Public Health Impacts of *Salmonella* from Raw Poultry: Prevalence in Retail Markets, Exposure Risk Factors During Handling and Epidemiological Links to Clinical Isolates.

Eyob Mazengia, M.S.

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Reading Committee:

John Scott Meschke, Chair

Stephen James Libby, Graduate School Representative

Ann M. Kimball

Judd L. Walson

Mansour Samadpour

Program Authorized to Offer Degree:

University of Washington Department of Environmental and Occupational Health Sciences

University of Washington

Abstract

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Eyob Mazengia, M.S.

Chair of the Supervisory Committee:

Associate Professor John Scott Meschke

Department of Environmental and Occupational Health Sciences

ABSTRACT:

Infections with *Salmonella* is a global public health concern. The majority of *Salmonella* infections are believed to be acquired as results of the consumption of contaminated foods. The ubiquitous nature of *Salmonella* in the environment, birds, amphibians, reptiles, farm and wild animals, have made source attribution challenging. *Salmonella* prevalence studies have

frequently reported poultry products to have several magnitudes of order higher contamination rates than other animal food products. Therefore, poultry products have been often cited as the major source of salmonellosis in the population and have resulted in numerous efforts to reduce the overall prevalence of *Salmonella* on raw poultry products through advancement in the industry and governmental initiatives. Despite substantial reduction in the prevalence of *Salmonella* on poultry products, the incidence of salmonellosis in the population has not declined. The lack of correlation between the prevalence of *Salmonella* on raw poultry products and that of the incidence of salmonellosis in the population has spurred serious discussion whether or not; there are other major sources of *Salmonella*, the utility of reducing the prevalence on raw poultry, the responsibility of consumers and whether or not direct links to the incidence of salmonellosis can be made. While there have been numerous prevalence studies conducted over the years, very few have had large sample size, focused on a particular geographical location, sampled over an extended period of time and most importantly were not able to quantify the levels of *Salmonella* on positive carcasses. Therefore, these studies have not been able to make direct associations between the serotypes and the genetic profiles of those isolates from raw poultry to those of clinical cases reported in the same geographical locations. Therefore, three independent studies were conducted to help bridge the current knowledge gaps. The objectives of the three studies were: **Study I**) Obtain the prevalence, concentration, antibiotic sensitivities, and the serotypes of *Salmonella* on raw poultry from retail stores in Seattle, Washington. **Study II**) Through direct observations, identify the major risk factors leading to cross contaminations during raw poultry handling in domestic kitchens. **Study III**) Characterize the genetic profiles of the *Salmonella* isolates recovered from raw poultry and evaluate their association with clinical cases submitted from the same geographical areas. The

outcomes of each of the listed objectives are presented in the following chapters in a manuscript format with introduction, method, analysis, results and discussion sections.

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Chapter One

INTRODUCTION

1.0: *Salmonella* Background

It is estimated that annually there are 9.6 million (90% CI: 6.6 – 12.7 million) domestically acquired foodborne illnesses resulting from 31 pathogens in the United States (Scallan *et al.*, 2011). These foodborne illnesses are believed to result in 55,961 hospitalizations and 1,351 deaths annually. Taking into consideration underreporting and infection acquired due to traveling outside the United States, about 1.0 million cases of foodborne illness are estimated to be caused by *Salmonella* spp., resulting in 168,000 physician visits, in 16,000 hospitalizations and about 400 deaths (Scallan *et al.*, 2011). According to the current estimates, infection with nontyphoidal *Salmonella* spp. is the leading cause of hospitalization (35%) and death (28%) of foodborne bacterial infections (Scallan *et al.*, 2011). The economic burden of *Salmonella* infections is estimated at 2.6 billion annually (ERS, 2009). Among the 1,097 reported foodborne outbreaks that occurred in 2007 in the United States, outbreaks resulting from *Salmonella* spp. accounts for the second most common cause followed by *Norovirus* (CDC-MMWR, 2010). Confirmed outbreaks underestimate the magnitude of *Salmonella* as one of the major food safety concerns, since more than 80% of all salmonellosis cases occur individually rather than as outbreaks (WHO, 2010). In addition to acute infections, some individuals develop long term sequelae of reactive arthritis and/or irritable bowel syndrome (CDC, 2010). In the rest of the world, the burden of salmonellosis is equally as concerning. Based on data from 4,093

internationally reported foodborne outbreaks between 1988 and 2007, 46.9% the outbreaks were attributed to *Salmonella* spp. (Greig and Ravel, 2009).

Salmonella incidence rate of 6.8 per 100,000 population had been set by The U.S. Department of Health and Human Services (DHHS) as the target for the Healthy People 2010 objectives (CDC-MMWR, 2010). However, based on the 17,468 laboratory confirmed cases of infections reported to CDC in 2009, the incidence of *Salmonella* per 100,000 population was 15.19 (CDC-MMWR, 2010). The age group with the highest incidence rate is children under the age of 4 years, and in 2009 the incidence of *Salmonella* per 100,000 individuals in this age group was 72.19. In 2006, 24% of the 40,666 isolates reported to the U.S., Centers for Disease Control were from children less than five years of age (DHHS, *Salmonella* Summary report 2006). In Washington State, the incidence rate in 2008 was 12.8 cases/100,000 individuals and has been stable at this rate for many years (WA Communicable Disease Report, 2008).

It is reported that anywhere between 55% to 96% of salmonellosis is acquired as results of the consumptions of contaminated foods (Havelaar *et al.*, 2008, Sumner *et al.*, 2003) and poultry has been identified as one of the major sources of *Salmonella* with estimates ranging from 10% to 22% of cases (Havelaar *et al.*, 2008, Mullner *et al.*, 2009, Pires *et al.*, 2010, Ravel *et al.*, 2009, USDA/FSIS 2008, Van Asselt *et al.*, 2009). Among the five major factors that were identified to contribute to foodborne illnesses, such as improper cooking, improper food storage conditions, lack of hygiene, cross contamination and acquiring food from unsafe sources, four of these factors directly point to the weakness in the final stages of food processing that take place in kitchens (WHO, 2006).

In general, despite the higher prevalence of *Salmonella* present on raw poultry carcasses, it is known that only a very small fraction of carcasses containing more than 3 log *Salmonella* contribute to 75% of the illnesses (Straver *et al.*, 2007). Current risk models show that lower contaminant levels do not significantly contribute to human salmonellosis and contaminant levels more than 10^4 *Salmonella* per carcass are responsible for increasing the risk of cross contamination and the likelihood of causing human salmonellosis (Straver *et al.*, 2007; Cox *et al.*, 2010). While the proportion of raw carcasses with high levels of *Salmonella* contamination is small, carcasses with higher *Salmonella* load are difficult to differentiate from those with none or lower levels of contaminations. Therefore, consumers need to be vigilant during handling of raw poultry. Identifying the major risk factors in the final stages of food handling that contribute to the spread of *Salmonella* from highly contaminated carcasses can improve food safety and reduce the risk of salmonellosis.

During the final stages of food handling practices, there are two major ways a contaminated raw poultry can result in salmonellosis. It is either as a result of undercooking or due to cross contamination of ready to eat foods (Fisher *et al.*, 2007). Since *Salmonella* contamination is found mainly on the outer surfaces of carcasses (Lumber, 2009, Brichta-Harhay *et al.*, 2008), it is believed that undercooking is not the major route of infection, but rather cross contamination of ready to eat foods (Luber P., 2009; Mylius *et al.*, 2007; Nauta *et al.*, 2007; Who/FAO 2003).

Currently, there are very limited quantitative microbial data available to help evaluate the risk introduced by some of the improper food handling practices (Smadi H. and Sargeant JM, 2013,

ASM, 2006, WHO, 2002), particularly resulting from events leading to cross contaminations and therefore unable to adequately evaluate the efficacy of current and proposed intervention procedures. The critical data gaps identified for risk assessment purpose include; the concentration of *Salmonella* on positive birds, quantitative data for several steps of poultry processing, quantitative data regarding the final food handling practices including cooking and handling and the concentration and prevalence of *Salmonella* in the final food handling stages (WHO/FAO, 2009; Cox *et al.*, 2010).

1.1: Epidemiology of *Salmonella*

There are more than 2500 serotypes of *Salmonella* identified thus far (WHO, 2010). Although most are capable of causing diseases in humans, some are known to be host specific (WHO, 2010, Hoelzer K. et al., 2011). The incidence of salmonellosis is higher during warmer months, which reflects *Salmonella*'s ability to proliferate in foods and the environment and thus cause diseases in humans. It is not clear whether the increase in salmonellosis during warmer months is a reflection of poor handling practices or an increase in the contamination of raw poultry during warmer months. Proper refrigeration (e.g., < 41F) of foods and proper cooking temperature of foods (e.g., > 165F) are known to minimize the risk of *Salmonella* infections from foods. Among other factors, the severity of infections depends on age, immune status, and the strain(s) of *Salmonella* involved. In addition, there are differences in their ability to survive stress, colonize animals, invade tissues and their ability to cause disease with varying outcomes (Cox *et al.*, 2010). The incubation period of *Salmonella* is between 1 to 3 days and symptoms include abdominal pains, diarrhea, fever and sometimes vomiting. While the majority of cases

recover within a 4 to 7 day period, some cases require hospitalization as a result of dehydration from severe diarrhea or the spread of infection into the blood (CDC, 2010).

Salmonella species are ubiquitous and are commonly found in the digestive tracts of diverse animals (Hoelzer K. et al., 2011, Pangloli, 2008; Sanchez S. *et al.*, 2002). Pangloli *et al.*, 2008 evaluated the occurrence and patterns of *Salmonella* spp. from dairy cows, calves and farm environment and *Salmonella* spp. were found in 38% (2,918/7680) of the samples tested. The samples that were positive for *Salmonella* included, animal swabs, bird droppings, feeds (mixed grain, silage), water, air (in milking parlor and calf barns), soil bedding materials, insects, bulk tank milk, and milking parlor (Pangloli, 2008). The prevalence of *Salmonella* contamination in foods of animal origin in the U.S. have been reported; 9.6% in retail market pork (Duffy EA, 2000), 4.2% in commercially produced ground beef (Bosilevac *et al.*, 2009); 61% (organic brand) and 44% (non-organic brand) of chickens from retail stores in Maryland (Cui *et al.*, 2005). The prevalence of *Salmonella* in various meat products analyzed by the Food Safety and Inspection Services (FSIS) for the year 2009 has shown a reduction over the previous years, particularly since 1996 when the agency required producers to use the Pathogen Reduction Hazard Analysis and Critical Control Points (PR/HACCP) (MMWR, 2010). In 2009, the U.S. Food Safety and Inspection Services tested a total of 29,116 various food samples and the percent positive rate of *Salmonella* per product class were: broilers (7.2%), market hog (2.3%), cow/bull (0.6%), steer/heifer (0.2%), ground beef (1.9%), ground chicken (18.2%), ground turkey (10.7%) and turkey (3.8%).

Salmonella infections and carriage are also common in wild and domestic animals (Hoelzer K., et al., 2011; Sanchez S. *et al.*, 2002). Contacts with domesticated and wild animals have been implicated in several salmonellosis outbreaks (Hoelzer K., et al., 2011). Cats and dogs are known to carry *Salmonella*. *Salmonella* carriage in healthy dogs is estimated to be between 1-36% and healthy cats 1-18% (Sanchez S. *et al.*, 2002). Some of the serotypes that are common among human salmonellosis cases such as *S.* Typhimurium, *S.* Heidelberg and *S.* Kentucky, have been isolated from dogs, particularly from dogs fed raw food diet (Hoelzer K., et al., 2011). Amphibians and reptiles, which are increasingly handled as pets, have been implicated in numerous outbreaks (CDC, 1990). Some of the serotypes that are more commonly cultured from reptiles include Java, Stanley, Marina, Poona, Pomona and sub-species Arizonae (Sanchez S. *et al.*, 2002) and serotypes often isolated from humans such are not often recovered from these reptiles.

In 2007, there were 136 single-etiology outbreaks that were due to *Salmonella* spp., accounting for 27% of all the reported outbreaks (MMWR, 2010). In the U.S., only 0.13% of sources of *Salmonella* is identified during outbreaks (Barber *et al.*, 2003), making it very difficult to summarize most of the sources contributing to salmonellosis. In the last few years, major outbreaks related to non-poultry products have been implicated including, jalapeno peppers, peanut butter, and tomatoes (CDC 2005, 2008, 2010).

Among all the serotypes reported in the U.S., the top most common *Salmonella* serotype was Enteritidis. In Washington State, the top ten most *Salmonella* serotypes recovered from clinical isolates in order of frequency are: Enteritidis, Typhimurium, Heidelberg, NewPort, Agona,

Branenderup, 4[5],12:i:-, Stanley , Montevideo, Hadar, Infantis. (WA-communicable Disease Report, 2011). Some of these serotypes are the same as the top ten most frequently isolated serotypes originating from non-clinical and non-human isolates (DHHS-*Salmonella* Summary 2006). The most common serotypes implicated in outbreaks in 2006 in the U.S., were Enteriditis, Typhimurium, Newport and Heidelberg (DHHS *Salmonella* Summary 2006). These common serotypes have been frequently isolated from various domestic, wild and farm animals including bovine and chickens. Nevertheless, it is not necessarily true that the most frequently isolated serotypes from meat products is also the same as those serotypes isolated from human cases. For example, serotype Kentucky makes up 50% of the NARMS retail chicken samples, yet it causes only 0.3% of human cases in the United States (Cox *et al.*, 2010).

1.2: Antibiotic Resistance

Multiple antimicrobial drug resistance among *Salmonella* serotypes from foods of animal origins is a food safety concern, because treatments of humans with multidrug resistance can be complicated and costly. Drug-resistant *Salmonella* spp. emerge in response to antimicrobial usage in food animals as well as some *Salmonella* spp. serotypes are more prone to develop resistance than others (WHO, 2010). In 2004, a national survey in the U.S. found 39% of *Salmonella* enterica serovar Typhimurium isolates from clinical isolates were resistant to one or more antimicrobial drugs and 23% showed resistant to five-drugs (CDC, 2003). In addition, 15% (28/290) of *Salmonella* enterica serovar Newport was reported to be resistant to multiple antimicrobial drugs including cephalosporins (CDC, 2003). *Salmonella* spp. isolated from chicken breast in 2007 showed resistant to multiple antibiotics in every class/subclass of antibiotics including aminoglycosides, aminopenicillins, beta-lactams, cephalosproins (3rd

generation), cephamycins, folate pathway inhibitors (give examples in parenthesis), phenicols, quinolones and tetracyclines (NARMS, 2007). The percent resistant *Salmonella* isolates from chicken breasts ranged from 1% for chloramphenicol to 41.4% for tetracycline. Similar levels of antimicrobial resistance patterns were also found for *Salmonella* isolates from ground turkey and pork chop (NARMS, 2007). *Salmonella* isolates from ground beef (n=13), were resistant for gentamicin and sulfisoxazole (NARMS, 2007). A national prevalence study of multi drug resistance of *Salmonella* isolated from commercially produced ground beef in the U.S. found 0.6%, with serotypes Dublin, Reading and Typhimurium exhibiting resistance to between two and ten antibiotics (Bosilevac *et al.*, 2009). A retrospective study of individuals with *Salmonella* serovar Newport infection showed that individuals with multidrug resistant infections were more likely to report exposure to cattle, farms, and unpasteurized milk (Karon *et al.*, 2007). The spread of multidrug-resistant *Salmonella* serovar Typhimurium phage type DT104 and others internationally in animals and humans underscores the seriousness of multidrug resistant organisms in foods (WHO, 2010; Aarestrup *et al.*, 2007).

1.3: Prevalence of *Salmonella* on Raw Poultry

Since 1994, the United States Department of Agriculture Food Safety and Inspection Services (USDA-FSIS) has monitored the prevalence of pathogenic and nonpathogenic microorganism on poultry carcasses as part of the Nationwide Young Chicken Microbiological Baseline Survey (YCBS) (USDA-FSIS, 2009). The sampling of carcasses is done while still in the processing plant. In 1995, the prevalence of *Salmonella* was about 20% and in 2000 it dropped to about 7.5%. Between 2000 and 2005, the prevalence steadily increased from 7.5% to 14%. For the years 2006 through 2008, the prevalence rates dropped once again to 7.5%. The latest data we

have is for 2007 and 2008, as part of the YCBS, FSIS analyzed 6,550 samples from 182 USDA inspected poultry establishment for *Salmonella*, *Campylobacter* and four non-pathogenic indicator organisms (generic *Escherichia* coli, total aerobic bacteria, *Enterobacteriaceae* and Coliforms) (USDA-FSIS, 2009). These samples included at the Rehang (prior to evisceration) and Post chill (post evisceration) of poultry processing stages. The prevalence rate of *Salmonella* at post-chill from the YCBS baseline data collected from July 2007 through June 2008 was 7.5% ± 0.43% (USDA-FSIS, 2009). While the decline in the prevalence rates between 14% in 2005 to 7.5% in 2008 is encouraging, the latest prevalence rate of 7.5% is similar to what was estimated in 2000.

A study by Brichta-Harhay et al (2007) from a single poultry abattoir found a much higher prevalence rate than the YCBS. The prevalence of *Salmonella* on post-chill carcasses was 41.7 ± 17.6% (Brichta-Harhay *et al.,* 2007). Other studies of processing plants include; in Georgia comparing between Organic vs. traditional commercial conditions found 37% and 31% respectively (Bailey *et al.,* 2005). The latest national market survey of the prevalence of *Salmonella* on raw poultry at retail levels in the United States ranged between 4.5% to 20%, with the national average of 12.1% (NARMS, 2008). Other previous studies have reported 33.9% in Georgia (Simmons *et al.* 2003), 43% in Ohio (Bokanyi et al, 1990), 15% nationwide with organic brands showing higher prevalence (Consumer reports, 2007). As for other developed countries, the prevalence rates at retail levels are, 2.4% in Australia, 16.3% in Belgium, 22.5% in Bulgaria, 15.0% in Germany, and 13.5% in Italy (Cox *et al.,* 2010). Differences in *Salmonella* prevalence between countries and even between studies need to be interpreted with extreme caution, since the differences can solely be the results of differences in sampling and laboratory

methods used. Because of difference in study design and detection methods employed, it is difficult to compare prevalence rates between studies (Cox *et al.*, 2010).

1.4: Prevalence vs. Incidence of salmonellosis

There is no established correlation between prevalence rate and that of the concentration of *Salmonella* on poultry carcasses. Neither is there a correlation between the prevalence rate of *Salmonella* on raw poultry at retail shops and that of the incidence of salmonellosis in the population (Cox *et al.*, 2010). The proposed standard by FSIS assumes that establishments with higher *Salmonella* prevalence place the public at risk than those of establishments with a lower prevalence rate. A risk assessment study conducted by Straver et al (2007) was able to show that over 75% of annual predicted illnesses are caused by a small fraction (0.8%) of poultry containing more than 3 log *Salmonella* at retail levels (Straver *et al.*, 2007).

Despite the reduction in the overall prevalence of *Salmonella* on poultry carcasses from reported pre HACCP rate of 20.0% (USDA-FSIS-199) to 7.3% in 2009 (USDA/FSIS 2009), the incidence of *Salmonella* in the population has not declined. There is no correlation between the prevalence rate of *Salmonella* on raw poultry to that of the incidence of salmonellosis in the population (Cox *et al.*, 2011). The lack of correlation between the prevalence of *Salmonella* on raw poultry and that of the incidence rate of salmonellosis has resulted some to question the utility of reducing the overall prevalence of *Salmonella* on raw poultry (Cason JA, 2012, Cox *et al.*, 2011) as a strategy to reducing the incidence of salmonellosis in the population.

Since only a small proportion of those infected individuals seek medical care and their source of infection ascertained (Hennessy WT, *et al.*, 2004), there have not been major sources of *Salmonella* infection revealed, other than poultry products, mainly based on the higher prevalence of *Salmonella* present on raw poultry compared to other food animal sources. Either the assumption that 95% of salmonellosis cases in the population is foodborne is an overestimate and there are multiple major non-food sources that have not been well understood. Or, if indeed 95% of salmonellosis infection is foodborne, the impact of poultry and other food animal sources should be closely examined to identify the risk factors starting from processing all the way to consumption.

1.5: Level of *Salmonella* on Raw Poultry Carcasses

Risk assessment studies have reported that the level of *Salmonella* contamination on raw poultry are better correlated (predictor) of the risk of human illnesses than prevalence (Cox *et al.*, 2011, Straver *et al.*, 2007). Nevertheless, due to the cumbersome nature of the enumerating methodologies available and the cost associated with them, very few studies have been conducted enumerating the concentrations of *Salmonella* on poultry both at the processing and retail levels**.** The YCBS study for the year between 2007 and 2008 from a total of 3,275 post chilled raw poultry tested, only 170 (5.19%) were enumerated and none of those enumerated exceeded 30 MPN/ml. Of those enumerated, 123 (46.1%) yielded from 0.0301 to 0.3 MPN/ml, 38 (14.2%) samples ranged from 0.301 to 3.0 MPN/ml and 9 (3.3%) had between 3.01 and 30 MPN/ml (USDA YCBS, 2008). The level of *Salmonella* on broiler carcasses from a single poultry processing establishment in the United States, using a membrane filtration method was evaluated (Brichta-Harhay *et al.*, 2007). Hundred Eighty broiler carcasses from pre-wash (60),

prechill (60) and Postchill (60) were included in the study. The geometric load of *Salmonella* was found to be 14,800 CFU/carcass for pre-wash carcasses, 2,240 CFU/carcass for prechill carcasses and 40 CFU/carcass for postchill carcasses. In England, 241 carcasses sampled from retail stores, using direct plating technique, yielded only two poultry samples found to contain 10^4 *Salmonella* per carcass (Jorgenson *et al.*, 2002).

Using data from the latest Young Chicken Baseline Study (YCBS) conducted between 2007/2008 (i.e., prevalence rate of 7.5%) and that of the *Salmonella* Verification Program data for the year 2008-2009, FSIS established a *Salmonella* performance Standard for poultry processors (USDA-FSIS, 2009). As part of the *Salmonella* performance Standard, FSIS classifies poultry processors into three categories, based on the *Salmonella* prevalence rates (USDA-FSIS, 2009). FSIS is proposing to change the current bench mark level of 23.5% to 7.5% based on the latest YCBS survey data. The proposed regulation calls for establishment in category one to maintain four or less *Salmonella* positives per 51 samples in the last two most recent completed sets. Establishments in category II are expected to maintain below the standard guidelines of five to ten *Salmonella* positive carcasses in the most recent completed samples. Those in category 3 are those establishments that exceed 11 or higher *Salmonella* positive carcasses. For each category there is a difference in the intensity of inspections and expected interventions that need to be implemented by the industry.

Using a risk model, FSIS predicted that implementation of the current proposed standard, hence lowering the standard guideline from 23.5% to 7.5% positive rate, which expects to result in 7 to 8% additional establishments meeting the proposed guidelines, can result in the reduction of an

estimated 12% of human salmonellosis in the population (USDA-FSIS, 2009). FSIS admits that, the risk model used to predict the estimated 12% reduction of human illnesses has a lot of uncertainty. One of the major shortfalls of the model is that, the YCBS survey data addresses the prevalence and concentrations of *Salmonella* in broiler chickens at stages removed from retail level, therefore the prevalence estimates may not be equivalent to what one will expect at retail levels. Therefore, the regulatory pressure to reduce *Salmonella* in processing plant samples would not improve public health unless *Salmonella* prevalence and concentration were also reduced at points closer to consumption (Fletcher, 2006). Furthermore, if reduction of the incidence of salmonellosis is the criteria used to evaluate the regulatory standards, the regulatory standards set need to have a direct link to that of public health outcomes.

1.6: Food Handling Practices in the Kitchens

While implementation of control efforts at both processing and final food preparation stages are critical at reducing the spread of *Salmonella*, given the low numbers of *Salmonella* on market ready poultry carcasses, the food safety gains may not be drastic if prevention efforts solely focus on poultry processing establishments.

Despite the level of *Salmonella* present on raw poultry upon arrival to the kitchens, if everyone follows effective food handling procedures and uses intervention efforts that are efficacious, the risk of salmonellosis should be reduced to a minimum. The high incident rates of salmonellosis in the population partly point to the failure of the final stages of food handling practices in domestic and commercial kitchens. It is reported that up to 87% of foodborne outbreaks, with many countries reporting between 10% to 50%, are associated with food prepared or consumed

in homes (van *et al.*, 2008; Redmond and Griffith, 2003), therefore intervention efforts that target the various risks associated with handling raw chicken by consumers can make significant impact. Improper food handling practices in the kitchens, can create conducive environments for the re-growth of *Salmonella* and/or the increase chance of cross contamination to other food types or the kitchen environment (Mylius *et al.*, 2007; van *et al.*, 2008, Luber, 2009), leading to the continued spread of *Salmonella* to the population. Given that the *Salmonella* levels on raw poultry are below those normally associated with human salmonellosis, a considerable mishandling must take place during the final food handling procedures to cause illnesses. On the other hand, if products with significantly higher contamination levels of *Salmonella* are handled, they may not require substantial level of mishandling to result in cross contaminations and infections.

For example, improper thawing of frozen chickens can increase the total bacterial load and therefore partly negate the intervention efforts at upstream poultry processing stages. Some typical food handling practices that can increase the chance of re-growth or create the opportunities for cross contamination in kitchens include: extended room temperature storage, thawing frozen poultry at room temperature or under warm running water, not following proper hand washing procedures after handling raw poultry, use of common equipment between raw and cooked products or other ready to eat foods (e.g., cutting boards, utensils), improper storage conditions of raw or partially cooked poultry and reuse of contaminated wiping cloths, which might be harboring microorganisms**.**

Among the many ways salmonellosis can be acquired from poultry, the two major pathways are believed to be either under cooking or cross contamination events (Lumber, 2009) with cross contamination events being the most dominant pathway (Nauta *et al.*, 2009;Brynestad et al, 2008; Nauta *et al.*, 2007). Bacterial contamination of carcasses is an outer surface phenomenon (Lumber *et al.*, 2007; Brichta-Harhay *et al.*, 2008), therefore the typical cooking temperature and durations are believed to minimize the risk associated with undercooking. On the other hand, cross contamination events are harder to monitor and assess their impacts, since there are many ways cross contamination can take place.

The Centers for Disease Control estimates 18% of foodborne diseases to be results of the use of contaminated equipment and 19% associated with poor hygiene practices (CDC, 2000). Cross-contamination activities can result from the re-use of equipment or utensils that are contaminated, lack or improper hand washing practice and improper food storage conditions.

1.7: Bacterial Transfer Rates and Cross Contamination

One of the pre-requisites for cross contamination risk is bacteria's ability to transfer between various environments. Several studies have evaluated the rates of bacterial transfers (Luber et al, 2006, Chen *et al.*, 2001; Cogan *et al.*, Kusumanngrum *et al.*, 2004). These studies have evaluated the rates of bacterial transfers between different environments including, from raw chicken to hands (Montville et al, 2001 and Chen *et al.*, 2001); from chicken to cutting boards (Kusumaningrum *et al.*, 2004; Chen *et al.*, 2001); from contaminated hands to ready to eat foods (Montville *et al.*, 2001; Chen *et al.*, 2001; Smith *et al.*, 2003); from hand to tap and vice versa

(Chen *et al.*, 2001); and from cutting board to salad (Kusumaningrum *et al.*, 2004; Chen *et al.*, 2001). In addition, some studies have evaluated the persistence on various environments, such as on hands (Chen *et al.*, 2001), on cutting boards (Cogan *et al.*, 2002) and persistence on contaminated salad (Smith *et al.*, 2003). A study by Luber *et al.*, found transfer rates from hands or kitchen utensils to ready to eat foods ranging from 2.9 to 27.5% (Luber *et al.*, 2006). The probabilities of transfer between various environments reported by the above cited studies include, 0.0415 (chicken to hands), 0.0125 (from chicken to cutting boards); 0.343 (from cutting board to salad); 0.0207 (from hand to salad); 0.0016 and 0.023 (from hand to tap and vice versa respectively). Due to similar adhesion characteristics to *Salmonella* spp., *Enterobacter aerogenes* has been used as a surrogate organism in place of *Salmonella* in some of the transfer studies (Chen *et al.*, 2001; Montville et al, 2001). The factors that influence rates of transfer include both intrinsic and environmental factors. The intrinsic factors include, the presence of exopolisacaride, biofilm, cluster formation and the presence of extracellular structures and the environmental factors are the amount of pressure applied, levels of moisture, roughness of surfaces, and contact time (Rodrigues *et al.*, 2008).

1.8: Food Attribution Approaches

Over the last few years, the importance of food attribution has been gaining momentum by governmental agencies, food industries and food safety specialists (Hald *et al.*, 2004, Batz *et al.*, 2004; Greig and Ravel, 2009). Effective risk based preventive measures are harder to implement unless there is an integrated and reliable quantitative estimate of the major sources of microbial contamination of foods (Havelaar *et al.*, 2007). If the public health professionals are unable to assign risks to the various food sources, it is difficult to expect consumers to differentiate the

different risks involved in handling various types of foods (Hoffmann *et al.*, 2006). The problem can be compounded because the governmental agencies and other food safety professionals are unable to design laws or implement food safety regulations to substantially reduce the risk of foodborne illnesses to the public (Hoffmann *et al.*, 2006). Therefore, the lack of clear quantitative data of the various sources of *Salmonella* contamination (e.g., through surveillance and outbreak investigations) impedes our ability to implement effective control measures and also evaluate the effectiveness of any implemented preventive measures (ICMSF, 2006).

There are multiple approaches used to measure food attribution (Batz *et al.*, 2004, Pires , 2009). Some of the methods include the use of outbreak data, epidemiological study (case control), risk assessments, food surveillance data and expert elicitation (Batz *et al.*, 2004, Pires, 2009). Batz *et al.* loosely termed these methods into two major approaches, epidemiological and microbiological approaches. Depending on the attribution approach implemented, the distribution of *Salmonella* can be described at the various stages in food processing (Pires *et al.*, 2009). The stages in food processing (e.g., reservoir, processing, distribution, consumption) where the attribution approach describes is called the point of attribution. Depending on what approaches are used, the point of attribution can be different (Pires *et al.*, 2009).

Unlike microbial subtyping and outbreak data approaches, risk assessment is a predictive tool that is very specific to particular food- pathogen combinations. Risk assessment follows a scientifically based process with four defined steps, which are hazard identification, hazard characterization, exposure assessment and risk characterization. In addition to predicting the possible outcomes, risk assessment can be used to identify steps in the food processing that can

significantly affect the risk to public health. For example, the risk assessment conducted by FDA on oysters for *Vibrio parahaemolyticus*, not only did it provide the public health risk of consuming oysters, but also identified factors such as temperature (air and water), time (harvest and chilling) and harvest techniques (regional differences) as key variables that influence the predicted outcomes (FDA, 2005). Like the risk assessment of *Vibrio parahaemolyticus* in oysters, most of the risk assessments conducted focus on a single food commodity (Havelaar *et al.*, 2007), such as *Salmonella* spp. in Egg products, E. coli O157:H7 in ground beef and beef trim, and *Listeria monocytogenes* in poultry deli and ready to eat meats (USDA-FSIS, 2001, 2009; FDA, 2005).

For risk assessment to be of use to compare food attribution results between food sources, the risk assessments conducted for each of the major food source/pathogens will need to be done comparably**.** This can be a challenge since risk assessments are conducted by various governmental and non-governmental groups, with different priorities, approaches and availability of data. In addition, there are only very few of them conducted. For example, we can compare the risk of *Salmonella* resulting from the consumption of eggs vs. beef, assuming the approaches used to conduct the risk assessments are comparable.

Unfortunately, microbial risk assessment is time consuming and fraught with knowledge gaps, such as quantitative pathogen specific contamination data, prevalence of contamination, food handling practices, susceptibility of host, dose response and the many environmental risk factors that determine the risk of infections. In addition, risk assessment relies heavily on past data sets, and is not directly linked to clinical outcomes, therefore it produces estimates that may be hard to

interpret, unlike the estimates derived from either outbreak or microbial subtyping food attribution approaches (Batz *et al.*, 2005; Havelaar *et al.* 2007). Nevertheless, microbial risk assessments do provide very useful information particularly when they are used to compare the relative influence of various processes. The following three studies were conducted to generate quantitative exposure assessment data for use in future microbial risk assessment studies to evaluate the contribution of various risk factors during raw poultry handling.

1.9: Objectives of Current Studies

Study one:

The overall objective of this study was to conduct a year-long market survey to help bridge some of the data gaps identified. The specific objectives of this study included: 1) Determining the prevalence and levels of *Salmonella* in retail chickens in Seattle, Washington; 2) Evaluating the existence of any significant difference in the prevalence and levels of *Salmonella* between various chicken products (i.e., breast, thighs, drums, wings, split breast, ground); 3) Determining the existence of any significant difference in the distribution of antibiotic resistance isolates among different processing plants; 4) Characterizing the *Salmonella* isolates through serotyping in order to compare them with most frequently isolated clinical serotypes in the State.

Study Two:

The objectives of this study are to conduct direct video based observational study of individuals handling raw poultry to prepare meals of their choice in their homes in order to quantify the various risk factors contributing to intra and inter meal cross-contaminations, and administer

questionnaires in order to determine the difference between subject's knowledge of reported food handling practices and their observed food handling behaviors, as well as capture the transportation and the storage conditions of raw poultry by subjects. The outcomes of interest are to identify and quantify the food handling activities that contribute to the risks of cross contamination events during the handling of raw poultry. In addition, kitchen environments that are more likely to contribute to inter-meal contaminations are identified.

Study three:

The main objectives of the current study are to genetically characterize the *Salmonella* isolates recovered from raw poultry products from local retail stores, followed by assessing their genetic relatedness to clinical isolates submitted to the Washington State Department of Health Laboratories (WA-DOH). In addition, PFGE profiles of *Salmonella* isolates from poultry are compared with those isolates recovered as part of the National Antibiotic Resistance Monitoring Study (NARMS). Some additional objectives were evaluating the genetic variability of the *Salmonella* isolates by product and processing establishments; determining the temporal and geographical distribution of indistinguishable PFGE clusters; and substantiating the strength of the association between the PFGE profiles from locally distributed poultry isolates and that of clinical isolates submitted to the health department in the same geographical area.

Chapter Two

Prevalence, Concentrations and Antibiotic Sensitivities of *Salmonella* Serovars in Poultry from Retail Establishments in Seattle, Washington, USA.

E. Mazengia[1], M. Samadpour[2]; H. W. Hill[2], K. Greeson[2], K. Tenney[2], G. Liao[1], X. Huang[1], J.S. Meschke[1*]

[1] University of Washington Department of Environmental and Occupational Health Sciences. Seattle, WA.

[2] Institute for Environmental Health, 15300 Bothell Way NE. Lake Forest Park, WA 98155

Key words: *Salmonella*, Poultry, Market survey, Prevalence, Antibiotic sensitivity

Abstract

Poultry have been identified as one of the major sources of salmonellosis, with estimates ranging from 10% to 22% of total cases. Despite several advances in the industry and new performance standards initiated in recent years, the incidence of salmonellosis in the population has not declined over the last fifteen years. *Salmonella* are pervasive in a wide variety of foods, thus estimating its burden resulting from specific food categories has been challenging and plagued with uncertainty due to critical data gaps. The objective of this study was to conduct a year-long market survey to help bridge the data gaps on the contamination rates and levels of *Salmonella* by product types (i.e., breast, thighs, drums, wings, split breast), and processing methods(conventional vs. organic). The recovered isolates were serotyped and tested for antibiotic sensitivities. Various chicken parts (1,322) were analyzed from April, 2011 through April, 2012. A polymerase chain reaction (PCR) method was utilized for initial screening of samples after an overnight enrichment in tryptic soy broth. Three tube most probable number assays (MPN) and anti-*Salmonella* immunomagnetic separation methods were utilized to determine the level of *Salmonella* and aid with the recovery of *Salmonella* species, respectively. Eleven percent of the samples were positive for *Salmonella*. Significant differences in percent positive rates by product types included up to a four-fold difference in percent positive rates between establishments ranging from 7% to 31%. Of those positive for *Salmonella* species, 94% had <30 MPN/100 grams. Processing types identified as organic or not using antibiotics, had significantly higher rates of recovery for *Salmonella*. On the other hand, all of the *Salmonella* isolates that were resistant to two or more antibiotics originated from conventional processing establishments where antibiotics were utilized. In addition, a significant proportion of isolates from conventionally processed products were serotypes clinically relevant to humans.

2.0: Introduction

Salmonella is a zoonotic pathogen with significant public health impact worldwide. In the United States, over 1.0 million cases of foodborne illness are caused by *Salmonella* spp., resulting in 168,000 physician visits, 19,336 hospitalizations and 378 deaths. According to the current estimates, infection with non-typhoidal *Salmonella* spp. is the leading cause of hospitalization and death for foodborne bacterial infections (Scallan *et al.*, 2011).

Despite several advances by the food industry and new performance standards initiated by the United States Department of Agriculture (USDA) in recent years to reduce the prevalence of *Salmonella* in raw poultry, salmonellosis in the United States has not declined over the last ten years when compared with other foodborne pathogens (CDC, 2011). Based on the 17,468 laboratory-confirmed cases of infections reported to CDC in 2009, the incidence rate of *Salmonella* per 100,000 persons was 15.19. The age group with the highest incidence rate is children under the age of 4 years, and in 2009 the incidence of *Salmonella* per 100,000 individuals in this age group was 72.19. In 2011, there were 589 cases reported in Washington State with two deaths (WA-DOH, 2011).

It is reported that between 55% and 96% of salmonellosis cases are acquired as the result of the consumption of a wide variety of contaminated foods (Havelaar A.H. *et al.*, 2008; Sumner J *et al.*, 2003;Batz BM *et al.*, 2012). There are significant differences in percent positive rates of *Salmonella* in various foods of animal origin, with poultry sources consistently having the highest rates (USFDA, 2010; Mullner, P. et al, 2009; Bosilevac, J.M. *et al.*, 2009; Cui, S. *et al.*,

2005). The 2010 retail meat report by the National Antimicrobial Resistance Monitoring System (NARMS) in the United States reports the following *Salmonella* percent positives: 13.0% for chicken breast and15.3% ground turkey (USFDA, 2010). Poultry has been identified as a major source of *Salmonella* with estimates ranging from 10% to 22% of total salmonellosis cases (Havelaar, A.H. et a, 2008l; Mullner, P. et al, 2009; Pires, S.M. et al, 2009;Ravel,A. et al, 2009; USDA, 2010;Van Asselt, E.D. et al,2009;Batz, B.M. et al, 2012). Poultry has been ranked as the top food item responsible for Quality Adjusted Life-Year (QALY) loss due to illnesses and hospitalization (Batz, B.M. et al, 2012). Among the top ten pathogen-food combinations, *Salmonella* in poultry was estimated to rank the third highest in the number of hospitalizations (4,159), death (81) and 4th in QALY loss (3,610) (Batz, B.M. et al, 2012).

Multiple antimicrobial drug resistance among *Salmonella* serotypes from foods of animal origin is a serious public health concern. The recall of 36 million pounds of ground turkey in 2011 in the United States due to multidrug resistant *Salmonella* Heidelberg underscores the gravity of the public health risk and the economic damage posed by multidrug resistant *Salmonella* in food products of animal origin.

Attribution studies have consistently ranked poultry as the highest contributor to the burden of salmonellosis in the population in the United States (Batz, B.M. et al, 201; Hoffman, S. et al, 2006). Unfortunately, because of the pervasive nature of *Salmonella* in a wide variety of foods, estimating its burden resulting from specific food categories has been challenging and plagued with uncertainty (Batz, B.M. et al, 2012; Pires, S.M. et al, 2009). Some of the main limitations of the data sources that were identified include: 1) Prevalence estimates were measured further away from point of consumption such as processing plants instead of point of sale; 2) Use of prevalence measures in risk estimates without data on the levels of *Salmonella*; 3) Use of

national prevalence studies which may lack specificity to any particular geographical locations due to differences in processing practices and other factors that influence the prevalence/level of *Salmonella*; 5) Limited availability of data on the serotypes and antibiotic susceptibility of *Salmonella* isolates.

The overall objective of this study was to conduct a year, long market survey to help bridge some of the data gaps identified. The specific objectives of this study included: 1) Determining the prevalence and levels of *Salmonella* in retail chickens in Seattle, Washington; 2) Evaluating the existence of any significant difference in the prevalence and levels of *Salmonella* between various chicken products (i.e., breast, thighs, drums, wings, split breast, ground); 3) Determining the existence of any significant difference in the distribution of antibiotic resistance isolates among different processing plants; 4) Characterizing the *Salmonella* isolates through serotyping in order to compare them with most frequently isolated clinical serotypes in the State.

2.1: Materials and Methods

2.1.1: Sampling and Design

From April 2011 through April 2012, various raw poultry parts were sampled from retail markets within the city of Seattle, Washington. Each week between 20-30 poultry samples were purchased from three to five grocery stores within a region of the city. All available brands were sampled from each store. Raw poultry products were purchased from fourteen major retail chain stores including wholesale, large and medium chain stores, and four independent stores. A total of 1322 raw chicken packages comprised of more than twenty five USDA permitted processing establishments were sampled. The 1322 samples included: 362 skinless/boneless breasts, 103 split breast, 293 thighs, 149 drumsticks, 101 wings, 97 ground chicken, 180 ground turkey and

37 gizzards/combo. A higher number of breast samples were collected because breasts are the most frequently consumed chicken part in the area (personal communications with local retail stores). There were 228 samples labeled, "USDA Certified Organic" and 1094 samples that were processed conventionally. Among the conventionally processed brands, 259 were from establishments that claimed not to use antibiotics during processing and were labeled as, "No Antibiotics used". All of the samples were transported in coolers on ice and stored at 4° C until they were processed. All samples were processed within 2-3 days of purchase and all were processed before their "use by" date.

Table 1: Poultry Sample Descriptions:

Processing Establishments	Product Types (#)	Process Types	Samples (#)
Est. A	Ground chicken Ground turkey	Conventional Conventional	48 81
Est. B	Ground turkey	Conventional	86
Est. C	Whole chicken	Conventional	144
Est. D	Whole chicken	No Antibiotics Organic	222 116
Est. E	Whole chicken	Organic	81
Est. F	Whole chicken	Conventional	352
Est. G	Whole chicken	No Antibiotics	32
Est. H	Whole chicken	Conventional	33
Est. I	Ground chicken	Conventional	36
Est. J	Whole chicken	Conventional	19
All Others	Whole chicken Ground chicken Ground Turkey	Conventional No Antibiotics Organic	43 13 13

2.1.2: Sample Processing and Isolation

Salmonella test system (AOAC 100701) for detection of *Salmonella* spp. on carcasses, which is an AOAC verified and certified method (Molecular Epidemiology, 2005) was used in the current study for the detection and isolation of *Salmonella* from poultry.

Enrichment:

Three hundred fifty grams of poultry sample were weighed in a sterile Whirl-Pak® bag (Midland Scientific, Omaha, NE) and mixed with 350 mL of 0.1% pre-warmed (42±2°C) peptone water. The poultry samples were massaged for 1 minute and vigorously shaken for 30 seconds. Fifty milliliters of the rinsate from the massaged chicken samples were transferred into sterile falcon tubes for enumeration (described later). Three hundred milliliters of modified Tryptic Soy Broth (mTSB) (Beckton Dickinson, Sparks, MD) was added to the remaining poultry sample in the Whirl-Pak, and was thoroughly mixed by hand for 30 seconds. The samples were incubated at 42± 2°C for 24 ± 2hrs. After an overnight incubation, the samples were thoroughly mixed and 1mL of enriched sample was transferred to a sterile 1.5mL microtube.

Screening:

All of the samples were screened for two *Salmonella* specific genes by transferring 2 µL of enriched sample to PCR tubes containing lysis and PCR reagents. The lysis protocol included a 10 minute proteinase K lysis step at 37°C and a 10 minute inactivation step at 95°C. Following lysis, 25 µL of lysate were transferred into tubes containing Taq polymerase. The Multiplex PCR (initial screening using PCR buffer ES6) included two *Salmonella* specific targets (Molecular Epidemiology Incorporated) (Sal_1^+ of 218bp and Sal_2^+ of 383bp) and an internal quality control target (16s rRNA). PCR products were resolved using gel electrophoresis as

previously described (Hill, Walter et al, 2011). A positive control (i.e., *Salmonella* target) and a Negative control were included with every PCR run. DNA ladders were loaded into each gel prior to analysis of PCR products. The Bio-Rad Gel Documentation System (Bio-Rad, CA, USA) was used to capture the images which were visually examined for the presence of amplified target gene bands.

Isolation:

An immuno-magnetic separation (IMS) procedure using anti-*Salmonella* antibodies (Dynal, Lake Success, NY) was employed as previously described (Cudjoe, S.K. et al, 1997) to recover and concentrate *Salmonella* spp. from those samples that were presumptive-positive during the initial sample screening by PCR in buffer ES6. A 15µL portion of anti-*Salmonella* antibody reagent 1% (W/V) in buffer was added to 500µL of enriched sample. After slow mixing for ten minutes at room temperature, a magnetic strip was used to facilitate the capture of anti-*Salmonella* beads. With the magnetic strip still in place, the supernatant was removed and rinsed twice with 500 µL of phosphate buffered saline containing 0.5% v/v Tween 20 (PBST, Sigma, St. Louis, MO)

The concentrated pellet was re-suspended in 20 µL of PCR grade sterile water. A 1:50 dilution was prepared using IMS concentrate and PCR grade water. Ten µL and 50 µL of the 1:50 dilution of the final concentrate were spread-plated onto Xylose Lysine Desoxycholate (XLD, Oxoid, Remel, Lenexa KS) and incubated at 35 $\pm$ 2°C for 24 $\pm$ 2hrs. Each plate was examined for typical pink colonies with and without black centers. Two typical colonies from each plate were picked for confirmation. In the absence of typical colonies, isolated colorless colonies were instead picked. Isolated colonies were streaked onto tryptic soy agar (TSA, Becton Dickinson,

Sparks, MD). All presumptive *Salmonella* positive colonies were confirmed using a multiplex PCR, for the detection of *Salmonella* using PCR buffers ES6 (Sal_1^+/ and/or Sal_2^+) and S7 (Sal_3^+).

2.1.3: Enumeration Procedure

For those samples that were PCR positive for *Salmonella*, their corresponding 50 mL rinsates stored at 4°C were used for enumeration purpose. Enumeration of *Salmonella* was conducted on the first 93 samples that were PCR positive during the initial screening procedure. The three tube Most Probable Number (MPN) procedure using 10g, 1g, and 0.1g samples in 0.1% peptone (1:1 ratio) was used to determine the concentration of *Salmonella*. The MPN tubes were incubated at 42°C for 24 $\pm$ 2hrs. Each row of dilution tubes was screened for *Salmonella* using the same PCR procedure as described above for initial screening. The final concentration of *Salmonella* was computed using online MPN calculator software (MPN Calculator, 2004). The results are reported as MPN/gram. The minimal detection limit (MDL) of the MPN procedure was <0.03 MPN/gram.

2.1.4: *Salmonella* Serotyping

The Kauffman- White scheme (KW) was used to determine the antigenic properties of the *Salmonella* isolates, as previously described (Grimont, P. et al, 2007). All of the serotyping was conducted in the U.S. FDA laboratory (Colorado).

2.1.5: Antibiotic Susceptibility Determination

Each isolate was first enriched in Brain Heart Infusion for 24 hours, and then isolated for purity. Colonies were emulsified in de-mineralized water to achieve a McFarland 0.5 suspension of

cells. Mueller Hinton Broth was inoculated with 10µl of the McFarland 0.5 suspension of cells. The broth was mixed and 50µl of broth was distributed into each well of a 96 well plate. The cells were then incubated for 18-24 hours in the presence of the selected antibiotics. After incubation, the plate was then read manually using the Sensititre® Vizion™ system (Sensititre, Trek Diagnostic Systems, Westlake, OH) to measure turbidity in each well.

Isolates were tested with 15 different antibiotics in decreasing concentrations. Results were reported as the Minimum Inhibitory Concentration (MIC) value, the lowest antibiotic concentration in which cells will not grow and an interpretation of Sensitive, Intermediate or Resistant was assigned to each antibiotic based on the breakpoint for that particular antibiotic. Defined breakpoints for each antibiotic were set by CLSI (Clinical and Laboratory Standards Institute). There were no breakpoints for Streptomycin, Azithromycin, Sulfisoxazole or Ceftiofur, and MIC values were recorded but they were reported as NI (no interpretation).

Four organisms were used as controls to ensure the system was working properly: E. coli ATCC 25922, E. faecalis ATCC 29212, S. aureus ATCC 29213 and P. aeruginosa ATCC 27853.

2.1.6: Statistical Analysis

The chi-square and Fisher Exact tests were used to determine the homogeneity of proportions and to determine the significance difference ($p \leq 0.05$) in percent positive rates between brand types, and product sources (GraphPad Calculator, GraphPad Software, Inc., San Diego, California). The statistical significance of multiple comparisons of proportions was assessed using the Marascuilo procedure, utilizing Microsoft Excel (Redmond, Washington).

Antibiotic sensitivity data was analyzed using the SWIN software (Sensititre, Thermo Fisher Scientific, Remel Products, USA).

2.2: Results

2.2.1: Percent Positive Rates of *Salmonella* by Product Types

A total of 150 samples were positive for *Salmonella*; of these, 127 were from chicken and 23 were from turkey. The overall *Salmonella* positive rate was 11.3 %. The lowest percent positive rate for *Salmonella* was found in breast samples (9.9%) and the highest in ground chicken samples (15.6%) (Table 2).

Table 2: Percent Positive Rates of *Salmonella* by Product Types.

Product types	# samples	# Positive	% Positive
All types	1322	150	11.3
Breast (Boneless/Skinless)	362	36	9.9
Split Breast	103	11	10.7
Thighs	293	30	10.2
Drums	149	18	12.0
Wings	101	10	10.0
Ground Chicken	97	18	15.6
Ground Turkey	180	23	13.3
Chicken Gizzards/ Combo	37	4	10.8

Ground chicken was significantly more contaminated with *Salmonella* than all whole muscle types: split breast, breasts, thighs, wings, drums and gizzards (χ^2 (1, N=1142) = 5.930, P=0.0149). There was no statistical difference in *Salmonella* contamination rates between ground chicken and ground turkey (χ^2 (1, N=277) = 1.337, P=0.2476).

Table 3: Percent Positive Rates of *Salmonella* by Process Type:

Process type	No of samples	# Positives	% Positives	P-values[†]
All types	1322	150	11.3	NA
Total Conventional*	1094	115	10.5	0.0394
W/Antibiotic	835	79	9.5	0.0108
W/O Antibiotic	259	36	13.9	0.7002
Organic	228	35	15.4	Ref.

*Conventional includes those with and without antibiotics.

[†]P-values of products labeled as Conventional compared to Organic.

There is a statistically significant difference in *Salmonella* contamination rates of products by process types; organically processed poultry were contaminated at a higher rate than that of conventionally processed products. When *Salmonella* contamination rates were compared between products labeled as organic and those of conventionally processed (i.e., includes products labeled as, "Antibiotics not used") the difference was statistically significant (Fisher Exact (1, N=1021), P=0.0394). A much higher statistical significance was also found when excluding products labeled as, "Antibiotics not used" (Fisher Exact (1, N=1077), P= 0.0108). On the other hand, there was no significant difference in contamination rates between products labeled organic and those products labeled, "antibiotic not used" (Fisher Exact (1, N= 487), P=0.70) (Table 3).

Table 4: Percent Positive Rates of *Salmonella* by Processing Establishment

Processing Establishments	No. of samples	# Positive	% Positive
All Est.	1322	150	11.3
Est. A	129	30	23.1*
Est. B	86	6	7.0
Est. C	144	10	6.9
Est. D	338	39	10.9
Est. E	81	24	29.6*
Est. F	355	28	7.9
Est. G	32	5	15.6*
Est. H	33	0	0
Est. I	36	0	0
Est. J	19	0	0
All other Est.	69	8	11.6

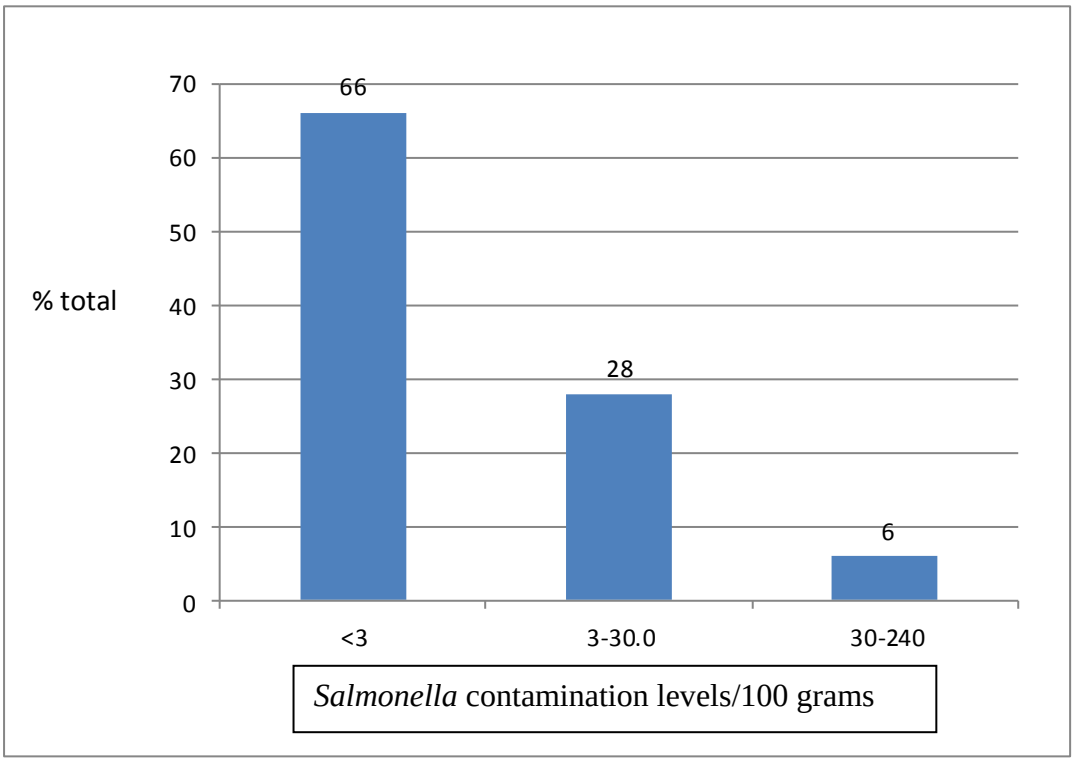
*Statistically significant when multiple proportions were compared using the Marascuilo procedure.

The percent positivity rates for *Salmonella* varied by establishment (Table Four). There was up to a four-fold difference in contamination rates of products between processing establishments. An overall homogeneity test of proportions showed significant inequalities among processing establishments ($\chi 2$ (10, N=1322) = 57.726, P=0.0001). To determine, which processing establishments had significantly higher rates of contamination, multiple proportions comparisons using the Marascuilo procedure was performed (excluding all other Est.). Processing establishments A, E, and G were found to have significantly higher rates of *Salmonella* contaminations than Products from other processing establishments.

2.2.2: Enumeration of *Salmonella* Levels:

Among the 150 *Salmonella* positive samples, the first 93 were enumerated using the three tube MPN technique. The concentrations of *Salmonella* among those that were PCR positive ranged from less than 3.0 MPN/100 grams to 240 MPN/100 grams. There was no statistical difference in the levels of *Salmonella* found on chicken products (i.e., < MDL and > MDL) by process types (i.e., conventional and organic) (Fisher Exact test (1, N= 93), P=1.0), and by product types (whole vs. ground) (Fisher Exact test (1, N=86), P=0.4230). Figure 1: summarizes the overall proportion of samples with different levels of *Salmonella* contamination.

Figure 1: Contamination Levels of *Salmonella*



2.2.3: Serotypes

The 106 of the *Salmonella* isolates serotyped were comprised of 11 serotypes (Table 5): Heidelberg, Enteriditis, Kentucky, Hadar, Schwarzengund, Agona, Senftenberg, Litchfield, Berta, Mbandaka, Typhimurium and Monophasic Typhimurium variants. All of the following serotypes were isolated from ground turkey products: *S*. Agona (2), *S*. Berta (1), *S*. Schwarzengund (5) and most of *S*. Hadar (5 out of 6). All of the *S*. Enteriditis, and *S*. Kentucky and 95% of *S*. Heidelberg were isolated from chicken products.

Table 5: The Top Ten *Salmonella* Serotypes Isolated From Poultry and Clinical Isolates:

Poultry		Clinical Isolates (2011)*	
Serotype	No. Isolates (%):		No. (%) Isolates
Heidelberg	37 (37)	Enteriditis	137 (23.3)
Enteriditis	24 (24)	Typhimurium	88 (14.9)
Kentucky	21 (20)	Heidelberg	27 (4.6)
Hadar	6 (6)	Newport	20 (3.4)
Schwarzengrund	5 (5)	Agona	18 (3.1)
Agona	2 (2)	Branenderup	17 (2.9)
Senftenberg	2 (2)	4[5],12:i:-	16 (2.7)
Berta	1 (1)	Stanley	14 (2.4)
Litchfield	1 (1)	Montevideo	13 (2.2)
Mbandaka	1 (1)	Hadar	12 (2.0)
Typhimurium	1 (1)	Infantis	11 (1.9)

*Clinical isolates submitted to the Washington State Public Health Laboratory (2011).

2.2.4: Antibiotic Sensitivities

A total of 106 *Salmonella* isolates were screened for antibiotic sensitivity to: Amoxicillin/Clavulanic Acid, Ampicillin, Cefoxitin, Ceftraxone, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Nalidixic Acid, Tetracycline, and Trimethoprim/Sulphamethoxazole. Tetracyline resistance was observed most frequently (1/3 of isolates). With the exceptions of Ciprofloxacin, Nalidixic Acid and Trimethoprim/Sulphamethoxazole, the rest of the antibiotics in the panel had one or more strains resistant to it. Of the *Salmonella* strains tested, about 40% were resistant to one or more antibiotics. Thirteen percent (14/106) showed either intermediate or full resistance to two or more antibiotics (Table 6). All of the strains resistant to two or more antibiotics were from an establishment producing products (turkey and chicken) under the same brand name, using conventional processing. Twenty four percent of the isolates (14/58) from these establishments were resistant (Intermediate/Resistant) to two or more antibiotics, whereas none of the strains

isolated from other establishments were resistant to two or more antibiotics ($\chi 2$ (1, N= 106 = 8.763, P= 0.0031)). The *Salmonella* serotype with the highest number of strains that were resistant or intermediate to two or more antibiotics was S. Heidelberg (22%), followed by *S.* Enteritidis (4%). The serotype with resistance to the highest number of antibiotics was *S.* Schwarzengrund showing resistance to six antibiotics (Table 7).

Table 6: Antibiotic Resistance by Establishment.

Process type	# Positive (tested)	# resistant (IN/R)* to ≥ 2 antibiotics.	Percent resistant (IN/R) to ≥ 2 antibiotics
All	106	14	13.2
Conventional (Est .A/ F)	58	14	24.0
All other Establishments	48	0	0

IN*= Intermediate, R*= Resistant

Table 7: *Salmonella* serotypes patterns with two or more resistant or intermediate antibiotics.

***Salmonella* Serotypes**	**AMC**	**AMP**	**FOX**	**AXO**	**CHL**	**OXA**	**GEN**	**KAN**	**NAL**	**TET**	**TRS**
Agona	s	R	s	s	s	s	R	R	s	R	s
Agona	s	I	s	s	s	s	s	s	s	R	s
Enteritidis	I	s	R	s	s	s	s	s	s	s	s
Hadar	s	s	s	s	s	s	s	R	s	R	s
Heidelberg	R	I	R	s	s	s	s	s	s	s	s
Heidelberg (3-strains)	R	R	R	I	s	s	s	s	s	s	s
Heidelberg	R	I	s	s	s	s	s	s	s	s	s
Heidelberg	R	R	R	s	s	s	s	s	s	s	s
Heidelberg	s	s	I	s	s	s	s	s	s	R	s
Heidelberg	s	s	s	s	s	s	s	R	s	R	s
Schwarzengrund	I	R	s	s	R	s	R	R	s	R	s
Schwarzengrund	s	s	R	s	s	s	s	s	s	R	s

R, resistant, I, intermediate. AMC (amoxicillin-clavulanic), AMP (ampicillin), FOX (cefoxitin), AXO (ceftriaxone), CHL (chloramphenicol), OXA (Ciprofloxacin),GEN (gentamicin), KAN (kanamycin), NAL (nalidixic acid), TET (tetracycline) and TRS (Trimethoprim/Sulphamethoxazole).

2.3: Discussion

2.3.1: Percent Positive by Sample Type

In our study the overall positive rate of *Salmonella* samples in the various poultry products tested was 11.3%. The percent positives for whole muscle chicken, ground chicken and ground turkey were 10.6, 15.6, and 13.3 respectively. Ground poultry product samples are statistically more contaminated than whole muscle products. It is no surprise that ground poultry were contaminated more frequently than whole muscle products, since ground products are comprise of parts from multiple carcasses. There was no statistical difference in *Salmonella* contamination rates between various whole muscle chicken parts. Our finding is similar to other studies (USFDA, 2010; Brichta-Harhay, M.D. et al, 2007; USDA, 2010). In 2010, the NARMS study focusing on the microbiological quality of retail meat found *Salmonella* in 13.0% of chicken breast and 15.3% of ground turkey samples (USFDA, 2010). In 2009, the U.S. Food Safety and Inspection Service tested 29,116 various food samples and the percent positive rates of *Salmonella* from poultry products were: 7.2% (broilers), 18.2% (ground chicken), 10.7% (ground turkey) and 3.8% turkey (USDA, 2010).

2.3.2: Prevalence by Process Type (Conventional vs. Organic)

To further characterize the distribution of contamination rates, our study looked at contamination rates by processing types. The processing types that were considered were “Conventional” where antibiotics are administered to the chickens during various phases of their growth; “No

Antibiotics" (products labeled no antibiotic used) and "Organic" (products labeled as USDA certified organic). Our study found significant differences in contamination rates between different processing types, with products from Conventional (9.3%) processors having the lowest contamination rate compared to poultry products labeled "USDA certified organic"(15.4%). There was no significant difference in contamination rates between those labeled, "No antibiotic" and "USDA certified organic" (i.e., 14.8% vs. 15.2%). Other studies had also found differences in contamination rates by process types, with Organic processors having higher contamination rates than Conventional processors (Bailey, J.S. et al, 2002; Cui, S. et al, 2005). On the other hand, a prevalence study of *Salmonella* from fecal samples of chicken while still in the farm reported a higher rate of *Salmonella* spp. from conventional farm (38.8%) than organic farm (5.6%) (Alai, W.Q. et al, 2010). The difference in reported study outcomes might have to do with samples taken at different stages in the poultry processing or differences in how poultry carcasses are handled (i.e., between organic and conventional) during slaughtering might influence the level of *Salmonella* contamination of finished products (e.g., use of disinfections).

2.3.3: Prevalence by processing establishment:

Our study found significant differences in percent positive rates between establishments, with contamination rates between 7 % and 31%. The three highest contamination rates by establishments were 23.1% (Est. A), 29.6% (Est. E), and 15.6% (Est. G). Establishment A produces both ground chicken and ground turkey, whereas establishments E and G produce whole muscle chicken. To avoid comparing products of processing establishments from different process types (e.g., Organic vs. conventional), percent positives are compared among

the same product types and with the same process type categories. In Establishment A, both ground chicken (12/52) and turkey (18/78) were equally contaminated (23%). When comparing percent *Salmonella* positive of ground chicken from establishment-A (12/52) and that of establishment-I (0/35), which also produces ground chicken conventionally, there is a statistical difference in the contamination rates (Fisher exact test (1, N= 87), P=0.0013). The same result was also found when comparing the contamination rates of ground turkey from establishment-A (18/78) to that of establishment-B (6/86), which also produces ground turkey conventionally (Fisher exact test (1, N= 164), P=0.0040).

On the other hand, there was no statistical difference when comparing whole muscle chicken labeled as "No Antibiotic" from processing establishment G (5/32) and that of D (31/285) (Fisher Exact test (1, N= 317), P=0.3855). The same non statistical difference was also found when comparing processing establishment labeled as "Organic" from establishment (25/81) E with that of D (4/31) (Fisher Exact test (1, N= 111), P=0.0878). Therefore, reporting results by using the USDA establishment number alone may not represent appropriate comparisons as multiple products may be processed in a single establishment.

2.3.4: Levels of *Salmonella* spp.

Critical data important to consider in microbial risk estimates are the levels of *Salmonella* in contaminated products. Due to the cumbersome nature of the enumerating methodologies and the cost associated with them, limited studies have been conducted to enumerate the concentrations of *Salmonella* on poultry both at the processing and retail levels (USDA, 2007/2008; Brichta-Harhay, M.D. et al, 2007; Jorgensen, F. et al, 2002). In our study, the concentrations of *Salmonella* among those samples that were screen-positive by PCR ranged

from less than 3.0MPN/100 gram to 240 MPN/100 grams. Ninety four percent (n=87) of those that were positive were found to have less than 30 MPN/100 grams and 6 % (6) had between 30 and 240 MPN/100 grams. Given that an average weight of a whole carcass is 1.5kg (broiler carcass weight, 3.3lbs), the upper estimated level of *Salmonella* this study found is 3,600 MPN/carcass. It had been reported that higher concentrations of *Salmonella* on carcasses are rare events and unpredictable (Brichta-Harhay, M.D. et al, 2007; Straver, J.M. et al, 2007), which is consistent with our finding of 3% of the positive samples exceeding an extrapolated concentration (1500g x MPN/g) of about 1300 MPN per whole carcass (1.5kg). This extrapolation assumes an even distribution of pathogens throughout the carcass. Other enumeration studies have found similar results (Brichta-Harhay, M.D. et al, 2007; USDA, 2010; Jorgensen, F. et al, 2002).

In our study, the maximum concentration of *Salmonella* was 3,600 MPN/carcass. The Young Chicken Baseline Survey study by FSIS for the year between 2007 and 2008 from a total of 3,275 post-chilled raw poultry tested, only 170 (5.19%) were enumerated and none of these exceeded 30 MPN/ml. Of those enumerated, 123 (46.1%) yielded from 0.0301 to 0.3 MPN/ml, 38 (14.2%) samples ranged from 0.301 to 3.0 MPN/ml and 9 (3.3%) had between 3.01 and 30 MPN/ml (USDA, 2007/2008). In England, 241 carcasses sampled from retail stores, using a direct plating technique, found 1% (2) samples to contain 10^4 *Salmonella* per carcass (Jorgensen, F. et al, 2002). Current risk models show that lower contaminant levels do not significantly contribute to human salmonellosis but that contaminant levels of more than 10^4 *Salmonella* per carcass are responsible for increasing the risk of cross-contamination and the likelihood of causing human salmonellosis (Cox, N.A. et al, 2010; Straver, J.M. et al, 2007). Nevertheless,

with an estimate of 8 billion birds slaughtered annually in the United States, the small percentage of carcasses with high *Salmonella* load can result in significant public health impact.

2.3.5: Serotypes

In this study, eleven distinct serotypes were identified. The three most predominant serotypes making up 85% of total were Heidelberg (37%), Enteriditis (24%), and Kentucky (21%). Serotypes Enteritidis and Heidelberg are among the top five most frequently recovered from clinical isolates in Washington State (WA-DOH, 2011) and both serotypes are among the top three recovered in this study. Nevertheless, it is not necessarily true that the most frequently isolated serotypes from meat products are also the same as those serotypes isolated from human cases. While serotype Kentucky is among the top three serotypes isolated from poultry in this study (21%), it is not among the top 20 *Salmonella* serotypes recovered from clinical isolates in Washington State (WA-DOH, 2011), hence contributing to less than 1% of the isolates submitted to the Washington State Department of Health laboratory. A similar finding was also reported by the NARMS study; serotype Kentucky makes up 50% of the NARMS retail chicken samples, yet it causes only 0.3% of human cases in the United States (Cox, N.A. et al, 2011). When we look at the distribution of serotypes by processing establishments, 80% of the serotype Kentucky samples originated from processing establishments that did not utilize antibiotics or from chicken products labeled as Organic. As for product type, all of the serotypes *S.* Agona (2), *S.* Berta (1), *S.* Schwarzengund (5) and most of *S.* Hadar (5 out of 6) were isolated from ground turkey products. All of the *S.* Enteriditis, and *S.* Kentucky and 95% of *S.* Heidelberg were isolated from only chicken products.

2.3.6: Antibiotic Resistance Profiles

Multidrug resistance among *Salmonella* serotypes from foods of animal origins is a serious public health concern. In this study, we screened 106 *Salmonella* isolates using a panel of eleven antibiotics (Amoxicillin/Clavulanic acid, Ampicillin, Cefoxitin, Ceftraxone, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Nalidixic Acid, Tetracycline and Trimethoprim/Sulphamethoxazole). As can be expected, this study showed products from establishments that utilize antibiotics have significantly higher rates of antibiotic resistant *Salmonella* than do products from establishments that are Organic or labeled as "No Antibiotics".

Overall, 40.0% (42/106) of the *Salmonella* isolates tested were resistant to at least one antibiotic. Thirty three percent of the *Salmonella* isolates tested were resistant to Tetracycline, making it the antibiotic to which most strains were resistant to. The current study results found six strains (5.6%) that were considered multi drug resistant (i.e., resistant to three or more antibiotics), including one strain of *S.* Agona, four strains of S. Heidelberg and one strain of *S.* Schwarzengrund. The latest NARMS study (i.e., 2011) reported substantially higher (44.9%) number of *Salmonella* strains that were resistant to three or more antibiotics (NARMS, 2011). The difference is due to the substantially higher percentage of *S.* Typhimurium (41.8%) represented in the NARMS study compared to the current study, which had only 1%. In 2011, *S.* Typhimurium accounted for larger proportion of the MDR in the NARMS study. On the other hand, the MDR found on poultry in the current study is higher than recently reported for ground beef in the United States, which was 0.6% (Bosilevac J. *et al.*, 2009). All of the *Salmonella* strains tested were susceptible to Ciprofloxacin, Nalidixic acid and Trimethoprim/Sulphamethoxazole).

Among the top three serotypes recovered, S. Heidelberg had a higher frequency of resistance. There were three strains of S. Heidelberg that showed full resistant or intermediate to two antibiotics 8.1% (3/37), two strains to three antibiotics 5.4% (2/37) and three strains to four antibiotics 8.1% (3/37). Compared to all serotypes that were resistant to two or more antibiotics, S. Heidelberg made up 57% (8/14) the resistant reported in this study. The percent resistant *Salmonella* isolates from chicken ranged from 1% for chloramphenicol to 33% for tetracycline. The *S.* Schwarzengrund serotype with resistance to the highest number of antibiotics was resistant to Ampicillin, Chloramphenicol, Gentamicin, Kanamycin, Tetracycline and showed intermediate resistant to Amoxicillin-Clavulanic.

All of the strains resistant to two or more antibiotics were from the same brand name (Est. A – ground chicken and Est. F-whole muscle). When compared with isolates from conventional processors, none of the S. Heidelberg isolates recovered from processing establishments that produced either organic or products with no antibiotics showed intermediate or full resistance to any of the antibiotics tested (($\chi 2$ (1, N= 37) = 5.709, P=0.0169). The serotype that was most frequently resistant to tetracycline was S. Kentucky [83% (19/23)].

2.4: Conclusion

Our study focused on poultry products distributed within the city of Seattle, Washington, over a one year period. Therefore our results can shed light on the distribution of *Salmonella* contamination rates by processing establishment and process types which national studies may not be able to accomplish, because of limited sampling per given geographical areas.

The major objective of this study was to provide data that can be used for microbial risk assessments of *Salmonella* in poultry. Microbial risk assessment studies should consider the following findings of this study: *Salmonella* contamination rates differ by product types, with higher contamination rates found in ground products. There does not appear to be difference in the levels of *Salmonella* found by process types and also product forms, although this can be due to only 6% of the positive samples were contaminated with greater than 30 MPN/carcasses. There are up to four-fold differences in *Salmonella* contamination rates between processors; establishments producing "Organic" and "No antibiotics" products have a higher rate. On the other hand, in this study, isolates with multidrug resistance were recovered only from processing establishments that utilized antibiotic-treated chicken. Multidrug resistant S. Heidelberg was recovered from various products, such as ground chicken, ground turkey and whole muscle chicken.

It has been reported that some serotypes are more likely to cause illnesses than others and therefore, microbial risk estimates should consider serotypes (Blaser, M.J. et al, 1981; Taylor, J.L. et al, 1993). Risk estimates that only consider the percent positive rates of *Salmonella*, without considering the serotypes present, might over or under estimate the microbial risk for humans. While "organic" and "no antibiotic" products have higher *Salmonella* contamination rates than conventionally processed products, a substantial proportion of isolates recovered from these products were S. Kentucky which is not among the serotypes considered to be highly infectious to humans when compared with *Salmonella* Enteriditis or Heidelberg. And, *S.* Kentucky is not among the top twenty isolates submitted to the Washington State Public Health Laboratories for serotyping (WA-DOH, 2011).

When microbial risk that can be attributed to consumption of poultry is estimated, one needs to consider processing types as part of the exposure assessment, as this will inform factors such as antibiotic susceptibilities, and differences in serotypes to which humans are exposed. Therefore, governmental agencies, such as USDA, will need to consider making serotype distributions as well as antibiotic susceptibility part of the pathogen reduction effort in poultry processing establishments. The mere measurement of and compliance with the USDA's poultry reduction rules may not necessarily translate into a reduction in the public health burden of *Salmonella* infection in humans. Future risk assessment studies should investigate the public health risks posed by reduction in the contamination rates of *Salmonella*, but also consider the increase in multidrug resistant *Salmonella* and serotypes of clinical concern. In addition, future studies should also focus on the major factors in the final stages of food handling practices of contaminated carcasses that contribute to the spread of *Salmonella*. Modification of these processes can improve food safety and reduce the risk of salmonellosis.

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Chapter Three

Direct Observational Study of the Risks of Cross Contaminations During Raw Poultry Handling: Practices in Private Homes.

E. Mazengia, C. Fisk, G. Liao, Huang H., and., J. Meschke*

[1] University of Washington Department of Environmental and Occupational Health Sciences. Seattle, Washington.

Key words: Observational study, Food safety, Cross contaminations, Hand washing, survey

Abstract:

Substantial proportion of foodborne illnesses have been associated with foods prepared in home kitchens, including salmonellosis. Among foods of animal origin, poultry products are by far more often contaminated with *Salmonella*. Therefore, improper raw poultry handling during meal preparation in individual homes, such as improper storage and lack of hand washing, can increase the risk of the transmission of salmonellosis or *Campylobacter*iosis. To determine the various risk factors that can contribute to the spread of pathogens in the domestic kitchen environments, direct observational study of individuals handling raw poultry in their homes was conducted. Fifty-six individual households were included in the study. Participating subjects prepared any meal of their choice, with 25 different poultry recipes represented in the study. Notational analysis was used to transcribe the observed food handling behavior into quantifiable risk factors. Following the videotaping questionnaires were administered to ascertain each individual's knowledge of safe poultry handling practices. Participating individuals were knowledgeable of poultry handling practices, but their observed poultry handling practices were significantly inferior to their knowledge of food safety. All of the individuals reported on the questionnaires that they wash their hands before and after handling raw poultry, while only 12% of the times hands were properly washed after handling raw poultry. Food handling practices leading to direct and/or indirect cross contaminations of hands, kitchen utensils, the kitchen environment, product containers (e.g., seasoning bottles) and devices (e.g., cell phones) were observed for 100% of the participating subjects. While all of the individuals checked for internal consistency of cooking, less than 5% of individuals measured final cook temperatures. Cross

contamination events are common during domestic poultry handling, increasing the potential risk of exposure to pathogens. People's knowledge of proper food handling was not translated fully into practice. Intervention efforts should focus on ways to minimize the risk of cross contaminations during poultry handling in homes.

3.0: Introduction

Foodborne illnesses continue to be a major public health burden in the United States (Batz M., *et al.*, 2011, Dewaal SC, *et al.*, 2013) and the rest of the world (WHO, 2012). Many countries report between 10% to 50% of foodborne outbreaks associated with foods prepared or consumed in homes (Van *et al.*, 2008; Redmond and Griffith, 2003, Bryan F., L, 1988; McCabe-Sellers and Beattie, 2004).

Improper food handling practices for raw poultry or other types of meats have been identified as risk factors for foodborne illnesses (Redmond C. E. and Griffith J. C. 2003, Redmond C.E., 2004, Phang S. H. and Bruhn M. C., 2011). In the United States, among the bacterial pathogens of concern, infection with non-typhoidal *Salmonella* spp. and *Campylobacter* spp. as results of contaminated foods, make up the majority of hospitalization and death (Scallan *et al.*, 2011). In the United States, compared to other major pathogens of concern, salmonellosis in the population has not declined in the last fifteen years (Batz M., 2012, CDC 2011).

Relative to other foods of animal origins, market prevalence studies have consistently found significantly higher rates *Salmonella* and *Campylobacter* contaminations of poultry products (FDA-NARMS 2010; Hill W. *et al.*, 2012). Implementations of safe food handling practices in food processing establishments and in the homes are both critical at reducing the spread of these

pathogens. Relaying solely on efforts made by poultry processors to reduce the contamination rates of raw poultry products, may not be successful at reducing the overall risk of infection in the population (USDA-FSIS, 2010). Therefore, given that highly contaminated raw products are infrequent and difficult to predict, safe food handling practices in homes becomes important at reducing the spread of these pathogens (Dufrenne J., 2001; Starvar, *et al.*, 2010).

Improper food handling practices in the kitchens can create conducive environments for the re-growth of *Salmonella* and/or the increase chance of cross contaminations (Mylius *et al.*, 2007; van *et al.*, 2008, Luber, 2009, Greig JD and Ravel A, 2009). It has been reported that food and non-food contact surfaces within domestic kitchens can readily be contaminated when contaminated raw poultry products are improperly handled (Zhao *et al.*, 1998, Cogan, T. A., *et al.*, 1999, Harrison, W. A., *et al.*, 2001). The Centers for Disease Control estimates 18% of foodborne diseases are the result of the use of contaminated equipment and 19% are associated with poor hygiene practices (CDC, 2000). Cross-contamination activities can result from the re-use of equipment or utensils that are contaminated, lack or improper hand washing practices, and improper food storage conditions.

For cross contamination to take place, pathogens have to be able to readily transfer between surfaces and also must be able to persist in the environment. Studies have evaluated the rates of bacterial transfers between different environments including, from raw chicken to hands (Montville et al, 2001 and Chen *et al.*, 2001); from chicken to cutting boards (Kusumaningrum *et al.*, 2004; Chen *et al.*, 2001); from contaminated hands to ready to eat foods (Montville *et al.*, 2001; Chen *et al.*, 2001; Smith *et al.*, 2003); from hand to tap and vice versa (Chen *et al.*, 2001); and from cutting board to salad (Kusumaningrum *et al.*, 2004; Chen *et al.*, 2001). These studies

have found bacteria do readily transfer between surfaces in food preparation environments (Luber et al, 2006, Chen *et al.*, 2001; Cogan *et al.*2002, Kusumanngrum *et al.*, 2004) and are also able to persistence on various environments, such as on hands (Chen *et al.*, 2001), on cutting boards (Cogan *et al.*, 2002) and ready to eat foods such as contaminated salad (Smith *et al.*, 2003).

Although about 73% of meals are prepared and consumed in homes in the United States (ADA, 2010), our understanding of how raw poultry products are handled in private homes is limited. Our current understanding of how foods are handled in private homes comes from questionnaire based and a handful of observational studies that were mostly conducted a decade ago (Redmond and Griffith, 2003. Worsfold and Griffity, 1997). Previous studies have found differences in what consumer say they do and what they actually practice when observed (Redmond and Griffith, 2003, Elizabeth Scott and Nancie Herbold, 2010). Therefore, direct observational studies are better able to evaluate individuals' food handling behaviors than surveys (Elizabeth Scott and Nancie Herbold, 2010; Kendall, P. A, *et al.*, 2004; Redmond and Griffith, 2003). This is particularly true since behaviors that contribute to the risk of cross contamination during food handling are difficult to ascertain through questionnaires or telephone interviews. Further, consumers are often unaware of how much their food handling practices contribute to the risk of cross contamination leading to illnesses. On the other hand, questionnaires allow an understanding of gaps in knowledge of poultry handling.

Thus far, we are not aware of studies that focused solely on poultry handling practices in domestic kitchens from the point of purchase to consumption, using a combination of questionnaires and direct observational studies. Studies have indicated that there is lack of

quantifiable data leading to cross contaminations (Mylius S. D., *et al.*, 2007, WHO/FAO, 2003). Data gaps on consumer practices in preparation and handling of poultry and quantifiable data on contributing pathways has made it difficult to build microbial exposure assessment models related to the variability of individuals food handling practices (Mylius S. D., *et al.*, 2007; David Vose, 2010; WHO/FAO, 2003; National Academy of Science, 2002).

Therefore, the objectives of this study are to conduct direct video based observational study of individuals handling raw poultry to prepare meals of their choice in their homes in order quantify the various risk factors contributing to intra and inter meal cross-contaminations. And administer questionnaires in order to determine the difference between subject's knowledge of reported food handling practices and their observed food handling behaviors, as well as capture the transportation and the storage conditions of raw poultry by subjects.

The outcomes of interest are to identify and quantify the food handling activities that contribute to the risk of cross contamination events during the handling of raw poultry. In addition, kitchen environments that are more likely to contribute to inter-meal contaminations are identified.

3.1: Materials and Methods

The research protocol and the administered questionnaires (Attachment A) were approved by the Institutional Review Board at the University of Washington.

3.1.1: Subject Recruiting

Fliers, postings and word of mouth were used to recruit a convenience sample of 56 households. Household subjects of 18 years or older, who regularly prepare meals starting with raw poultry for themselves or others were enrolled in the study. Fliers were posted at the university and e-

mails sent to students and university employees. Additional subjects were recruited by word of mouth from researchers' circle of friends within and outside the university, and family members. Written description of the study was handed to potential participants and researchers were available to answer any questions. Subjects who were interested to participate were required to sign the consent form before they were allowed into the study. Participants were scheduled at their convenience.

3.1.2: Video Recording

Prior to starting video recording, the research assistant reviewed the study objectives with the subject and answered questions subjects had. Video recording was initiated on subject's indication that they were ready to begin meal preparation. Subject's food handling procedures during meal preparation were digitally recorded using hand held video recording (Cisco, Flip Video). All activities related to the preparation of the meal, such as preparing side dishes, or ware washing activities were recorded. In the events, subjects place the poultry in the oven for cooking and were not doing anything related to the preparation of the final meals, video recording was paused and then resumed at the time the poultry product was removed from the oven and handling resumed. The video recordings concluded when subjects were done with all food preparation steps and were ready to serve or consume the prepared meals. All video recordings were transferred to external hard drives for viewing on a computer monitor. For ease of tracking, the video recordings were labeled with the name of researcher followed by the subject ID, which is a numerical number assigned to the subject (e.g., Eyob_01).

3.1.3: Administering Questionnaires

The written questionnaires were administered after completing the video recordings (Attachment A) to minimize the impact of the questionnaires on the observed behaviors. The questionnaires consisted of a total of 51 questions in six distinct sections: 1) Consumption/preparation frequencies, 2) Product Transportation, 3) Storage/handling temperatures, 4) Poultry handling/Sanitation, 5) Hand washing and 6) Final cook temperatures. Each questionnaire package was labeled with the same labeling convention used for the video recordings.

3.1.4: Notational Analysis

A modified version of a notational analysis form as described by Lubran, M.B *et al.*, 2010 (Attachment B), was used to annotate subject videos. In brief, notational forms were used to keep a detailed track of each activity a subject preparing meal performed from the beginning to the end of the recording. Information recorded included the type of activity (e.g., hands washed), how the activity was done (e.g., soap, hot water, and paper towel used), how long the activity was done (e.g., 15 seconds) and how hands were dried (e.g., cloth towel). In addition, the notational form we used has columns for tracking specific cross contamination activities (e.g., cloth towels contaminated), activities related to sanitation of food contact surfaces (e.g., cutting board washed, rinsed and sanitized before re-use), and a column for documenting activities that could compromise the final cooked product (e.g., tasted food with unwashed hands) or contamination activities that could potentially compromise future food handling (e.g., salt shaker contaminated). The determination of whether or not an activity met proper food handling practice was determined prior to reviewing the recorded videos based on criteria adapted from the United Stated Food and Drug Administration, Retail Food Code, 2009 (Table 8).

Each video recording was viewed by two researchers on separate notational analysis forms. Observations between forms were then reconciled and used to assess quantifiable risk factors. To ensure consistency between researchers, a margin of 10% was set as the allowable maximum divergence in measured activities (e.g., number of required hand washes). If divergence in excess of 10% between reviewers' recorded observations was found, the recorded videos were jointly reviewed and differences harmonized.

Table 8: Criteria Used to Score Lack of Proper Food Handling Practices

Activities or Risk factors	Expected proper food handling practices
Before beginning food handling	*Proper hand washing prior to starting food handling.
Bare hand contact with raw poultry (e.g., unpacking or processing)	Proper hand washing prior to handling anything that will continue to be used during the course of cooking (e.g., utensils) or will not be discarded right away (e.g., packaging materials).
Washing dishes or cleaning counter tops contaminated with raw poultry.	Food utensils should be washed, rinsed and sanitized before reuse. Contaminated food contact surfaces should be cleaned and properly sanitized. Proper hand washing after thoroughly cleaning or sanitizing dishes or contaminated surfaces.
Activities contributing to cross contaminations	If hands or utensils (e.g., spoons) have been used to handle raw poultry, they should be thoroughly washed.
Handling of reusable food containers (e.g. seasoning bottles)	Prior to handling re-usable food containers, hands should be properly washed or in an event the food containers are cross contaminated, they should be thoroughly washed.
Cleaning contaminated food contact surfaces	All contact surfaces that are contaminated (e.g., sink contaminated with chicken juice or counter surfaces), should be thoroughly cleaned (e.g., using sanitizer) right after contamination or at the completion of cooking session.
Contamination of cloth towels.	Cloth towels should not be used to dry hands that are not properly washed or used to dry contaminated surfaces. Single use paper towels recommended.
Proper storage of foods	Raw poultry products should be stored on the lowest shelf in the cooler.
Thawing of frozen poultry	Frozen raw poultry should be thawed using one of the following methods: refrigerator, microwave, thawed under cold running water.
Checking chicken doneness	Use a thermometer
Serving/Eating	Proper hand washing after completing meal preparation, before handling items that will be eaten (e.g., salad) or used (e.g., serving spoons).

*Proper hand washing: Hand washing with soap and hot water with 15 minutes of actively rubbing and thoroughly rinsing.

3.2: Data Analysis

Microsoft Excel (Microsoft Corporation, Redmond, Washington) was used to compile, summarize data points, and calculate basic descriptive statistics. Mean and percentages of responses and observations were calculated and presented in tables. Mean and standard deviation values were also computed and used to describe the distribution of responses and observed data points.

Fisher exact test was used to measure significant difference between reported and observed food handling practices (GraphPad Calculator, GraphPad Software, Inc., San Diego, California). T-test results were used to evaluate the difference in the number of cross contamination episodes based when side dishes were prepared.

3.3: Results

3.3.1: Questionnaire Results

Demographics

Starting on November 01, 2011 to December 15, 2012, 56 subjects were recruited to participate in the current study. The ages of subjects in the study range between 18 years to 65 years old, with a median age of 21 years old. Fifty three percent of subjects were female. Thirty five percent of the participants had (17/48) had their Bachelor's degree, 62.5% (30/48) had some college education and 2.1% had High School education (1/48). Some participants left a few of the questionnaires unanswered, therefore total number of respondents per questions are less than 56.

Poultry consumption

All study subjects reported to regularly buying chicken, with 14% reporting to also buying ground turkey products. Among those that reported to regularly buying chicken products, eighty six percent (86%) of subjects reported buying breasts. Study participants reported to consuming meals with poultry anywhere from once a month to thirty times per month. On average, participants reported preparing meals with poultry five times per month (SD = 4). Study subjects reported to preparing most or all of the meals they consume in homes.

Transportation and Storage

To find out how long poultry products were stored at room temperature during transportation and after arriving home, subjects were asked the average transport time from store to home and how soon after arriving at home, poultry products were stored either in the refrigerator or in the freezer. According to the survey results, 99% subjects reported arriving home within less than thirty minutes and ninety eight percent of them reported storing poultry either in the refrigerator or freezer within fifteen minutes after arriving (Table 9). Sixty one percent (n= 27) of subjects reported storing poultry products in the refrigerator on the bottom shelf, whereas the remainder reported storing products on either the top or middle shelf. Among those reported storing poultry products in the refrigerator (N=39), subjects reported storing raw poultry products in the refrigerator between one to seven days before use (Avg. 2.7 days, SD=1.4 days). Most of them (49%) reported to using products within two days after storage in the refrigerator.

Table 9: Time to Cold Storage:

Time	Store to home	Time before storage*
< 15 minutes	**47 (84%)**	**56 (98%)**
15-30 Minutes	**9 (15%)**	**1 (2%)**
>30 minutes	**1(1%)**	**0**

*Length of time before proper storage after arriving home.

Raw Poultry handling

The questionnaires ascertained several aspects related to food preparation and handling of poultry, including whether or not raw chicken was washed, if separate cutting boards were used, when side dishes were prepared (i.e., before, during or after poultry handling), whether wash rags were used and how often they were cleaned or replaced, and whether disinfectants to sanitize food contact surfaces were used after poultry products were handled. Table 10 summarizes the results of the survey study pertaining to poultry handling, hand washing, final cook temperature, and sanitation.

Four different methods of thawing frozen poultry products were reported by subjects, with 72% (n= 34) reporting either thawing in the refrigerator or using microwave. Forty five percent (n=21) of subjects reported washing raw poultry under running water. Forty five percent of subjects (n=21) reported not having separate cutting boards for handling raw poultry and other non-poultry food items. Of those that do not have separate cutting boards, ninety five percent of the subjects (N=20/21) reported to washing the cutting board before it is used to handle other food items. Sixty four percent (n=30) of subjects reported preparing side dishes either during or after raw poultry is handled.

Hand washing

Subject's hand washing practices before, during, and after food handling was asked. All of the subjects reported to washing their hands with hot water and soap before food preparation begins. Overall, high compliance with good hand washing practices was reported by most subjects. Subjects reported washing their hands 92% to 100% of the time before, during and after handling poultry. Additionally, 90% (N=42) reported washing their hands before preparing side dishes (e.g., salad).

Final cook Temperatures

In response to questions regarding final cook temperature, seventy five percent (n=35) of subjects reported not taking final cook temperature of cooked poultry. However all subjects reported relying on look and feel to determine adequacy of cooking. Further, thirty eight percent (n=18) reported not knowing the appropriate final cook temperature (165°F) for poultry, with one subject reporting 100°F as final cook temperature for poultry.

Table 10: Summary of Questionnaire Results:

Activities (# responded)	% of Responses (No./total response)			
Where do you store raw poultry (47)	In the refrigerator	In the freezer	Use the same day purchased	
	58	40	2	
Where poultry is stored in the refrigerator (44)	Bottom shelf	Middle shelf	Top shelf	
	61	34	5	
# Days raw poultry is stored in the refrigerator (39)	≤ 2 days	3 days	4 days	≥ 5 days
	49	31	10	10
Do you have thermometer? (47)	Yes		No	
	42.5		57.5	
Thawing Procedure (47)	Refrigerator	Under running water	Microwave	Room temperature
	48	14	24	14
Hand washing (47)	Wash hands before	Wash hands after touching raw poultry	Wash hands when done	
	100	98	92	
Do you wash raw chicken (47)	Yes		No	
	45		55	
Cutting board usage (47)	Don't have separate cutting boards		Don't reuse without properly washing	
	45 (21/47)		95 (20/21)	
Use of wash rags	Use wash rags	Washed daily	Replace wash rags > monthly	
	79	40 (17/43)	34 (14/41)	
Do you use sanitizers (47)	Yes		No	
	77		23	
Final cook temperature (48)	Don't check final temp.	Final temp. not known	Think final cook temp is <165F	Think final cook temp is > 165F
	75(35/47)	37.5	10	17
When side dishes are prepared (47)	Before	During	After	N/A
	23	45	19	13

N/A: Not Applicable

3.3.2: Results of Video Observations

A total of 1880 minutes (31.3 hrs.) of direct observations of raw poultry handling was recorded and analyzed. The length of video observation per household was 33.6 minutes long (SD= 20.0 minutes). A combined total of 9,166 activities during the course of the observational study were recorded in notational forms for analysis of hand washing activities, poultry handling and cross contamination, sanitation of food contact surfaces, and handling of final cook products. The average number of activities per meal preparation was 164 activities (SD = 92.0 activities). The number of activities per household (per meal prepared) ranged from as low as 54 to as high as 473 activities.

Poultry storage

Forty two percent (16/38) of households were observed storing raw poultry either on the top shelf (n=13) or middle shelf (n=3).

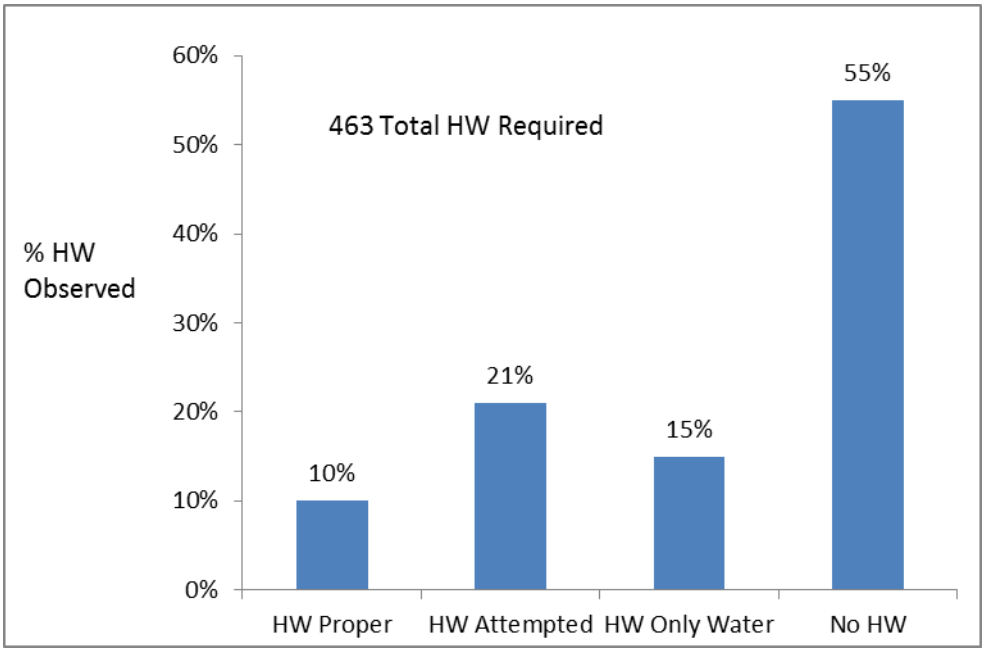
Thawing procedure

Seventy one percent of subjects (40/55) prepared meals starting from fresh raw poultry; therefore thawing procedures were not observed for the majority of the households. However among those that started with frozen raw poultry, fifty three percent of them (8/15) thawed in the microwave, twenty seven percent under running water (4/15) and twenty percent (3/15) thawed in the refrigerator overnight.

Hand Washing

There were a total of 463 hand washes required throughout the study, making up about 5% of the total activities. Hand washing practices were specifically assessed prior to starting to handle foods, after touching raw poultry, after washing dishes used for raw poultry and prior to handling either side dishes or final meal with bare hands. Eighty percent (44/56) subjects did not wash their hands with soap and water at all prior to starting to prepare meals. Overall, fifty five percent (253/463) of the activities that were required to be followed by a hand wash were not washed at all. The mean duration of hand washing with soap and water when hand washing was required was 13 seconds (SD=7 seconds). Figure 2 summarizes the frequency of the different types of hand washing observed before, during and after meal preparations.

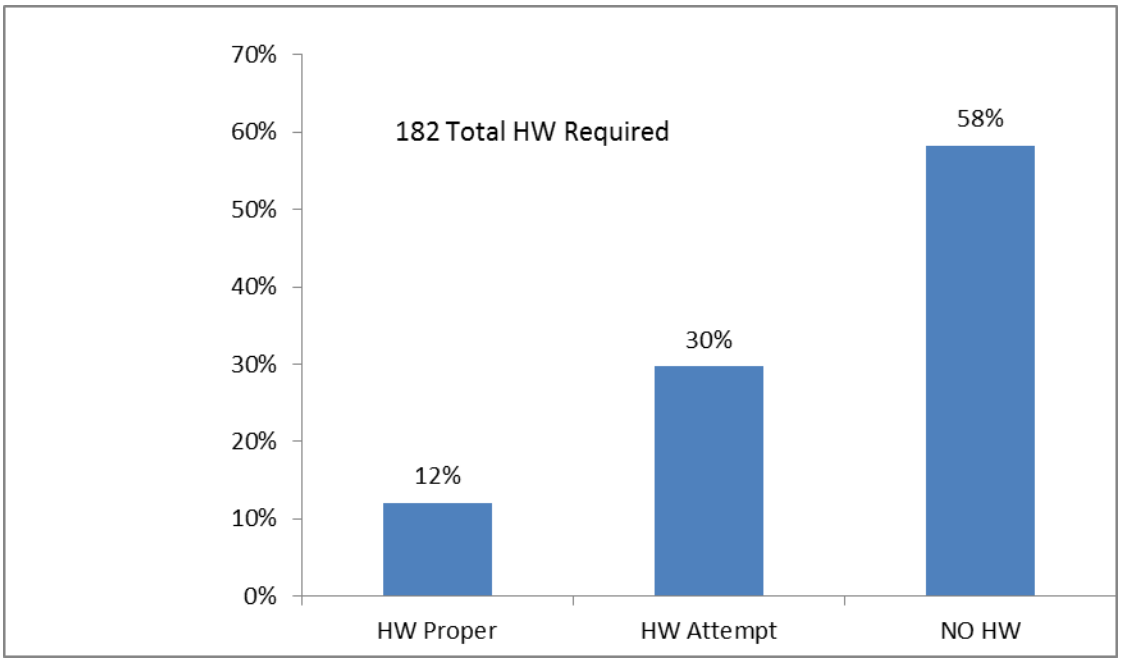
Figure 2: Total Hand Wash Required and Frequency of Hand Wash Types



HW with soap and water for > 15S; HW attempted: HW with soap and water for < 15S; HW only water: HW with only water; NO HW: No HW after the activity.

Among the 463 total hand washes expected, 182 (39%) were after hands were used to directly handle raw poultry or handle utensils soiled with raw poultry (e.g., washing plates that were used to handle raw poultry). Among the 182 expected hand washes after handling raw poultry, 58% (106/182) of subjects did not wash hands at all. Figure 3 summarizes the frequencies of hand washing right after handling raw poultry.

Figure 3: Total Hand Wash Required After Touching Raw Poultry:



Cross contaminations

Cross contaminations of food and contact kitchen surfaces resulting from handling raw poultry were observed.

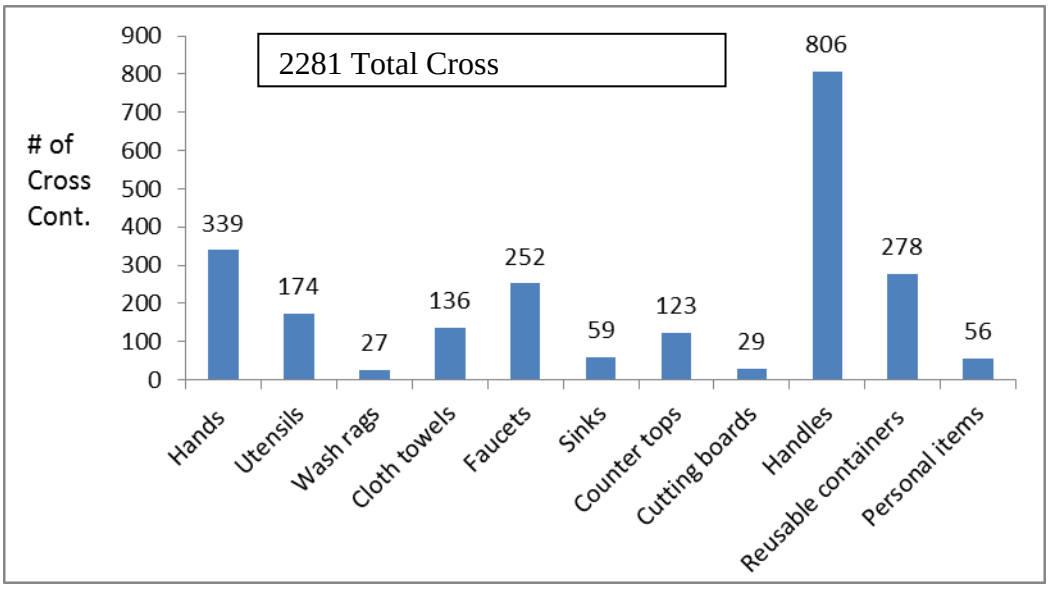
Of the total 9,166 activities annotated in the observational study, 2, 281 (25%) of those activities resulted in cross contamination events. Numerous kitchen environments were contaminated, such as counter tops, utensils, appliances, cabinet handles, knobs, reusable food containers (e.g., salt shakers, oil bottle), wash rags, and cloth hand towels. Twenty nine percent (16/56) of subjects initially started meal preparation by first washing the raw poultry in the sink. Each of these washes resulted in contaminations of hands, sinks and the surrounding counter top surfaces. Figure 4 summarizes the frequency and items cross contaminated during raw poultry handling. Personal items such as computers, cell phones, and recipe books were among those

items that were observed to be contaminated. Among the items that were most frequently cross contaminated by hands were handles of cabinets, drawers or appliances (e.g., microwaves), making up 35% (806/2281) of total cross contaminations. The next four most frequently contaminated items were hands (15%), Reusable food containers (12%), faucet handles (11%) and utensils (7.6%). Lack of proper hand washing contributed to the cross contamination of some of the utensils, cloth towels, faucet handles, cabinet handles, reusable food containers and personal items making up 85% of total cross contaminations (1947/2281).

While cutting boards are often suspected of contributing to cross contamination episodes, none of the observed subjects reused cutting boards that were initially used to prepare raw poultry for other food preparation purposes without first washing cutting boards. On the other hands, 5% (3/56) of subjects were observed either placing a reusable food container on contaminated cutting board or directly contaminating counter tops with previously used cutting boards.

During the course of meal preparations, there were 355 instances where subjects were observed touching their hair, mouth, face or other body parts. Seventy one percent of participants were observed touching bare body parts during the course of meal preparation (40/56), with frequency of touching ranging from 1 to 47 times (Avg. 17, SD=12)

Figure 4: Frequencies of observed cross contamination events.



Final Meal and Cook Temperature Monitoring

Five percent (3/56) of subjects took final cook temperature of cooked meals; despite 43% of the households reporting to owning a thermometer and 25% (12/46) reported checking final cook temperatures. Nevertheless, all subjects checked the inside of the meat for color and consistency before eating or serving. Over all, eighty two percent (46/56) of the meals were directly or indirectly handled with contaminated hands or utensils. In addition, during the course of preparing meals, Forty three percent of subjects (24/56) were observed eating or drinking foods while preparing meals (e.g., chips) without first properly washing their hands or after touching contaminated surfaces. Among those households that also prepared side dishes (n=26), 58% prepared their side dishes during the same time the main meals were being prepared and 35% (9/26) prepared side dishes after the main meals were prepared. The mean number of cross contaminations for those that did not prepare side dish at all (n=30), those that prepared before

(N=2), during (N=15) and after (N=9) were, 35, 30, 53 and 70 respectively. There is a significant statistical difference in the frequency of cross contaminations between those that did not prepare side dishes at all (N=30) and those that prepare side dishes during or after the main meals (T-test, P=0.026). There was no statistical significant difference in the frequency of cross contaminations observed between those that prepared side dishes during and after the main meals (T-test, P= 0.438).

Cleaning/Sanitizing

During the recorded meal preparations, 62.5% (35/56) of subjects cross contaminated their counter tops, whereas only 7% (4/56) of subjects were observed properly cleaning or sanitizing the contaminated counter top surfaces. Twenty five percent of subjects (14/56) used dish wash rags to wipe counter surfaces and other contact surfaces.

3.3.3: Reported vs. Observed Food Handling Practices

There were differences in participants response to the questionnaires and were observed practicing. Overall participants reported more safe food handling practices than were observed practicing. The most striking differences are the percent of individuals reported to wash their hands before starting to prepare meals (Fisher exact, P=<0.0001) and use of sanitizer to disinfect contact surfaces (Fisher exact, P=<0.0001). Table 11 summarizes some of the food handling practices reported vs. observed.

Table 11: Reported vs. Observed Food Handling Practices

Safe Food Handling Practices	Reported (%)	Observed (%)	Fisher Exact test
Poultry storage (Bottom shelf)	61	42	0.0693
Raw poultry not washed	53	71	0.1027
Use sanitizer to disinfect contact surfaces.	77	12.5	<0.001
Wash hands before beginning of meal preparation.	99	5	<0.001

3.4: Discussion:

Our study identified and quantified a number of risk factors that can potentially increase the risk of infection with *Salmonella* or *Campylobacter* that are often found on raw poultry products.

Transportation and storage:

According to the survey results, 99% of raw poultry products are put away in the refrigerator or freezer within 60 minutes after purchase. Extended room temperature storage after purchase does not appear to be a significant risk factor among households in the study. Nevertheless, a larger proportion of households (42%), continue to store raw poultry products either on the top or middle shelves of the refrigerator, potentially increasing the risk of contamination of other food products that are stored on the bottom shelves.

Fifty one percent of the subjects in the study reported storing raw poultry in the refrigerator for three or more days, with ten percent of them keeping raw poultry in the refrigerator for more than five days. The recommended storage time for raw poultry products in home refrigerators is less than two days (USDA-FSIS, 2013). Extended refrigeration storage of raw poultry in poorly cooled refrigerators can potentially increase the risk of re-growth of pathogenic organisms

resulting in an increased risk of cross contaminations and spoilage of products. An observational study of domestic refrigeration temperature had found 59% of refrigerators holding foods at greater than the recommended cold holding temperature of 41°F (5°C) (Kennedy J., *et al.*, 2005). Therefore, if poultry products are not going to be used within two days after purchase, consumers should consider freezing the products.

Poultry Handling

Almost half of the households reported regularly washing raw poultry (45%) before use, which is higher than the data from the observational study (29%). Either way, a substantial proportion of households continue to wash raw poultry, whereby contaminating their hands, sinks, and the counter top around the sink. All of the households that washed raw poultry were observed contaminating various items, including the tap handle, sinks, hands, utensils and the counter top around the sink. Due to its risk of increasing cross contaminations, washing of meats is not recommended (USDA-FSIS, 2013).

Proper thawing of frozen raw poultry can minimize the risk of the regrowth of bacterial pathogens and reducing the risk of cross contaminations. Seventy two percent of households reported to thawing frozen raw poultry either in the microwave or in the refrigerator. Fourteen percent of them reported to thawing at room temperature and another 14% reported to thawing under warm running water. Neither of the latter methods are recommended for thawing poultry, since both can increase the risk of the regrowth of *Salmonella*, hence increasing the risk of cross contamination. Among the fifteen participants in the study that were observed thawing poultry, microwave thawing was performed fifty three percent of the time, while thawing under running water was performed 27% of the time. A similar result was reported by an observational study

whereby 16% of households thawed frozen burgers at room temperature on the counter, while fifty six percent of households thawed in the refrigerator (Phang *et al.*, 2011).

Hand washing

While 100% of subjects reported washing their hands before beginning to prepare meals, only 5% of the observed subjects washed their hands properly before beginning to prepare meals. While 98% of subjects reported to washing their hands with soap and hot water after handling raw poultry, our observation found only 12% of the times hands were properly washed after directly contaminated with raw poultry.

Previous observational studies have reported lack of proper hand washing practices among food handlers (Scott E. and N. Herbold, 2010, Kendall P. *et al.*, 2004, Redmond *et al.*, 2004). A similar microbiological observational study, evaluating frequency of hand washing before, during and after poultry meal preparation found only 5% of households washed before beginning meal preparation, 35% after contact with raw poultry and 5% after completing meal preparation (Hoelzl C. *et al.*, 2012). Our study found about one third the frequency of proper hand washing reported by Hoelzl C, 2012.

In addition to lack of hand washing, the recommended length of time contaminated hands are expected to be washed with soap and water are not often met. In the current study, the length of times hands were washed with soap and hot water greatly varied from as short as two seconds to as long as 33 seconds, with an overall average of 13 seconds (SD =7 seconds). Other observational studies evaluating raw burger handling had reported lack of compliance to the recommended hand washing with 15 seconds of active rubbing (Scott E. and N. Herbold, 2010, Phang *et al.*, 2011).

An observational study by Redmond *et al.*, reported 100% of participants failed to carry out adequate and immediate hand washing and drying after handling raw chicken on at least one occasion. The risk posed by lack of proper hand washing is compounded by the fact that 46% (26/56) of subjects in the current study were observed snacking or tasting cooked meals with fingers that were directly or indirectly contaminated.

Unfortunately, there continues to be a substantial difference in what people claim they practice and what they actually do when observed. This may be due to higher consumer's knowledge of food safety which they fail to practice (Scott E. and N. Herbold, 2010, Redmond C. E. and Griffith J. C., 2003).

Cross contaminations:

Among the many ways salmonellosis can be acquired from poultry, the two major pathways are believed to be either under cooking or cross contamination events (Luber, 2009), with cross contamination events being the most dominant pathway (Nauta *et al.*, 2009; Luber 2009). An observational study coupled with microbiological sampling of contact surfaces had found 80-86% all unsafe food-handling behaviors were associated with cross contamination (Redmond C. E and Griffith J. C., 2004). Other similar studies have reported the ease by which pathogens can transfer from naturally contaminated raw poultry to various surfaces, including ready to eat foods (Ravishankar S. *et al.*, 2010; Luber *et al.*, 2006).

Unwashed hands contributed to 85% of all of the cross contamination episodes observed in current study, underscoring the importance of proper hand washing. Numerous kitchen environments were directly or indirectly contaminated. By far the most frequently contaminated non-food contact surfaces are handles of cabinets and appliances. In addition, food containers

such as salt shakers, oil bottles and other reusable food containers are also among the most commonly contaminated products. Subjects were observed directly handling these items without first washing their hands after handling raw poultry. This is consistent with previous observational studies (Redmond C. E. and Griffith J. C., 2003; Phang S. H. and Bruhn M. C., 2011). Contaminated food containers can potentially increase the risk of infections with *Salmonella* from future meals.

In our study, a substantial proportion of households reported not having separate cutting boards (45%) and almost all reported never reusing cutting boards without first adequately cleaning. When observed, none of them reused contaminated cutting boards to process other food items. This might be a reflection of only 21% of households using cutting boards during recorded meal preparations. However, compared to a survey done by the U.S. Food and Drug Administration, more than two decades ago, 26% of American consumers did not clean cutting boards after it was used to cut raw meat or chicken (Bryan, F. L. 1988). If this is an actual shift in food handling practices, it is encouraging since cross contaminations due to improper use or cleaning of cutting boards have been implicated in foodborne outbreaks (Cliver O. D, *et al.*, 2006).

Hand towels were frequently observed being used to clean contaminated unwashed hands, clean counter tops, and also to dry clean utensils. Contamination of hand towels made up 6% of the contamination episodes. Hand towels were being used for multiple purposes, including drying hands, wiping counter tops, and drying utensils and plates. *Campylobacter* isolation from hand towels that were suspected of being used to dry inadequately washed hands and wipe kitchen work surface had been previously reported (Redmond C. E., *et al.*, 2004).

Final cook temperature

Bacterial contamination of carcasses is an outer surface phenomenon (Lumber *et al.*, 2007; Brichta-Harhay *et al.*, 2008), therefore the typical cooking temperature and durations are believed to minimize the risk associated with undercooking. The only certain way to monitor final cook temperature is to use a thermometer, but small proportion of the households reported and were observed using thermometers. According to the response to the questionnaires, twenty five percent of households reported regularly using thermometer to check final cook temperatures of cooked poultry. On the other hand, only three percent of the households were observed using the thermometer to monitor final cook temperatures. In addition, almost half of the households reported to ether not knowing the final cook temperature (37%) or reported final cook temperature to be less than 165°F (10%). This number is lower than what was reported by Phang *et al.*, 2011, where by about 65% of subjects responded they did not know the final cook temperature for burgers (Phang S. Ho and Bruhn C., 2011). Despite its unreliableness, all of the households were observed checking the inside of the chicken for doneness before consuming. Lack of thermometer use to check the final cook temperature has similarly been reported in previous studies (Heelzl C. *et al.*, 2012, Kendall, P.A., *et al.*, 2004).

The order of when different types of foods are prepared can have an impact on the overall risk of cross contamination. Preparation of side dishes during or after raw poultry handling can potentially increase the risk of cross contamination. Among those in the study that were observed preparing side dishes, 93% percent prepared side dishes either during or after raw poultry were handled. Another observational study had reported 100% of individuals in the study were observed touching side dishes (i.e., ready to eat foods) after handling raw poultry (Redmond CE *et al.*, 2004). As can be expected, individuals who prepared side dishes before

handling raw poultry or not at all prepared side dishes had significantly lower number of cross contaminations when compared with those preparing during or after. Therefore, as simple as encouraging consumers to prepare side dishes before raw poultry products are handled, can significantly reduce the risk of acquiring foodborne illnesses.

Cleaning/Sanitizing

None of the subjects in our current study were observed sanitizing food contact surfaces both before starting and after completing meal preparations. During the course of meal preparations, 62.5% (35/56) of subjects cross contaminated their counter tops, whereas only 7% (4/56) of subjects were observed properly cleaning or sanitizing the contaminated counter top surfaces. By contrast, 77% percent of households reported to using sanitizers on a regular basis. Some bacterial pathogens such as *Salmonella* are known to attach and survive on different kitchen surfaces (Ravishankar S. *et al.*, 2010; Zhao P., *et al.*, 1998). Therefore, sanitizing or cleaning of food and non-food contact surfaces is important in preventing cross contamination events. Twenty five percent of the households were observed using wash rags to wipe surfaces contaminated with raw poultry. In some instances, the same wash rags were also used to clean counter tops.

3.5: Conclusions

Because direct observational studies are costly and time consuming, large numbers of questionnaire based studies have been used to understand people's food handling practices. However this study and others have demonstrated that people's knowledge of safe food handling practices drastically differs from their actual food handling behavior. Therefore, results of questionnaire based studies should be interpreted with caution. In particular, reported hand

washing frequency and the extent of cleaning and sanitizing, differ from recorded observations. These poor practices were observed facilitating cross contaminations of multiple kitchen surfaces and potentially exposing the study population to bacterial pathogens. On the other hand, cutting boards which are often implicated in foodborne illnesses did not appear to be risk factors in the current study.

Still there were several activities leading to cross contaminations observed that can contribute to not only to intra-meal infections, such as handling final cook products with contaminated hands and utensils, but also to inter-meal infections, resulting from contaminated items such as, handles, counter surfaces, appliances, personal devices (e.g., cell phones) and seasoning food containers. In households with multiple persons sharing the same kitchen, there were multiple pathways for cross contaminations. Lack of sanitizing of contact surfaces right after use were observed as potential reasons for inter meal contaminations.

Consumers are the last line of defense in the fight against foodborne illnesses. Therefore, interventions to reduce the burden of foodborne illnesses should as equally be focused on people's food handling practices as raw poultry processors. The recent multistate foodborne outbreaks, due to *Salmonella* Heidelberg from raw poultry, resulting in more than 128 illnesses and 34 hospitalizations underscores the impact of poor poultry handling practices in homes. Consumers should be educated to treat each package of raw poultry product as potentially containing *Salmonella* and should handle each package with caution to minimize cross-contaminations. Frequent proper hand washing after handling raw poultry and sanitizing common contact surfaces throughout the kitchen on a regular basis can minimize the frequency of cross contaminations, hence reducing the risk of infections.

Despite what most consumers believe and report (U.S. FDA, 2006), foods prepared in homes may not be any safer than commercially prepared foods. If the same food inspection criteria used to evaluate commercial retail establishments were applied to households in this study, as many or more deficiencies in proper food handling practices would have been cited. Food inspection results are often reported as Satisfactory or Unsatisfactory. Satisfactory inspection is given to establishments with no or some low risk violations (e.g., sanitation solution too week), whereas Unsatisfactory is given to establishments with violations deemed high risk (e.g., lack of hand washing, cross contamination events). There are many food safety controls commercial retail establishments have individual households don't have. Some of the main controls include; designated hand washing stations, segregated cold holding storage, a basin or proper fresh produce washing equipment, use of disposable paper towels, use of sanitizers to disinfect surfaces, and some food safety trainings. Therefore, based on the current observations made, 100% of the households in this study would have received unsatisfactory inspections.

In addition to focused consumer food handling educations to the identified risk factors, design based interventions should also be considered. Future studies should also focus on ways the kitchen environments can be designed to minimize the frequency of hand contacts or cleaning of common contact surfaces in the home kitchens and use of surfaces with minimal risk of bacterial transfer.

Chapter Four

Comparisons of Pulsed Field Gel Electrophoresis of *Salmonella* spp. isolated from Raw Poultry from Local Retail Stores to Human cases from the same Geographic Areas

E. Mazengia[1], J. Meschke[1], K. Greeson[2], M. Samadpour[2]*

[1] University of Washington Department of Environmental and Occupational Health Sciences. Seattle, WA.

[2]Institute for Environmental Health, 15300 Bothell Way NE. Lake Forest Park, WA 98155

Key Words: PFGE, *Salmonella*, salmonellosis,

Abstract

Foods of animal origin, such as poultry, eggs and pork, are recognized sources of infection with *Salmonella* and other human pathogens. Determining the proportion of foodborne infections resulting from different food sources has been challenging. In the current study, hundred thirty three *Salmonella* species isolated from poultry products purchased from retail stores were subtyped by Pulsed Field Gel Electrophoresis following restriction with *Xba*I. The main objective of the study was to determine the degree of association between PFGE *Xba*I profiles of *Salmonella* isolated from locally distributed poultry products to that of clinical isolates submitted to the Washington State Department of Health Laboratories. Furthermore, the unique PFGE profiles were also compared for matches with *Salmonella* isolate from various food animals from multiple states as part of the National Antimicrobial Resistance Monitoring System. The PFGE clusters were analyzed for their longitudinal distribution throughout the sampling period and their clonality within and between processing establishments. There were a total of 47 unique clusters with 49% belonging to one of the four predominantly circulating clusters. There were eight indistinguishable PFGE profiles between our isolates and those from the National Antibiotic Resistance Monitoring System. Matches as far back as 2002 from multiple states were found. The PFGE matches were recovered from chicken, turkey and beef products throughout the years from multiple states. Serovars Kentucky from chickens (n=153) and Hadar from turkey (n=99) made up 79% the total matches. There were twelve indistinguishable PFGE profiles between those that were isolated from locally purchased poultry products and clinical isolates submitted to the Washington State Department of Health Laboratories. The twelve PFGE clusters represented a total of 580 clinical isolates submitted to the health department

since the year 2000. Serovars *Salmonella* Heidelberg (n=51) and *Salmonella* Enteriditis (n=21) made up 70% (72/103) of the total matches. Most of the PFGE clusters were recovered from multiple processing establishments throughout the sampling period of one year. Some clones persist over multiple years in multiple processing establishments and various product types. While this study finds a strong association between PFGE clusters found on raw poultry products from local grocery stores and that of clinical isolates from the same geographical area, it does not claim that all of those clinical isolates were cases infected as results of the consumption or handling of poultry. Nevertheless, it substantiates the potential of poultry as the main source of human infections in the study area.

4.0: Introduction:

Salmonella is a zoonotic pathogen with significant public health impact worldwide. In the United States, over 1.0 million cases of foodborne illness are caused by *Salmonella* spp., resulting in 168,000 physician visits, 19,336 hospitalizations and 378 deaths. According to the current estimates, infection with non-typhoidal *Salmonella* spp. is the leading cause of hospitalization and death of foodborne bacterial infections (Scallan *et al.*, 2011).
In 2010, the incidence rate of salmonellosis in the United States, based on confirmed *Salmonella* culture, is 17.6 per 100,000, which is about three times higher than the target incidence rate set for Healthy People 2010 (Cummings PL., 2012). The age group with the highest incidence rate is children under the age of 4 years, and in 2009 the incidence of *Salmonella* per 100,000 individuals in this age group was 72.19. In Washington State, the incidence rate has been about 12.8 cases/100,000 individuals for many years (WA-DOH, 2011).

Multiple foods, including fruits and vegetables (i.e., sprouts, mangoes, cantaloupe, tomatoes, cucumbers, jalapeno, peppers), non-poultry meats (i.e., ground beef, pork) and animals/reptiles (i.e., hedgehogs, turtles, frogs and rodents), and dog foods have been implicated in numerous recent foodborne outbreaks (CDC, 2013). By far the most frequently epidemiologically linked food products to multistate outbreaks are poultry products (CDC, 2013). Source attribution studies have indicated poultry products contribute a substantial proportion of salmonellosis in the population in the United States (Batz *et al.*, 2012; Hoffmann *et al.*, 2006).

Over the last several years, the incident rate of salmonellosis in the United States has not drastically changed, despite the numerous initiatives and programs that have been implemented. In 2009, the U.S. Department of Agriculture proposed tighter pathogen reduction performance standards for *Salmonella* and *Campylobacter* on chilled broiler and turkey carcasses (FSIS, 2009). Lack of correlation between reduction in the prevalence of *Salmonella* on raw poultry and lower incidence of salmonellosis in the population has caused some to question the utility of efforts to control the prevalence of *Salmonella* on poultry.

There are many reasons why the prevalence of *Salmonella* on raw poultry products may not correlate well with reported human illnesses. First, prevalence rates include *Salmonella* species that are found on raw poultry that may not have as much public health impact. For example, while *Salmonella* Kentucky makes up substantial proportion (~30-42%) of the prevalence rate on raw poultry (Cox *et al.*, 2011), it is often not among the top twenty *Salmonella* serotypes responsible for clinical cases (NARMS, 2011), therefore obscuring the relationship between the prevalence and that of incidence rates in humans. Second, risk assessment studies have reported that, the contamination level of *Salmonella* on raw carcasses has an increase risk to human

illness than the mere presence of *Salmonella* (WHO, 2002, Straver *et al.*, 2007, Callicott K. *et al.*, 2012). Therefore, the presence of *Salmonella* on raw carcasses alone does not translate to an increase risk of human infection. More appropriately, *Salmonella* contamination levels might be strongly correlated with incidence rate of *Salmonella* in the population. Third, there are differences in the risk of infection by serotypes. Some strains are able to better persist in the environment, invade cells (Sanchez S. *et al.*, 2002, Cox *et al.*, 2011). While prevalence rate treats all *Salmonella* as equal, there are differences in the risk posed by serotypes, hence the incidence rate of salmonellosis. Forth, prevalence rates do not account for differences in the level of antibiotic resistance. For instance, a chicken carcass that is positive for multidrug resistance S. Heidelberg has a higher infectivity risk than one that is sensitive. Fifth, there are multiple food handling processes that happen between when prevalence rates are determined (often in-plant sampling, right after post chilling) and consumption. Activities such as packaging, transportation, storage, and final food handling practices greatly affect the potential risk of a *Salmonella* positive carcass. The in-between activities can modify (i.e. increase or decrease) the risk of a *Salmonella* positive carcass, which prevalence rates do not account for. Our own previous study of individuals handling raw poultry in their home kitchens revealed many risk factors such as lack of proper hand washing (88%) after directly handling raw poultry (Mazengia E. *et al.*, 2013b). Sixth, the number of samples collected on a monthly basis to establish a prevalence rate per new FSIS directive (Pathogen Reduction for *Salmonella* from Broiler carcasses), compared to the amount of poultry processed and available for consumption is insignificant. In the United States, there are about 9 billion birds slaughtered annually, with a modern processing plan handling more than 200,000 birds per day (Kiepper, B, 2011), but the average number of samples taken per plant per Pathogen Reduction for *Salmonella* is, are

51/month? (i.e., about 9 samples for every 1000,000 carcasses processed). Seventh, the microbiological laboratory methods used to concentrate, detect and isolate *Salmonella* from raw poultry are different from the methods used in clinical laboratories, therefore influencing the rate of detection, and the serotypes recovered. Finally, it has been established that human acquire salmonellosis from multiple food and non-food sources (CDC, 2013a). Reptiles and amphibians (e.g., frogs, toads, newts, and salamanders) have been implicated in a number of large *Salmonella* outbreaks (CDC, 2013a), yet those human cases are not excluded when a correlation between raw poultry and human salmonellosis cases is established, hence potentially over estimating the impact of poultry have in human infections.

Since only a small proportion of those infected individuals seek medical care and their source of infection ascertained (Hennessy WT, *et al.*, 2004), there have not been major sources of *Salmonella* infection revealed, other than poultry products, mainly based on the higher prevalence of *Salmonella* present on raw poultry compared to other food animal sources. Either the assumption that 95% of salmonellosis cases in the population is foodborne is an overestimate and there are multiple major non-food sources that have not been well understood. Or, if indeed 95% of salmonellosis infection is foodborne, the impact of poultry and other food animal sources should be closely examined the risk factors starting from processing to all the way to consumption.

Multiple risk factors have been suggested as to why the burden of salmonellosis the United States has remained high, including aging population, increase global market, and population with increased comorbid conditions (Cummings PL *et al.*, 2012). Compared to other foods of

animal origin, the prevalence of *Salmonella* on raw poultry products is by far the highest. The 2010 retail meat report by the National Antimicrobial Resistance Monitoring System (NARMS) in the United States reports the following *Salmonella* percent positives by products: 13.0% for chicken breast, 14.6% ground turkey; 1.1% ground beef and pork chops 2.2 (NARMS retail meat report, 2011).

While it is well studied fact that improper food handling practices of *Salmonella* contaminated poultry or other food items can result in the infection of humans (Rosenquist, H, 2003), due to the ubiquitous nature of *Salmonella* in various food and non-food sources, there is uncertainty in estimating the overall public health burden of salmonellosis from raw poultry. In order to better evaluate the impact of control efforts (e.g., reducing the prevalence), a direct link to human illnesses has to be established.

The current study is the third in a serious of studies looking at the overall contribution of poultry handling to the risk of salmonellosis. The first study looked at the prevalence of *Salmonella* found in locally (Seattle, Washington) distributed raw poultry products (Mazengia, *et al.*, 2013a). It was reported that, serotypes such as *S.* Heidlberg and *S.* Enteriditis were recovered from multiple poultry processing establishments over the study period. While serotype *S.* Kentucky was among the top three recovered from raw poultry, it was not among the top 20 clinical isolates reported (WA-DOH, 2011). In addition, it was reported that all of the antibiotic resistance *Salmonella* isolates were recovered from processing establishments that utilized antibiotics in their processing. The second study looked at how raw poultry products are transported and handled in individual homes (Mazengia, *et al.*, 2013b). It was reported that there were multiple risk factors identified that are known to increase the risk of contamination with

Salmonella during raw poultry handling. Observed food handling practices were inferior to reported knowledge of food safety in the study group. The main objectives of the current study are to genetically characterize the *Salmonella* isolates recovered from raw poultry products from local retail stores, followed by assessing their genetic relatedness to clinical isolates submitted to the Washington State Department of Health Laboratories (WA-DOH). In addition, PFGE profiles of *Salmonella* isolates from poultry are compared with those isolates recovered as part of the National Antibiotic Resistance Monitoring Study (NARMS). Some additional objectives were evaluating the genetic variability of the *Salmonella* isolates by product and processing establishments; determining the temporal and geographical distribution of indistinguishable PFGE clusters; and substantiating the strength of the association between the PFGE profiles from locally distributed poultry isolates and that of clinical isolates submitted to the health department in the same geographical area.

4.1: Materials and Methods

4.1.1: Poultry Meat Sampling

In 2011/2012 a market survey study quantifying the prevalence of *Salmonella* species on poultry from local grocery stores was conducted. In the course of a year, a total of 1322 poultry products, representing several processing establishments, were purchased from local grocery stores in Seattle, Washington and analyzed for *Salmonella*. Poultry samples including whole muscle and ground products were included in the sampling.

4.1.2: Isolation and Characterization of *Salmonella* spp. from Poultry

The isolation and characterization of the *Salmonella* isolates from local poultry products were discussed previously (Mazengia, *et al.*, 2013a). Briefly, 350 g of poultry products was mixed with 350mL of 0.1% prewarmed (42±2°C) peptone water. After thorough mixing, 50 mL of rinsate was removed for enumeration and 300 mL of modified TSB (mTSB) (Beckton Dickinson, Sparks, MD) added. After an overnight incubation at 42$\pm$ 2°C for 24 $\pm$ 2hrs, 2 µL of enriched sample was screened for two *Salmonella* specific genes (Sal_1^+/Sal_2^+) using multiplex Polymerase Chain Reaction (PCR) (Molecular Epidemiology Incorporated, Bothell, Washington). Those samples that were positive for either or both Sal_1^+/Sal_2^+, their corresponding enriched samples were subjected to immune magnetic separation procedure using anti *Salmonella* antibodies (Dynal, Lake Success, NY)) to aid with the concentration of the *Salmonella* species. The concentrates were both spread plated and also streaked onto Xylose Lysine Desoxycholate (XLD) (Oxoid, Remel, Lenexa KS) and incubated at 35 $\pm$ 2°C for 24 $\pm$ 2hrs. Typical colonies were picked for further testing. One hundred fifty samples were positive for *Salmonella* (11.3%).

One hundred six of those isolates were serotyped following the procedure of Kauffman-White scheme (KW) by FDA laboratory (FDA, Colorado laboratory). Eleven serotypes with the following proportions were obtained, 37% Heidelberg, 24% Enteriditis, 20% Kentucky, 6% Hadar, 5% Schwarzengrund, 2% Agona, 2% Senftenberg, 1% Berta, 1% Litchfield, 1% Mbandaka and 1% Typhimurium. All of the isolates were also screened for antibiotic susceptibilities to a panel of 15 antibiotics in the same laboratory.

4.1.3: Pulsed Field Gel Electrophoresis (PFGE)

A total of 141 *Salmonella* isolates were subtyped following the standardized PFGE protocol developed by the Centers for Disease Control and used by PulseNet-USA (Anonymous, 2004; Graves and Swaminathan, 2001; CDC, 2009). Briefly, bacterial DNA was prepared and digested using restriction enzyme *Xba*I (Takara, Shiga, Japan). *Salmonella* enterica Braenderup (ATCC BAA-664) was also digested with *Xba*I and used as the DNA size marker. The digested DNA was separated with a contour-clamped homogeneous electric field (CHEF Mapper, BioRad, Hercules, CA) in 1% SeaKem agarose gels (Lonza, Basel, Switzerland) at a 2.2 to 54.2 second pulse over 18 hours, and stained with ethidium bromide.

4.1.4: Comparison to the NARMS PFGE Database

The National Antimicrobial Resistance Monitoring System (NARMS) is a national public health surveillance system that tracks antibiotic resistance in foodborne bacteria. NARMS retail meat monitoring is an ongoing multi-agency collaboration work involving the U.S. Food and Drug Association Center for Veterinary Medicine, multiple public health laboratories (FoodNet sites) and the Centers for Disease Control (CDC). Each FoodNet site submits 40 retail meat samples every month for analysis of *Salmonella*. The forty samples are comprised of ten of each of the following meat products: chicken breast, ground turkey, ground beef and pork chop. The *Salmonella* isolates are all subtyped following the PFGE laboratory protocol developed by the CDC. Detailed description of the samples and analysis is previously published (Zhao S. *et al.*, 2008). NARMS has therefore an extensive data base of PFGE profiles representing these foods of animal origin since starting the retail meat program in 2002. All of the unique PFGE patterns

generated in the current study were submitted to NARMS for comparison to PFGE profiles of *Salmonella* isolated from various food animal sources.

4.1.5: Comparison to the Washington State Health Department Laboratories

The Washington State Department of Health receives between 589 to 850 clinical *Salmonella* isolates each year. All of the *Salmonella* isolates are subtyped using standardized PFGE protocol developed by the CDC and used by PulseNet-USA (Ribot, EM, *et al.*, 2006). While *Xba*I is used as the primary enzyme, a limited number of the isolates are additionally restricted using *Bln*I (e.g., if requested by CDC). Therefore, most of the PFGE patterns stored in the database can only be compared for similarities with *Xba*I restricted profiles. The state laboratory has thousands of *Salmonella* PFGE profiles in its database spanning more than fifteen years. All of the unique PFGE patterns generated in the current study were submitted to WA-DOH for comparison to PFGE profiles of *Salmonella* isolated from clinical cases in the database.

4.2: Analysis

Samples and laboratory data were entered into Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA).

Gels that have been generated using the standardized PFGE protocols were digitally saved as a TIFF file. Gel files were imported into BioNumerics; normalized using a global standard and bands were scored and quantified. PFGE patterns were analyzed using BioNumerics software (Applied Maths, Inc., Austin, Texas). Cluster analysis was performed using the Dice similarity

coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic mean) algorithm to construct a dendrogram.

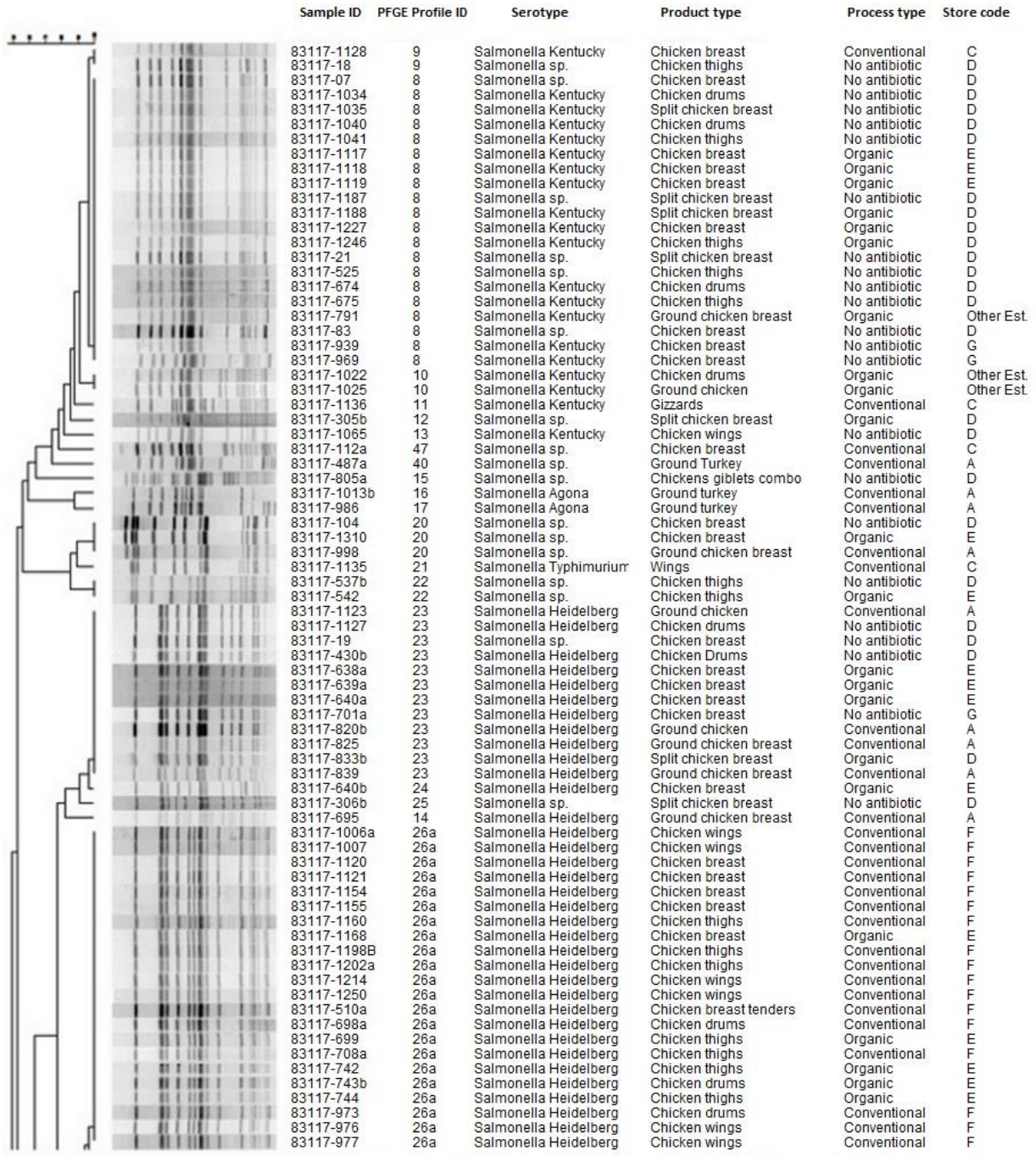
4.3: Results

4.3.1: Pulsed Field Gel Electrophoresis

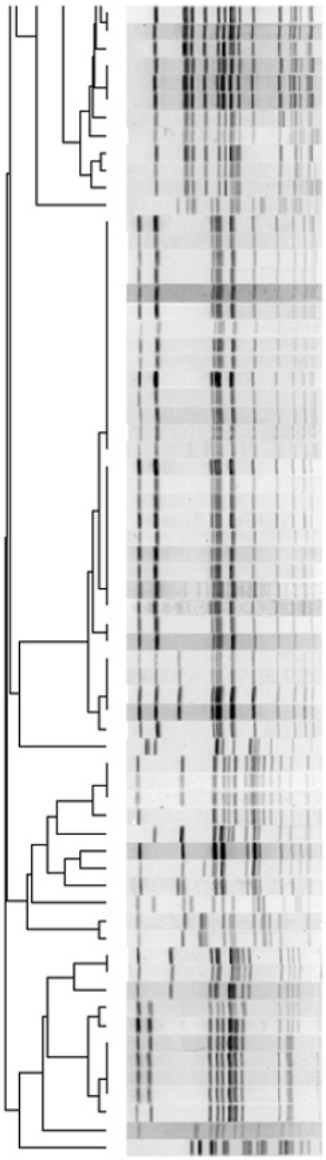
One hundred forty one of the total 150 *Salmonella* positive isolates were subtyped following DNA restriction with *Xba*I enzyme.

The *Salmonella* isolates in the current study had a high degree of variability with Simpson's index of diversity of **D=0.85.** Fifty one percent of the unique PFGE profiles were recovered only once during the sampling period. A total of 47 distinct profiles (based on 100% similarity) were found (Fig. 5) and unique PFGE patterns were numerically labeled from 1 to 47. There were four (1, 8, 23 and 26) predominant circulating strains. These four predominant strains were mainly represented by the following serotypes: Enteriditis (profile 1), Kentucky (profile 8), Heidelberg (profiles 23, and 26). These four profiles comprised 56% (79/141) the total isolates subtyped. Some clustering by serotype and by processing establishment was evident.

Figure 5: Dendrogram of PFGE *Xba*I Profile of *Salmonella* From Raw Poultry.



Sample ID	PFGE Profile ID	Serotype	Product type	Process type	Store code
83117-1128	9	Salmonella Kentucky	Chicken breast	Conventional	C
83117-18	9	Salmonella sp.	Chicken thighs	No antibiotic	D
83117-07	8	Salmonella sp.	Chicken breast	No antibiotic	D
83117-1034	8	Salmonella Kentucky	Chicken drums	No antibiotic	D
83117-1035	8	Salmonella Kentucky	Split chicken breast	No antibiotic	D
83117-1040	8	Salmonella Kentucky	Chicken drums	No antibiotic	D
83117-1041	8	Salmonella Kentucky	Chicken thighs	No antibiotic	D
83117-1117	8	Salmonella Kentucky	Chicken breast	Organic	E
83117-1118	8	Salmonella Kentucky	Chicken breast	Organic	E
83117-1119	8	Salmonella Kentucky	Chicken breast	Organic	E
83117-1187	8	Salmonella sp.	Split chicken breast	No antibiotic	D
83117-1188	8	Salmonella Kentucky	Split chicken breast	Organic	D
83117-1227	8	Salmonella Kentucky	Chicken breast	Organic	D
83117-1246	8	Salmonella Kentucky	Chicken thighs	Organic	D
83117-21	8	Salmonella sp.	Split chicken breast	No antibiotic	D
83117-525	8	Salmonella sp.	Chicken thighs	No antibiotic	D
83117-674	8	Salmonella Kentucky	Chicken drums	No antibiotic	D
83117-675	8	Salmonella Kentucky	Chicken thighs	No antibiotic	D
83117-791	8	Salmonella Kentucky	Ground chicken breast	Organic	Other Est.
83117-83	8	Salmonella sp.	Chicken breast	No antibiotic	D
83117-939	8	Salmonella Kentucky	Chicken breast	No antibiotic	G
83117-969	8	Salmonella Kentucky	Chicken breast	No antibiotic	G
83117-1022	10	Salmonella Kentucky	Chicken drums	Organic	Other Est.
83117-1025	10	Salmonella Kentucky	Ground chicken	Organic	Other Est.
83117-1136	11	Salmonella Kentucky	Gizzards	Conventional	C
83117-305b	12	Salmonella sp.	Split chicken breast	Organic	D
83117-1065	13	Salmonella Kentucky	Chicken wings	No antibiotic	D
83117-112a	47	Salmonella sp.	Chicken breast	Conventional	C
83117-487a	40	Salmonella sp.	Ground Turkey	Conventional	A
83117-805a	15	Salmonella sp.	Chickens giblets combo	No antibiotic	D
83117-1013b	16	Salmonella Agona	Ground turkey	Conventional	A
83117-986	17	Salmonella Agona	Ground turkey	Conventional	A
83117-104	20	Salmonella sp.	Chicken breast	No antibiotic	D
83117-1310	20	Salmonella sp.	Chicken breast	Organic	E
83117-998	20	Salmonella sp.	Ground chicken breast	Conventional	A
83117-1135	21	Salmonella Typhimurium	Wings	Conventional	C
83117-537b	22	Salmonella sp.	Chicken thighs	No antibiotic	D
83117-542	22	Salmonella sp.	Chicken thighs	Organic	E
83117-1123	23	Salmonella Heidelberg	Ground chicken	Conventional	A
83117-1127	23	Salmonella Heidelberg	Chicken drums	No antibiotic	D
83117-19	23	Salmonella sp.	Chicken breast	No antibiotic	D
83117-430b	23	Salmonella Heidelberg	Chicken Drums	No antibiotic	D
83117-638a	23	Salmonella Heidelberg	Chicken breast	Organic	E
83117-639a	23	Salmonella Heidelberg	Chicken breast	Organic	E
83117-640a	23	Salmonella Heidelberg	Chicken breast	Organic	E
83117-701a	23	Salmonella Heidelberg	Chicken breast	No antibiotic	G
83117-820b	23	Salmonella Heidelberg	Ground chicken	Conventional	A
83117-825	23	Salmonella Heidelberg	Ground chicken breast	Conventional	A
83117-833b	23	Salmonella Heidelberg	Split chicken breast	Organic	D
83117-839	23	Salmonella Heidelberg	Ground chicken breast	Conventional	A
83117-640b	24	Salmonella Heidelberg	Chicken breast	Organic	E
83117-306b	25	Salmonella sp.	Split chicken breast	No antibiotic	D
83117-695	14	Salmonella Heidelberg	Ground chicken breast	Conventional	A
83117-1006a	26a	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-1007	26a	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-1120	26a	Salmonella Heidelberg	Chicken breast	Conventional	F
83117-1121	26a	Salmonella Heidelberg	Chicken breast	Conventional	F
83117-1154	26a	Salmonella Heidelberg	Chicken breast	Conventional	F
83117-1155	26a	Salmonella Heidelberg	Chicken breast	Conventional	F
83117-1160	26a	Salmonella Heidelberg	Chicken thighs	Conventional	F
83117-1168	26a	Salmonella Heidelberg	Chicken breast	Organic	E
83117-1198B	26a	Salmonella Heidelberg	Chicken thighs	Conventional	F
83117-1202a	26a	Salmonella Heidelberg	Chicken thighs	Conventional	F
83117-1214	26a	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-1250	26a	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-510a	26a	Salmonella Heidelberg	Chicken breast tenders	Conventional	F
83117-698a	26a	Salmonella Heidelberg	Chicken drums	Conventional	F
83117-699	26a	Salmonella Heidelberg	Chicken thighs	Organic	E
83117-708a	26a	Salmonella Heidelberg	Chicken thighs	Conventional	F
83117-742	26a	Salmonella Heidelberg	Chicken thighs	Organic	E
83117-743b	26a	Salmonella Heidelberg	Chicken drums	Organic	E
83117-744	26a	Salmonella Heidelberg	Chicken thighs	Organic	E
83117-973	26a	Salmonella Heidelberg	Chicken drums	Conventional	F
83117-976	26a	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-977	26a	Salmonella Heidelberg	Chicken wings	Conventional	F



Sample ID	PFGE Profile ID	Serotype	Product type	Process type	Store code
83117-01	30	Salmonella Heidelberg	Chicken breast tenders	Conventional	F
83117-50	30	Salmonella sp.	Chicken thighs	Conventional	F
83117-662a	28	Salmonella Heidelberg	Ground turkey	Conventional	B
83117-34	26b	Salmonella sp.	Chicken thighs	Conventional	F
83117-37	26b	Salmonella sp.	Chicken thighs	Conventional	F
83117-41	26b	Salmonella sp.	Chicken thighs	Conventional	F
83117-945a	27	Salmonella Heidelberg	Chicken wings	Conventional	F
83117-1317	29	Salmonella Heidelberg	Chicken drums	Conventional	F
83117-1225	31b	Salmonella Heidelberg	Chicken thighs	Organic	Other Est.
83117-949a	31a	Salmonella Heidelberg	Ground turkey breast	Conventional	A
83117-1248	32	Salmonella sp.	Chicken breasts with rib meat	Conventional	C
83117-948a	19	Salmonella Heidelberg	Ground turkey	Conventional	A
83117-1030	1	Salmonella Enteriditis	Chicken drums	Organic	E
83117-1305	1	Salmonella Enteriditis	Chicken breast	Organic	E
83117-1306	1	Salmonella Enteriditis	Chicken breast	Organic	E
83117-1307	1	Salmonella Enteriditis	Chicken breast	Organic	E
83117-653a	1	Salmonella Enteriditis	Chicken thighs	Organic	E
83117-706a	1	Salmonella Enteriditis	Ground chicken	Conventional	A
83117-722b	1	Salmonella sp.	Ground chicken	Conventional	A
83117-726b	1	Salmonella sp.	Chicken breast	Organic	E
83117-733	1	Salmonella Enteriditis	Ground chicken	Conventional	A
83117-776a	1	Salmonella Enteriditis	Chicken drums	Organic	E
83117-777	1	Salmonella Enteriditis	Chicken thighs	Organic	E
83117-829	1	Salmonella Enteriditis	Chicken thighs	Organic	E
83117-865	1	Salmonella Enteriditis	Ground turkey	Conventional	A
83117-870	1	Salmonella Enteriditis	Chicken drums	Conventional	Other Est.
83117-09	2	Salmonella sp.	Chicken thigh fillets	Conventional	F
83117-1184	2	Salmonella Enteriditis	Ground chicken sausage	Conventional	Other Est.
83117-1195	3	Salmonella Enteriditis	Chicken breast	Conventional	C
83117-22	2	Salmonella sp.	Chicken thighs	No antibiotic	D
83117-497b	2	Salmonella sp.	Grouond chicken	Conventional	A
83117-584b	2	Salmonella Enteriditis	Chicken drums	Organic	D
83117-686a	2	Salmonella Enteriditis	Chicken thighs	Conventional	F
83117-854a	2	Salmonella Enteriditis	Ground chicken	Organic	Other Est.
83117-343	2	Salmonella sp.	Chicken drums	No antibiotic	D
83117-1196	3	Salmonella Enteriditis	Chicken breast	Conventional	F
83117-518a	3	Salmonella sp.	Chicken Drums	No antibiotic	D
83117-1132	5	Salmonella Enteriditis	Chicken breast	Conventional	C
83117-1133	5	Salmonella Enteriditis	Chicken breast	Conventional	C
83117-1285a	5	Salmonella Enteriditis	Ground chicken	Conventional	C
83117-531a	5	Salmonella sp.	Chicken giblets	No antibiotic	D
83117-888	6	Salmonella Enteriditis	Chicken thighs	Organic	E
83117-1107	7	Salmonella Litchfield	Split chicken breast	No antibiotic	D
83117-1095	33	Salmonella Schwarzengrund	Ground turkey	Conventional	A
83117-422a	33	Salmonella sp.	Ground Turkey breast	Conventional	A
83117-429a	33	Salmonella sp.	Ground Turkey breast	Conventional	A
83117-953a	34	Salmonella Schwarzengrund	Ground turkey	Conventional	A
83117-985	35	Salmonella Schwarzengrund	Ground turkey	Conventional	A
83117-522b	36	Salmonella sp.	Ground turkey	Conventional	B
83117-993	4	Salmonella Schwarzengrund	Ground turkey	Conventional	B
83117-1124	37	Salmonella Schwarzengrund	Ground turkey	Conventional	A
83117-1183	41	Salmonella Senftenberg	Ground chicken sausage	Conventional	Other Est.
83117-1039	38a	Salmonella Mbandaka	Chicken giblets combo	No antibiotic	D
83117-841	38b	Salmonella sp.	Chicken breast	No antibiotic	G
83117-658	39a	Salmonella Hadar	Ground turkey	Conventional	A
83117-659	39a	Salmonella Hadar	Ground Turkey	Conventional	A
83117-473a	39b	Salmonella sp.	Ground Turkey	Conventional	A
83117-139b	42	Salmonella sp.	Split chicken breast	Conventional	F
83117-573b	44	Salmonella sp.	Turkey burger patties	Conventional	B
83117-487b	43	Salmonella sp.	Ground Turkey	Conventional	A
83117-868	43	Salmonella Hadar	Ground turkey	Conventional	A
83117-923	43	Salmonella Hadar	Split chicken breast	No antibiotic	D
83117-867a	43	Salmonella Hadar	Ground turkey	Conventional	A
83117-1094	45	Salmonella Hadar	Ground turkey	Conventional	A
83117-663b	46	Salmonella sp.	Ground turkey	Conventional	B
83117-583	18	Salmonella sp.	Chicken thighs	Organic	D

4.3.2.Distribution of PFGE profiles by processing establishments and process types:

There were twelve PFGE profiles that were found in multiple establishments making up 26% (12/47) of the total unique PFGE patters found. Some of the PFGE profiles (1, 2, 8, 23) were found in four or more different establishments (Table 12). Establishment B did not share any common PFGE profiles with other establishments and it is a conventional processing

establishment that produces ground turkey. Some of the PFGE profiles (e.g., 1, 8), were found in multiple product types (e.g., ground turkey and chicken parts), but also were found in different processing types (e.g., organic and conventional processors). PFGE profiles 1, 8, and 23 correspond to serotypes Enteriditis, Kentucky and Heidelberg respectively.

Table 12: PFGE Profiles by Establishment and Product Types/Process Types

PFGE Profiles	**Processing Establishments**							
Est.	A	B	C	D	E	F	G	Other
Products	GT,GC	GT	Ck	Ck	Ck	Ck	Ck	GC/Ck
Process	C	C	C	AF	O	C	AF	C/O
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
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26								
27								
28								
29								
30								
31a/b*	a							b
32								

33								
34								
35								
36								
37								
38								
39								
40								
41								
42								
43								
44								
45								
46								
47								

AF: Antibiotics Free; C: Conventional; O: Organic
Ck: Chicken parts; GT: Ground Turkey; GC: Ground Chicken
a/b*:similar but not indistinguishable.

4.3.3: Distribution of PFGE Clusters Over Sampling Period

The four predominant unique PFGE profiles (1, 8, 23, 26) were recovered from raw poultry samples throughout the year. Not only were these PFGE profiles isolated throughout the year within a processing establishment, but also were recovered from multiple processing establishments. Table 13 summarizes when these predominant PFGE profiles were recovered during the sampling period, starting on April 2011 through April 2012.

Table 13: Temporal Distribution of the Predominant Circulating PFGE Profiles

PFGE Profiles	# w/profiles	# est. w/profiles	Est.(counts)	April/2011	May/2011	June/2011	July/2011	Aug/2011	Sep/2011	Oct/2011	Nov/2011	Dec/2011	Jan/2012	Feb/2012	Mar/2012	April/2012
1	14	3	E (9)	1	0	0	0	0	1	1	5	3	2	0	2	3
8	20	4	D(16)	2	1	0	0	0	2	0	2	1	0	2	9	3
23	10	4	A(4) D(3)	1	0	1	0	1	0	3	2	4	0	0	2	0
26	26	2	F(21)	4	0	0	0	1	0	6	0	0	0	4	5	4

4.3.4: PFGE Comparisons to NARMS Database

All of the unique PFGE subtypes from the current study were submitted to NARMS for comparisons to the PFGE subtypes generated from poultry, ground turkey, ground beef and pork chop samples from multiple states since 2002. There were eight indistinguishable PFGE matches (n=55 in the current study) made within those in the NARMS PFGE database (Table 14). There were 320 isolates in the NARMS data base representing these eight unique PFGE profiles recovered from chicken, turkey and beef. A substantial proportion (64%) (210/320) of the 320 samples that had indistinguishable PFGE profile matches with the current study were isolated from chicken (63%), followed by turkey (34.5%) and beef (1.5%).

Some of the profile matches had been seen in NARMS database as early as the year 2002 (e.g., 8, 21). There were six different serotypes represented from the eight PFGE matches. *Salmonella* Kentucky (50%) and *Salmonella* Hadar (38%) were the two serotypes with the most matches.

All of the *Salmonella* Hadar matches were from ground turkey and had an overall similarity of >80%.

Table 14: Comparisons with Isolates Submitted to NARMS by Product Types

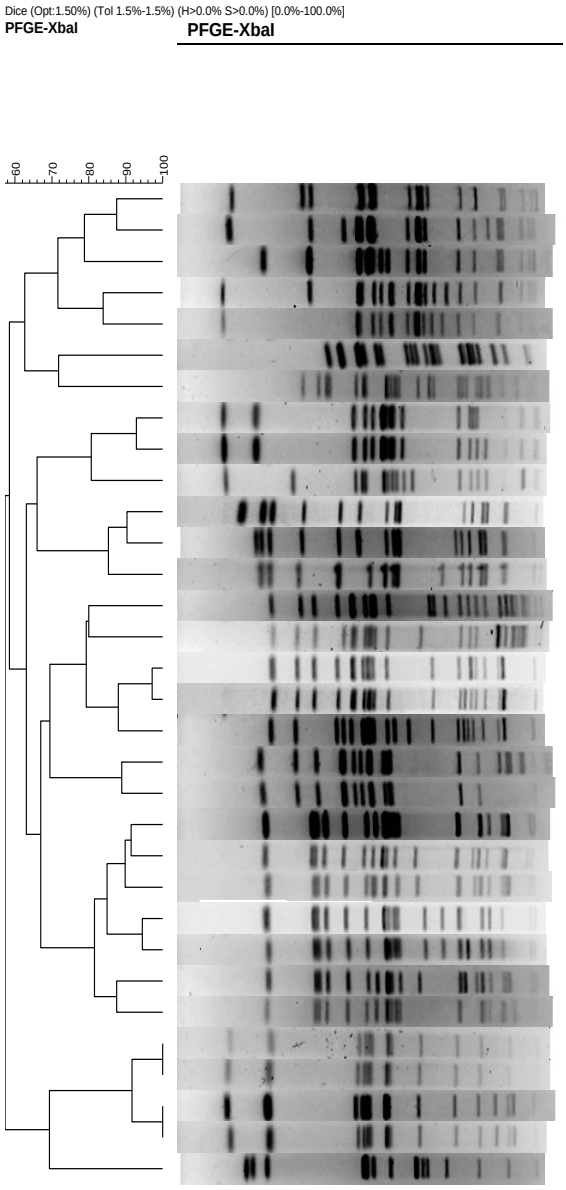
Study Sample ID	PFGE ID-(n*)	Serotype	# Chicken	Turkey (#)	Beef (#)	# matched	Earliest found	# States
77	8 (20)	Kentucky	153	3	4	160	2002	12
37	26 (26)	Heidelberg	7	0	0	7	2007	3
104	20 (3)	Typimurium/1,4(5),12:i-	12	0	0	12	2002	6
139	42 (1)	Hadar	10	45	0	55	2002	8
473	39 (1)	Hadar	0	15	0	15	2006	9
573b	44 (1)	Hadar	14	39	0	53	2002	7
1013b	16 (1)	Agona	1	9	1	11	2007	5
1039	38 (2)	Mbandaka	7	0	0	7	2002	8

*n: number of isolates associated to that PFGE profiles in the current study.

4.3.5: PFGE Comparisons with Clinical Isolates from WA-DOH

Forty seven unique PFGE patterns representing 141 total *Salmonella* isolates were compared to *Salmonella* isolates submitted to the Washington State Department of Health laboratories. Thirty five of the forty seven PFGE patterns submitted to the state health department laboratory met the quality control standards set by the state laboratory for inclusion in the comparisons. Twelve PFGE patterns were indistinguishable from clinical isolates submitted since the year 2000. These twelve PFGE profiles represented a total of 580clinical cases. Figure 6 displays the thirty five unique PFGE patterns that matched the twelve clinical isolates and the serotypes associated with each of the matching sets.

Fig. 6: Unique PFGE Patterns From Poultry Compared With WA Clinical Isolates



Key	WA-XbaI-Pattern	Unique Pattern	Serotype
Eyob_1124		XbaI	Schwarzengrund
Eyob_993		XbaI	Schwarzengrund
Eyob_985		XbaI	Schwarzengrund
Eyob_1095		XbaI	Schwarzengrund
Eyob_953b		XbaI	Schwarzengrund
Eyob_583			
Eyob_948b		XbaI	Heidelberg
Eyob_481b	HA027		Hadar
Eyob_573b		XbaI	Hadar
Eyob_659	HA018		Hadar
Eyob_104	Sub08		I,4,4,12i
Eyob_1135		XbaI	Typhimurium
Eyob_537b			
Eyob_112a			
Eyob_481a			
Eyob_18		XbaI	Kentucky
Eyob_83	KEN15		Kentucky
Eyob_1136		XbaI	Kentucky
Eyob_986			
Eyob_1013b	AG041		Agona
Eyob_1225	HE001		Heidelberg
Eyob_50	HE026		Heidelberg
Eyob_34	HE082		Heidelberg
Eyob_19			
Eyob_640b		XbaI	Heidelberg
Eyob_945a		XbaI	Heidelberg
Eyob_949b	HE011		Heidelberg
Eyob_518a	SE033		
Eyob_518b	SE033		Enteritidis
Eyob_1030b	SE032		Enteritidis
Eyob_584b	SE032		Enteritidis
Eyob_1107	LIT08		Litchfield

4.3.6: Temporal Distribution of the Indistinguishable PFGE Subtypes

There were a total of 580 clinical isolates with matching PFGE patterns for *Salmonella* isolated from raw poultry from the current study. Some of the matched PFGE profiles were from as far back as the year 2000, with ten of the matches presenting over multiple years. The number of clinical cases associated with each matching PFGE pattern from raw poultry ranged from 1 to 209 cases (Table 15). PFGE profiles 20 (DOH_Sub08) and 16 (DOH_Ag041) were also found in the NARMS comparison.

Table 15: Number of Indistinguishable PFGE Subtypes by Year

Sample ID	WA-DOH-ID	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
34	HE082											5	1			**6**
50	HE026			3	6	27	6	11	5		38	28	7	67		**198**
83	Ken15												1			**1**
104	Sub08			5	3	5	6	7	8	8	6	3	5	4		**60**
481	HA027										1	2	2			**5**
518a/b	SE033				1		2	5	2	4	10	10	3	14	1	**52**
584b/1030b	SE032				1		2		1	5	4	2	4	1	1	**21**
659	HA018								1	2				1		**4**
949b	HE011			1												**1**
1107	LIT08									14		3	1			**18**
1013b	Ag041								1				2	2		**5**
1225	HE001	2	13	10	30	21	29	17	21	24	10	9	9	13	1	**209**

4.3.7: Indistinguishable PFGE Profiles From Clinical Cases and Raw Poultry by Serotypes and Poultry Processing Establishments

Sixty six percent (61/93) of the *Salmonella* isolates from raw poultry were represented in the twelve indistinguishable PFGE profiles that were found when comparing with clinical cases (Table 16). There were seven serotypes represented in the twelve PFGE profiles with both serotypes *S*. Heidelberg (n=51) and *S*. Enteriditis (n=21) making up 70% (72/103) of the matching clinical isolates. The twelve PFGE profiles were found in ten different poultry processing establishments and on multiple product types (e.g., ground turkey and chicken pieces).

Table 16: Indistinguishable PFGE Subtypes by Serotypes and Establishments

Sample ID	# of samples w/ indistinguishable Poultry PFGE subtypes	# of samples w/ indistinguishable Clinical PFGE subtypes*	Serotypes	Processing Est. or number of est.	Product type
34	25	6	Heidelberg	D, E, F	CK
50	23	198	Heidelberg	F	Ck
83	22	1	Kentucky	D, G, Other	CK, Gck
104	3	60	1:4:4:12J	A, D	GcK, CK
481	1	5	Hadar	A	GT
518a/ 518b+	3	52	Enteriditis	C, D, F	CK
584b/ 1030b+	18	21	Enteriditis	A, E, D, Other	Gck, Ck
659	3	4	Hadar	A	GT
949b	2	1	Heidelberg	A	Gck,
1107	1	18	Litchfield	D	Ck
1013b	1	5	Agona	A	GT
1225	1	209	Heidelberg	Other	Ck

*Laboratory confirmed clinical cases (2000-2012) with indistinguishable PFGE subtypes from poultry samples. Gck: Ground chicken, GT: Ground turkey, CK: Chicken pieces. ++518a/b and 584b/1030b had indistinguishable PFGE profiles per PFGE analysis by WA-DOH.

4.4: Discussion:

4.4.1 PFGE comparisons by establishments, product and process types:

While PFGE profile clusters are mainly grouped by establishment, there are several profiles that were found in multiple products from different processing establishments. In particular, PFGE profiles 1, 2, 8, 20 and 23, were found in three or more different processing establishments. These profiles not only were found in different processing establishments, but also on different product types and processing types. For instance, profile 08 was recovered from raw chicken products processed in conventional, antibiotic free and organic establishments. Furthermore, these profiles were also present in various product types (ground turkey, ground chicken and chicken pieces). Other studies comparing the genetic profiles of *Salmonella* isolated from foods and that of humans, have also found significant overlaps not only between humans and foods of animal origin, but also across different animal species (Han J., *et al.*, 2010, Wasyl D., *et al.*, 2012).

In the current study, there were several indistinguishable PFGE profiles recovered multiple times from the same processing establishments spanning as long as a 12 month period in the current study. For instance, profiles (1, 8, 23 and 26) were recovered from products processed in the same establishments, representing different batches, over more than six months. These profiles represent clinically relevant serotypes such as *S*. Heidelberg and *S*. Enteritidis. The persistent recovery of these profiles is an indication of inadequate control of *Salmonella* in the processing establishments, persistent carriage in flocks, or common sources of exposure (e.g., feeds). Our finding is similar to other studies, where by *Salmonella* species were recovered multiple times over a long period of time (Patchanee P., *et al.*, 2008, Wasyl D. *et al.*, 2012). A study by Wasyl

D. *et al.*, (2012), reported recovery of *S*. Saintpaul from foods and humans within and between different European countries over a period of five years.

The overlap in PFGE profiles observed in this study was not limited to only processing establishments that supplied poultry products in the current study area, but also, there were eight indistinguishable PFGE profiles that were found in multiple poultry products that were produced and distributed in other states over several years. For instance, PFGE profiles (8, 20, 42 and 44) were found in the NARMS database as far back as 2002. The presence of common PFGE profiles across processing establishments, across multiple states and years can be due to either the genetic stability of these profiles allowing them to be widely distributed or the presence of common exposure sources, such as a common source of feed. Other studies have also reported the presence of indistinguishable PFGE profiles not only between poultry establishments, but also between different food animals (Patchanee P., *et al.*, 2008) and food products from different regions (Wasyl D. *et al.*, 2012).

4.4.2 PFGE comparisons with another animal food sources from NARMS

The two most common serotypes from raw poultry that were also found in the NARMS database were *S*. Kentucky (n=160) and *S*. Hadar (n=123). While *S*. Kentucky was isolated mainly from chicken (n=153), *S*. Hadar was isolated from ground turkey products. Compared to chicken and ground turkey, ground beef had two common PFGE profiles (8, 17) it shared with the current study, representing five samples. The serotypes that were associated with the beef samples were *S*. Kentucky and *S*. Hadar. While Kentucky is among the most frequently isolated serotypes from raw poultry products, it is not among the top twenty or so most commonly isolated clinical isolates (Cox *et al.*, 2011). In addition, the prevalence of *Salmonella* on beef products is

substantially lower than what is found on poultry products (Bosilevac JM *et al.*, 2009; Hill w. *et al.*, 2011, NARMS, 2010). Therefore, the contribution of raw beef products to the overall burden of Salmonellosis in the population may not be substantial, when compared to poultry products. Although the NARMS database also includes pork chops, there was no identical PFGE reported.

4.4.3 PFGE comparisons with Clinical Isolates from WA-DOH

Out of thirty five unique PFGE profiles that were compared to those in the database of the state laboratory, there were twelve indistinguishable matches. *S.* Heidelberg and *S.* Enteriditis were the two most commonly found isolates, representing sixty six percent of the cases. Four of the twelve profiles that matched belonged to S. Heidelberg and two matched *S.* Enteriditis. These two serotypes are among the top three most commonly seen clinical isolates in Washington State (WA-DOH, 2011). Some of these PFGE matches were seen as far back as the year 2000. Out of the twelve PFGE profiles that were found, there were two profiles (20 and 16) that were also found in the NARMS data base. Profile 20 has been seen in Washington State every year since 2002. An association between *Salmonella* genetic profiles from human salmonellosis cases and that of poultry or other animal sources have been previously reported (Han J. *et al.*, 2010, Patchanee P. *et al.*, 2008). Genetic profile overlap has also been reported between *Campylobacter* species isolated from raw poultry and that of campylobacteriosis cases (Callicott K. *et al.*, 2012).

Between April 2011 and April 2012, we isolated *Salmonella* Heidelberg from raw poultry before the poultry borne outbreak. The *Salmonella* isolates were recovered from a single processing establishment (Est F), which was eventually implicated in the current outbreak. The current outbreak has been responsible for more than 128 illnesses resulting in 32 hospitalizations (CDC, 2013). This particular profile (ID#50 matching HE026) was isolated in our study two times from raw poultry from the same implicated processing establishment as early as April, 2011, and this profile has been seen in clinical samples submitted to the state laboratory every year since 2002 (except for 2008). It is important to remember that the current study only used a single enzyme based analysis therefore; restriction with multiple enzymes or DNA sequencing might have revealed that some of those indistinguishable profiles might be different.

4.5: Conclusion

It has been challenging to show the correlation between the prevalence of *Salmonella* on raw poultry products and the incidence of salmonellosis in the population (Cason J., 2012; Callicott K. *et al.*, 2012). This is corroborated by the lack of correlation between the reduction in the overall prevalence of *Salmonella* in raw poultry and that of the incidence of salmonellosis in the population in the United States (Cason J., 2012).

While it has been reported that the link between human illnesses and *Salmonella* in meat products is not clear (Cason J., 2012a,b), the link between the recent poultry borne outbreak mainly occurring in the Northwest (WA, OR) of the United States, and that of the implicated samples in the current study make a strong case. Some have suggested that reducing the prevalence of *Salmonella* on raw poultry, as is currently required under the Pathogen Reduction

Performance Standards for *Salmonella* and *Campylobacter* on chilled broiler and turkey carcasses by the USDA, will not result in the reduction of salmonellosis in the population and have suggested that there might be other major sources of *Salmonella* that should be controlled for (Cason J., 2012). Part of the challenge in establishing the link to public health comes from the lack of substantial data, since only about 2.6% of illnesses due to *Salmonella* infection are culturally confirmed (Hennessy WT, *et al.*, 2004). In addition, majority of the salmonellosis infections are reported in sporadic cases, compounding the difficulty in establishing a direct link between food sources and the public health impact. In the absence of a large scale prospective study including sampling of multiple foods, and consumers for multiple years, which is unrealistic, the next best option is to establish a link to illnesses through genetic matches. While multiple food items including fruits and vegetables (i.e., sprouts, mangoes, cantaloupe, tomatoes, cucumbers), other meat sources (i.e., ground beef, pork) and non-food sources (e.g., hedgehogs, dry dog foods, turtles, frogs and rodents) have been linked to salmonellosis, by far the most frequently epidemiologically linked food products to multistate outbreaks have been poultry products (CDC, 2013b). Furthermore, while reptiles and amphibians are often implicated in human salmonellosis, less than 1% of human salmonellosis cases are caused by reptile-associated serotypes (Hoelzer K. et al., 2011).

While there might be multiple sources *Salmonella* infection, the current study does show that there is substantial overlap between the PFGE profiles of *Salmonella* isolates recovered from raw poultry products distributed in the Seattle, Washington area and the clinical isolates submitted to the state laboratory from the same geographic areas. The *Salmonella* strain responsible for the large poultry borne *Salmonella* outbreak in the North West that was also isolated during raw

poultry sampling in the previous study makes a strong case directly linking contamination of poultry products to that of human illnesses (Personal communication with WA-DOH, Figure 2). *S*. Heidelberg and *S*. Enteriditis were most frequently isolated from raw poultry in the current study and were also the most frequently matched profiles to clinical isolates submitted to the state laboratory. Hence, substantiating the public health implications of *S*. Heidelberg and *S*. Enteriditis directly related to contamination of poultry. While this study finds a strong association between PFGE clusters found on raw poultry products from local grocery stores and that of clinical isolates from the same geographical area, it does not claim that all of those clinical isolates were cases infected as results of the consumption or handling of poultry. Nevertheless, it substantiates the potential of poultry as the main source of human infections in the study area.

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Chapter Five

CONCLUSION

The presence of *Salmonella* on raw poultry products is well established. Over the last several years, multiple efforts have been made by the industry to control the level and the prevalence of *Salmonella* on raw carcasses. As a result of these control efforts and regulatory directives (e.g., PR/HACCP), on average, the prevalence of *Salmonella* has declined. Nevertheless focused efforts to reduce the overall prevalence of *Salmonella* on raw carcasses, has not resulted in the reduction of the incidence of salmonellosis. Change in the prevalence of *Salmonella* serotypes resulting in salmonellosis in the population over the years is a reflection of the continued challenge poultry and other meat processors are facing. Change in control efforts such as type of antibiotics used or change in the type of disinfection products used for sanitation, does ultimately affect the serotypes, antibiotic resistance and distribution of *Salmonella* in the poultry industry and ultimately on raw poultry products potentially resulting in salmonellosis.

Compared to other food animal products, raw poultry products have much higher *Salmonella* percent positives. According to the 2010 NARMS Retail Meat study, while chicken breasts have an overall percent positive (2002 through 2010) rate of 13.3% (chicken breast) and 14.9% (ground turkey), the overall percent positive rates for ground beef and pork chops were 1.2% and 1.1% respectively (NARMS, 2010). Except for ground turkey, much higher percent positives of *Campylobacter* was found in chicken breasts. The following *Campylobacter* percent positives were found on retail meats: 46.7% (chicken breast), 1.7% (ground turkey), 0.1% (ground beef) and 0.3% (pork chop).

In 2011, FSIS analyzed 26,345 samples of food animals. The percent positive for *Salmonella* were as follows: young chicken (6.5%), market hog (3.3%), cow/bull (0.8%), steer/heifer (0.5%), ground beef (2.4%), ground chicken (30.9%), ground turkey (12.3%) and turkey (2.4%) (USDA-FSIS, 2013)

There are many reasons why the prevalence of *Salmonella* on raw poultry products may not correlate well with reported human illnesses. First, prevalence rates include *Salmonella* species that are found on raw poultry that may not have as much public health impact. For example, while *Salmonella* Kentucky makes up a substantial proportion (~42% in 2004) of the prevalence rate on raw poultry, it is often not among the top twenty *Salmonella* serotypes (~0.2% in 2004) responsible for clinical cases (Cason J, 2012, Cox NA 2011), therefore obscuring the relationship between the prevalence and that of incidence rates in humans. In the current study, S. Kentucky was found in 21% of the carcasses that were found to be positive for *Salmonella*, but this serotype was not among the top twenty serotypes isolated from cases in the region. Second, risk assessment studies have reported that, the contamination level of *Salmonella* on raw carcasses is a stronger predictor of human illness than the mere presence of *Salmonella* (WHO, 2002, Straver *et al.*, 2007). Therefore, the presence of *Salmonella* on raw carcasses alone does not translate to an increased risk of human infection. Third, there are differences in the risk of infection by serotypes. Some strains are able to better persist in the environment, invade tissues and cause different degrees of damage to their hosts (Sanchez S. *et al.*, 2002, Cox *et al.*, 2011). While prevalence rate treats all *Salmonella* as equal, there are differences in the risk posed by serotypes, hence the incidence rate of salmonellosis. Forth, prevalence rates do not account for differences in the level of antibiotic resistance. For instance, a person infected with multi-drug

resistant *S*. Heidelberg is more likely to seek medical care than other serotypes and or strains that are antibiotic sensitive, hence influencing the incidence rate of salmonellosis. In the current study poultry products that were conventionally processed (i.e., those that were not labeled as, "Organic" or "Antibiotic Free") were found to contain all of the *Salmonella* strains with multiple antibiotic resistant. Fifth, there are multiple food handling processes that happen between when prevalence rates are determined (often in-plant sampling, right after post chilling) and consumption. Activities such as packaging, transportation, storage, and final food handling practices greatly affect the potential risk of a *Salmonella* positive carcass. The in-between activities can modify (i.e. increase or decrease) the risk of a *Salmonella* positive carcass, which prevalence rates do not account for. Sixth, the number of samples collected on a monthly basis to establish a prevalence rate per new FSIS directive (Pathogen Reduction for *Salmonella* from Broiler carcasses/HACCP), compared to the amount of poultry processed and available for consumption is insignificant. In the United States, there are about 8 billion birds slaughtered annually, with a modern processing plant handling more than 200,000 birds per day (Kiepper, B, 2011), but the average number of samples taken per plant per Pathogen Reduction for *Salmonella*, which is 51 carcasses/session. This level of sampling is unable to detect the small percentage of carcasses with higher bacterial count that are believed to increase the risk of infections. Seventh, the microbiological laboratory methods used to concentrate, detect and isolate *Salmonella* from raw poultry are often different from the methods used in clinical laboratories, therefore influencing the rate of detection, and the serotypes recovered (Fletcher *et al.*, 2006; Gorski Lisa, 2012). The average number of different serotypes found on raw carcasses is not well understood, although more often than not one serotype is reported per sample. This is most likely a reflection of the limitation of the methodology used than the reality

(e.g., number of colonies picked). Finally, it has been established that humans acquire salmonellosis from multiple food and non-food sources (CDC, 2013a). Reptiles and amphibians (e.g., frogs, toads, newts, and salamanders) have been implicated in a number of large *Salmonella* outbreaks (CDC, 2013a), yet those human cases are not excluded when a correlation between raw poultry and human salmonellosis case is established, hence potentially over estimating the impact of poultry or non-poultry food items have in human infections.

Regardless of the challenges in being able to directly correlate the prevalence rate of *Salmonella* in raw poultry and that of the incidence rate of salmonellosis, it is still accepted that substantial proportion of salmonellosis infections are acquired through ingestion. It is widely agreed that prevalence rates shed limited light on the contribution of raw poultry to the incidence of salmonellosis in the population. On the other hand, the limited number of large studies with large to moderate sample sizes, including the current study, provide us with strong evidence that raw poultry products are still distributed to consumers with a substantial number of *Salmonella* cells on them. It has been reported that enumeration methods often underreport the total number of cells present, because they are unable to extract significant proportion of the cells that are present even after multiple rinses (Cox *et al.*, 2011). In the current study, the most contaminated sample contained 3,600 MPN/carcasses. While this number is most likely a portion of the total *Salmonella* that was present on the carcass tested, even at the current level, its potential impact of direct or indirect (i.e., via cross contamination) infection to humans might be significant, particularly if improper poultry handling by consumers is considered. Keeping in mind the billions of poultry products slaughtered annually in the United States, there might be a few

million poultry products distributed with significantly higher level of *Salmonella* contamination than is considered acceptable.

To establish direct quantitative links between human cases of salmonellosis and food products, one will have to conduct a large prospective study sampling multiple food products, both animal and non-animal sources and a comprehensive epidemiological investigations (i.e., follow up, testing, considering both symptomatic and asymptomatic individuals) of hundreds of thousands of consumers will have to be conducted for multiple years. It is unlikely a study of this magnitude will be conducted in any foreseeable future. Therefore, to bridge the data gaps identified, the current three independent studies were conducted. Study one focused on characterizing the prevalence, concentration, antibiotic susceptibilities and serotypes of *Salmonella* from 1,322 raw poultry products purchased from retail stores from Seattle, Washington. The second study evaluated and identified risk factors during raw poultry handling in domestic kitchens by consumers in the Seattle area through direct observational study. The third study attempted to establish epidemiological links through genetic match between *Salmonella* isolates from raw poultry and that of cases from the same geographic areas.

In the current study, *Salmonella* contamination rates differ by product types, with higher contamination rates found in ground products. There were differences in the prevalence rates of *Salmonella* by processing establishments, with some products having up to four-fold higher prevalence rates. As can potentially be expected, some of those establishments with higher prevalence rates were found to be establishments producing "Organic" and "No antibiotics" products. Nevertheless, all of the multi-drug resistance isolates were recovered from processing establishments using antibiotics in their process. Multi-drug resistant S. Heidelberg was

recovered from various product types, such as ground chicken, ground turkey and whole muscle chicken. Furthermore, while a higher number of carcasses were found to be *Salmonella* positives from establishments producing labeled "Organic" and "No antibiotics", 90.5% (19/21) of the *S.* Kentucky, 50% (12/24) *S.* Enteritidis and 32% (12/37) *S.* Heidelberg were recovered from these establishments. Nevertheless, *S.* Kentucky was not among the top twenty serotypes recovered from cases in the region. If prevalence rates alone were considered in assessing the impact to public health, a greater level of risk would have been assumed to come from establishments with higher prevalence rate, which may not be accurate. Therefore, governmental agencies, such as USDA-FSIS, will need to consider making serotype distributions as well as antibiotic susceptibility part of the pathogen reduction effort in poultry processing establishments.

In the current study, the concentrations of *Salmonella* among those that were PCR positive ranged from less than 3.0 MPN/100 grams to 240 MPN/100 grams. Although the contamination levels of raw poultry did not differ by product and process types in the current study, it is considered to be critical in determining the potential risk of products (Straver *et al.*, 2007, Cason J. *et al.*, 2007, Cox *et al.*, 2011). It is important to consider, while a small percentage of raw poultry products are believed to have in excess of 10^4 MPN/carcass, given that more than 8 billion birds are slaughtered in the United States annually, a substantial number of carcasses are potentially released to consumers with high levels of *Salmonella* contamination. From the perspective of poultry processors, carcasses with high *Salmonella* load are difficult to predict and identify. Therefore, while 1-2% of raw poultry products with high *Salmonella* load may be challenging to control in typical modern processors that slaughter 200,000 birds per day, its public health impact can be substantial. In events the poultry processing establishments are not

able to control and predict products with high *Salmonella* bacterial load, food handling practices by consumers becomes the most critical last step in the defense against salmonellosis.

Food handling practices in both commercial food processing establishments and domestic kitchens are part of the multi-hurdle efforts to control the transmission of *Salmonella* from raw poultry to consumers. In the foreseeable future, consumers will need to handle raw poultry products with extra caution and expect raw poultry products that can potentially contain large number of multi-drug resistant *Salmonella* organisms. The current study confirmed previous studies that reported that people's knowledge of safe food handling practices differs from their actual food handling practices (Redmond C. E. and Griffith J. C. 2003, Redmond C.E., 2004, Phang S. H. and Bruhn M. C., 2011).

There were several raw poultry handling practices that were observed potentially leading to intra-and inter meal cross-contaminations. By far the highest risk factor observed was the lack of hand washing after handling raw poultry, with only 12% (22/182) of instances where proper hand washing with soap and hot water was observed. Numerous common kitchen surfaces were contaminated with either unwashed or improperly washed hands right after contamination with raw poultry. Some of the most contaminated surfaces included: handles, counter surfaces, appliances, personal devices (e.g., cell phones) and reusable food containers (e.g., salt shakers). In households with multiple persons sharing the same kitchen, there were multiple pathways for cross contaminations. Lack of sanitizing of contact surfaces right after use were observed as potential sources of inter meal contaminations. The overall lack of proper food handling, including hand washing, and sanitizing is compounded with lack of final cook temperature monitoring. In light of the findings of study one, consumers should be made aware of the potential risks of acquiring salmonellosis resulting from improper food handling practices,

particularly from lack of proper hand washing practices. The frequency of hand washing, frequency of activities leading to cross contaminations and identifying common contact surfaces, should be able to bridge the knowledge gap related to cross contaminations in domestic kitchens. Future studies should evaluate the level of *Salmonella* contamination the "typical" food handling practices are able to safely handle without excess risk of direct or indirect contaminations. Although consumers believe their food handling practices are safer than those of commercial food establishments (US-FDA, 2006), if the same food inspection criteria used to evaluate commercial retail establishments were applied to households in this study, 100% of the households in this study would have received unsatisfactory inspections.

Among the challenges identified in numerous studies is the lack of direct links between the control efforts in processing establishments and that of human illnesses (Cason J. *et al.*, 2012, Cox *et al.*, 2011). Part of the challenge in establishing the link to public health comes from the lack of substantial data, since only about 2.6% of the illnesses due to *Salmonella* infection are culturally confirmed (Hennessy WT, *et al.*, 2004) and the majority of salmonellosis infections are reported in sporadic cases with no particular source implicated. In an attempt to establish a direct link between raw poultry products distributed in the Seattle area and that of potential public health implications, PFGE profiles generated from raw poultry products were compared with NARMS database and WA-DOH database.

In the current study, PFGE profile matches were found across processing establishments, transcending product and process types over the sampling period. In addition, there were some (eight) PFGE profiles that were also recovered in the NARMS study over the last ten years. These profiles were seen mainly in chicken breast and ground turkey samples collected as far back as 2002 from multiple states (Foodnet sites). *S.* Kentucky from chicken breasts and *S*

Hadar from ground turkey were the two serotypes with the majority of the match. The wide distributions and persistence of these profiles might be either there is a common source of contamination (e.g., feed, layer flocks) that continuously re-introduce these profiles into the poultry processing establishment or these profiles have been able to establish stable residence in the poultry processing environments. Neither *S.* Kentucky nor *S.* Hadar are among the top ten serotypes from salmonellosis cases in Washington (WA-DOH, 2011).

More importantly, there was substantial overlap between the PFGE profiles of *Salmonella* isolates recovered from raw poultry products distributed in the Seattle, Washington area and the clinical isolates submitted to the state laboratory from the same geographic areas (12 PFGE profiles). Some of the PFGE profiles matched the clinical isolates recovered as far back as the year 2000, indicating the persistence of some of the strains in poultry products. In addition, some of the profiles were seen over several years in a row, potentially pointing to raw poultry as the potential source of infection. *S.* Heidelberg and *S.* Enteriditis were most frequently isolated from raw poultry in the current study and were also the most frequently matched profiles to clinical isolates submitted to the state laboratory. Hence, substantiating the public health implications of *S.* Heidelberg and *S.* Enteriditis directly related to contamination of poultry. Furthermore, our study was able to recover *S.* Heidelberg from raw poultry which was linked to a large poultry borne *Salmonella* outbreak in the Northwest, resulting in 128 infections and 37 hospitalizations (CDC, 2013). While this study finds a strong association between PFGE clusters found on raw poultry products from local grocery stores and that of clinical isolates from the same geographical area, it does not claim that all of those clinical isolates were cases infected as results of the consumption or handling of poultry. Nevertheless, it substantiates the potential of poultry as the main source of human infections in the study area.

Table 17: Food Handling Behavior Survey Questionnaires

Food Handling Behavior Survey Questionnaires							
Demographic							
1	Gender	Male	Female				
2	Age Group						
3	Educational Level	< HS	HS	Some college	BS or greater		
Frequency of raw chicken contact							
4	On average, how many packages of fresh chicken do you buy per month? (Note: the answer will be used in subsequent questions). Referred to "**P**"						
5	On average, how many packages of fresh ground turkey do you buy per month?						
6	Out of a total of "**P**" fresh raw chickens you purchase in a month, how many are ground?						
7	Where do you normally buy your raw chicken from?						
8	How much chicken (lb.) do you buy each time you purchase fresh chicken? If both, what proportion of each?	Small/medium Pack (1lb-2lbs)		Family pack (>2lbs)			
9	What part(s) of chicken do you buy the most? (If multiple parts, out of "**P**" purchases per month, how many of each type do you buy?)	Breast	Thighs	Drums	Wings	Split breast	Whole
10	How many times per month do you prepare meals using raw chicken? (This value "**M**" is used in subsequent questions). Referred to "**M**"						

11	Out of the "**P**" chicken packages you purchase per month, how many of them do you prepare?						
Transportation							
13	What is the average travel time from the retail store where you buy the chicken from to your home?	<15 min	30 min	60 min	90 min	120 min	Write___
14	On average, how long after you arrive at home do you put away the purchased fresh chicken?	<15 min	30 min	60 min	90 min	120 min	Write___
Raw chicken room temperature storage							
15	How soon after purchase do you use the raw chicken to prepare meals? Break down total purchases into each category.	Same day	within two days	within three days	four days	Write___	
17	For chickens to be used the same day, do you store the chicken anywhere else other than in the refrigerator or freezer? If yes, where and how long?	Yes	No	If Yes, where and how long?____			
19	If the fresh chicken is not used the same day it was purchased, how do you store the chicken for later use? If multiple answers, you can break down the "P" purchases into each method.	Room temp. storage		Refrigerator		Freezer	
20	If chicken is stored in the refrigerator, on average how long do you store it before use? (in days)						
21	If fresh chickens are stored in the refrigerator, where in the refrigerator do you store the chicken? (If multiple answers, pick the one that is most common).	Top	Middle	Bottom			
22	Do you have an operating thermometer in the	Yes	No				

	refrigerator? (yes or no)						
23	If chickens are stored in the freezer for later use, what procedure do you use to thaw the frozen chicken? If multiple methods are used, mark all those that apply.	Room temp. storage	Transfer to refrigerator	Run under hot/cold water	Microwave	Do not thaw	
24	If the fresh chicken is thawed at room temperature, how long do you leave the chicken at that temperature?						
Preparation:							
25	Do you normally wash fresh chicken pieces prior to using them to prepare meals?	Yes	No				
26	Do you have separate cutting boards for cutting raw chickens versus none meat products?	Yes	No				
	If you have separate cutting boards, do you ever reuse the same cutting board without washing to prepare other foods such as fruit, salad, and bread? How many times in the last month?	Yes	No	If yes, how many times in the last month?			
27	If you do not have separate cutting boards, do you ever reuse the same cutting board without washing to prepare other foods such as fruit, salad, and bread? How many times in the last month?	Yes	No	If yes, how many times in the last month?			
28	With how many of the "M" chicken meals (total number of chicken meals in a month time) did you also have side dishes (e.g., fruit, salad, and						

	bread)?						
29	Of those meals you had a side dish with your chicken (e.g., fruit, bread, green salad), when did you most often prepare the side dish(es)? (If multiple answers, choose the one most often followed).	Before preparing chicken	At the same time I prepare chicken	After I prepare chicken	Do not prepare side dishes		
30	After you use cutting boards to prepare fresh chicken, how do you wash the cutting board before it is used again? If multiple methods are used, pick one that you follow the most.	Rinse with cold water	Rinse with hot water	Wash with hot water and soap	Wash with hot water, soap and brush	Wash in the dishwasher	Other:
31	In the last "M" times you prepared chicken meals, how many times did you reuse the knife to prepare cooked foods or other ready to eat foods (such as bread, fruit, green salad).						
32	Do you use wash rags or sponges to clean kitchen counters and other kitchen surfaces?	Yes	No				
33	If you use wash rags, out of the "M" times you prepared meals with fresh chicken in the last month, how many times did you clean the counter tops using wash rags?						
34	How often do you wash the rag?						
	How often do you replace wash rags?						
35	Do you use disinfectants to clean counter surfaces?	Yes	No				

36	After "**M**" times you prepared meals with fresh chicken in the last month, how many times were the counter tops cleaned using disinfectants?						
37	What type (s) of disinfectant is used to clean counter tops?						Don't know
Hand washing							
38	Do you wash your hands before preparing meals?	Yes	No				
39	Prior to how many of the "M" chicken meals you prepared in the last month, did you wash your hands?						
40	How do you normally wash your hands before preparing meals?	With cold water	With hot water	With cold water and soap	With hot water and soap	Use Sanitizer	Don't wash hands
41	Do you wash your hands with hot water and soap after touching fresh chicken/turkey prior to handling anything else?	Yes	No				
42	During the preparation of the "M" meals in the last month, how many times did you wash your hands with hot water and soap after handling raw chicken?						
43	Do you wash hands with hot water and soap prior to preparing side dish(s)?	Yes	No				
45	Do you wash your hands with hot water and soap after you are done preparing chicken meals?	Yes	No				
Final cook							
46	How do you determine when the chicken is cooked?	Look	Touch	Taste	Take final cook temp.	Measure Time	

47	Do you sometimes check final meat temperature using a thermometer?	Yes	No	
48	Do you handle the cooked chicken with your bare hands when serving?	Yes	No	
49	What temperature should a fresh chicken be cooked to?		Don't know	
50	Do you reuse any of the utensils (cutting board, knives, spoons, plates) that were used to handle fresh chicken for the cooked chicken?	Yes	No	
51	In the last month, how many times did you wash your kitchen sink?			

Table 18: Notational Analysis Form:

Date	Analyst				Subject?	Gender	Age	Duration	# of actions	Product typ	Partners?	Cooking dur	Temp cook	Name of m	
	Hand Washing				Cross contamination								Cleaning of food contact				Other
Actions	Required	Adequate	Attempted? Soap used?	Length (sec.)	Cutting Board/Counter , towels (Surface)	Utensil	Wash rags	Side dish	Final cooked product	Hands contaminated	touched the faucet	Other(touching self, cabintets, knob, other	Required?	Adequate	Attempted	Sanitizer used or not?	Other major observation (e.g., injested potential contamination?)

Table 19: Risk factors or Activities Transcribed from Observational Videos

General Information	
	Subject ID:
	Gender
	Age:
	Total number of activities:
	Duration:
	Where was the poultry stored (top, middle, bottom)
	Was Chicken/Turkey washed
	Final Cook Temperature taken?
	Thawing procedure used:
	How many people in the kitchen?
	Eating or drinking while still cooking?
	Separate cutting boards used for chicken?
Hand Washing	When were side dishes prepared? Before/During/After?
	Proper hand-wash before food handling? (Y/N)
	Cloth towels used or paper towel to dry hands or both:
	Total number of hand-washes required:
	Total proper number of hand-washes (soap, water for >15 seconds):
	Total number of hand-washes attempted (soap and water <15 min):
	Total number of hand-washes required that was only washed with just only water:
	Total number of hand washes required but not washed at all:
	Required number of hand washes after handling poultry
	Proper number of hand washes after direct cross contamination of hands with poultry
	Attempted HW after direct c-c with poultry hands
	Hands washed w/soap and water before food handling begins (Y/N)
	Average length of times hands washed with soap and water?
g to Cross Contamination	Average length of times hands washed with only water, when HW was required.
	Number of times Hands are cross contaminated (directly from Poultry or indirectly from utensils)

	# CC: utensils
	# CC of Wash Rags
	Number of times touched face, hair, clothing (i.e. self)
	Number of cross contaminations: counter top surfaces
	Number of cross contaminations: faucets
	Number of cross contaminations: cloths towels
	Number of cross contaminations: Hands to appliances (IPod, phone, computer, watch, glasses
	Number of cross contaminations: Hands to recipe book
	Number of cross contaminations: Hands to handles (refer, cabinets, knobs, timers)
	Number of cross contaminations: Cutting board to anything else
	Number of cross contaminations: Cutting board to final cooked products
	Number of direct cross contaminations: Poultry to counter surfaces
	Number of cross contaminations: reusable food product containers (e.g., seasoning, oil bottle)
	Total number of cross contaminations (All)
Food Contact Surfaces	Counter top sanitized or cleaned before use?
	Total number of times counter tops was cross contaminated?
	Total number of times counter tops was sanitized properly after cross contaminated?
	Total number of times sinks were cross contaminated?
	Total number of times sinks were properly sanitized or cleaned after cross contaminated?
	Was wash rags used to clean surface contact surfaces?
	Cooking utensils properly washed, rinsed and sanitized? (Before use or put away). If not observed, put N/O
Final cook	Total number of times ate something with contaminated hands? (e.g., chips)
	Was there anything used to handle final cooked products cross contaminated (e.g., hands, contaminated plates or utensils)? (directly or indirectly)
	Took final cook temp? (Y/N)
	Duration of Cooking (in minutes)
	Checked for cooking completion by look and feel: (Y/N)
	Name of meal prepared:

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