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Research Article

A Study on Foodborne Bacterial Cross-contamination During Fresh Chicken Preparation

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Abstract

Background: Cross-contamination of foodborne pathogens from undercooked poultry meat to ready-to-eat food has been shown to be responsible for a number of foodborne disease outbreaks. Various studies have indicated that bacterial cross-contamination occurs during food preparation where bacteria present on food contact other surfaces and cause illness. **Objectives:** This study evaluated the ability of bacteria to survive and cross-contaminate other foods during the preparation of fresh chicken. *Salmonella spp.* cross-contamination from chicken to cucumber and utensils under various food handling scenarios was determined. **Methods:** Two scenarios were tested: in scenario 1, cutting board and knife used for cutting chicken without washing step were sampled. In scenario 2, cutting board and knife was washed with tap water separately after cutting chicken, and subsequently used for cutting cucumber. In scenario 1, chicken, cutting board, knife, and hands were sampled, and in scenario 2, cucumber was tested. Fluorescent *in situ* hybridization (FISH) method, using published Salmonella specific gene probes was used for *Salmonella* detection in samples taken from cross-contamination scenarios. A culture-based detection by Hektoen enteric agar was used for the confirmation of *Salmonella* species. **Results:** All the samples analyzed were found to be positive for *Salmonella spp.* with different contamination levels. These results were further confirmed by culture based method. In scenario 1, *Salmonella spp.* was detected by Sal-1 and Salm-63 oligonucleotide probes in all four samples (chicken, cutting board, knife and hands). A high contamination level was observed in chicken samples in comparison to samples collected from cutting board, knife and hands. In scenario 2, *Salmonella spp.* was detected by Sal-1 and Salm-63 oligonucleotide probes in the cucumber with very low contamination level. *Salmonella Enterica* was also detected by Sal-3 and Sapath-3 in both scenarios but the contamination level was not high as compared to *Salmonella spp.* **Conclusion:** In conclusion, *Salmonella spp.* cross-contamination during fresh chicken preparation to read-to-eat-food (cucumber) was confirmed by this study. The experimental data obtained in this study clearly suggest that it is extremely difficult to prevent the spread of *Salmonella spp.* during the preparation of raw poultry-based meals. Therefore, extreme precautions such as proper cleaning and sanitization of utensils, equipment and surfaces should be carefully followed during the preparation of fresh poultry meat-based food items.

Keywords: Cross-contamination; foodborne pathogens; *Salmonella*; chicken

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1. Introduction

The importance of food for human health has been widely recognized; eating safe food and optimal quantities of nutritious is a basic human right and a major precondition for a happy and productive life [17]. Therefore, the prevention of diseases and improvement of human health is of paramount significance, not only for governments but also for consumers themselves. Additionally, the production of safe food is based on the implementation of general preventive measures such as Good Hygiene Practices (GHP) and such application of measure represents the pre-requisite conditions required in preparing safe food [18, 19]. Cross contamination is a general term which refers to the transfer, direct or indirect, of bacteria or viruses from a contaminated product or surfaces to non-contaminated products" [2]. Cross contamination plays a significant role in transferring harmful pathogens such as *Campylobacter* and *Salmonella* to fresh produce [5, 16, 22].

Several studies have been conducted in households' kitchen settings to determine the effect of microbial cross-contamination via chopping board, cutlery, and hands on the microbiological quality of a realistically prepared meal ready to eat food [4, 6, 12, 20]. These and several other closely related studies indicated that microbial contamination of kitchen surfaces during food preparation due to foodborne bacteria present in foodstuff is one of the main causes of foodborne outbreaks. Many investigators revealed that bacteria cells adhered to those surfaces of domestic kitchens are not easily removed by normal cleaning procedures [3, 5, 22] and various pathogenic bacteria like *Campylobacter and Salmonella* survive in kitchen utensils, hands and possibly cross contaminate other food Several studies indicate that various pathogenic bacteria like *Campylobacter and Salmonella* survive in kitchen utensils, hands and possibly cross contaminate other food [7, 13, 21]. Therefore, it is very important to understand the adhesion of pathogenic bacteria to kitchen utensils that are used in the preparation of meal. Microbial contamination, can be easily passed from kitchen items to the food when contacting food, this normally happens when equipments are not efficiently cleaned and sanitized between each use [5, 22]. Microbial contamination from food to food occurs mainly when raw foods, especially poultry meat comes into contact with cooked or ready to eat foods through chopping board and contaminated utensils [11, 13]. A number of survey studies have reported on the unsafe practices common among consumers in the kitchen [5, 16]. Because of the inefficient use of cutting surfaces, as well as the applied cleaning methods may lead to the cross contamination of ready to eat food with the *Salmonella* spp.

As indicated in the several studies published over the past several years that food-borne illness remains a significant worldwide problem, and cross-contamination is believed to play a key role in the transmission of foodborne pathogens, especially *Salmonella* spp., which is one of the major causative organisms for the food-borne diarrheal disease locally and globally [2, 20]. The transfer of *Salmonella* is possible from naturally contaminated chicken to different cutting surfaces and then to cucumber handled on the common food cutting surfaces, as these kinds of food practices are very common in the household conditions. The present study was designed to closely mimic the cross-contamination scenarios of *Salmonella* from naturally contaminated fresh chicken to ready to eat food (cucumber) via cutting board, knife, and gloved hands. Two cross contamination scenarios were simulated, and Fluorescent *in situ* hybridization (FISH) and culture based methods were used to evaluate *Salmonella* cross contamination in this study. *Salmonella* spp. is the foodborne bacteria, which was chosen as a model bacterium in this study, as it belongs to the most foodborne bacteria causing infections worldwide. The main objective of the study was to evaluate the ability of *Salmonella* spp. to survive and cross-contaminate ready to eat food (cucumber) during the preparation of fresh chicken.

2. Materials and Methods

2.1. Sample Collection

The fresh chicken carcass was purchased from the slaughterhouse on the same day of the experiment to increase the probability of obtaining a true Salmonella positive sample and to decrease the chance of contamination and subsequent false positive results. The chicken carcass obtained from the slaughterhouse was immediately placed in a plastic bag and transported to the microbiology laboratory in an ice filled container. The sample was processed within one hour of being collected. Fresh and damage-free cucumber was also brought from the market. Clean and sterilized cutting board and knife were placed in the lab as well.

2.2. Cross-Contamination Scenarios

To evaluate the cross-contamination of *Salmonella*, two different cross-contamination scenarios were simulated. The experiment started with sterilizing the location of the experiment by using 70% of ethanol. The cutting board and knife were scrubbed and

rinsed thoroughly using commercial detergent and tap water, and then dried thoroughly with paper towels. Sterile Gloves were used throughout the experiment and 70% ethanol was used for further sterilization of hands. The following scenarios were tested.

2.3. Scenario 1 (Cross-Contamination of Cutting Board, Knife Blade and Hands)

The chicken sample was placed on the detergent washed cutting board. The chicken was first sampled directly before preparation by using sterile cotton swabs. This sampling was done based on preliminary experiments which have shown that all parts of the chicken have approximately equal number of bacterial load [12]. Thus, the sampling of the chicken was taken from different parts of the chicken, assuming that each part carried the same number of bacteria. The household kitchen scenario was simulated for the preparation of chicken by cutting chicken into small pieces with a detergent sterilized knife. The chicken pieces were turned once to simulate the amount of handling. Afterwards, the cutting board, the blade of the knife and both hands were sampled (Fig. 1).

2.4. Scenario 2 (Cross-Contamination of Cucumber)

A similar experiment as the one described above was conducted, with the exception that after simulating the preparation of chicken in scenario 1, the gloves were changed and both hands were washed thoroughly with soap and tap water, and then dried with paper towels. The cutting board and knife were rinsed just with tap water without using commercial detergent. Therefore, cutting board and knife remained unsterilized. Fresh cucumber was placed on the cutting board after rinsing it by tap water, and cut into pieces using the knife. This was to simulate the slicing of salad ingredients (cucumber) without sterilizing the cutting board and knife. The cucumber slices were then sampled by using sterile cotton swabs (Fig. 1). In total ten samples obtained from the 2 cross contamination scenarios were used for detection of *Salmonella*.

2.5. Detection of Salmonella Species

Molecular-based method such as fluorescent *in situ* hybridization (FISH) is a sensitive and robust assay applied for whole-cell detection via hybridization with nucleic acids within the target cell and without altering the morphological integrity of the cells [23].

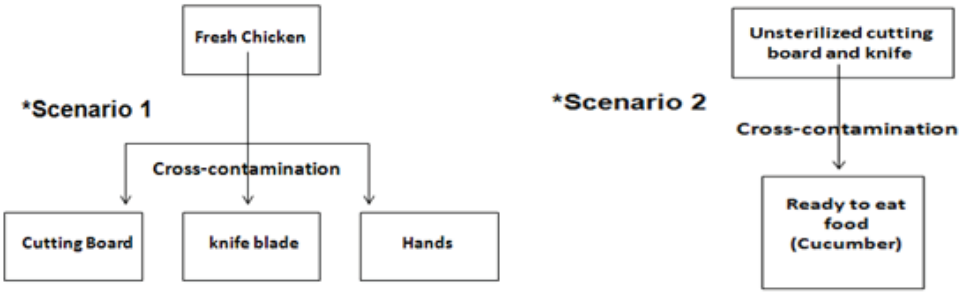

Figure 1: Cross-contamination scenarios determined in this study.

FISH is based on the hybridization of a genomic sequence characteristic of food-borne bacteria which specifically immobilized and label whole bacterial cells by fluorescently labeled rRNA oligonucleotide probes [1]. FISH assay was performed on obtained samples by following these common steps: fixation, sample preparation, hybridization of the probe and the target sequence, washing off the excess unbound probe and finally detecting and visualizing the labeled cells by the application fluorescent microscopy as described previously [14, 15]. Four oligonucleotide probes were used: two *Salmonella* spp. probe, Sal1 [10], Salm-63 [9]; a *Salmonella enterica* oligonucleotide probe, Sal-3 [15]; the *Salmonella enteric* subsp. probe (Sapath3) [10]. For further detection and confirmation of the presence of *Salmonella*, classical culture based method was preformed to support the results obtained from the rapid detection by FISH assay. Selective and differential agar (Hektoen Enteric Agar) was used to isolate and differentiate *Salmonella*. The swab samples from Chicken, cutting board, knife, hands and cucumber were streaked onto the surface of the plates. The plates were incubated for 24-48 hours and then viewed to check for growth of dark greenish colonies of *Salmonella*.

3. Results and Discussion

The hybridization conditions for the oligonucleotide probes used in this study (Sal1, Sal3, Salm-63 and Sapath3) were optimized to obtain a positive fluorescence signal for the detection of previously isolated *Salmonella* strains from the chicken samples [8]. These probes gave negative results with the non-target organisms. In most cases, hybridization with Sal1, Sal3, and Sapath3 probes showed best possible fluorescence signal, when 25% formamide was used in the hybridization solution. However, 35% formamide concentration was found to be the best for the Salm-63 probe. The results of FISH based analysis of the samples obtained from two cross contamination scenarios are summarized in table 1.

Samples	Oligonucleotide probes used for the detection of Salmonella			
	Sal1 (*Salmonella* spp)	**Sal3** *(Salmonella enterica)*	**Salm-63** (*Salmonella* spp)	**Sapath3** (*Salmonella enteric* subsp)
	25% of formamide	25% of formamide	35% of formamide	25% of formamide
CK	+++	+/-	+++	+++
B	++	++	++	++
K	+	+	+	+
H	+++	++	++	+++
Cucm	+/-	++	+/-	+++

Table 1: Detection of *Salmonella* in samples of food handling scenarios by FISH technique. *Abbreviation used: **CK:** Chicken, **B:** Cutting Board, **K:** Knife, **H:** Hands, **Cucm:** Cucumber, **(+++):** Signal with high contamination, **(++):** Signal with moderate contamination, **(+):** Signal with low contamination, **(+/-):** Contamination with weak signal.

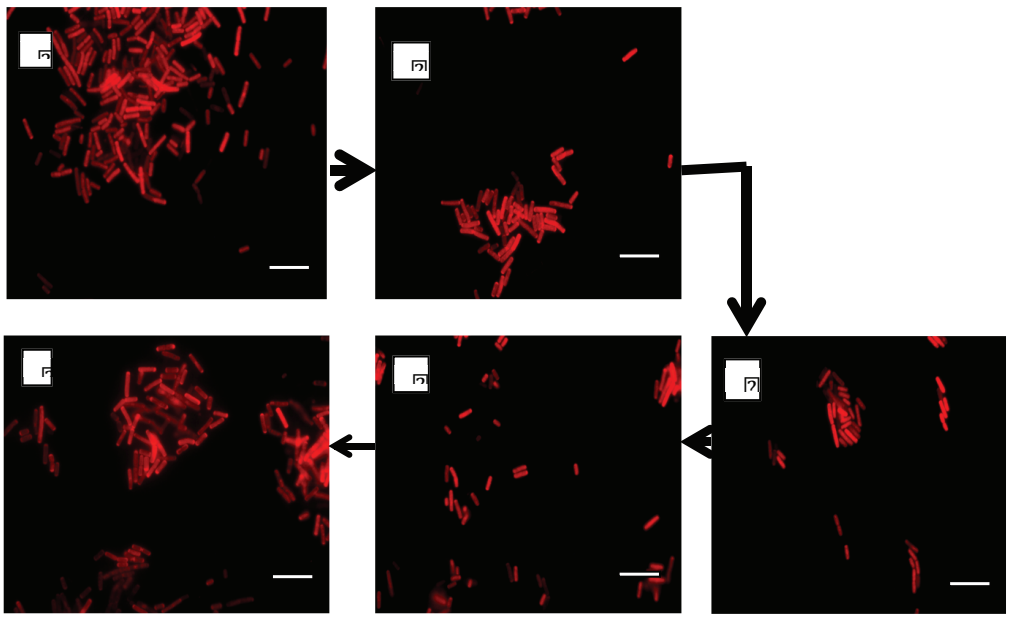

Figure 2: In situ hybridization of samples (chicken, cutting board, Knife, hands and cucumber): *Salmonella* species hybridized by TRITC–labeled Sal-1 probe. **A)** Chicken sample; **B)** Cutting board; **C)** Knife; **D)** Hands; **E)** Cutting board. Bar =10 μm and applies to all photomicrographs. Original magnification: 1000X.

All oligonucleotide probes included in this study detected *Salmonella* in all samples directly obtained from the five items. The probes Sal3 and Sapath3 were specifically hybridized with strain of *S. enterica* in the five samples, and the probes Sal1 and Salm-63 were specifically hybridized with *Salmonella* spp. in general. However, different fluorescence signals were observed from the probes indicating different levels of Salmonella spp. contamination. The representative florescence microscopy photomicrographs showing detection of Salmonella species targeted by each oligonucleotide probe are shown in (Fig. 2).

The results of this study have shown the presence of *Salmonella* spp. in all samples using four *Salmonella* specific oligonucleotide probes by employing FISH assay. All of the analyzed samples such as chicken, cutting board, knife, hands and cucumber was found positive for *Salmonella* spp. with different contamination levels. A strong positive hybridization signal with Sapathe3 probe was observed in three samples (Chicken, hands and cucumber). This observation indicates that these three samples were heavily contaminated with *S. enterica* subsp., as this oligonucleotide probe targeted 16S rRNA of *Salmonella enterica* subspp. A closely related study conducted by Zadernowska and Chajecka [8] detected *Salmonella* spp. in food products by FISH method. According to this study, 56 out of 86 food samples were found positive for *Salmonella* using FISH assay. This study suggested that the reason behind a huge number of positive results of *Salmonella* detection by using the FISH method is because this assay seems to be less prone to diverse physical and chemical properties of food products such as temperature, concentration of NaCl, and pH; which can work as stress factors for *Salmonella* spp. Additionally, the chicken samples were observed to have a sufficient number of ribosomes detectable by hybridization with Sal1 and Sal3 probes in comparison to cutting board, knife, hands and cucumber samples. A closely related study by Van asslt et al. [22] evaluated the overall transfer rates of *Campylobacter* for cutting board, knife and hands in the same range as observed in this study.

In this study, no clear correlation was found between the presence of *Salmonella* and the risk of Salmonellosis and this could be due to lack of quantitative data. Nevertheless, the obtained results gave an insight into the overall transfer of *Salmonella*. This indicated that one mistake (e.g. not washing, cutting board) could lead to severe consequences. The fact that transfer of *Salmonella* spp. via various contact surfaces are comparable was also found by Luber et al [12] and this study suggested average transfer of *Campylobacter* from hands or kitchen utensils to ready-to-eat foods ranged from 2.9 -27.5% (moderate contamination). In comparison, in this study, high to moderate level contamination with the *S. enterica* subspp. was observed using two specific probes which targeted this particular *Salmonella* subspecies. The transfer rate of foodborne bacteria (*Salmonella* and *Campylobacter*) from kitchen utensils or hands to ready-to-eat foods such as cucumber slices in this study and in the previous study [12], gave a good indication of the variability of the different surface cross-contamination levels that can be expected in a varied kitchen environment. Three samples (cutting board, knife and cucumber) were found with a low level of *S. enterica* subspp. contamination and this was determined by hybridization of the samples with Sal3 probe using the FISH technique. Furthermore, hybridization with another Salm-63 probe found low levels of *Salmonella* spp. contamination in the three enriched samples (Chicken, cutting

board and knife). Luber et al. [12] reported that cross-contamination was most likely to occur via the hands of the cook. Nevertheless, in this study hands were found with low to moderate level contamination as compared to the other samples. Moreover, the behavioral variation among consumers like washing hands, cutting board, and knife, also play a vital role in various levels of contamination. For instance, [5, 16] demonstrated that consumers followed unsafe practices during the preparation of meals which could lead to Salmonellosis and other foodborne outbreaks. In general, the idea of cross-contamination and its possible consequences are unknown to the most of the food handlers. Therefore, this study was designed to indicate the incidence of *Salmonella* spp. cross contamination during the preparation of fresh chicken under various food handling scenarios. The adherence of *Salmonella* on critical surfaces in the kitchen environment such as cutting board, blades of knife, hands and ready to eat food such as cucumber during and after the preparation of chicken was detected by four *Salmonella* species and subspecies specific gene probes employing FISH assay.

The identity of *Salmonella* detected by FISH assay using specific oligonucleotide probes was further confirmed by the growth of *Salmonella* isolates on Hektoen enteric agar (HEA). All *Salmonella* isolates developed characteristic dark green colonies with black center on the HEA. The overall results of this study indicated varied level of *Salmonella* cross-contamination in the items used for the fresh chicken preparation. This finding proved the occurrence of microbial cross-contamination during the fresh chicken preparation. The transfer of *Salmonella* from chicken to items and from items to cucumber provided an overestimation of Salmonellosis. This study demonstrated that contamination occurs frequently through the use of contaminated utensils in preparing other foods. Furthermore, *Salmonella* has potential to spread from chicken samples to the cutting board and other utensils. The consumer behavior in the household or food restaurant kitchen settings is one of the intrinsic factors which could lead to cross-contamination and foodborne illness.

4. Conclusions

This study provided an overall insight about how cross contamination could happen during the preparation of fresh chicken, and the ability of foodborne bacteria to survive on the food contact surfaces and cross contaminate ready to eat food. In conclusion, the results of this study clearly indicated that the raw chicken contaminated with *Salmonella* could cross-contaminate a large number of utensils in the kitchen environment. All of the analyzed samples (chicken, cutting board, knife, hands and cucumber) were found positive for *Salmonella* spp. with different contamination levels. The

experimental data obtained in this study clearly suggest that it is extremely difficult to prevent the spread of *Salmonella* spp. during the preparation of raw poultry-based meals. This study recommends that effective measures should be taken to avoid cross-contamination in the kitchen environment. For example, raw and ready to eat food should be handled separately; the same cutting board and chopping knife should not be used for both raw and ready to eat food. Furthermore, hands and utensils that come into contact with raw foodstuffs must be properly washed and sanitized. This study also has found that even after casual rinsing of the cutting board and knife with tap water, a larger number of *Salmonella* cells remain attached to these critical surfaces. In order to minimize the chances of Salmonellosis, good hygiene practices should be followed by food handling personnel. Further studies are recommended on the consumer behavior in the domestic kitchen environment and more specifically to evaluate the extent of accurate sanitary measures practiced by consumers to prevent cross-contamination. In addition, a study on the effect of hygienic measures on the foodborne bacterial cross-contamination in the UAE is highly recommended.

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