp. according to the American Public Health Association (APHA) (Andrews et al., 2001). Analyses were carried out in the Laboratory of Microbiological Control of Animal Products, Department of Food Technology, Veterinary Medicine College, Universidade Federal Fluminense (UFF).

Samples were collected in an industrial kitchen that prepares meals in a university restaurant, in the city of Niteroi, State of Rio de Janeiro, Brazil. The kitchen produces approximately 6,000 meals a day, 4,200 at lunch and 1,800 at dinner, consumed by students and employees in general of the University, in site and in other restaurant units of the University. Dinner is offered in the main unit and in a secondary unit.

The university kitchen was chosen because it is considered a large production center of meals for the academic community, to improve students' performance and because the kitchen does not have a microbiological control program for the meals it serves.

The investigation was carried out between April 2012 and January 2013, and was divided in two phases. The first phase included the collection of all samples, with no interference to researcher. The second took place after the results obtained in the first stage were presented to the manager and the employees of the kitchen, with the subsequent implementation of improvements in the work process in light of the results obtained during the first phase.

The meetings to present the results of the analyses were carried out using the quality tools of the plan, do, check, action (PDCA) cycle, the Cause and Effect Diagram and the Technical Regulation of Best Practices in Production. At the end of this process, new operational methods were described directed to the safe production of foods in the kitchen.

## Bacteriological analyses

### *Sample collection*

Thirty-six chicken meat samples were randomly collected (18 in each stage) in the kitchen: three samples during preparation and three samples during distribution and consumption. The chicken cuts sampled were: deboned chicken breast (DCB), deboned skinless whole leg (DSL), and whole leg (with bones and skin, WL).

Collection of raw chicken samples was carried out during preparation. This stage of the processing of chicken included the thawing, the rinsing in running water and vinegar, and the addition of a seasoning mix prepared with vinegar, salt, garlic, onion and bay leaves. Collection of finished food took place during the distribution of meals, in a university restaurant that receives the transported meals. The raw and cooked food samples were from the same batch of meals distributed in the menu. There, three containers with chicken food are delivered daily. One sample was retrieved from each container.

In stages 1 and , the deboned chicken breast was minced and cooked in a steam cooker for 60 min on average, while the whole leg (with bone and skin) and the deboned and skinned whole leg were prepared in a combined oven for 55 min, on average.

Bacteriological analyses were carried out using 250 g samples of chicken meat (for both raw and cooked chicken), as recommended in the official literature (Brasil, 2001). Samples were randomly collected and placed in sterile polyethylene bags. All samples were transferred to the laboratory immediately after collection in a thermal plastic reusable bag containing ice. Collection of samples for the bacteriological analyses followed the procedures outlined by LACEN (2010).

Besides, and during the two stages of the study, six samples were collected from hands and of personal protective equipment (four rubber gloves, two from mail gloves, two from silicon gloves, six from aprons and two from masks) of the employees working in preparation, cooking and distribution areas, were collected using sterile swabs, totaling 22 samples. Workers were randomly selected. Personal protective equipment is individually used and is compulsory, for the safety and health of workers (Brasil, 2010). Additionally, samples were collected from utensils (two from the plastic chopping boards, two from containers and respective covers, two from stainless steel spatulas) as specified by APHA (Andrews et al., 2001), totaling eight samples, in the two stages of this study. These utensils were chosen because they are used in the preparation and distribution of chicken. These swab samples were collected during the food processing activities, with no previous hygiene, except for the samples collected from the hands of preparation workers. The samples collected from the containers and lids were obtained before they were used. The analysis of hands, personal protective equipment and kitchen utensils and followed the same methodology, but samples were collected using a disposable sterile swab as described by APHA (Andrews et al., 2011) LACEN (2010).

Collection of all samples, at the different stages of meat production, was preceded by the observation of procedures at the different stages of meal production, according to a predefined program of routine inspection and description (Lakatos and Marconi, 2011). The chicken meat cuts were randomly retrieved throughout the production process. During observation of the procedures, temperatures of chicken meat lots used for sample collection were measured, at the different steps, using a specific meat thermometer (Incoterme™).

In the laboratory, a 25 g portion was obtained from each chicken sample using sterile instruments and homogenized in a stomacher with 225 ml buffered peptone saline 1%. Then, the mixture was incubated at 37ºC for 24 h. Then, two 1 ml aliquots were retrieved and seeded in two separate tubes, one containing 10 ml Rappaport Vassiliadis (RV) broth (Himedia M880-500 g) and one containing 10 mL EE Mossel (M) broth (Himedia M287-500 g). The tubes were then incubated at 37 and 41ºC, respectively, for 24 h.

Each suspension was then seeded on disposable sterile Petri dishes containing the following selective media: Hektoen (H) Agar (Himedia M467-500 g), Brilliant Green Agar Base (BPLS) (MicroMED 2164), and *Salmonella* Differential Agar (SS) (Himedia M1078-500g) and the dishes were incubated upside down for 18-24 h at 36°C.

The dishes presenting typical *Salmonella* spp. colonies were picked, and five colonies obtained (from each culture medium) were streaked on Triple Sugar Iron (TSI) agar (Himedia M021-500 g) and immediately incubated 36ºC for 24 h at 36°C. Typical *Salmonella* spp. growths were selected and an inoculum was obtained from the center of the dish using a needle and seeded on separate tubes containing Phenylalanine agar (Himedia M281-500 g) and Nutrient agar (Himedia M090-500 g), and incubated for 24 h at 35°C. Then, 3 to 5 drops of ferric chloride 10% were added to the tubes containing the Phenylalanine agar. Sterile saline was added to the positive *Salmonella* spp. cultures the Nutrient agar and one drop of the suspension was retrieved and transferred to a glass slide for serological test to ascertain the self-aglutination capacity of the isolate. One drop of *Salmonella* Polyvalent serum (Probac do Brasil™) was then added and confirmation of a positive result was obtained by agglutination of serum in contact with the suspension analyzed.

The analyses of hand, personal protective equipment and utensils followed the same methodology. The only difference was the initial dilution of these samples, which was carried out in a test tube containing 90 mL buffered peptone saline 1%, and homogenized. Samples were diluted by adding buffered peptone saline 1% and homogenize.

## Statistical analyses

The data obtained were analyzed by the Student's t test to assess

**Table 1.** Number and percentage of positive samples for *Salmonella* spp. in each chicken cut, in preparation and distribution, in each of the stages.

| Cut | Preparation | | | | Distribution | | | |
|---|---|---|---|---|---|---|---|---|
| | Stage 1 | | Stage 2 | | Stage 1 | | Stage 2 | |
| | N | % | N | % | N | % | N | % |
| Deboned chicken breast (DCB) | 1 | 33 | 2 | 67 | 2 | 67 | 0 | - |
| Deboned skinless whole leg (DSL) | 1 | 33 | 0 | - | 0 | - | 0 | - |
| Whole leg (WL) | 3 | 100 | 2 | 67 | 1 | 33 | 1 | 33 |
| Mean (%) | 55.6 % | | 44.5% | | 33.4 % | | 11.2 % | |

significant differences between mean results independently for each sample type (meat and surfaces). Statistical significance was $\alpha = 0.05$.

#### Ethics approval

This research, which is part of a PhD dissertation, was approved by the Ethics Committee of the Medicine College, Teaching Hospital Antônio Pedro, Universidade Federal Fluminense (protocol CAAE 0417.0.258.000-11).

## RESULTS

The results are presented for the two stages including those for chicken meat samples (biological analyses) and hands, personal protective equipment, kitchen utensils and equipment (surfaces). Of the 18 chicken meat samples analyzed in the first stage, eight (44.4%) were positive for *Salmonella* spp. in the chicken meat cuts analyzed, except the deboned skinless whole leg (DSL) in distribution, which were negative for *Salmonella* spp. (Table 1).

In the first stage, the samples presenting the highest number of positive results were whole leg (with bone and skin, WL), with three positive samples (100%). In distribution, the highest percentage of isolation occurred in DCB (deboned chicken breast), with two (67%) of positive samples (Table 1).

In the second stage, of the 18 samples analyzed, in preparation and in distribution, five (27.8%) were positive for *Salmonella* spp. (Table 1). The samples with the highest prevalence of positive results were deboned chicken breast (DCB), with two (67%) positive samples, and whole leg (WL), with two (67%) positive samples, in preparation. In distribution, only one (33%) sample of whole leg (WL) was positive for *Salmonella* spp. (Table 1).

A reduction in microbial load was observed in food, between the stages; however, the Student's t test showed that there were no significant differences between mean results in the two stages, in spite of the changes implemented in the operational process, both in preparation and distribution, with significance level $\alpha = 0.05$.

In the analysis of hands, protective equipment and kitchen utensils, the following results were obtained: in stage 1, in the preparation area, *Salmonella* spp. were isolated from hands, apron and mail gloves; in the cooking area, the bacteria were isolated in the rubber gloves a and b; in the distribution area, it was isolated in masks, totaling six (40.0%) positive samples of samples (Table 2).

In stage 2, *Salmonella* spp. were isolated in the preparation area, in the following samples: hands, rubber gloves, apron, mail glove and chopping board. In the cooking area, the bacteria were isolated in aprons. In the distribution area, it was isolated in hands a and b. In total, eight (53.33%) of samples were positive (Table 2).

A difference was observed in microbial loads between hands, personal protective equipment and utensils, in the two stages. However, the Student's t test revealed no significant differences between mean results of the two stages (1 and 2) with significance level $\alpha = 0.05$.

The results of the means of the temperatures measured in the preparations that presented positive *Salmonella* results are presented in Table 3. These data are relevant for the comprehension of the process of cross-contamination in terms of the operational flow.

In stages 1 and 2 the deboned chicken breast was minced and cooked in a steam cooker for 60 min on average, while the whole leg (with bone and skin) and the deboned and skinned whole leg were prepared in a combined oven for 55 minutes, on average. Between stages 1 and 2, meetings were held with the manager, nutritionists and workers to analyze and discuss the results of the microbiological analyses and observations carried out, followed by the presentation of new work methodologies. At the end, several suggestions were carried out, to improve quality of the techniques used and consequently of the final product. The PDCA cycle was used to define objectives and a new systematization of the procedures (Figure 1). The cause and effect diagram was essential to identify and analyze problems and propose suggestions.

Table 4 presents the mistakes observed and the solutions implemented. The mistakes observed and for which no solutions were implemented are listed at the end of the table. The results of the observational analysis

**Table 2.** Number of positive samples positive for *Salmonella* spp. In hands, personal protective equipment and utensils in preparation and distribution, in each of the stages.

| Section | Surface | Stage 1 | Stage 2 | Sections |
|---|---|---|---|---|
| **Preparation** | Hand | 02 | positive | positive |
| | Apron | 02 | positive | positive |
| | Rubber glove | 02 | absence | positive |
| | Mail glove | 02 | positive | positive |
| | Chopping board | 02 | absence | positive |
| **Cooking** | Rubber glove (a) | 01 | positive | No personal equipment in this stage |
| | Rubber glove (b) | 01 | positive | No personal equipment in this stage |
| | Silicon glove | 01 | No personal equipment in this stage | absence |
| | Silicon glove | 01 | No personal equipment in this stage | absence |
| | Apron | 02 | absence | positive |
| | Stainless steel container | 02 | absence | absence |
| | Cover | 02 | absence | absence |
| | Stainless steel spatula | 02 | absence | absence |
| **Distribution** | Hand (a) | 02 | absence | positive |
| | Hand (b) | 02 | absence | positive |
| | Apron | 02 | absence | absence |
| | Mask | 02 | positive | absence |
| **Mean (%)** | | | 40% | 53.3 % |

**Table 3.** Mean temperatures of prepared and cooked chicken cuts, with positive results for *Salmonella* spp. in the distinct stages of the production cycle.

| Cut | Stage 1 | | | Stage 2 | | |
|---|---|---|---|---|---|---|
| | Preparation (°C) | Main kitchen (°C) | Distribution (°C) | Preparation (°C) | Main kitchen (°C) | Distribution (°C) |
| **DCB** | 10.6 | 82.8 | 63.4 | 9.5 | 81.0 | 63.4 |
| **WL** | 5.9 | 85.5 | 64.6 | 8.34 | 87.8 | 70 |
| **DSL** | 8.1 | 93.5 | 75.7 | 13.2 | 90.8 | 76.5 |

DCB: Deboned skinless chicken breast, DSL: Deboned skinless whole leg (in combined oven), WL: Whole leg

| | |
|---|---|
| **4 Act:** Definition of new proposals in terms of monitoring, with a view to continuous improvement | **1 Plan:** Assessment of the operational model used and implementation of the required changes; meetings with the workers involved |
| **3 Check:** The proposals implemented are evaluated systematically? The evaluation of results is carried out? | **2 Do:** Meetings with workers; data analysis, discussions and proposals; implementation of proposals |

**Figure 1.** Analysis of the production process in the area of meat preparation based on the PDCA cycle.

**Table 4.** Problems identified in the observational analysis with implications for the microbiological analyses, and solutions proposed by managers and kitchen workers.

| Area | Problems identified | Solutions proposed and implemented |
|---|---|---|
| PREPARATION | Mistakes in operational flow | Establishment of new operational flows to prevent crossings and backflows |
| PREPARATION | Problems in physical-functional structure | Works to correct physical problems |
| COOKING | Use of rubber gloves in cooking | This item will not be allowed in this area; only disposable gloves will be used |
| COOKING | Inappropriate personal protective equipment and utensils | Purchase of silicon gloves and of specific utensils for the area |
| COOKING | Mistakes in operational flow | Establishment of new operational flows to prevent crossings and backflows |
| PREPARATION/ COOKING/ DISTRIBUTION | Mistakes in hand hygiene | Training on the correct procedure |
| PREPARATION/ COOKING/ DISTRIBUTION | Mistakes in procedures of hygiene and sanitation of the physical area, utensils and equipment | Definition of a standardized operational program for hygiene and sanitation. Purchase of appropriate equipment and hygiene and sanitation products |
| PREPARATION/ COOKING/ DISTRIBUTION | Circulation of people who do not work in the area | The circulation of people who do not work in the area should be restricted |
| PREPARATION/ COOKING/ DISTRIBUTION | Problems in the division of utensils for the areas, such as knives, containers, etc. | Purchase and identification of utensils for each area |
| | **Problems that were identified but were not solved** | **No correction** |
| PREPARATION/COOKING | Mistakes in conformity with physico-structural structure | |
| PREPARATION | Absence of a walk-in refrigerator for the thawing of meat and for prepared meat<br>Structural problems in the existing walk-in refrigerators | |
| PREPARATION/ COOKING/ DISTRIBUTION | Mistakes in the supervision of people along the production chain and in the detailing of responsibilities | |
| PREPARATION/COOKING/ DISTRIBUTION | Mistakes in the systematic microbiological control of foods and surfaces | |
| PREPARATION/COOKING/ DISTRIBUTION | Deficiency in the technical training of kitchen workers | |

were not quantified, only described in a report, for the two stages.

## DISCUSSION

In the present study, in food preparation, *Salmonella* spp. was isolated during both stage 1 and stage 2. In stage 1, results were positive for deboned chicken breast (DCB), deboned skinless whole leg (DSL), and whole leg (with bones and skin, WL). In stage 2, positive results were observed for deboned chicken breast (DCB), deboned skinless whole leg (DSL). This may indicate cross-contamination during any stage of the chicken production

chain, but it may also be due to contamination by spices, since the samples analyzed at this stage were already seasoned. However, it should be remembered that *Salmonella* spp. is not significantly implicated in the contamination of spices *in natura.*

Contamination of chicken meat by *Salmonella* spp. may indicate hygienic and sanitary issues in breeding sites, during slaughter or during handling of animals thereafter, as reported by several authors. In fact, Duarte et al. (2009), in a study that analyzed 260 chicken carcasses from five different processing plants, identified *Salmonella* spp. in 9.6% of tested samples. Contamination with *Salmonella* spp. was also detected by Borsoi et al. (2010), in a study that analyzed 180 chilled chicken carcasses, the pathogen was identified in 12.2% of samples. However, Nierop et al. (2005) report a higher value, 19.25% of 99 chicken carcasses were contaminated by *Salmonella* spp.

Duarte et al. (2009) and Nde et al. (2007), underline the fact that there is *Salmonella* spp. contamination in chicken breeding sites and flocks, and warn of the subsequent introduction of the bacterium the slaughter houses. In fact, the pathogen present in feathers and skin may contaminate the meat during slaughter, raising the bacterial counts on structures, equipment, utensils and even in scalding water, in a cross-contamination process that extends throughout the production chain. The endresult of such unsafe process is food with an inappropriate degree of microbiological quality, which represents a health hazard for the end consumer.

Franco (2012), underline that the investigation of *Salmonella* spp. is very important, since the bacterium plays a crucial role in the epidemiology of food poisonings due to its more severe etiopathogeny. For this reason, the analytical sampling is more representative (25 g), with acceptable threshold defined as the absence of the pathogen in this sample, while the threshold established for other microorganisms is given based on 1 g of the food matrix.

Indu et al. (2006), Pereira et al. (2006), and Castanha et al. (2010) investigated the antibacterial action of extracts as potential inhibitors of pathogenic agents, even of *Salmonella* spp. However, Fuselli et al. (2004) also pointed to the problems with–microbiological quality in terms of contamination by bacteria commonly observed in the environment, such as spores, fungi, some micrococci and mycobacteria.

It should be emphasized that hands, personal protective equipment used in the preparation of food were positive for *Salmonella* spp. (Table 3). This result may be associated with contamination of chicken meat with the bacterium, lending strength to the occurrence of cross-contamination in utensils and workers, and vice-versa. Nde et al. (2007) investigated the cross-contamination by *Salmonella* spp. in an abattoir and reported the event when hygienic and sanitary controls are ineffective. Malatova et al. (2009), in a study carried out in a commercial restaurant, identified the presence of *Salmonella* spp. in the hands of kitchen staff after processing foods that were positive for the pathogen. In a set of recommenddations on the prevention of *Salmonella* spp. Contamination, CDC (2013d) underlines the importance of the care taken with surfaces involved in food preparation.

In the industrial kitchen investigated in the present study, during the observation of the activities in the preparation area, both in stages 1 and 2, even after the implementation of new procedures, mistakes were identified concerning hand sanitation, use of protective equipment well as sanitation of the work area and in the management procedures in general. It was observed that preparation staff constantly circulated in other areas of the food production chain, with no due care to cleaning hands or removing personal protective equipment.

In the preparation area, workers hygienized their hands before collection, but positive results were observed for samples in the two stages (Table 2), which indicates the inadequacy of the process. A similar result was obtained by Gonçalves et al. (2013), in a study on the microbiological quality of hands of food preparation workers after hygienization, where the authors detected the presence of enteropathogenic bacteria.

In a study on the hygiene of hands, Cruz et al. (2009) indicate, as the likely causes for low adherence to the practice, the role of behavior, habits, the environment and deliberate intention. The managers of the kitchen carry out yearly training on several themes associated with safe food preparation. However, mistakes in hand hygiene persist, which may indicate issues concerning the adherence to this practice, since the facilities are equipped with the physical installations for the purpose.

Regarding the work methods in the kitchen investigated in the present study, in the preparation area, chicken meat was thawed in a chamber with no refrigeration, or in the area where meat is going to be prepared, the air conditioner on and set at 18ºC. After the chicken meat was removed from the package, it was left there for between 5 and 6 h on average, until seasoned and stored at 4ºC.

Franco (2012) reported that time and temperature control is essential to prevent the growth of *Salmonella* spp., since it does not thrive successfully in temperatures under 5ºC and above 47ºC. The samples analyzed in the present study were not kept under temperatures considered safe for preparation of chicken meat (Table 4), raising microbiological contamination concerns.

Considering that several studies address the occurrence of *Salmonella* spp. in chicken meat, it can be inferred that, in the event of an initial contamination the bacterium may develop where inappropriate time-temperature conditions associated with other factors like environment, equipment, hand hygiene and cross-contamination, prevalent.

The objective of the present study was to investigate the presence of *Salmonella* spp. during the different stages

of the processing of the food matrix, and to identify hygienic and sanitary conditions as of its consumption, but not to trace the microorganism presence from the farming unit and, transportation to the industrial processing plant. Therefore, it is not possible to ascertain that the positive results for *Salmonella* spp. reported here are due to problems happening in the farm or during transportation, since the equipment and utensils employed in the production chain and the workers may have carried the bacterium. Yet, Duarte et al. (2009) and Nde et al. (2007) described that poultry may be asymptomatic carriers of the pathogen.

Regarding the chicken prepared for consumption, positive results for *Salmonella* spp. were observed in three samples (DCB and WL) in stage 1 and in one sample in stage 2 (WL) (Table 2). These results configure the inadequacy of technical procedures of handling of foods after cooking, since during this process the foods were exposed to temperatures and times considered high and long enough to eliminate the bacterium (Table 4). According to Franco (2012), the minimum temperature to reduce viable cells (in a 6 log scale) is 70ºC for 2 min in a humid environment. However, according to a recommendation by ICMSF (1996), *Salmonella* spp. may be eliminated at 60ºC for 15 to 60 min, since these microorganisms are non-sporulated and are thermoliable.

The observation of processes during sample collection affords to conclude that; (i) the time between preparation of chicken in the kitchen and distribution was 4 h; (ii) to be transported, the prepared food is placed in a stainless steel container, which is placed in a hot box, (iii) the hot box is open only at the moment of distribution.

It is possible to conjecture that the contamination of the food ready for consumption takes place because of the cross-flow of the product, promoted by staff, from the preparation, the cooking a and distribution areas, due to problems in the handling of the food after cooking. This fact is confirmed by the positive results for *Salmonella* spp. in the hands and personal protective equipment of workers in the cooking and distribution areas, both in stages 1 and 2 (Table 2), even after the implementation of the new work procedures (Table 4). Another aspect that reinforces this hypothesis is the fact that the temperature reached by the cooked chicken may ensure the elimination of the pathogen.

The problems observed may have linked direct relationship with the microbiological results obtained in this study. In stage 1, one worker handled chicken ready for consumption wearing a reusable rubber glove, circulating in the kitchen; after they were removed from the ovens, trays were placed on top of one another, containing the food and with no protection in between; the preparation staff circulated in the cooking area wearing the same personal protective equipment; the raw chicken meat was kept near the cooked chicken in the cooking area; the gloves worn to handle raw chicken and those worn to handle the product ready for consumption were placed side by side, in an inappropriate place.

Concerning the circulation of staff, it should be stressed that this was a recurring habit in the kitchen, even after the implementation of the new procedures, defined between the two stages of this research. It was noticed that no instruction or restriction is in place for this matter: people circulate between the different work areas wearing the same protective equipment. This behavior promotes cross-contamination, since it carries microbial loads between work spaces.

After training, technical procedures were corrected. However, old, inappropriate habits in the handling of foods reemerged, with positive results for the etiological agent investigated, in food, in hands and on surfaces in stage 2. Concerning the food, possible to observe that, during the collection of samples, of DCB and DSL, in distribution, in stage 2, it was workers followed the new procedures strictly. However, in the WL samples, old mistakes were made, like touching the cooked food with the hands and piling up trays after removing them from ovens with no physical protection in between.

The results obtained in the present study were similar to the findings by Chan and Chan (2008), in a study carried out in Japan, between 1996 and 2005. The authors reported the occurrence of 5,967 outbreaks of food poisonings, of which 6.47% were due to poor hygiene of workers, and restaurants were the main sources of these infections.

A similar result was obtained by Guimarães et al. (2001), who investigated the microbiological quality of ready meals and the implications concerning the people handling these foods. The authors detected *Salmonella* spp. in beans and sautéed cassava. *Salmonella* Typhy, *Salmonella* Enteritidis and *Salmonella* spp. strains were isolated from staff handling these foods. In like manner, Madalosso et al. (2008) in a similar study in a commercial restaurant, associated the cause of the outbreak of food poisoning by *Salmonella* Enteritidis with the blender used in food preparation.

Considering cross-contamination as an important factor in the maintenance of *Salmonella* in food raw materials, Rubin et al. (2012) isolated *Salmonella* spp. from the hands of staff in charge of preparing food, from different surfaces, from a cloth used to tap-dry surfaces and from a sponge in an area used for food production.

Opposite results to those obtained in the present study were reported by Mesquita et al. (2006), who analyzed the microbiological quality of roast chicken in a food unit and obtained positive *Salmonella* spp. results in roast chicken, in hands, in containers or in counters. The same analytical profile was assessed by Vasconcelos and Filho (2010) in a study about the microbiological quality of meals prepared in the kitchens of commercial restaurants.

However, CDC (2013d) recommends the prevention to the contamination by *Salmonella* spp. based on the correct hand hygiene practices during preparation and

cooking procedures and during the handling of different food items, the hygiene of surfaces after contact with raw foods, mainly meat, and the avoidance of the contact of raw foods with cooked dishes, preventing cross-contamination.

In the present study, a decrease in the studied pathogen counts was observed after the training program and the implementation of procedural changes (Table 1). Changes were made in the operational flow, in the processing of chicken meat, in hygiene and sanitation procedures, in time and temperature control, and in the handling of cooked chicken, to improve service quality (Table 4). Nevertheless, this is not an acceptable result, since in Brazil the microbiological threshold is zero *Salmonella* spp. counts in 25 g of cooked meat and in the present study the pathogenic agent was isolated in the WL samples and in hands and personal protective equipment (Table 2), after the changes implemented. I can be concluded that the mistakes in the management of the service and in the handling of food remain.

It should be said that control measures have to be in place to monitor food processing, starting at the purchase of raw material of better quality and ending at the distribution of the food prepared, so as to reduce risks to the nutritional, sensory, physical, chemical and microbiological quality of cooked meals. It is only through these measures that it is possible to guarantee the production of safe foods.

## Conclusion

The high number of samples positive for *Salmonella* spp. *in natura* chicken meat, in the meat ready for consumption and on the surfaces analyzed in the present study showed that the procedures adopted in preparation and cooking are inappropriate.

*Salmonella* spp. is a fecal bacterium, and therefore it is essential to control the quality of the raw material used, the preparation procedures, and the hygienic practices of staff in working areas, utensils and personal protective equipment. Services presenting these kinds of problems should review or implement control protocols and redefine procedures and techniques to minimize and correct these issues.

It is only with the adoption of a mindset that aims to protect the worker and the end consumer that it will be possible to meet the requirements defined according to the right to healthy, quality nourishment.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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