SALMONELLA TRANSFER POTENTIAL DURING HAND HARVESTING OF TOMATOES UNDER LABORATORY CONDITIONS

By

PARDEEPINDER KAUR BRAR

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2011

I would like to dedicate this work to my family and friends for being the source of inspiration and pride for me.

ACKNOWLEDGMENTS

For their support and guidance through my graduate studies, I wish to express sincere appreciation to my graduate committee: Dr. Michelle Danyluk, Dr. Keith Schneider, and Dr. Max Teplitski. Especially, I would like to thank Dr. Michelle Danyluk for accepting me as graduate research assistant, encouraging and believing in me, and providing a great environment during my education in Food Safety. I thank all who helped me with my research, including Rachel McEgan, Lorrie Freidrich, Gwen Lundy, Joshua Vandamm, and Luis Martinez. Thanks a lot for helping me in learning various laboratory skills and giving me tons of moral support and appreciation time to time. I really appreciate all your time and support.

This research would not have possible without the help of Citrus Research and Education Center (CREC). I want to thanks the entire CREC group for their open doors and assistance. Finally, I would like to extend my special appreciation to my parents, my fiancée Prabhjot, my uncle Mr. Amarjit Singh Dhaliwal, and my great friends (Maninder and Sushila) for their love and support.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

SALMONELLA TRANSFER DURING HAND HARVESTING OF TOMATOES UNDER LABORATORY CONDITIONS

By

Pardeepinder Kaur Brar

August 2011

Chair: Michelle Danyluk
Major: Food Science and Human Nutrition

Tomatoes have associated with many multistate outbreaks of *Salmonella* and harvesting is suspected as a potential source of contamination. Workers prefer to wear gloves during tomato harvesting and may reuse them multiple times. Experiments were performed using mature green, round tomatoes with two types of gloves (reusable and single-use) and two hygienic conditions of reusable glove (clean and dirty). Transfer scenarios used during experiments were glove (clean and dirty) to tomato, tomato to glove (clean only) and glove (clean and dirty) to many tomatoes. Dirty gloves (reusable only) were prepared by rubbing with a fresh tomato leaf for 20 s. Uninoculated surface was touched with inoculated surface (6 log CFU/surface) for 5 s following 0 h, 1 h and 24 h drying. Tomatoes, 25 for clean and 10 for dirty gloves, were also touched subsequently with gloves after 0 h and 1 h drying time. All the clean glove samples were placed in sterile sampling bags with 20 ml of Butterfield's Phosphate Buffer; 0.1% Tween-20 was added to the dirty reusable glove samples. Following stomaching (gloves) or rubbing (tomatoes), samples were enumerated on non-selective and selective agar having rifampicin. Enrichments were performed when samples fell below

the limit of detection. No significant differences in TCs were obtained between clean reusable and single-use as well as clean and dirty reusable gloves at three drying times (0 h, 1 h and 24 h), on touching single tomato with an inoculated glove surface. Similarly, no significant differences were observed upon touching gloves with inoculated tomatoes at 0 h and 1 h drying time. Drying the inoculum on tomato surface for 24 h results in significantly more number of *Salmonella* positive reusable glove samples. Differences between 0 h and 1 h TCs were significant only when tomatoes were touched with inoculated gloves. When 25 tomatoes were touched with clean reusable and single-use gloves, TCs reduced significantly from third tomato and seventh tomato, respectively, while in case of dirty reusable gloves, no significant reductions were observed for all the 10 tomatoes. This study provides valuable insight into *Salmonella* transfer between gloves and mature, green tomatoes.

CHAPTER 1
INTRODUCTION

Consumption of fresh produce has increased in comparison to previous years (USDA 2008a,b). Health consciousness is one of the main driving forces behind this trend (National Cancer Institute, 1991). Availability of produce has also increased due to better transportation, storage facilities and import from other countries. (Ahvenainen, 1999). Along with increasing consumption, increase in produce-linked outbreaks has also been reported (Mead et al., 1999; Scallan et al., 2011). Produce is available in many forms, examples include raw produce, minimally processed produce, or fresh cut produce, which is harvested, packed and shipped to the stores without undergoing any kind of pathogen reduction step. Minimally processed produce and fresh cut produce, also known as 'ready to use' produce, is a growing segment in grocery stores and restaurants (Tauxe et al., 1997).

Fresh fruits and vegetables are minimally processed and typically consumed raw. Once contaminated, removal of pathogens from fresh produce is a difficult task, attention at all points from farm to fork is critical to prevent contamination of fresh produce. Risk of contamination is linked to fresh produce while still on plants, in fields or orchards, or during harvesting, transport, processing, distribution, marketing, at retail, or in home while preparing. A variety of fresh fruits and vegetables have been involved in foodborne outbreaks. Performing trace-back investigations is also difficult in produce outbreaks due to short shelf-life. Prevention of contamination is believed to be the key for controlling produce-linked outbreaks. "Guide to minimize microbial food safety hazards for fresh fruits and vegetables" is a guiding document issued by Food and Drug Administration (FDA), which covers the general safety features that can be adopted by

industry to reduce the microbial risk. Despite the availability of guiding documents, foodborne outbreaks related to fresh produce still occur, infecting thousands and causing deaths.

Non-typhoidal *Salmonella* (NTS) is the leading cause of food-related hospitalization and deaths in the U.S.; occurring due to foodborne infections (Scallan et al., 2011). Approximately 11% (1.03 million) of the foodborne illnesses, 35% (19,586) of hospitalizations and 28% (378) of deaths in the U.S. (Scallan et al., 2011). *Salmonella* has been linked to many produce items including tomatoes. Outbreaks have found to be associated with cut tomatoes as well as intact tomatoes. In 1990, 176 illnesses caused by *Salmonella* Javiana were reported in four states. These illnesses were linked to raw tomatoes (CDC, 1991; Hedberg et al., 1999). In 1993, 100 reported cases of salmonellosis caused by sv. Montevideo were part of a three state outbreak that was linked to raw tomatoes (Hedberg et al., 1999). In January 1999, *Salmonella* Baildon was recovered from 86 infected persons in eight states. Many restaurants across several states were involved, suggesting the tomatoes were likely contaminated in the beginning, at the farm or during packing, and before distribution (Cummings et al., 2001). In July 2002, an outbreak of salmonellosis caused by *Salmonella* Javiana occurred associated with attendance at the 2002 U.S. Transplant Games held in Orlando, Florida during late June of that year. The outbreak investigation ultimately identified 141 ill persons. The epidemiological investigation implicated fresh, pre-package diced Roma tomatoes as the probable vehicle for the outbreak (CDC, 2002).

During August and September 2002, an outbreak of salmonellosis cause by sv. Newport affected the East Coast. Approximately 404 confirmed cases were identified in

over 22 states. Epidemiological analysis indicated that tomatoes were the most likely vehicle, and were traced back to the tomato packing facility in the mid-atlantic region. Inspections of that packing facility revealed numerous violations of the Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) published by the FDA (Greene et al., 2008). In early July 2004, an outbreak of salmonellosis associated with Roma tomatoes, occurred in many states of U.S. and Canada. Five separate serotypes of *Salmonella* were eventually associated with the outbreak and those were *Salmonella* Javiana, *Salmonella* Typhimurium, *Salmonella* Anatum, *Salmonella* Thompson, and *Salmonella* Muenchen (CDC, 2005). In 2005-2006 four large multistate outbreaks associated with *Salmonella* and tomatoes occurred and were linked to cut tomatoes served in restaurants. Investigations of this outbreak confirmed that tomatoes involved were supplied either whole or pre-cut from tomato fields in Florida, Ohio and Virginia (CDC, 2007). In 2008, an outbreak of salmonellosis caused by sv. Sanitpaul infected numerous people. Investigators suspected raw tomatoes as a source of outbreak in the beginning and warn the consumers against raw tomatoes, which was later found to be associated with jalapeño and Serrano peppers. This cost Florida tomato industry c.a. $100 million (CDC, 2008). All the above outbreaks showed that *Salmonella* Tomato outbreaks have infected many people all over the country in past two decades.

Most of the tomatoes involved in the outbreaks discussed above came from either Florida or Virginia. Florida is the main producer of tomatoes in the U.S. due to its warm climate (VanSickle and Hodges, 2008). The occurrence of many multistate outbreaks led the Florida tomato industry, in cooperation with the Florida Department of Agriculture and Consumer Services (FDACS) to develop and enforce Tomato Good

Agricultural Practices (T-GAPs) Tomato Best Management Practices (T-BMPs) for the safe production, harvesting, processing and distribution of tomatoes. (DACS, 2007) These practices are mandatory in Florida for growers, packers, repackers and other agencies involved in tomatoes production.

Salmonella contamination of tomatoes and other fresh produce has been suspected to occur through contact with contaminated irrigation water, manure, animal intrusion in field, worker's hands, contaminated utensils, clothes and others (Beuchat, 1996; Sargent et al., 1989; Bloomfield and Scott, 1997; Scott and Bloomfield, 1993). All these sources provide a potential route for *Salmonella* contamination during tomato production. *Salmonella* has shown survival on the surface of tomatoes and inside tomatoes, provided adequate time and appropriate conditions exist (Shi et al., 2007; Wei et al., 1995, Asplund and Nurmi, 1991). *Salmonella* is believed to enter the tomato plant through flowers, cracks and crevices on the surface of plant and tomatoes (Guo et al., 2001). Harvesting is one of the different potential sources of tomatoes contamination. Mechanical harvesting is not common in Florida due to high cost and less expertise, thus most tomatoes are harvested manually (Zahara and Johnson, 1981) As per T-GAPs, workers are required either to wear gloves during harvesting or wash their hands frequently to prevent potential contamination or spread of contamination. Tomato fields are sprayed with different pesticides during growing phase. To reduce the exposure of skin to various pesticides present on the tomato surface and to protect cuts on hands while harvesting, most of the workers prefer to wear gloves. As the harvesting progress, workers can use the same gloves for whole day and wash them with water

and sanitizer at the end of the day. The same gloves are then used on the next day of harvesting.

This research focuses on the risk of *Salmonella* cross-contamination between gloves and mature green, round tomatoes during hand harvesting. Clean and dirty reusable gloves were selected to perform experiments. Experiments were also performed with clean single-use gloves used in tomato packinghouses to sort and pack tomatoes in cartons, after washing with sanitizer. Comparison between two different types of gloves (reusable and single-use) and two different hygienic conditions of reusable gloves (clean and dirty) will help in quantifying the risk of *Salmonella* cross-contamination during harvesting and post-harvesting of mature green, round tomatoes.

CHAPTER 2
LITERATURE REVIEW

Fresh Produce

Consumption of Fresh Produce

Demand for fresh fruits and vegetables has increased significantly in the U.S. in past decade. Between 1970 and 2008, U.S. per capita consumption of fresh vegetables increased approximately 67%, from 49 to 82 kg (107.9 to 180.5 lb) per year (USDA 2008a) and from 1976 to 2007, U.S. per capita consumption of fresh fruit increased approximately 19%, from 38.2 to 45.5 kg (84.2 to 100.2 lb) per year (USDA 2008b). Per capita expenditure on fresh produce is expected to increase more than per capita expenditure on any other foods by 2020 (Clemens, 2004).

The driving force behind the increase in consumption of fresh produce is health. The National Cancer Institute's (NCI) and Produce for Better Health Foundation (PBHF) "five a day" program encourages Americans to consume at least five servings of fruits and vegetables per day (National Cancer Institute, 1991). This program increased the consumption of produce by Americans from 23% to 26% from 1991 to 1998. To strengthen the program, NCI formed a partnership with U. S. Department of Agriculture (USDA) in April 2002 (National Cancer Institute, 1991). This partnership resulted in increased consumer awareness about the benefits of fresh produce, promotion of healthier lifestyles by public health officials, and the presence of new produce items, not previously available; all having an impact in changing the diet of the American people (Berger et al., 2010; Beuchat, 1996). Consumption of fruits and vegetables is encouraged in many parts of the world due to the high fiber, vitamins, minerals and low fat content found in these items which is believed to help in preventing cancer and

cardiovascular diseases (Ness and Powles, 1997). New techniques to improve shelf life of fresh produce as better storage facilities, improved marketing facilities and innovative packaging facilities have made availability of fresh produce feasible throughout the year (Ahvenainen, 1999).

To meet the growing demand of fresh produce, the U.S. has signed trade agreements with other countries to import fresh produce (Clemens, 2004). As a result, between the period of 1980 to 2001, import of fresh fruits and vegetables increased by 155 and 265 percent, respectively (Clemens, 2004). In 2001, U.S. fresh fruit imports accounted for 38.9%, up from 24.2% in 1980 and fresh vegetables accounted for 11.6%, up from 5.5% in 1980 (Clemens, 2004). In 2002, about 27% (859, 502 metric tons) of tomatoes consumed in U.S. were imported from two countries, Mexico and Canada. Mexico (84.2% i.e., 723, 425 metric tons) was the largest supplier of tomatoes to U.S. in 2002 (11.7% i.e., 100, 499 metric tons) (Clemens, 2004). As is true with tomatoes, the import of other fruits and vegetables has also increased since 1980. Produce from different countries of the world (Ecuador, Guatemala, Mexico, Canada, Chile, Costa Rica and others) are commonly available in U.S. (Clemens, 2004).

Fresh Produce Related Outbreaks

A foodborne outbreak is defined as ‘an incident in which two or more persons experience a similar illness after ingesting a common food, which epidemiological analysis implicates as the source of the illness’ (FDA, 2006). Historically, fresh fruits and vegetables were never believed to support growth of bacteria; currently fresh produce are associated with a number of foodborne outbreaks annually (Scallan et al., 2011; NACMC Food, 1999; De Roever, 1998; Francis et al., 1999; Nguyen-the, 1994; Tauxe, 1997). The reason for an increased number of produce linked foodborne illness

outbreaks occurring in summer months is not yet clear, but it may be attributed to the fact that more and more fresh fruits and vegetables are consumed in hot weather and the presence of high temperature conditions in summer also favors the growth of pathogens (Hedberg et al., 1994).

From 1987 to 1992, a constant number of produce related outbreaks was discovered annually, but between 1993 and 1997, a five-fold increase in the number of produce related outbreaks was observed (Bean et al., 1997). Etiological agents could not be determined from more than 50% of the outbreaks during these periods and the identified etiological agents outbreaks were predominantly due to bacterial pathogens, primarily *Salmonella* (Bean et al., 1997). During the period of 1995 and 1998, nine outbreaks occurred from the consumption of fresh vegetable sprouts. *Salmonella* and *Escherichia coli* O157:H7 were the causative agents behind these foodborne illnesses (NACMCF, 1999).

The Center for Science in Public Interest (CSPI), performed a comprehensive survey of all the outbreaks with identified food vehicle in U.S., and found produce accounts for 13% of outbreaks and 21% of illnesses during the period of 1990 to 2005 (CSPI, 2005). CSPI Internet's database of 5,000 foodborne illness outbreaks, indicates that between 1990 and 2003, 554 outbreaks with nearly 28,315 cases have been linked to produce and produce dishes. Among these, vegetables were linked to 205 outbreaks with 10,358 cases, while fruits were identified as the vehicle in 93 outbreaks with 7,799 cases. Of the 93 fruit-associated outbreaks, 15 were linked to berries and 25 were linked to melons. Produce dishes were implicated in 256 outbreaks involving 10,158 cases.

According to the estimate of Scallan et al. (2011), contaminated food including fresh produce is responsible for 9.4 million illnesses, 55,961 hospitalization, 1,351 deaths annually in the U.S. They further estimate that produce related outbreaks have decreased by 20% during the past decade (Scallan et al., 2011). The various reasons for observing decreased produce linked outbreaks may be different analysis method by Scallan et al. in comparison with the previous Mead et al. (1999) study, a smaller sample size used for study ($1/5^{th}$ of previous study), a smaller proportion of Norovirus outbreaks estimated as foodborne and of course the safer food supply. The current estimate of foodborne illnesses was done by considering 31 known agents of foodborne illnesses (Scallan et al., 2011). The data were collected from the period of 2000-2008 and estimates were based on U.S. population in the year of 2006 (Scallan et al., 2011).

Pathogens Associated with Raw Produce Outbreaks

Produce associated outbreaks can be caused by bacteria, viruses and parasites. From 1973-1987, around 90% of the foodborne illnesses from all sources, including produce, were reported to be associated with bacterial pathogens (Bean et al., 1990). Similarly in the next decade (1990-2000), bacterial pathogens causes maximum foodborne illnesses associated with all the sources (Mead et al., 1999). On the contrary, the recent analysis done by Scallan et al. (2011) have estimated that Norovirus (58%) is the etiological agent behind most of the foodborne illnesses in the U.S. The current estimate of number of foodborne illnesses caused by Norovirus is lower than the previous estimate (40% to 26%) as less proportion of norovirus outbreaks are estimated to be foodborne under current estimate (Scallan et al., 2011).

Specifically emphasizing all produce linked outbreaks, as per the investigations done by CSPI, from 1990 to 2005, Norovirus was the top cause of outbreaks (40%),

followed by *Salmonella* (18%), *Escherichia coli* (8%) and *Clostridium* (6%). The main hazards associated with fruits were Norovirus (39%), *Salmonella* (28%), and *Cyclospora* (8%). Among vegetable outbreaks, the major pathogens were Norovirus (26%), *Salmonella* (21%), and *Clostridium* (12%). The major pathogens in produce dish outbreaks were Norovirus (51%), *Salmonella* (13%), *E. coli* (6%) and *Shigella* (6%). According to the above data, Norovirus and *Salmonella* were more frequently associated with produce-linked outbreaks than other pathogens during 1990-2005. Specific produce items reported to be involved with salmonellosis outbreak are alfalfa sprouts (Proctor et al., 2001; CDC, 2009), jalapeno pepper (Klontz et al., 2010), tomatoes (Cumming et al., 2001), bean sprouts (O'Mahony et al., 1990), cantaloupes (Mohle-Boetani et al., 1999), and watermelons (Gayler et al., 1955).

The risk of foodborne pathogen transmission from fresh produce is associated with imported as well as domestic produce. In March 1999, FDA analyzed 1000 imported and domestic fresh produce commodities (Broccoli, cantaloupe, celery, cilantro, culantro, loose-leaf lettuce, parsley, green onion, strawberries and tomatoes) for *Salmonella*, *E. coli* O157:H7 and some for *Shigella* spp. Among all the imported samples, 44/1003 was found positive for either *Salmonella* (35/44) or *E. coli* O157:H7 (9/44) while 11/1028 domestic samples were found positive for either *Shigella* spp. (5/11) or *Salmonella* (6/11) (FDA, 2001; FDA 2003). Many bacterial pathogens can be isolated from raw produce (Beuchat, 1996). *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes* are the bacteria which are normally present in soil whereas *Salmonella, Shigella*, *E. coli* and *Campylobacter* are believed to originate from enteric environment and contaminate raw produce through feces, sewage water, or untreated

irrigation water contact. Enteric pathogens (primarily *Salmonella* and *E. coli* O157:H7) are among the greatest concern as they may grow in or on fresh fruits and vegetables under favorable conditions.

Growth and survival of pathogens on fresh produce depends upon factors like the type of organism, type of produce, pH, moisture, oxygen, acid, and various other intrinsic and extrinsic factors. Foodborne illness occurs when produce is contaminated with pathogen and sufficient number of pathogen cells remaining viable until consumption of produce (Harris et al., 2003). The minimum number of cells required to cause illness in humans depends upon the health, susceptibility and age of individual, amongst other factors. Hence, the term infective dose is very relative, and rather dose response models should be used to predict the probability of risk. A dose response model for *Salmonella* was prepared by FAO/WHO in 2002, and describes the relationship between number of bacteria or dose ingested and likelihood of infection. The elderly, children, the women who are pregnant and immunocompromised individuals are more prone to infections. Some pathogens, like *Salmonella*, multiply inside the human body and then cause infection; this is called a food infection (Harris et al., 2003). To prevent outbreaks associated with fresh produce, there is need to do research with various potential pathogens, sources and other variables influencing contamination of fresh produce. Many pathogenic microbes have been isolated from variety of fresh fruits and vegetables at variable frequency. Prevention of contamination is the likely key required to control produce-linked outbreaks.

Sources of Raw Produce Contamination

Produce comes from different sources and countries and is often eaten without further processing. It is quite susceptible to contamination and must be handled

carefully during production, harvesting, packaging, transportation, storage and consumption. In most of the produce-associated outbreaks, the exact source of contamination is not identified and different pre and post-harvest sources are suspected to be the most likely causes of contamination.

Pre-harvest

Among the pre harvest sources, soil, feces, irrigation water, green or inadequately composted manure, air, wild and domestic animals are considered as likely sources of contamination (Beuchat, 1996; Doyle and Erickson, 2008). The land or soil where the crop is grown can harbor pathogens from previous crop and can potentially transmit these pathogens to the next crop. The literature also suggests that moist soil can harbor *Salmonella* for 45 days (Guo et al., 2002). Animal or human feces containing pathogens can likely contaminate crop fields through contact with irrigation water and manure applied in fields (Cox et al., 2005; Holley et al., 2006; Marina and Odumeru, 2004). Properly treated or composted manure from animals is applied as fertilizers to improve and maintain productive soils and helps in stimulating plant health. On the contrary, inadequately decomposed manure applied to soil can possibly contaminate the crops grown in the same soil (Solomon et al., 2002). *Salmonella* inoculated into hog manure treated soil at 5 log cfu/g survived up to 180 days. This observation supports the hypothesis that human pathogens can persist in animal manure (Holley et al., 2006). Irrigation water is another potential route through which microbial contaminants can come in contact with fruits and vegetables. Applying pesticides with contaminated water can highly likely contaminate plants to which it is applied. Contamination of plants through airborne transmission is not well studied. However, in livestock sheds, transfer of pathogens through air has been documented which supports the concept of plant

contamination through airborne transmission (Dowd et al., 2004). Along with airborne transmission, transmission of pathogens through animals, birds, insects contacting the outside grown produce are also possible sources (Beuchat, 1996).

Harvesting

Human contacts (Montville et al., 2001), gloves (Jimenez et al., 2007), shirts, handling containers (Sargent et al., 1989), clothes (Bloomfield and Scott, 1997; Scott and Bloomfield, 1993) used in harvesting are the various potential sources of contamination during harvesting. Infected or ill workers can transfer pathogens directly to produce by touching (Todd et al., 2009). Unclean hands or harvesting tools used by healthy workers can also contaminate produce. Gloves, which are used as barrier for preventing contamination of food by handlers, can become permeable to bacteria through their subsequent use and can spread the contamination (Montville et al., 2001). According to a study, gloved hands transfer *Salmonella* to and from fresh green bell peppers due to smooth surface of both (Jimenez et al., 2007). A worker's shirt that touches produce during or immediately after harvest can be a potential source of cross-contamination. Additionally, dirty bins and buckets used to hold fresh produce like tomatoes can be additional potential sources of cross-contamination during harvesting (Sargent et al., 1989).

Post-harvest

Depending upon the commodity, produce is either packed in the field and sent to destination market or it is placed in bins and sent to a packing facility. Employees, equipment, cold storage units, packaging material, transport container, wash/rinse water used for washing or cross-contamination from other food items stored in same storage unit are the various sources that can potentially contaminate produce after harvesting

(Beuchat, 1997). Although, gloves, hair nets are worn by employees working in packing houses, ill workers, improper boot cleaning station, inadequate bathroom facilities are sources that can potentially contaminate the produce in a packinghouse. Food contact surfaces such as conveyor belts and dump tanks, can contaminate produce if not sanitized properly. Improper storage conditions (improper temperature and moisture) used to hold or transport fresh produce can be considered a point where pathogens may grow on product (USDA, 1997). In the food preparation environment, cross-contamination of salads with *Campylobacter* and *Salmonella* from chicken carcass via. kitchen surfaces are documented (Kusumaningrum et al., 2004). The use of same utensils for fresh produce and meat products highly increases the chances of cross-contamination (Chen et al., 2001).

Possible Reasons behind Increase in Raw Produce Outbreaks

Madden (1992) categorizes the fresh produce in the list of potentially hazardous food which includes “food products that contain the nutrients necessary to support rapid and progressive growth of infectious or toxigenic microorganisms.” Despite the numerous prevention efforts by industry, foodborne outbreaks associated with raw produce remain persistent problem in the U.S. Increased importation of fresh produce items, increased consumption of fresh produce, globalization of food supply, change in agronomic practices, change in eating habits, increase in susceptibility of individuals, and improved surveillance are all believed to contribute to fresh produce being linked foodborne illness outbreaks (Guo et al., 2000; Nyachuba, 2010; Beuchat, 2002). In 2002, consumers spent ca. 46.1% of their food income outside home due to busy schedules (Marriott and Gravani, 2006). Nearly 70% of illnesses associated with tomatoes were found to be linked with restaurants where tomatoes were used primarily

in salads (Klein et al., 2009). Increase in fresh produce consumption is observed in all the seasons, which led to an increase in importation of products from different parts of world where sometimes the safety standards are below U.S. safety standards. An increase in outbreaks may also be linked to various unhygienic practices followed from field through consumption (Beuchat, 1996; Beuchat and Ryu, 1997; D'Aoust, 1994).

The identification process, or traceback, for the source and pathogen responsible for produce related outbreak is slow and can lead to an increased number infected person due to continued consumption of the contaminated crop. The foremost reason for the delay in identifying and controlling produce related outbreaks is the complex distribution system of produce. The complicated distribution system and the perishable nature of fresh produce, made the traceability of outbreak very difficult (Harris et al., 2003). By the time the outbreak is confirmed, the produce associated with the outbreak is rarely available to verify pathogen presence. In addition, produce is often served along with other ingredients that make the search of finding exact source even more difficult (Tauxe et al., 1997). On an average, it generally takes several weeks to identify the source of outbreak can lead to an increased number of persons getting ill (Harris et al., 2003).

Cost Associated with Foodborne Outbreaks

Foodborne outbreaks do not merely affect the people who suffer from infection but also cause huge loss to the responsible food company in terms of money and reputation. The costs involved in foodborne diseases are the medical treatment costs (hospital services, physician services and drugs), business losses, loss of productivity, loss of quality of life (death, pain, suffering and functional disability) and costs to others in society (e.g., costs to insurance companies that pay medical expenses) (Dalton et al.,

1996; Scharff, 2010). The recent estimate of economic cost of foodborne illness in the U.S. is quite comprehensive and included all the sources of illnesses. Approximately, $152 billion is estimated to be the cost associated with foodborne illnesses, with an average cost per case being $1,850 (Scharff, 2010). Data from Foodborne Disease Outbreak Surveillance system estimated that produce is responsible for $39 billion of health-related costs in the U.S. every year. One illness case from contaminated produce costs on an average of $1,960, which is more than cost associated with one illness case from any other source ($1,850). The cost of one foodborne illness case involving non-typhoidal Salmonella is $9,146, while the overall burden of Salmonella outbreaks in U.S. in one year is estimated at $14 billion (Scharrf, 2010). The ten states having highest cost per case are Hawaii, Florida, Connecticut, Pennsylvania, South Carolina, the District of Columbia, Mississippi, New York, Massachusetts, and New Jersey (Scharff, 2010). This cost estimate illustrates the magnitude of problem and burden of foodborne illnesses on the U.S. and the need for more efforts and research in the field of food safety.

Salmonella

Salmonella belongs to family *Enterobacteriaceae*, and was named after Daniel E. Salmon, an American bacteriologist who isolated strain enterica or choleraesuis from intestine of pigs suffering from a disease with symptoms similar to those of human cholera in 1885 (Bell and Kyriadides, 2000). *Salmonella* is a gram negative, rod shaped (0.7-1.5 × 2.0-5.0 μm), non-spore forming, oxidase-negative and catalase-positive bacteria that can grow under both aerobic and anaerobic conditions (Maurer and D'Aoust, 2007). Most of the strains are motile in nature. Salmonellae reside in the intestinal tract of warm and cold blooded animals. This enteric bacterium catabolizes D-

glucose and other carbohydrates and produces acid and gas (Maurer and D'Aoust, 2007).

Salmonella Nomenclature

Salmonella species have been known for more than 100 years and are prevalent worldwide. Nomenclature which is widely used these days is based on biochemical testing and recognizes only two species of *Salmonella* i.e *Salmonella enteric*a and *Salmonella bongori,* with *Salmonella enterica* having six subspecies, *Salmonella enterica* subsp. *enterica* (I)*, Salmonella enterica* subsp. *salmonae* (II), *Salmonella enterica* subsp. *arizonae* (IIIa), *Salmonella enterica* subsp. *diarizonae* (IIIb), *Salmonella enterica* subsp*. houtenae* (IV) and *Salmonella enterica* subsp. *indica* (VI). Subspecies of *Salmonella enterica* are designated by Roman numerals as specified. Further sub-classification into serotypes (serovars) is done at state public health laboratories (D'Aoust, 2007; Tindall, 2005). The serovars of *Salmonella* were initially named on the basis of their specific host e.g., Typhimurium causes mouse typhoid fever. After recognizing that host specificity did not exist for many serovars, new strains were named based on the location from where they were isolated. To overcome the problem of long names, serovars are abbreviated, however this nomenclature is also no longer used.

Kauffmann-White scheme, also known as antigenic formula for *Salmonella* serovars, was the first attempt to systematically classify *Salmonella* into serovars or serotypes. *Salmonella* strains are classified into 'serovars' or 'serotypes' based on antigens they possess. As of 2002, more than 2,541 serovars of *Salmonella* have been differentiated as per this scheme and the number is increasing every year (D'Aoust, 1989). Different *Salmonella* antigens include: O antigen determined on the basis of

lipolysaccharide, present on the outer membrane of bacteria, H antigen, determined on basis of peritrichous flagella, Vi antigen, also known as capsule antigen occurs only in serovars Typhi, Paratyphi C and Dublin (Maurer and D'Aoust, 2007). Serotype is mainly based on the immunoreactivity of two surface structures, O antigen and H antigen. O antigen is a carbohydrate (also called a polysaccharide). It is a polymer of O subunits; each O subunit is typically composed of four to six sugars depending on the O antigen. Different sugars and different linkages between sugars produce the different antigen and are designated by mainly numbers (or letters). H antigen is the filamentous portion of the bacterial flagella. H antigen is made up of protein subunits called flagellin. The ends of flagellin are conserved and give the filament its characteristic structure. H antigens are typically designated by lower case letter and numbered z's. Each serovar possess a unique combination of antigens, known as antigenic formula. Apart from serological and biochemical classification, phage typing is also gaining importance. The serovars are further differentiated based on their cell sensitivity to lytic activity of selected bacteriophages into phage type (PT) or definitive type (DT) (Maurer and D'Aoust, 2007; Bell and Kyriakides, 2000).

Most (nearly 1,500) of the *Salmonella* strains associated with foodborne illness are present under *Salmonella enterica* subsp. *enterica,* (Maurer and D'Aoust, 2007; Brenner et al., 2000) only 20 serovars are present under *Salmonella bongori.* Some serovars of *Salmonella* are ubiquitous, while some are rare (Maurer and D'Aoust, 2007). For example, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common serovars associated with human illness while *Salmonella* Mjordan is rarely reported (Maurer and D'Aoust, 2007; D'Aoust, 1989). Some *Salmonella* serovars cause diseases

in one host only, for example, *Salmonella* Gallinarum is prevalent in poultry, *Salmonella* Cholerasuis in swine and *Salmonella* Typhi and *Salmonella* Paratyphi A are common among humans (Bell and Kyriadides, 2000). The serovars which are restricted to one host only, are known as host restricted, e.g., *Salmonella* Gallinarum and *Salmonella* Typhi; serovars that are common in one host but has potential to cause illness in others also, are called host adapted, e.g., *Salmonella* Dublin. Those serotypes who have broad range of hosts are called unrestricted, e.g., *Salmonella* Typhimurium and *Salmonella* Enteritidis (Uzzau et al., 2000).

Growth and Survival Characteristics

Salmonella can adapt to wide range of growth conditions. It can grow at low temperature conditions of 2°C and elevated temperature of 54°C, although most of the serotypes are unable to grow below 7.0°C (Baker et al., 1986; Droffner and Yamamoto, 1992; Bell and Kyriadides, 2000). The growth is slow below 10°C and optimum around 35-37°C. Another study showed that viable cells of *Salmonella* species can be isolated from milk at 60-67.5°C (D'Aoust, 1994). *Salmonella* has shown potential to tolerate high temperature conditions in low water activity or high fat content food materials (Bell and Kyriakides, 2000). The range of pH at which *Salmonella* can grow is also very wide i.e., 3.8 (Asplund and Nurmi, 1991) to 9.5 (Holley and Proulx, 1986), however, very few serotypes grow below pH 4.5. The optimum growth of *Salmonella* is observed in the pH range of 6.5 to 7.5. The water activity required for the growth of *Salmonella* ranges from 0.94 to 0.99 or greater. Some salmonellae are found to tolerate dry environments e.g., *S.* Agona (D'Aoust, 1989). The addition of 3-4% sodium chloride generally inhibits the growth of *Salmonella*, although salt tolerance is found to increase with increase in temperature (Maurer and D'Aoust, 2007). All the above facts show that *Salmonella* has

potential to grow under poor climatic conditions contribute to it as an important foodborne pathogen. *Salmonella* also exhibit ability to survive in frozen or low water activity products for months and sometime years, although growth is rarely observed under such conditions but viable cells can be obtained (Bell and Kyriakides, 2000). Research has shown the persistence of *Salmonella* in dry environments and outbreak with chocolates is one of many examples (Werber et al., 2004).

Diseases Caused by *Salmonella*

Salmonella can cause two main illnesses in humans, one being typhoid or enteric fever, occurred due to ingestion of *Salmonella* in bloodstream and is caused by *Salmonella* Typhi or *Salmonella* Paratyphi. The other is gastroenteritis or non-bloody diarrheal disease, occurred due to foodborne infection. It has reported in many *Salmonella* linked multistate outbreaks. *Salmonella* mainly causes self-limiting illnesses, but can also cause chronic diseases like reactive arthritis, osteomyelitis, enteric fever, cardiac inflammation or neural disorders (D'Aoust, 1994).

Typhoid *Salmonella*

Typhoid fever occurs due to the ingestion of food or water contaminated with *S*. Typhi and is mainly prevalent in developing countries. Approximately, 400 typhoidal cases occur in the U.S. and nearly 70% of these cases are associated with international travel to developing countries of Asia, Africa or Latin America (Schneider and Goodrich, 2008). Typhoidal fever is typically associated with humans. Humans (nearly 4%) can be unapparent chronic carriers and can shed this bacterium in their stool. The only source of typhoid fever is through fecal-oral route (Schneider and Goodrich, 2008). S. Typhi has potential to overcome the defense mechanism of human intestine. Upon ingestion of contaminated food or water, *Salmonella* starts multiplying inside the body with the

phagocytes (Immune system cells responsible for killing bacteria), spread into the bloodstream and cause destruction of internal organs of the body as liver, spleen, bone marrow, intestines and the mesenteric lymph nodes which can potentially lead to death in humans. Symptoms of typhoid fever include fever, abdominal cramps in the first week of infection and watery diarrhea, abdominal pain, headache, nausea, loss of appetite, rose spots across abdomen, constipation in the second week. The use of antibiotics can gradually resolve the symptoms in subsequent weeks but infection can also turn into meningitis, osteomyelitis or other serious problems (D'Aoust, 1994). Incubation period for typhoid fever ranges from 8 to 28 days and symptoms generally persists for 4 to 8 weeks approximately in untreated individuals (D'Aoust, 1994; Schneider and Goodrich, 2008).

Non-typhoid *Salmonella*

Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. It is quite prevalent in many countries (Notermans et al., 1992; Sewell et al., 2001; Todd et al., 2009; Werber et al., 2005). Non-typhoid *Salmonella* (NTS) refers to any serotype under *Salmonella,* except Typhi and Paratyphi, is responsible for salmonellosis. Although NTS infections mainly cause mild to moderate self limiting gastroenteritis, serious cases can result in death. Most (90%) of the illnesses, hospitalizations and deaths in the U.S. are estimated to be caused by seven major pathogens (*Salmonella*, Norovirus, *Campylobacter*, Toxoplasma, *E. coli* O157, *Listeria, Clostridium perfringens*). Among above seven, around 11% (1.03 million) of foodborne illnesses, 35% (19,586) of hospitalizations and 28% (378) of deaths in the U.S. were caused by non-typhoidal *Salmonella* (Scallan et al., 2011). Hence, NTS is the leading cause of estimated hospitalization and deaths in U.S. as per the recent report on

foodborne illnesses (Scallan et al., 2011). NTS pass to humans through contaminated food and water contact, just as *S.* Typhi does. *Salmonella* is often believed to spread through the fecal-oral route (Maurer and D'Aoust, 2007). It can cause serious infection among children and persons greater than 65 years of age. Individuals recovering from *Salmonella* have potential to shed the bacteria up to three months. Non-typhoid *Salmonella* has been linked to animal contact, animal based foods (eggs, beef especially undercooked meat products, dairy products), fresh produce and others. Many *Salmonella* serovars are found to be associated with produce related outbreaks (Beuchat, 1996). The incubation period for salmonellosis ranges from 6–72 hours (usually 12-36 hours), however, incubation periods longer than three days have been documented (Rafatellu, 2008). The duration of disease is approximately less than 10 days unlike typhoid fever. Salmonellosis is localized infection colonizing intestine and the mesenteric lymph nodes as NTS rarely overcome the intestinal defense (Rafatellu, 2008). Once NTS overcomes the intestinal defense, it can cause systemic and deadly infection. It leads to chronic clinical condition similar to typhoidal infection after migrating from intestine to other organs of the body (D'Aoust, 2004).

Salmonella in Non-host Environment

Salmonella resides mainly in the gastrointestinal tracts of animal hosts including animals, birds, reptiles and humans for long period of time in their life (Maurer and D'Aoust, 2007; Sanyal et al., 1997). Animal hosts are considered as the primary habitat in the life of this bacterium which provides all the required conditions (sugars and free amino acids) for the survival and growth of *Salmonella.* After being shed by its host species, *Salmonella* comes into environment and can pass on to different things. The wide host range of bacterium aids its persistence in the environment. *Salmonella* has

shown ability to survive on fresh produce (Beuchat, 1996), in water (Boring et al., 1971; Cherry et al., 1997), soil, manure (Natvig et al., 2002) and dry food products (Bell and Kyriakides, 2002). Studies have demonstrated the persistence of *Salmonella* in poultry, pig and cow farms. It has been collected from cow herd, ground meat of cow, cattle feed, raw materials, waste slurry from infected pigs and from various other sites (Baloda et al., 2001; Hurd et al., 2002; Millemann et al., 2000). The rate of survival in aquatic conditions and saline conditions is quite high *Salmonella* (Chao et al., 1987). *Salmonella* has been found in bathrooms, toilets and have infected people indicating the potential of transmission and survival in air (Barker, 2000). In addition, pigs have also shown symptoms of salmonellosis after inhaling *Salmonella* (Fedorka-Cray, 1995). *Salmonella* has also been shown to adhere to mineral particles (Stenstorm et al., 1989). During its life cycle, salmonellae actively move between its host species and non-host environment (Winfield and Groisman, 2003). *Salmonella* can survive in non-host environment for long period of time (Winfield and Groisman, 2003).

Salmonella experiences harsh physiochemical conditions on plant surfaces. Temperature, moisture, osmotic conditions fluctuate very frequently in short period of time, unlike its host environment. Lack of nutrients availability and exposure to ultraviolet radiations makes its survival even more difficult (Hirano et al., 2000; Lindow et al., 2003). A study conducted to compare the population of human enteric pathogens with the population of leaf associated bacteria, shows that under wet and warm conditions, human enteric pathogens can grow at higher rate than leaf associated pathogens (Brandl, 2002; O'Brien et al., 1989). Apart from constantly fluctuating conditions experienced on plants, carbon sources found on plants help in the survival of

enteric pathogens. Different fruits and vegetables provide unique ecological atmosphere due to differences in surface morphology, internal tissue composition, metabolic activities of leaves, stem, flower, roots, and tubers, allowing wide variety of microbes to survive and grow on different produce (Beuchat, 2002). Intrinsic and extrinsic ecological factors naturally present in produce or imposed during different operation, greatly affect the potential of pathogens to stay and manifest their capabilities (Beuchat, 2002). The knowledge and understanding of microbial ecology on fresh produce is very important for the development of strategies to prevent pathogen contamination of produce. All the above factors show the ubiquitous nature of *Salmonella* and its ability to survive or persist in non-host environment.

Salmonella and Tomatoes

In the list of top ten riskiest produce items in the U.S., tomatoes stand at eighth place (Klein et al., 2009). Tomatoes have been implicated in 14 multistate foodborne outbreaks of *Salmonella* recently from the period of 1996 to 2008 (CDC, 2007; CDC, 2009; Cummings et al., 2001; Greene et al., 2008; Hedberg et al., 1999; Tauxe, 1997). According to USDA ARS, nearly 1,840 cases of *Salmonella* have been associated with tomatoes consumption during the period of 1998 to 2006 and approximately, 1,503 cases of salmonellosis were associated with both tomatoes and peppers consumption in 2008 (Hedberg et al., 1999). Most of the outbreaks linked to tomatoes have been traced to the product originating from Virginia or Florida, according to FDA Tomato Safety Initiative, 2007. *Salmonella* strains involved in these outbreaks include *Salmonella* Baildon, *Salmonella* Thompson, *Salmonella* Montevideo, *Salmonella* Anatum, *Salmonella* Braenderup, *Salmonella* Javiana, *Salmonella* Newport, *Salmonella* Typhimurium and *Salmonella* Saintpaul (CDC, 2008a). The trace-back investigations

could not find the exact source of contamination and pre-harvest contamination is thought to be a probable source for these outbreaks. Various suspected pre-harvest sources of *Salmonella* contamination in tomatoes are irrigation water, soil, manure, insects, flowers and human sources (Beuchat, 1996). Contamination during harvesting is also suspected as a potential source of outbreaks. Field packed tomatoes have higher chances of contamination due to direct human contact (Sargent et al., 1989). The behavior of *Salmonella* on the surface of tomato, inside the tomato and decontamination with different sanitizers has been described in numerous studies.

Salmonella on surface of tomato

The behavior of *Salmonella* is different on the surface of tomato and inside tomato. Outside tomato, it has to tolerate the harsh environmental conditions that do not support the growth of *Salmonella* in most of the times. Previous research *Salmonella* survival and growth on tomato surface under suitable temperature, relative humidity, atmospheric gas composition, and other required conditions, has been documented. Maintaining high humidity and temperature during tomato storage has shown increase in growth of *Salmonella* on the surface of tomatoes (Iturriaga et al., 2003). The persistence and growth of *Salmonella* on intact tomatoes depends upon serovar (Shi et al., 2007), inoculation dose (Wei et al., 1995), inoculation site (Das et al., 2006), temperature (Zhuang et al., 1995), medium used to deliver bacterial cells (Wei et al., 1995) and method and drying time of inoculation (Lang et al., 2004). Some serovars persist longer than others (Shi et al., 2007). Serovars like Hadar, Montevideo or Newport showed more persistence than Enteritidis, Typhimurium and Dublin on the surface of ripened or red tomatoes, while in case of immature or green tomatoes, all the above mentioned serovars showed same persistence. Some of above serovars are

from tomato-associated outbreaks and others are from animal or clinical isolates (Shi et al., 2007). According to one study, on the surface of mature, green tomatoes, *Salmonella* Montevideo showed significant growth within 7 days and 1 day at 25°C and 30°C storage temperature, respectively, while at 10°C, it persisted on tomato surface for 18 days without any significant reduction in population (Zhuang, 1995). *Salmonella* was found to survive on tomato skin for approximately 20 hours in distilled water (Wei et al., 1995). Surface inoculation of *Salmonella* on intact tomatoes through spot inoculation method was reported as the best method when comparing dip, spot and spray inoculation. Same researchers has reported better *Salmonella* recovery after 1 h drying of inoculated tomatoes as compared to 24 h drying indicating that method of inoculation and drying time does affect the recovery of *Salmonella* from tomato surface (Lang et al., 2004). Inoculation site also effect the survival and growth of *Salmonella*. *Salmonella* inoculated at stem scars and growth cracks showed better survival than on tomato skin (Wei et al., 1995). Stem scars are considered the location on the tomato plant where the most *Salmonella* infiltration can occur (Das et al., 2006).

Along with, intact tomatoes, *Salmonella* serovars showed persistence on dry tomato leaflets and tomato stem, as well (Guo et al., 2001; Rathinasabapathi, 2004). *Salmonella* Montevideo showed no significant reduction in population from 6.6 log CFU per leaflet on providing hydroponic nutrient and 100% relative humidity. Although, approximately, 3.0 to 3.5 log reduction in population was observed, when leaflets were dried after 48 h incubation at 60% relative humidity (Rathinasabapathi, 2004). Studies related to sources of intact tomato contamination with *Salmonella* reveal that the transfer and growth of *Salmonella* on the tomato surface is possible through contact

with contaminated moist soil (Guo et al., 2002). Controlled atmosphere packaging and modified atmosphere packaging have proved quite effective in reducing *Salmonella* population from the surface of tomatoes (Das et al., 2006).

Salmonella in tomatoes

The exterior of produce is thought to be a physical barrier, preventing bacteria from penetrating to the inside of the product (Tauxe et al., 1997). The outer surface of plant has a protective layer made up of cutin and wax called cuticle that is first defense of microbial pathogens (Beldin et al., 1998). The presence of outer layers including, the skin, rind, or peel on the fruit makes the permeability of pathogens difficult (Tauxe et al., 1997). Once inside the produce, pathogens are exposed to nutrient rich environment in opposite to the surface of produce. The growth of *Salmonella* has been reported in many produce items like in papaya, mangoes, watermelons, pineapples (Penteado and Leitao, 2004; Strawn and Danyluk, 2010). *Salmonella* internalization and colonization of tomato fruit is possible through its contact with tomato stem, flowers, and cuts on skin surface (Guo et al., 2001). A study conducted to identify the irrigation water and seed stock as the possible sources of internal contamination of tomato fruit have demonstrated that contaminated irrigation water (7 log CFU/ml of *Salmonella* Montevideo) and seed stock (seeds soaked in 8 log CFU/ml of *Salmonella* Montevideo for 24 h) were not able to contaminate tomato fruit (Miles et al., 2009). Similar results with contaminated irrigation water (5 Log CFU/ml) were obtained by Jablasone et al. (2004). Conversely, hydroponically grown tomatoes have shown *Salmonella* presence inside tomato after sucking up *Salmonella* contaminated water (Guo et al., 2002) and international outbreak of *Salmonella* with alfalfa sprouts was reported to be caused by

seed contamination (Mahon et al., 1997). This shows that more research and work is required to find out the ways and the sources of contamination of tomato fruit.

Once *Salmonella* internalized tomato plant, its elimination is very difficult except cooking or any other similar kill step. Fully ripe tomatoes have low pH 3.9-4.4 which is unsupportive for the growth of *Salmonella* (Beuchat, 2002). When inside the tomato, *Salmonella* has to combat with its low pH for its sustenance. The potential of this bacterium to survive as well as grow in tomatoes under optimum temperature and moisture conditions has been documented several times and was firstly reported by Asplund and Nurmi (1991). *Salmonella enterica* serovars Typhimurium, Infantis, Baildon have been shown to grow in tomatoes (Asplund and Nurmi, 1991; Zhuamg et al., 1995; Weissinger et al., 2000). When the surface of produce is cut or bruised, fluids containing nutrients or antimicrobials are excreted, this can enhance or retard the growth of pathogens (Beuchat, 2002). In case of cut or diced tomatoes, the temperature plays an important role in the growth of bacteria e.g., *Salmonella* Montevideo population in tomatoes at 5°C, does not change for 9 days but increased significantly at 20°C or 30°C in 22 hours (Zhuang et al., 1995). Similarly, *Salmonella* Baildon showed growth on diced tomatoes at temperature conditions of 21°C or 30°C, and died when stored at 4°C (Weissinger et al., 2000). Growth of *Salmonella* in tomatoes was found to be enhanced by the presence of proteolytic mold that increases the pH of tomatoes and gives better environment for bacteria to proliferate (Wade et al., 2003; Wells et al., 1999). Once inside the tomatoes, *Salmonella* was found to resist sanitizer treatments (Burnett and Beuchat, 2007).

Salmonella Disinfection on Produce

Once the produce is infected with *Salmonella*, it becomes very difficult or almost impossible to eliminate contamination until cooked or any such heat treatment is given. Implementation of decontamination steps is imperative to reduce the risk from little to great extent. Fresh produce does not undergo pathogen reduction steps unless they are irradiated (Saroj et al., 2006). The use of hot water to remove pathogenic microbes from the surface of whole or cut fresh produce has been practiced by food industry but the adverse effects on color, texture and flavor limit the use of this treatment. The efficacy of disinfectants depends upon the nature of fruits and vegetables treated, pH and temperature of solution, bacteria targeted, concentration and time of exposure of disinfectant (Parish et al., 2003). Many disinfectants with varied success have been investigated on tomatoes. Irradiation is an effective tool in controlling pathogens on the surface of raw fruits and vegetables (Saroj et al., 2006). Pulse UV-light has been tried and tested on raspberries and strawberries with positive outcome against enteric bacteria, *E. coli O*157:H7 and *Salmonella*, with no adverse effect on fruits (Bialka and Demirci, 2008). Although many researchers have emphasized the use of irradiation for decontaminating raw produce, yet the reluctance of consumers and high cost required for irradiations are the factors affecting its popularity in food industry.

Chlorine remains a convenient and inexpensive sanitizer, and is quite commonly used in food environments. It is available in three forms sodium hypochlorite, calcium hypochlorite and chlorine gas. The recommendation for chlorine is 100 to 150 ppm free chlorine at pH 6.5-7.5 (Ritenour et al., 2002; Parish et al., 2003). At pH 7, 80% of chlorine is in hypochlorous form (the active form that actually kills bacteria) (Ritenour et al., 2002). The pH of fresh Florida waters is 8 and is decreased by adding citric acid

(mainly) before adding chlorine to it (Ritenour et al., 2002). Depending upon concentration of hypochlorous acid, exposure time required to obtain reduction in microbial population varies, typically increasing the concentration, decreases the exposure time required and vice-versa. Keeping the concentrations of hypochlorous acid high is considered effective in controlling cross-contamination even when exposure time is less than 1 min (Ritenour et al., 2002; Felkey, 2006). According to Wei et al. (1995), *Salmonella* inoculated at stem scars, unbroken surface and wounds was not disinfected with the application of 100 ppm of chlorine for 2 min. Zhuang et al. (1995), however, reported the effectiveness of chlorine treatment in reducing bacterial population and concluded that dipping mature green tomatoes in 60 to 110 ppm chlorine solution for 2 min can significantly reduce the *Salmonella* population from the surface and the core tissue. Although, increasing the concentration of chlorine did not result in complete inactivation. Similarly, trisodium phosphate was found to be very effective in controlling *Salmonella* on the surface and core tissues of mature, green tomatoes according to a study by Zhuang and Beuchat (1996). Significant reductions were found from the surface and core tissues of tomatoes on dipped in 1% and 4-15% solution of trisodium phosphate, respectively. Complete elimination of *Salmonella* from surface was obtained by dipping tomatoes in 15% solution (Zhuang and Beuchat, 1996).

Chlorine was found effective in reducing *Salmonella* population on seeds as well. Alfalfa sprout seeds inoculated with *Salmonella* Stanley showed reduction in population at chlorine concentrations of 100 and 290 ppm in 10 min. More reduction was observed in case of higher chlorine concentration. However, further increase in concentration did not led to any further reduction in population of *Salmonella* (Jaquette et al., 1996). In

another study, alfalfa sprouts were inoculated with a five-strain mixture of *Salmonella* and dipped in chlorine solution of concentration 200, 500 or 2,000 ppm. Population of *Salmonella* was reduced to undetectable levels after treatment with 2,000 ppm chlorine. Reduction in population was similar with the use of 200 and 500 ppm of chlorine or 2% and 5% of hydrogen peroxide, respectively when dipped for 2 min (Beuchat, 1997).

The major challenge in chlorine use is to maintain its concentration in a free form. Presence of high organic matter in wash water and inaccurate pH can decrease the effectiveness of chlorine and cause increase in microbial population (Ritenour et al., 2002). A study was conducted by Senter at al.(1985) to determine the microbiological changes in fresh market tomatoes during packing operations. They observed higher population of *Salmonella* on tomatoes washed in 114 ppm chlorine solution as compared to the controls indicating that degradation of chlorine can lead to increase in bacterial population. However, increase in concentration of chlorine to 226 ppm did decrease the enterobacteriacae count. An issue raised by Garg et al. (1990) is the difference in effectiveness of chlorine rinses in laboratory and industrial environment which can also be attributed to the fact of inadequacy of chlorine in industrial environment at certain moments. Above all, the formation of chlorinated organic compounds such as trihalomethanes from chlorine have raised safety concerns (Parish et al., 2003).

Due to all of these issues with the use of chlorine, alternate treatments have been studied to eliminate microbial population with different rate of success on fresh produce. Antimicrobial activity was found to increase with the addition of bromine into solution containing chlorine (Shere et al., 1962). Use of ozone in the elimination of

Salmonella from fruit, eggshell and berry surfaces is also an effective treatment (Rodriguez et al., 2004). Another finding by Perry et al. (2008) emphasized the sequential use of heat and ozone as they proved to be more effective than either of them alone. Ozone is also found to be effective for the treatment of fruit juices and water (Patil et al., 2010; Restaino et al., 1995). Mattson et al. (2010) studied the efficacy of four plant-derived antimicrobials namely, carvacrol, *trans*-cinnamaldehyde, eugenol and β-resorcylic acid in tomato wash solutions against *Salmonella spp*. Although, sensory or quality analyses were not performed during this study, reductions in *Salmonella* populations were observed with the use of plant-derived antimicrobials (Mattson et al., 2010).

After harvesting tomatoes are dumped in water to remove the field heat. Tomatoes have potential to uptake *Salmonella* cells and water from dump tank (Bartz and Showalter, 1981). Infiltration is observed to be more from stem end scar. It occurs only when water pressure overcomes the internal gas pressure (Zhuang et al., 1995). To avoid infiltration of *Salmonella* cells by tomatoes, the temperature of dump tank is recommended to be 10°F greater than the fruit pulp temperature. Zhuang et al. observed significantly high uptake by core tissue of tomato (at 25°C) on dipping in 10°C as compared to 25 or 37°C (Zhuang et al., 1995). As the temperature difference between harvested tomatoes and water is responsible for this water movement, maintaining water temperature higher than fruit pulp temperature helps in preventing this problem.

Salmonella can escape disinfectants by various ways. Oliver et al. (2005), determined chlorination can induce viable but not culturable state (VBNC) in bacterial

cells which can pose serious threat to wastewater decontamination methods used. Both uncombined (free) and combined chlorine were tested on *E. coli* and *S.* Typhimurium and VBNC cells were obtained (Oliver et al., 2005). In addition to the potential for VNBC cells, microbes can also dwell in cracks, crevices, pockets, inaccessible to chlorine and add to lack of chlorine's effectiveness. Another important issue is ingestion of bacterial cells by protozoa. *Salmonella* resistance to free chlorine is increased after getting ingested by protozoa. *T. pyriformis* provided 50 fold more resistance to many bacterial pathogens upon ingestion; bacterial pathogens can survive in chlorinated water if they are inside protozoa (King et al., 1988). Infiltration of bacterial pathogens into raw produce also provides shield to bacterial pathogens and they can potentially escape disinfection (Ritenour et al., 2002).

Bacterial Transfer

Most of the bacterial transfer studies performed to date have been conducted in processing and handling environments considering different food contact surfaces as a potential source to transfer pathogens to and from food. Microbial transfer to any surface depends upon many factors like bacteria involved (Mackintosh et al., 1984), type of surfaces (Chen et al., 2001), moisture level (Gill et al., 2002) and inoculation dose involved (Montville et al., 2003). All the mentioned factors affect the cross-contamination rates between surfaces. A study conducted by Lin et al. (1997) demonstrated a strong relationship between inoculation dose of bacteria used and their transfer to knife used to cut tomatoes. *Salmonella* (Rifampicin resistant) inoculated with high dose onto the stem scar end was able to contaminate the center and the bottom of the tomato, while low inoculum dose was unable to contaminate the center of tomato. The knife used to cut the tomatoes was found to be contaminated as well and had

potential to transfer this contamination to subsequent tomatoes cut with the knife (Lin et al., 1997). Another study conducted by Fravalo et al. (2009) regarding *Campylobacter* transfer from chicken thighs to cutting board demonstrates that the transfer of *Campylobacter* from naturally contaminated chicken thighs to cutting boards is inversely related to the initial load. Transfer rates for *Campylobacter* to cutting boards were determined by dividing the ratio of bacterial cells present on the blade to the bacterial cells naturally present on the skinned poultry (Fravalo et al., 2009). *Escherichia coli* transfer to iceberg lettuce through blade portion of field coring device (Taormina et al., 2009) and *Listeria monocytogenes* transfer to salami through slicer (Aarnisalo et al., 2007; Sheen, 2008) also demonstrated bacterial transfer potential between food and food contact surfaces. The study on transfer of *Listeria* to salmon during slicing was conducted by Aarnisalo et al. (2007) and after observing all the possible patterns of surface transfer, empirical equations were developed which depends on inoculation dose and slicing number. Sheen (2008) developed a model to predict the transfer of *Listeria monocytogenges* to salami during slicing with a blade.

Bacterial transfer studies related to gloves have also been documented. Montville et al. (2001) used a model for obtaining bacterial transfer rates between chicken and bare hands, chicken and gloved hands, bare hands to lettuce and hands to lettuce with gloves on. The transfer rate observed from chicken to bare hands was in between 0.61 to 10.43% and chicken to gloved hands was from 0.0001 to 0.24%. The transfer rate from gloved hands to lettuce ranged from 0.0003 to 0.0545%. This research also illustrated a point that bacteria can transfer from food to gloved hands and then again to

food from those gloved hands, which is in agreement with other findings that gloves are permeable to bacteria and have potential to transfer pathogens.

Tomato Production

Tomatoes (*Solanum lycopersicum*) belong to the family *Solanaceae* and originate from the west coast of South America in the areas of Peru and Ecuador (Olson, 2009). Botanically, tomato is a fruit because it is a mature ovary, but has always considered as vegetable because of its use in meals or as salad instead of desert. In 1893, U. S. Supreme Court declared it a vegetable. Tomatoes are mainly water, in 100 g of tomato fruit, 93.76 g is water. Tomatoes are rich in magnesium, potassium and phosphorous. They are considered as good source of lycopene, vitamin C, vitamin A and folate (UDSA, 2004). One medium sized tomato provides 40% of the RDA of vitamin C (ascorbic acid), 20% of the RDA of vitamin A, significant amounts of potassium, dietary fiber, calcium, plus some other vitamins in lesser amount with as few as 35 calories (Sargent, 1989). Hence, tomatoes are considered as highly nutritious vegetable.

The U.S. is one of the top leading tomato producing countries. The production of tomatoes in the U. S. increased by 25% from 1991 to 2007 (VanSickle and Hodges, 2008). Approximately 4.1 billion lb of fresh tomatoes were produced in the U.S. for domestic supply in 2007 (Vansickle and Hodges, 2008). Along with increase in production, the consumption of tomatoes has also increased in the U.S. According to USDA Economic Research Services, the consumption of fresh tomatoes increased by 40% in the period of 1997-1999 over the 1977-1978 period (Lucier et al., 2000). Per capita consumption of raw and processed tomatoes estimated for the decade of 1990-2000 in U.S. is 16.7 lb and 75.2 lb, respectively. Processed tomato products, including

sauces, catsup, pastes, and salsa, account for 81% of the total tomato consumption (Lucier et al., 2000). Increase in domestic consumption has increased importation of fresh produce, which accounts for 56% of total domestic consumption. Importation of tomatoes is mainly from Mexico and Canada, with Mexico being the main supplier after petitioning U.S. International Trade Commission in 1996 (Vansickle, 2007). Switch in demand was observed in 2005, when the demand of greenhouse tomatoes increased among consumers. Green house tomatoes have become more popular than field grown tomatoes and make up the majority of imported tomatoes.

The southeastern areas of U.S. (Alabama, Florida, Georgia, North Carolina, South Carolina, Tennessee, and Virginia) and California are the main tomato producing areas. The southeastern areas produces more than half (57%) of the tomatoes of the Unites States which is around 2.11 million lb, as per 2006 data, whereas California produces 1.23 million lb which is 33% of total tomato production in the U.S. (VanSickle, 2008). These data showed that around 90% of the tomato production in the U.S. is concentrated in above mentioned regions, while the rest of the nation produces less than 10% of total tomatoes of U.S. The seasons for tomatoes production in southeast U.S. and California are fall, winter, spring and spring, and summer and fall, respectively (VanSickle, 2008).

Florida is the largest producer of fresh market tomatoes and produced approximately 1.45 billion lb of fresh tomatoes in 2007 (34% of U.S. total production) (Vansickle and Hodges, 2008*).* Among all the vegetable crops grown in Florida, tomatoes are the third most important crop after potato and lettuce, accounting for more than 22% of total cash receipt (FASD, 2008). Round tomatoes are more popular in

Florida but along with round tomatoes, Roma tomatoes, cherry tomatoes and grape tomatoes are also grown on significant acreage in Florida (Roka, 2010; Van Sickle, 2008). Florida exports around 1.1 billion lb of tomatoes of fresh produce to the Canada and other countries. In late 1980s and early 1990s, land under tomato cultivation was more than 50,000 ac that decreased to in between 43 to 45,000 ac until after 2004-05 and further to 31,500 ac in 2007-08 (FTC, 1992; FTC, 2008). Florida tomato industry contributes $997 million annually to the state economy including both direct tomato sale ($464 million) and indirect and induced effect from these sales (FASD, 2008). It creates 8,231 direct and indirect jobs and around $299 million goes to tomato labor income in Florida (VanSickle, 2008). The main tomato growing areas in Florida are: Miami-Dade county, Southwest Florida, Palm Beach Fort-Pierce regions, Tampa bay area and Florida Panhandle area west of Tallahassee (Sargent, 1989). The average yield in Florida is about 1,400 25-lb carton per ac (Olson, 2009).

Tomato Growing in Florida

In Florida, tomato plants are first planted in greenhouses and about five weeks later, seedlings are transplanted to fields. One pound of seeds produces ca. 140,000 tomato plants. The planting period for Florida tomatoes specifically is July-Aug15 and Feb-Apr15 in North Florida, Aug-Sept and Jan-Feb in central Florida and Aug-Feb in South Florida (Oslon, 2009). Distance between rows and plants kept in Florida for tomato production is 48-72 in and 12-31 in, respectively. Optimum plant to plant distance is 27 in, decreasing the distance can overcrowd the plants (Olson, 2009). Most of the production is through indeterminate varieties and 7 to 8 ft stakes are used for the tall plants. Though staking increases the production costs, it improves the yield and overall quality of fruit as well. Selection of proper variety is very important to obtain good

yield. The required characteristics of good cultivar are: it should be high yielding and disease resistant. Along with this, horticultural quality of fruit like shape, size, color, smoothness, resistance to defects, adaptability and market acceptance which includes ripening ability, firmness and flavor of tomato fruit are also considered (Olson, 2009). Different cultural practices like bed preparation, fumigation, nutrient management, pruning, staking are required for better yield (Sargent, 1989).

Tomatoes are harvested around 100 to 120 days after the seeds are planted. The six different maturity and ripening stages as per U.S. standards for mature tomatoes: green, breaker, turning, pink, light red and red. Tomatoes are considered green when the tomato surface is green. The shade of green can vary from light to dark. Green tomatoes are not very sweet and require ripening to develop the red color and sweetness. Being a climacteric fruit, similar to cantaloupe, avocado, mango, and papaya; tomatoes can ripen after harvesting. Taking advantage of this property of tomatoes, harvested mature green tomato can be kept in specialized storage room with the supply of natural hormone ethylene that aids in ripening of tomatoes (Sargent, 1989). If harvested at proper mature green stage and given proper treatment, high quality tomatoes are developed. Mature green, round tomato is the main variety of tomato harvested in Florida, representing 90% of total tomato production. In 2002-03, 73% of Florida field tomato sales (by weight) were mature green tomatoes, down from 86 percent in 1997. The decrease of market share of mature, green tomatoes is due to availability of Canadian and European tomato products (VanSickle, 2008). The second ripening stage is called breaker stage where 10% or less of tomato surface show definite break of color from green to tannish-yellow, pink or red. Third stage is called

Turning stage, when greater than 10% but less than 30% of tomato surface show Tannish-yellow, pink or red color. Fourth stage is pink when greater than 30% but less than 60% of tomato surface show pink or red color. Fifth stage is light red where more than 60% and less than 90% of tomato surface show red color. The final stage is red when more than 90% of surface shows a red color (Sargent, 1989). Tomatoes harvested at red stage are very vulnerable to bruises and damages and have a shorter shelf life. Harvesting of tomatoes at a stage from breaker to red is called as vine-ripened tomatoes (Sargent, 1989).

Tomato Harvesting in Florida

Fresh tomatoes are handpicked in Florida and transferred to buckets and then to field crates. Various parameters to check the ripening of fruit are: position on the plant, size, shape, surface appearance, and presence of brown corky tissue on the stem scar. Apart from these external parameters, checking internal condition of a few tomatoes is another parameter. A few tomatoes should be picked from field prior to harvesting and sliced to check its internal condition (Sargent, 1989). Harvesting starts at late morning to protect crop from disease spread. Depending upon the market conditions, length of harvesting day can be more or less (Roka, 2010). Harvesting is done by crews, with one crew having 6 categories of workers: pickers, checkers, bucket handlers, dumpers, row boss, and tractor drivers (Zahara and Johnson, 1981). Pickers pick the right fruit and put them in buckets. After filling two buckets, tomatoes are shifted to gondolas, handlers take buckets and dump them and return the empty buckets to pickers (Zahara and Johnson, 1981). Gondolas or bins should be placed close to workers to avoid the walking distance and to reduce chances of injury. Gondolas should not be overfilled as that can impart bruises or other defects in tomatoes. Bins, gondolas, buckets or other

equipment used during harvesting should be sanitized quickly to avoid cross-contamination. Highly trained workers are required to do harvesting at proper stage and cause minimum damage to tomatoes. Immature, dead, decayed, defective tomatoes should be left in the field only (Roka, 2010; Sargent 1989). After picking, tomatoes are divided on the basis of size and color. Mechanical harvesting started in 1977, but is not popular among harvesters in Florida due to the high cost involved, more incidences of injury of tomatoes and dirt problems on the conveyor belt (Zahara and Johnson, 1981). Tomato fruit has very thin epidermis that increases its chances of getting bruised, wounded or punctured (Bartz and Showlater, 1981).

After harvesting, tomatoes are brought to packinghouse and dumped into chlorinated dump tanks. Adequacy of chlorine in dump tank is important to prevent cross-contamination. Bruised or punctured tomatoes can suck up water from dump tank if the temperature of dump tank water is lower than the temperature of pulp of tomatoes. Maintaining a dump tank's water temperature 10°C higher than the tomato pulp temperature will help to control this problem (Sargent, 1989). The rate dumping of tomatoes is also important as it determines the effective removal of all the dead, bruised or unwanted tomatoes from the lot. Three different classifications are used for Florida tomatoes 6×7, 6×6 and 5×6 and larger. The minimum and maximum sizes under these classifications are 2 9/32 in and 2 19/32 in, 2 17/32 in and 2 29/32 in, 2 25/32 in and none, respectively (FTC, 2004).

After sorting, green tomatoes are packed in fiberboard carton (single or double layer) with net weight of 25 lb. All the cartons are staked on standard pallets, with 80, 25-lb carton on each pallet (Sargent, 1989). Pallets are then shifted to ripening rooms

with controlled temperature (68 to 72°F) and relative humidity (85 to 95%) conditions that facilitate ripening. Proper temperature maintenance is very important for good color development. Higher temperature inhibits the red color and low temperature slows down the process. Lower temperatures can also results in chilling injury (Sargent, 1989). Ethylene, a natural plant hormone that helps in ripening is used at the concentration of 150 ppm. Proper air circulation is must in ripening room to avoid accumulation of carbon dioxide. Accumulation of more than 1% carbon dioxide can adversely affect the ripening process. Around 24-72 h of ethylene exposure is required by green tomatoes to develop the appropriate color. Immature fruit sometimes do not develop the desired red color even after 5 days of exposure (Sargent, 1989). The recommended storage temperature of tomatoes is 55°F; storing tomatoes below this temperature can result in poor quality (Sargent, 1989).

Tomato Good Agricultural Practices and Best Management Practices

Tomatoes related outbreaks in the U.S. raised a question on the safety procedures adopted by tomato growers, packers and re-packers. Contamination of tomatoes is believed to occur at every step of production. Therefore, in order to minimize the likelihood of contamination of fresh-market tomatoes by human pathogens, several preventive steps are required to be taken. Keeping this in mind, T-GAPs and T-BMPs were developed to help the Florida tomato industry in producing safe tomato crop. T-GAPs and T-BMPs are considered mandatory by the State of Florida and are still voluntary in California.

Florida is the first state in U.S. to implement frequent mandatory government inspection and audit of tomato handling, production and packing to verify adherence to T-GAP and T-BMP practices. T-GAPs and T-BMPs are mandatory standards imposed

by the state of Florida used to ensure the safety of fresh tomatoes produced, packed, repacked, distributed and sold in Florida or from Florida (DACS, 2007). The main purposes of these practices are enhancing safety of tomatoes by safely doing different operations and educating and training the workers at all levels. T-GAPs and T-BMPs are enforced by the co-operative effort between Florida Department of Agricultural and Consumer Services (FDACS) and Florida Tomato industry and supported by scientific research (DACS, 2007). Good agricultural practices courses, sanitation of dump tanks and packing lines workshop, workshop on in-plant sanitation, worker hygiene and field and plant sanitation are the major courses recommended for growers, packers, re-packers and workers under T-GAPs and T-BMPs. These practices became effective from July 1, 2008 (DACS, 2007).

T-GAPs are specifically used for field and green house production of tomatoes. T-GAPs require safe distance of fields from any animal operation or animal farms, where tomatoes are grown. Keeping a safe distance from animal farms reduce the risk of runoff and possible chances of contamination. Domestic animals are required to be excluded from the tomato field during growing and harvesting as they can shed pathogens in fields. Any kind of residual material that can harbor insects or pests should be excluded as well. Fields or green houses where tomatoes are grown are monitored on a regular basis and records are kept. Along with the safe field conditions, all the inputs that are given to field to grow tomatoes are also regulated under GAPs. Irrigation water used for crops is required to be non-contaminated and in case it is contaminated then proper treatments should be done before application to fields. These steps will eliminate any chance of contamination of field through irrigation water that is highly

suspected source of tomatoes contamination. Water used for pesticides spray is required to be potable water having appropriate microbial standards. All the tests are recorded and given to inspectors on request. GAPs have considered worker's hygiene as a crucial part for the safety of crops. Worker's hygiene and training is a big part of these practices. Workers suffering from illnesses are not allowed to do activities involving food contact and proper monitoring and documentation of worker's hygiene, training and education is also required. Pesticides and fertilizers are used according to their instructions and requirements only. Manure used in field should be properly composted as inefficiently composted manure can harbor pathogens and contaminate the crop. Record of date of compositing, methods used for compositing and date of application of manure to field are kept for future uses under T-GAPs (DACS, 2007).

Harvesting of tomatoes is an operation where workers come into direct contact with the tomato fruit. Presence of pathogens on raw produce during the time of harvest can directly cause human infection (Beuchat, 2008). Along with the worker hygiene, all the equipment they use is also required to be free of pathogens. Harvesting crews are required to sanitize the harvesting containers at least weekly or more frequently, if necessary and the use of final pack containers in the field is prohibited. The food contact surface "any equipment, container that touches the produce" is cleaned and sanitized routinely with permitted sanitizers. Good sanitation not only prevents infection of crops, but also reduces decay during shipping and storage. Keeping records of good sanitation practices is also crucial to show adherence to a food safety plan and to help identify potential problem areas. Good recordkeeping helps in trace-back investigations and quick actions, if necessary. During harvesting, harvesting crews are required to

remove any kind of dirt, debris associated with the tomatoes and dead, decayed or damaged fruit are left in fields only (DACS, 2007).

Tomato Best Management Practices (T-BMPs) are used for packinghouse operations and post-harvest handling of tomatoes. Packing houses and processing facilities are required to be constructed according to the requirements. The only difference between packing and processing facility is packing facility does not require closed structures as with processing facilities. The use of potable water meeting all the microbial standards with daily change and cleaning of dump tank is recommended under these practices. The temperature of dump tank is required to be maintained 10°F higher than temperature of fruit pulp to minimize the water and microorganisms intrusions into the fruit. Diligently removal of all the dead, damaged and decayed tomatoes is mandatory. The disinfectants should be used at proper concentration and for proper time to achieve maximum possible decontamination in processing facilities. Timely monitoring of disinfectants is required to ensure that the adequacy of disinfectant is maintained. Ensure proper hand washing and toilet facility for workers that are required to be cleaned and sanitized regularly. T-BMPs require proper storage area for tomatoes with written sanitation procedure for coolers and storage space. Animal and pest exclusion from the area where tomatoes are handled and packed is also required. Proper pest control programs are required to be documented. Only permitted chemicals and pesticides are to be used inside the facility. Record keeping of all the products packed, shipped, handled, stored, transported, standard operating procedures, sanitation standard operating procedures, sanitization monitoring records, sanitation monitoring records is also required. Packing containers are required to have the

address of the packer or grower and tomatoes are required to have positive lot identification number. Packing of fruit in unsanitized or the incoming containers and mixing of produce from different producers is prohibited in repacking facilities. The cleanliness of transporting vehicles needs to be ensured before loading and requires transporters to have positive lot identification required for trace-back. T-BMPs require trace-back provision from all who handle tomatoes at certain point during the whole process (DACS, 2007).

Exemption from T-GAPs and T-BMPs Requirements

An individual grower producing tomatoes no more than two twenty-five pound boxes for one customer or selling these boxes to local farmers market are not required to follow T-GAPs and T-BMPs by law. Along with this, charitable contributions are also exempted from these practices (DACS, 2007).

Gloves

Hands are a potential intermediate source for spreading the pathogens to food items. A survey of 81 foodborne illnesses linked to food workers conducted by Guzewich and Ross (1999) illustrates that 89% of the outbreaks are due to transmission of pathogens through hands. Another survey by Bean et al. in 1997 found around 36% of all the foodborne disease that occurred during between 1988-1992 (2,423 outbreaks) were due to poor personal hygiene of workers. Workers can transmit pathogens to food from a contaminated surface, from another food, or from hands contaminated with organisms from their gastrointestinal tract. Workers who no longer show symptoms of salmonellosis can continue shedding *Salmonella* in their stools for five weeks, as asymptomatic carrier, and may constitute significant risk. The resident microflora that is almost always present on the skin is not of major concern in term of human infections,

however, transient pathogenic microorganisms that temporarily stay on the hands or the skin are of greater concern (Paulson, 1996). Pathogens believed to transmit through workers are *E. coli*, Hepatitis A virus, *Salmonella* spp., *Shigella* spp., and *Clostridium perfringens* (Restaino and Wind, 1990; Synder, 1997). *Salmonella* present on surface even in very low number (120 organisms/cm^2) has potential to transfer to fingertips of workers and further to food touched with contaminated fingers (Scott and Bloomfield, 1990; Pether and Glibert, 1971). After contamination from worker hands, multiplication of pathogens can occur under favorable conditions of temperature or moisture.

To prevent the cross-contamination, gloves are recommended by researchers to act as a barrier to bare-hand contact with food. Research on gloves is mainly conducted in healthcare settings that are not similar to the food service or food production and processing. In food establishments, gloves are used for the purpose of preventing pathogens from transferring to food via hand's contact from workers (Paulson, 1996). Gloved hands are considered more protective than bare hands in transferring bacteria as per the various studies conducted. A study conducted by Montville at al. (2001) emphasized the importance of gloves in reducing microbial transfer from bare hands to chicken and from chicken to bare hands. During harvest, gloves also help in protecting harvesting crew from coming in contact with the different chemicals sprayed on tomato crop. Many pesticides, insecticides and fungicides are sprayed to tomato crop during production period and most workers wish to limit their exposure to these chemicals. Gloves are recommended for use during picking or harvesting of tomatoes.

Although gloves are found to be more protective as compared to bare hands, they cannot be considered completely safe. Transfer of pathogens from food to gloves

hands has also been documented (Montville et al., 2001). This shows that gloves are permeable and bacteria have the potential to pass through them. In addition, gloves promote a false sense of security among workers. Contaminated gloves transfer pathogens to different patients in hospital settings (Ehrenkranz, 1992). Glove use can also promote poor hand washing and more accumulation of pathogens on hands. Washing the hands prior to and after glove use is highly recommended. Wearing gloves without hand washing may lead to the contamination of both the inner and the outer part of glove. Avoiding washing hands and not wearing gloves can provide good moist and warm environment for the transient pathogens present on the surface of hands and can lead to their growth (Larson et al., 1989). Loose fitting and very tight gloves enhances the chances of growth and more transfer of microorganisms (Larson et al., 1989).

Different types of gloves are used in food establishments, including disposable and reusable gloves. Disposable gloves are typically very thin, 4-8 mils thick. They provide poor chemical resistance but better touch sensitivity. They should never be reused and changed frequently. They are not very protective against hazardous chemicals and bio hazardous material. Disposable gloves can tear very easily. Reusable gloves, on the other hand are typically 18-28 mils thick. They are much better than disposable gloves against strong chemicals and hazards. Reusable gloves have a long cuff made of same kind of material which can provide extra protection. Reusable gloves should be washed and sanitized properly before reused. Reusable rubber gloves are mainly used for cleaning purposes (Imperial College London, 2005). Latex gloves use in medical facilities has higher chances of retaining the microorganism because of its three dimensional lattice structure responsible for elasticity also. Washing with

several different antimicrobials has little effect in the removal of bacteria from latex surface. This provides very useful information that microorganisms can adhere to the latex gloves and cannot be easily removed from them (Doebbeling et al., 1992).

Tomatoes have been linked to several outbreaks in the recent years. Transfer of human pathogens following human contact (infected employees) during harvesting has been documented (Todd et al., 2009). Setting up and maintaining an appropriate sanitation program throughout handling is important. Under GAPs, glove use (Disposable or Reusable) or bare hands with frequent hand washing is recommended during tomato harvesting. When single-use or disposable gloves are used, the washing and sanitizing prior to the use of gloves is suggested (FDA, 1998). In Florida tomato packing house, following T-BMPs, disposable gloves are mainly preferred and changed very frequently, for example after touching hair, mouth or any other surface except tomato. Disposable gloves are not reused in tomato packinghouses in Florida. Reusable gloves are required to be made of easily cleaned or sanitized material as pre FDA guidelines and are required to be cleaned and sanitized when required. Reusable gloves are required to be kept at safe and sanitized place when not used by employees. Reusable gloves are used in Florida during harvesting of tomatoes and reused next day after sanitizing (FDA, 1998). T-GAPs do not have any recommendation to change the gloves during harvesting, in turn, permitting workers to keep using gloves until torn.

Focus of Research

The level of *Salmonella* transfer from harvesting surfaces to or from tomatoes is an understudied topic needing more attention and research. In this research, we have tried to quantify the risk associated with gloves, which is considered as the potential routes of cross-contamination during tomato harvest. The two types of gloves were

tested, single-use latex gloves and reusable rubber gloves. Reusable gloves were also made dirty to evaluate if the risk associated with glove use changes over the course of the day as gloves become soiled. The outcomes obtained from this work are helpful in providing science based recommendations to tomato growers and packers regarding their gloves use policies.

CHAPTER 3
MATERIAL AND METHODS

Preliminary Experiments

Preliminary experiments were performed to gain insight into experimental procedures and to further define variables. Preliminary experiments were performed to determine the carrier medium required for preparing *Salmonella* cocktail, the drying time for inoculum on the surface, the culture media for recovering *Salmonella* cells and to make gloves dirty similar to real tomato harvesting situation.

Carrier Medium

The following carrier mediums were evaluated: water, 0.1% Peptone water (Difco, Becton Dickinson, MD), Tryptic Soy Broth (TSB) (Difco, Becton Dickinson, Sparks, MD), 5% Horse serum (HS) and Butterfield's phosphate buffer (BPB) (Hardy Diagnostics, CA). The carrier mediums were used to prepare *Salmonella* cocktail (see below) and experiments were performed by inoculating single-use gloves and touching with mature green, round tomatoes, immediately and after 1 h inoculum drying. Each group consisted of three samples, and only one replication was performed for all the carrier mediums (n=3) except for 0.1% peptone water, which was replicated for four times (n=12).

Inoculum Drying Times

To evaluate the inoculum drying time on surface of gloves, different drying times were selected including immediate sampling, 30 min, 1 h, 1 h 30 min, 2 h and 3 h. Experiments were performed by inoculating single-use gloves (see below) and touching with tomatoes. Peptone water (0.1%) was used as a carrier medium for the trial. Each

replication consisted of three samples and two replications (n=6) were performed at each drying time except 1 h, for which four replications were performed (n=12).

Culture Media

Trial experiments were also performed to determine the suitable culture media for the experiments. Tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD; non-selective), Bismuth sulfite agar (BSA; Difco, Becton Dickinson, Sparks, MD; selective) and Xylose lysine deoxycholate agar (XLD; Difco, Becton Dickinson, Sparks, MD; selective) supplemented with rifampicin (100 µg/ml; TSAR, BSAR, XLDR) (Fisher Bioreagents, Fair Lawn, NJ) were used to enumerate bacterial population during trial. The main comparison was being made between the two selective media, BSAR and XLDR. Single-use gloves, mature green, round tomatoes and water (as a carrier medium) were used to obtain the data. One replication with three samples was performed for all the culture media (n=3).

The inoculum was dried in bio safety cabinet for 0 min, 15 min, 30 min, 45 min and 60 min. The purpose was to evaluate the two selective media types (BSAR and TSAR) under the combination of carrier medium, and inoculum drying time, to evaluate where maximum *Salmonella* transfer and subsequent enumeration occur.

Dirty Reusable Gloves

To soil reusable gloves similar to real tomato harvesting field, different quantities of soil (0.5 g, 0.3 g, 0.1 g), different volumes of tomato internal tissue (1/4 TSP, 1/8 TSP, a drop) and a tomato leaf were rubbed on glove piece for 5 s to 30 s with 5 s interval. Based on appearance, 0.1 g of soil, one drop of tomato internal tissue and a tomato leaf were selected to rub on glove piece for 20 s. Experiments were also performed with differently inoculated dirty glove pieces. One set was immediately

inoculated after making dirty, while other set was dried for half an hour and then inoculated. The purpose was to observe, if any, differences in *Salmonella* transfer from differently dried and inoculated gloves to tomatoes.

Tomatoes

Round, mature green tomatoes were purchased from local sources and stored at 40°F before use. After washing with cold water, tomatoes were stored overnight at room temperature prior to inoculation. A circle of 2 to 3 cm diameter was drawn on the surface of tomatoes on the day of inoculation.

Gloves

To perform experiments with gloves, clean gloves (Reusable or Single-use) were purchased from local sources and were cut into (5 cm × 5 cm) square size pieces with sterile scissors. For experiments with dirty reusable gloves, clean reusable gloves were cut into (5 cm × 5 cm) and rubbed with a tomato leaf for 20 s.

Salmonella Strains

Five rifampicin resistant *Salmonella* strains used in experiments were: *Salmonella* Michigan (MDD 251;Cantaloupe), *Salmonella* Montevideo (MDD 236;Almond survey-Danyluk et al. 2007), *Salmonella* Newport (MDD 314; Tomato outbreak-Greene et al., 2008), *Salmonella* Poona (MDD 237; Cantaloupe outbreak-CDC, 1991), and *Salmonella* Saintpaul (MDD 295; Orange juice). A stock solution of rifampicin was prepared by adding 1 g of rifampicin in 20 ml of methanol. The solution was then filter sterilized (Nalgene (0.20 µm pore size), Rochester, NY), wrapped in foil and stored in refrigerator at 4±2°C until used. In 1 L media, 2 ml of stock solution of rifampicin was added to obtain the final concentration.

Cocktail Preparation

Salmonella cultures, stored at -80°C in cryogenic vials were obtained from Danyluk laboratory culture collection prior to each experiment, thawed and then were streaked onto Tryptic Soy Agar plates supplemented with rifampicin (100 µg/ml; TSAR) followed by overnight incubation (24 ± 2 h) at 37±2°C. One isolated colony was transferred to 10 ml of tryptic soy broth supplemented with rifampicin (100 µg/ml; TSBR), and incubated for 24 h at 37±2°C. Following this incubation, a 10 µl loopful was transferred into 10 ml of fresh TSBR and incubated for 24 h at 37±2°C. Cultures were collected by centrifugation (Allegra X-12, Beckman Coulter, Fullerton, CA) at 3,000 rpm for 10 min. The cells were washed two times by pouring the supernatant and suspending in 10 ml of BPB. Washed cells were then suspended in BPB with half the initial volume (5 ml). The resultant cultures had ca. 10^9 CFU/ml bacterial population. Serial dilutions were performed twice in 9 ml of 0.1% peptone to achieve concentration of ca. 10^7 CFU/ml. Equal volume of each *Salmonella* strain was collected into a cocktail and vortexed. The *Salmonella* cocktail was then suspended in 1 ml size micropipettes and microcentrifuged (Eppendorf, Minispin, Hauppauge, NY) at 13,400 rpm for 10 min. The supernatant was dumped off from all micropipettes and 1 ml of 0.1 % peptone water (carrier medium) was added to each. The cocktail was vortexed and stored on ice for up to 1 h, prior to inoculating gloves or tomatoes.

Inoculum Procedures

Tomatoes or glove pieces were inoculated in bio safety cabinet with the *Salmonella* cocktail. A 100 µl cocktail of bacterial cells were distributed in 6 to 8 drops on the glove pieces or circled area of tomatoes (Iturriaga et al., 2006). Inoculum was dried for 0 h, 1 h or 24 h prior to transfers.

Transfer Scenarios

Clean (Reusable or Single-use) or Dirty (Reusable) Gloves to Tomatoes

The uninoculated tomatoes were touched with the inoculated glove surfaces, for less than 5 s and placed into a sterile sampling bag (17.78 cm × 30.48 cm) (Fisher brand, Pittsburg, PA). Samples were taken at three different drying times: 0 h (wet inoculum), 1 h and 24 h along with one control sample. Each replication consisted of three inoculated gloves samples for each drying time and three samples for control. Three additional glove samples were placed in each group and sampled with similar protocol as tomatoes, to determine the population of *Salmonella* on inoculated surface. A total of six replications were performed (n=18).

Tomatoes to Clean (Reusable or Single-use) Gloves

Inoculated tomatoes were touched with glove pieces, for less than 5 s and placed in sterile sampling bags in similar way as explained in the above transfer scenario. Sampling method, sampling time and number of replications were also similar to the gloves to tomato scenario described above.

Clean (Reusable or Single-use) or Dirty (Reusable) to Many Tomatoes

Inoculated gloves were touched with 25 sequential tomatoes (clean gloves) and 10 sequential tomatoes (dirty gloves), one after the other and placed in sampling bags. Samples were taken immediately after inoculation (0 h) and after 1 h drying of inoculum. Experiments were replicated three times with three samples at one time point (n=9).

Enumeration of Pathogen

Twenty milliliters of BPB was added to the sampling bags containing the originally inoculated surface and the transfer surface with clean gloves were used. For all work involving dirty gloves, 20 ml BPB with 0.1% Tween-20 was added to the sampling bags.

Tween-20 was added to help in removal of cells from soiled surfaces (Raiden et al., 2003). Sampling bags with tomato samples subjected to a gentle rub-shake-rub for 1 min and glove samples were stomached (Smasher, AES Lab, Cranbury, NJ) for 1 min prior to enumeration. Serial dilutions were prepared using 0.1% peptone water (9ml) and liquid in bags was surface plated (0.25 ml in quadruplicate and 0.1 ml in duplicate) onto both TSAR and BSAR media to increase the limit of detection. The colonies present after incubation at 37±2°C for 24±2 h (TSAR) or 48±2 h (BSAR) incubations were counted.

Enrichment

When counts fell below the limit of detection (1.3 log CFU/surface), enrichment for *Salmonella* was conducted by the US Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) protocol for produce (FDA, 2007). Briefly for *Salmonella*, 20 ml of double strength lactose broth (Difco, Becton Dickinson) was added to the sterile sampling bags and incubated at 37 ± 2°C for 24 h. One hundred microliters and 1 ml of mixture was then transferred to 9.9 ml tubes of Rappaport-Vassiliadis R10 (RV; Difco, Becton Dickinson) and tetrathionate (TT; Difco, Becton Dickinson) broths, respectively. Test tubes were incubated for 48 h at 42 ± 2°C for RV and 24 h at 37 ± 2°C for TT. A 10 µl loopful was streaked onto BSA, XLD, and Hektoen Enteric agar (HE; Difco, Becton Dickinson), and incubated at 37 ± 2°C for 24 h. *Salmonella* positive colonies are black with metallic sheen on BSA, red with black centers on XLD, and blue-green with or without a black center on HE. Positive colonies were selected and transferred (10 µl needle) to triple sugar iron agar (TSI; Difco, Becton Dickinson) slants and lysine iron agar (LIA; Difco, Becton Dickinson). Slants were incubated at 37 ± 2°C

for 24 h. A confirmed *Salmonella* enrichment results in TSI slants have a pink top and black bottom with gas formation and LIA tubes are black or no color change.

Transfer Coefficients

Transfer coefficients are calculated by the following equation:

$$TC = \frac{P_t}{P_i} \text{TC} = \text{P}$$

Where P_t = *Salmonella* population on touched surface which can be tomato or glove (CFU/surface), P_i = *Salmonella* population on inoculated surface (CFU/surface).

Statistics

All the results obtained were appropriately averaged to get the final counts. Transfer coefficients obtained were analyzed using Statistical analysis system (SAS 9.2; SAS institute inc., Cary, NC, USA) for analysis of variance (ANNOVA) and significance was determined by least square significant test at $p<0.05$.

CHAPTER 4
RESULTS

Preliminary Experiments

Carrier Media

The population of *Salmonella* obtained immediately after inoculation and after 1 h of inoculum drying with 0.1% peptone water as 'carrier medium' were 5.1±0.2 log CFU/tomato and 5.0±0.0 log CFU/tomato, which were higher than 5% Horse Serum (3.0±0.6 log CFU/tomato and 2.5±0.0 log CFU/tomato) and TSB (4.9±0.2 log CFU/tomato and 4.8±0.0 log CFU/tomato) at both drying times (Table 4-1; Figure 4-1). The population of *Salmonella* obtained from 0.1% peptone water was similar to water (5.1±0.2 log CFU/tomato) when inoculum was wet, however, after drying the inoculum for 1 h, *Salmonella* population obtained from water (4.9±0.1 log CFU/tomato) was lower than the population obtained from 0.1% peptone water (Table 4-1; Figure 4-1). Comparing BPB (5.0±0.1 log CFU/tomato and 5.0±0.1 log CFU/tomato) and 0.1% peptone water demonstrated that population of *Salmonella* obtained from wet inoculum was lower in case of BPB. Hence, 0.1 % peptone water was selected as carrier medium for further experiments.

Drying Time

Transfer of *Salmonella* to tomatoes was not observed after 1 h of inoculum drying on gloves (as it dried out) with the exception of one experiment with 5% Horse Serum, where it transferred up to 2 h (Table 4-2; Figure 4-2). *Salmonella* population obtained after 15 min of inoculum drying (5.2±0.2 log CFU/tomato), 30 min (5.1±0.2 log CFU/tomato) and 45 min (5.0±0.2 log CFU/tomato) was almost equal to the population obtained from wet inoculum (5.1±0.2 log CFU/tomato) (Table 4-2; Figure 4-2). However,

drying the inoculum for 1 h showed lesser *Salmonella* recovery (4.8±0.2 log CFU/tomato). Thus, future experiments included immediate sampling upon inoculation and 1 h of inoculum drying.

Culture Media

On comparing results obtained from TSAR, BSAR and XLDR (Table 4-3; Figure 4-3). XLDR media showed almost 1 log lower *Salmonella* recovery from wet inoculum (4.6±0.3 log CFU/tomato) as well as 1 h dried inoculum (4.5±0.3 log CFU/tomato) as compared to TSAR (5.1±0.2 log CFU/tomato and 5.1±0.1 log CFU/tomato) and BSAR (4.9±0.5 log CFU/tomato and 4.9±0.4 log CFU/tomato). TSAR and BSAR were selected as culture media for the research.

Dirty Glove Protocol

There were no significant differences in the recovery of *Salmonella* (log CFU/tomato) from tomatoes touched with four different dirty gloves (D1, D2, D3 and D4) (Table 4-4) or from two different dirty gloves (D1 and D2) touched with inoculated tomatoes ($p \geq 0.05$) (Table 4-5). For further experiments gloves were made dirty by rubbing a tomato leaf with it for 20 s. After one hour of inoculum drying, the population of *Salmonella* recovered from tomatoes touched with inoculated dirty gloves was below the detection limit in all the four cases (D1, D2, D3 and D4). However, the population of *Salmonella* obtained from two dirty gloves (Log CFU/glove) touched with inoculated tomatoes did not differ significantly between wet and dry inoculum conditions ($p \geq 0.05$).

Experiments were also performed with two differently inoculated dirty gloves. One set of dirty gloves were made dirty by rubbing a tomato leaf for 20 s and then immediately inoculated (Table 4-6), while others were made dirty in similar pattern and dried at room temperature for half an hour before inoculating (Table 4-7). No significant

differences were observed in recovery of *Salmonella* from tomatoes touched with differently inoculated dirty gloves ($p \geq 0.05$). All the further experiments were performed without drying the dirty gloves for 30 min.

Salmonella Transfer from Clean Reusable Gloves to Tomatoes

When the inoculum was wet, *Salmonella* population obtained from inoculated clean reusable gloves was 5.9±0.1 log CFU/glove, while 5.2±0.1 log CFU/tomato was obtained from tomatoes touched with gloves (Table 4-8; Figure 4-4). Transfer coefficients obtained from wet inoculum were 0.25±0.1 (i.e., 25%). On drying the inoculum for 1 h, ca 5.4±0.2 log CFU/glove and 5.1±0.1 log CFU/tomato were obtained from inoculated clean reusable gloves and tomatoes, respectively. Transfer coefficients obtained after 1 h of inoculum drying were 0.48±0.5 (i.e., 48%). TCs obtained after 1 h were significantly different than TCs obtained from wet inoculum ($p \leq 0.05$). Distribution of TCs can be seen in tables x-y, demonstrating a near normal distribution for results received. Statistical analysis performed for TCs obtained from two different media (TSAR and BSAR) at different inoculum drying times, showed no significant differences ($p \geq 0.05$). Hence, all the rest of analysis was performed using TSAR values only. Drying the inoculum for 24 h, recovered <2.2±0.7 log CFU/glove and <1.4±0.2 log CFU/tomato of *Salmonella* population from inoculated clean reusable gloves and tomatoes, respectively. Among all samples, one inoculated glove sample and six tomato samples were below the detection limit after 24 h of inoculum drying.

Salmonella Transfer from Single-use Gloves to Tomatoes

Salmonella population transferred from wet (0 h) inoculated single-use gloves and tomatoes was 6.0±0.1 log CFU/glove and 5.4±0.3 log CFU/tomato and the transfer coefficients were 0.32±0.1 (i.e., 32%); (Table 4-9; Figure 4-27). After drying the

inoculum in biosafety cabinet for 1 h, 5.9±0.4 log CFU/glove and 5.4±0.1 log CFU/tomato of *Salmonella* population was obtained from inoculated single-use gloves and tomatoes, respectively. Transfer coefficients obtained after 1 h inoculum drying were 0.29±0.2 (i.e., 29%) and were significantly different than TCs obtained from wet inoculum ($p \leq 0.05$). After drying the inoculum on glove surface for 24 h, 2.5±0.5 log CFU/glove of *Salmonella* population was obtained from single-use gloves, while all the tomato samples touched with inoculated gloves were below the detection limit upon enumeration (Table 4-9; Figure 4-27).

There were no significant differences between TCs obtained from tomatoes touched with clean reusable and single-use gloves at different drying times (0 h and 1 h) ($p \geq 0.05$) (Table 4-8; Figure 4-4; Table 4-9; Figure 4-27). Drying the inoculum on glove surface for 24 h, showed similar results, no significant differences in number of *Salmonella* positive tomato samples were obtained between clean reusable and single-use gloves after 24 h of inoculum drying ($p \geq 0.05$). However, TCs obtained from 1 h dried inoculum were significantly higher than TCs obtained from wet inoculum ($p \leq 0.05$), which declined after 24 h drying, when statistical analysis was performed with both the gloves together.

Salmonella Transfer from Dirty Gloves to Tomatoes

When the inoculum was wet (0 h drying), *Salmonella* population obtained from inoculated dirty gloves and tomatoes were 5.5±0.2 log CFU/glove and 5.0±0.2 log CFU/tomato, respectively (Table 4-10; Figure 4-14). TCs obtained from wet inoculum were 0.41±0.3 (i.e., 41%) (Table 4-10). Drying the inoculum on dirty glove surface for 1 h reduced the recovery of *Salmonella* from inoculated gloves as well as from tomatoes touched with dirty gloves. Population obtained from dirty gloves after 1 h inoculum

drying was 3.4±0.2 log CFU/glove, while for tomato samples, enrichments were performed and five tomato samples touched with inoculated gloves came out positive (Table 4-10; Figure 4-14).

There were no significant differences in TCs calculated for dirty and clean reusable gloves, when the inoculum was wet ($p \geq 0.05$) (Table 4-8; Figure 4-4; Table 4-10; Figure 4-14). Drying the inoculum on clean and dirty glove surface for 1 h gave similar results. No significant differences in number of *Salmonella* positive tomato samples were obtained between inoculated clean and dirty reusable gloves ($p \geq 0.05$).

Salmonella Transfer from Tomatoes to Clean Reusable Gloves

Salmonella population obtained from inoculated tomatoes and transferred to clean reusable gloves on immediate (0 h) sampling was 6.0±0.1 log CFU/tomato and 5.4±0.3 log CFU/glove, respectively (Table 4-11; Figure 4-17). TCs obtained from wet inoculum were 0.18±0.0 (i.e., 18%) (Table 4-11). After drying the inoculum on tomato surface for 1 h, 5.6±0.4 log CFU/tomato and 5.4±0.1 log CFU/glove of *Salmonella* was obtained from tomatoes and clean reusable gloves. TCs after 1 h of inoculum drying were 0.38±0.2 (38%) and was not significantly different from TCs from wet inoculum ($p \geq 0.05$) (Table 4-11). After drying the inoculum on tomato surface for 24 h, 4.1±0.2 log CFU/tomato of *Salmonella* was obtained from inoculated tomatoes, while <2.8±0.1 log CFU/glove was found to transfer to glove surface (Table 4-11; Figure 4-17). Two glove samples out of total 9 samples were below the limit of detection upon enumeration after 24 h of drying (Table 4-11).

Salmonella Transfer from Tomato to Single-use Gloves

Salmonella population obtained from inoculated tomatoes at 0 h was 5.7±0.4 log CFU/tomato and that recovered from gloves was 5.4±0.2 log CFU/glove (Table 4-12;

Figure 4-22). TCs obtained from wet inoculum were 0.37±0.2 (i.e., 37%) (Table 4-12). After drying the inoculum for 1 h, *Salmonella* recovered from inoculated tomato and gloves were 5.4±0.4 log CFU/tomato and 5.2±0.5 log CFU/glove, respectively (Table 4-12; Figure 4-22). TCs obtained after 1 h drying (0.39±0.2) were not significantly different from those obtained from wet inoculum ($p \geq 0.05$). When the inoculum was dried for 24 h on tomato surface, 3.4±0.6 log CFU/tomato of *Salmonella* population was obtained from inoculated tomato surface, while all the glove samples were below the limit of detection upon enumeration (Table 4-12; Figure 4-22)

There were no significant differences in TCs obtained from clean reusable and single-use gloves touched with inoculated tomatoes at different drying times (0 h and 1 h) ($p \geq 0.05$) (Table 4-11; Figure 4-17; Table 4-12; Figure 4-22). However, after drying the inoculum on tomato surface for 24 h, significant differences in number of *Salmonella* positives were observed between clean reusable and single-use glove samples, with clean reusable gloves showing more positives ($p \leq 0.05$). Drying the inoculum on tomato surface for 1 h did not significantly affect the *Salmonella* transfer from inoculated tomatoes to clean gloves ($p \geq 0.05$).

Salmonella Transfer from Clean Reusable Gloves to Twenty-five Tomatoes

Transfer coefficients obtained from first tomato touched with inoculated clean reusable gloves wet inoculum were 0.61±0.6 and decreased continuously up to ninth tomato (0.01±0.0) (Table 4-13; Figure 4-27). The remaining tomatoes touched with inoculated clean reusable gloves wet inoculum yield <0.01 transfer. From tomato 10 to tomato 15, six or seven samples out of nine total samples were positive upon enrichment for *Salmonella* (Table 4-13; Figure 4-27). From the 16^{th} to 23^{rd} tomato, at least two and at most five samples were positive upon enrichment (Table 4-13; Figure

4-27) and for last two tomatoes (24th and 25th) touched with gloves, none of the sample came out positive. Transfer of *Salmonella* was observed up to 23 tomatoes touched subsequently with inoculated clean reusable gloves wet inoculum. Significant differences in TCs were observed between first tomato touched with gloves and third tomato up to ninth tomato, when the inoculum was wet ($p \leq 0.05$).

On drying the inoculum for 1 h, TCs obtained from first tomato were 0.86±1.7 and decreased for subsequently touched tomatoes (Table 4-14; Figure 4-27). TCs obtained from the fifth tomato touched with one hour dried inoculum were 0.12±0.3 (Table 4-14; Figure 4-27). From tomatoes six to 16 tomato, at least three and at most seven samples out of nine were positive upon enrichment (Table 4-14; Figure 4-27). Tomatoes 17 to 19 showed two positive samples (Table 4-14; Figure 4-27). The remaining tomatoes touched with inoculated clean reusable gloves (tomatoes 20-25), only the only positive enrichment was one of nine samples from the 22nd tomato (Table 4-14; Figure 4-27). There were no significant differences between TCs obtained from tomatoes touched with wet (0 h) and dry (1 h) *Salmonella* inoculum, comparing TCs obtained from first five tomatoes only ($p \geq 0.05$) (Table 4-13; Figure 4-27; Table 4-14).

Salmonella Transfer from Single-use Gloves to Twenty-five Tomatoes

When the inoculum was wet on single-use gloves, TCs obtained from first to tenth tomato, decreased from 0.33±0.4 to 0.01±0.0 (Table 4-15; Figure 4-28). From the 11th to 15th tomato, at least five and at most eight samples were positive upon enrichment. From the 16th to 23rd tomato, at least two and at most four samples were positive for *Salmonella*. The last two tomatoes touched with inoculated single-use gloves were positive with one out of nine samples (Table 4-15; Figure 4-28). Significant differences

in TCs were obtained between first tomato and seventh tomato up to ninth tomato touched with inoculated gloves ($p \leq 0.05$).

When the inoculum was dried for 1 h, TCs obtained from first tomato were 0.49±0.3 and decreased to 0.01±0.0 up to 8th tomato (Table 4-16; Figure 4-28). For the ninth tomato, eight out of nine, and for tenth tomato, six out of nine samples, were positive for *Salmonella* upon enrichment. For all the rest of the tomatoes (11 to 25), at least one sample and at most four samples were positive upon *Salmonella* enrichment (Table 4-16; Figure 4-28). There were no significant differences in TCs obtained from wet and 1 h dried inoculum, comparing TCs obtained from five tomatoes only ($p \geq 0.05$) (Table 4-15; Figure 4-28; Table 4-16). Since no significant differences were obtained between wet and dry inocula, comparisons between clean reusable and single-use gloves were made using wet inoculum TCs only.

Statistical analysis showed that TCs obtained from first two tomatoes (T1 and T2) touched with clean inoculated reusable gloves were significantly higher than all the tomatoes touched with inoculated single-use gloves, expect first tomato (Table 4-13; Figure 4-27; Table 4-15; Figure 4-28). This implies that *Salmonella* transfer from clean reusable gloves to first two tomatoes was higher, which after that was similar as single-use gloves. However, from single-use gloves, statistically insignificant TCs were obtained up to seventh tomato. Thus, single-use gloves transfer less *Salmonella* (ca. 33%) to more number of tomatoes touched, while clean reusable gloves transferred more *Salmonella* (61%) to fewer tomatoes. *Salmonella* positive tomato samples obtained after enrichment of the 10th to 25th tomato from both clean reusable and single-use gloves were not significantly different from each other ($p \geq 0.05$).

Salmonella Transfer from Dirty Reusable Gloves to Ten Tomatoes

From wet inoculum on dirty reusable gloves, TCs obtained decreased from 0.22±0.2 to <0.01±0.02 for first tomato up to ninth tomato (Table 4-17; Figure 4-29). For the tenth tomato touched with inoculated wet dirty reusable gloves, 8 samples out of 9 were positive for *Salmonella* upon enrichment. There were no significant differences in TCs obtained from all the ten tomatoes touched subsequently with inoculated dirty reusable gloves wet inoculum ($p \geq 0.05$) (Table 4-17; Figure 4-29). After drying the inoculum on glove surface for 1 h, at least two and at most five samples came out positive upon *Salmonella* enrichment for all the ten tomatoes touched subsequently (Table 4-18; Figure 4-29). Drying the inoculum on dirty reusable gloves for 1 h showed almost equal chances of *Salmonella* transfer to all the ten tomatoes touched subsequently.

Significant differences were observed between TCs obtained from tomatoes contacted subsequently with inoculated clean and dirty reusable gloves wet inoculum ($p \leq 0.05$). (Table 4-13; Figure 4-27; Table 4-18; Figure 4-29). Similarly to the case of single-use gloves, the transfer coefficients obtained from first two tomatoes touched with clean reusable gloves were significantly different from the TCs obtained from all ten tomatoes touched with dirty reusable glove ($p \leq 0.05$). When the inoculum was wet, clean reusable gloves transfer more *Salmonella* (0.61±0.6) to first two touched tomatoes, while the *Salmonella* transfer to next eight tomatoes touched with clean reusable gloves and all the ten tomatoes touched with dirty reusable gloves, is similar. Comparing clean and dirty reusable gloves after 1 h of inoculum drying showed significant differences in number of *Salmonella* positive tomato samples, with clean reusable gloves showing more transfer ($p \leq 0.05$).

Table 4-1: *Salmonella* transfer from inoculated single-use glove to tomato using different carrier mediums (n = 3-12).

	Salmonella population (log CFU/tomato)	
Carrier Media	0 min. (wet inoculum)	1 h (dry inoculum)
Water	5.1±0.2	4.9±0.1
0.1% Peptone water	5.1±0.2	5.0±0.0
Butterfield's Phosphate Buffer	5.0±0.1	5.0±0.1
5% Horse Serum	3.0±0.6	2.5±0.0
Tryptic Soy Broth	4.9±0.2	4.8±0.0

Table 4-2: *Salmonella* transfer from inoculated single-use glove to tomatoes at different drying times using 0.1% peptone as a carrier media (n=6-12).

Drying time (min.)	TSAR	BSAR
0	5.1±0.2	4.9±0.1
15	5.2±0.2	5.0±0.1
30	5.1±0.2	4.6±0.1
45	5.0±0.2	4.4±0.0
60	4.8±0.2	4.7±0.2
90	<2.0	<2.0
120	<2.0	<2.0
180	<2.0	<2.0

Table 4-3: *Salmonella* transfer from inoculated single-use glove to tomatoes using TSAR, BSAR and XLDR media with water as a carrier medium (n=3).

Drying time (min.)	TSAR	BSAR	XLDR
0	5.1±0.2	4.9±0.5	4.6±0.3
15	4.7±0.0	4.8±0.0	4.5±0.0
30	4.9±0.0	4.7±0.0	4.5±0.0
45	4.6±0.0	4.3±0.0	3.7±0.0
60	5.1±0.1	4.9±0.4	4.5±0.3

Table 4-4: *Salmonella* transfer from inoculated dirty gloves to tomatoes at 0 h, 1 h and 24 h of inoculum drying, following a 5 s touch (n=3).

	0 h		1 h		24 h	
	TSAR	BSAR	TSAR	BSAR	TSAR	BSAR
Dirty1[a]	5.2±0.1	5.2±0.2	<1.3	<1.3	<1.3	≤1.3
Dirty2[b]	5.2±0.1	4.5±0.6	<1.3	<1.3	<1.3	<1.3
Dirty3[c]	5.0±0.1	4.9±0.1	<1.3	<1.3	<1.3	<1.3
Dirty4[d]	5.3±0.3	5.2±0.1	<1.3	<1.3	<1.3	<1.3

[a]0.1g soil, a leaf and a drop of tomato gut was rubbed on glove for 20 s

[b]0.1g soil, a leaf was rubbed on glove for 20 s

[c]0.1g soil, a leaf and a drop of tomato gut was rubbed on glove for 20 s and dried for half an hour

[d]0.1g soil, a leaf rubbed on gloves for 20 s and dried for half an hour

Table 4-5: *Salmonella* transfer from inoculated tomatoes to dirty gloves at 0 h, 1h and 24 h of inoculum drying, following a 5 s touch (n=3).

	0 h		1 h		24 h	
	TSAR	BSAR	TSAR	BSAR	TSAR	BSAR
Dirty1[a]	5.3±0.2	5.1±0.1	5.2±0.0	5.1±0.1	<1.9	<1.3
Dirty2[b]	5.3±0.2	5.1±0.1	5.2±0.1	5.0±0.2	<2.3	<1.3

[a]0.1g soil, a leaf and a drop of tomato gut was rubbed on glove for 20 s
[b]0.1g soil, a leaf was rubbed on glove for 20 s

Table 4-6: *Salmonella* transfer from inoculated dirty gloves to tomatoes at 0 h and 1 h inoculum drying, following 5 s touch (n=3).

	Dirty glove[a]		Tomato	
Drying time (h)	TSAR	BSAR	TSAR	BSAR
0	5.4±0.9	5.6±0.9	5.0±0.2	5.0±0.1
1	3.3±0.1	1.5±0.2	<1.3	<1.3

[a]gloves were made dirty by rubbing tomato leaf with it for 20 s and 0.1% tween-20 was added to buffer for better recovery

Table 4-7: *Salmonella* transfer from inoculated dirty gloves to tomatoes at 0 h and 1 h inoculum drying, following 5 s touch (n=3).

	Dirty glove[a]		Tomato	
Drying time (h)	TSAR	BSAR	TSAR	BSAR
0	5.9±0.1	5.7±0.3	5.0±0.2	5.0±0.1
1	4.1±0.3	3.1±0.6	<1.3	<1.3

[a]gloves were made dirty by rubbing tomato leaf with it for 20 s and were dried for half an hour before inoculating, 0.1% tween-20 was added to buffer

Table 4-8: *Salmonella* transfer from inoculated clean reusable gloves to tomatoes after 0 h, 1 h and 24 h of inoculum drying, following a 5 s touch (n=9-18).

	Clean reusable glove		Tomato		TC	
Time (h)	TSAR	BSAR	TSAR	BSAR	Average	Range
0	5.9±0.1	5.8±0.1	5.2±0.1	5.2±0.1	0.25±0.1	0.14-0.36
1	5.4±0.2	5.1±0.4	5.1±0.1	4.9±0.2	0.48±0.5	0.22-0.86
24	<2.2±0.7[a]	<1.3[b]	<1.4±0.2[c]	<1.4±0.2[c]	3/9	

[a]n=9, one replication was below the limit of detection
[b]n=9, all nine replications were below the limit of detection
[c]n=9, six replications were below the limit of detection

Table 4-9: *Salmonella* transfer from inoculated single-use gloves to tomatoes after 0 h, 1 h and 24 h of inoculum drying, following a 5 s touch (n=9-18).

	Single-use glove		Tomato		TC	
Time (h)	TSAR	BSAR	TSAR	BSAR	Average	Range
0	6.0±0.1	5.8±0.1	5.4±0.3	5.4±0.2	0.32±0.1	0.11-0.47
1	5.9±0.4	5.1±0.4	5.4±0.1	5.3±0.2	0.29±0.2	0.05-0.66
24	2.5±0.5	2.2±0.7	<1.3[a]	<1.3[b]	0/9	

[a]n=9, all the replications were below the limit of detection

Table 4-10: *Salmonella* transfer from inoculated dirty reusable gloves to tomatoes after 0 h and 1 h of inoculum drying, following a 5 s touch (n=9).

	Dirty reusable glove		Tomato		TC	
Time (h)	TSAR	BSAR	TSAR	BSAR	Average	Range
0	5.5±0.2	5.1±0.2	5.0±0.2	5.0±0.2	0.41±0.3	0.14-0.86
1	3.4±0.2	2.1±0.5	≤1.3[a]	≤1.3[b]	5/9	

[a]four replications were negative upon enrichment
[b]all nine replications were below the limit of detection

Table 4-11: *Salmonella* transfer from inoculated tomatoes to clean reusable gloves after 0 h, 1 h and 24 h of inoculum drying, following a 5 s touch (n=9-18).

	Tomato		Clean reusable glove		TC	
Time (h)	TSAR	BSAR	TSAR	BSAR	Average	Range
0	6.0±0.1	6.0±0.2	5.4±0.3	5.4±0.2	0.18±0.0	0.12-0.21
1	5.6±0.4	5.6±0.4	5.4±0.1	5.3±0.2	0.38±0.2	0.11-0.76
24	4.1±0.2	3.8±0.3	<2.8±1.0[a]	<2.7±0.9[b]	7/9	

[a]n=9, two replications were below the limit of detection
[b]n=9, one replication was below the limit of detection

Table 4-12: *Salmonella* transfer from inoculated tomatoes to single-use gloves after 0 h, 1 h and 24 h of inoculum drying, following a 5 s touch (n=9-18).

	Tomato		Clean reusable glove		TC	
Time (h)	TSAR	BSAR	TSAR	BSAR	Average	Range
0	5.7±0.4	5.7±0.4	5.4±0.2	5.2±0.5	0.37±0.2	0.22-0.87
1	5.4±0.4	5.3±0.5	5.2±0.5	5.1±0.5	0.39±0.2	0.11-0.62
24	3.4±0.6	3.1±0.7	<1.3[a]	<1.3[a]	0/9	

[a]n=9, all the replications were below the detection limit

Table 4-13: *Salmonella* transfer from inoculated clean reusable glove to twenty-five tomatoes touched subsequently with wet inoculum (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	5.7±0.4	5.9±0.1		
T-1	5.3±0.2	5.2±0.4	0.61±0.6	0.11-1.8
T-2	4.6±1.2	4.4±1.3	0.46±0.6	0.00-1.8
T-3	4.3±1.2	4.1±1.4	0.27±0.3	0.00-5.1
T-4	4.0±1.2	3.8±1.5	0.26±0.6	0.00-1.8
T-5	3.5±1.2	3.2±1.3	0.06±0.1	0.00-0.34
T-6	3.1±1.4	3.1±1.4	0.03±0.1	0.00-0.06
T-7	3.1±1.1	3.0±1.1	0.02±0.0	0.00-0.02
T-8	2.5±1.5	2.7±1.3	0.02±0.0	0.00-0.08
T-9	2.6±1.2	<2.9±1.1[c]	0.01±0.0	0.00-0.08
T-10	<2.1±0.9[b]	6/9	6/9	
T-11	<1.8±1.1[a]	7/9	7/9	
T-12	<1.6±0.5[a]	7/9	7/9	
T-13	<1.9±0.9[a]	7/9	7/9	
T-14	<1.8±0.7[a]	7/9	7/9	
T-15	<2.1±0.8[a]	7/9	7/9	
T-16	5/9	5/9	5/9	
T-17	4/9	4/9	4/9	
T-18	3/9	3/9	3/9	
T-19	5/9	5/9	5/9	
T-20	4/9	4/9	4/9	
T-21	2/9	2/9	2/9	
T-22	2/9	2/9	2/9	
T-23	2/9	2/9	2/9	
T-24	0/9	0/9	0/9	
T-25	0/9	0/9	0/9	

[a]two replications were negative upon enrichment
[b]three replications were negative upon enrichment
[c]three replications were below the limit of detection

Table 4-14: *Salmonella* transfer from inoculated clean reusable glove to twenty-five tomatoes touched subsequently with an hour dried inoculums (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	5.3±0.3	5.2±0.4		
T-1	4.6±0.7	4.6±0.7	0.86±1.7	0.02-4.9
T-2	3.5±1.7	3.2±1.7	0.87±2.2	0.00-2.2
T-3	3.6±1.2	3.3±1.4	0.16±0.3	0.00-0.85
T-4	3.3±1.6	<3.5±1.4[c]	0.35±0.7	0.00-1.9
T-5	2.8±1.5	<3.1±1.5[c]	0.12±0.3	0.00-0.78
T-6	<2.3±1.6[a]	<2.5±1.3[c]	7/9	
T-7	<2.3±1.5[a]	7/9	7/9	
T-8	<2.1±1.3[b]	6/9	6/9	
T-9	4/9	4/9	4/9	
T-10	5/9	5/9	5/9	
T-11	4/9	4/9	4/9	
T-12	4/9	4/9	4/9	
T-13	4/9	4/9	4/9	
T-14	3/9	3/9	3/9	
T-15	4/9	4/9	4/9	
T-16	3/9	3/9	3/9	
T-17	2/9	2/9	2/9	
T-18	2/9	2/9	2/9	
T-19	2/9	2/9	2/9	
T-20	0/9	0/9	0/9	
T-21	0/9	0/9	0/9	
T-22	1/9	1/9	1/9	
T-23	0/9	0/9	0/9	
T-24	0/9	0/9	0/9	
T-25	0/9	0/9	0/9	

[a] two replication were negative upon enrichment
[b] three replications were negative upon enrichment
[c] two or three replications were below the limit of detection

Table 4-15: *Salmonella* transfer from inoculated single-use glove to twenty-five tomatoes touched subsequently with wet inoculums (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	5.8±0.5	5.8±0.2		
T-1	5.1±0.1	5.0±0.1	0.33±0.4	0.04-1.2
T-2	4.8±0.4	4.6±0.7	0.18±0.2	0.01-0.17
T-3	4.6±0.4	4.4±0.9	0.13±0.1	0.01-0.35
T-4	4.3±0.5	4.0±1.2	0.05±0.0	0.00-0.09
T-5	4.0±1.1	<4.2±0.6[d]	0.05±0.1	0.00-0.09
T-6	3.8±0.8	3.2±1.2	0.02±0.0	0.00-0.03
T-7	3.5±1.0	<3.4±1.0[d]	0.01±0.0	0.00-0.02
T-8	3.0±1.4	<3.5±0.6[d]	0.01±0.0	0.00-0.04
T-9	3.0±1.0	<2.9±0.9[d]	0.01±0.0	0.00-0.03
T-10	2.5±1.0	<2.7±1.1[d]	0.01±0.0	0.00-0.06
T-11	5/9	5/9	5/9	
T-12	<1.4±0.8[a]	8/9	8/9	
T-13	<1.8±0.8[b]	7/9	7/9	
T-14	<1.7±0.8[b]	7/9	7/9	
T-15	<1.7±0.8[c]	6/9	6/9	
T-16	4/9	4/9	4/9	
T-17	2/9	2/9	2/9	
T-18	2/9	2/9	2/9	
T-19	4/9	4/9	4/9	
T-20	2/9	2/9	2/9	
T-21	2/9	2/9	2/9	
T-22	3/9	3/9	3/9	
T-23	3/9	3/9	3/9	
T-24	1/9	1/9	1/9	
T-25	1/9	1/9	1/9	

[a]one replication was negative upon enrichment
[b]two replications were negative upon enrichment
[c]three replications were negative upon enrichment
[d]one or two replications were below the limit of detection

Table 4-16: *Salmonella* transfer from inoculated single-use glove to twenty-five tomatoes touched subsequently with an hour dry inoculums (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	5.4±0.1	5.2±0.2		
T-1	5.0±0.3	4.7±0.5	0.49±0.3	0.1-1.1
T-2	4.0±1.3	3.8±1.2	0.30±0.4	0.03-0.98
T-3	4.0±1.0	<4.1±0.7[a]	0.26±0.5	0.00-1.5
T-4	3.5±1.3	3.2±1.4	0.37±0.6	0.00-1.4
T-5	2.9±1.0	<3.0±1.1[a]	0.05±0.1	0.00-0.31
T-6	2.5±1.1	2.5±1.0	0.01±0.0	0.00-0.05
T-7	2.2±1.1	<1.9±0.7[b]	0.01±0.0	0.00-0.01
T-8	2.2±1.1	<1.9±0.7[b]	0.01±0.0	0.00-0.05
T-9	8/9	8/9	8/9	
T-10	6/9	6/9	6/9	
T-11	3/9	3/9	3/9	
T-12	4/9	4/9	4/9	
T-13	3/9	3/9	3/9	
T-14	3/9	3/9	3/9	
T-15	4/9	4/9	4/9	
T-16	1/9	1/9	1/9	
T-17	3/9	3/9	3/9	
T-18	2/9	2/9	2/9	
T-19	2/9	2/9	2/9	
T-20	1/9	1/9	1/9	
T-21	1/9	1/9	1/9	
T-22	1/9	1/9	1/9	
T-23	1/9	1/9	1/9	
T-24	1/9	1/9	1/9	
T-25	1/9	1/9	1/9	

[a]one replication were below the limit of detection
[b]three replications were below the limit of detection

Table 4-17: *Salmonella* transfer from inoculated dirty reusable glove to ten tomatoes touched subsequently with wet inoculum (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	5.7±0.2	5.3±0.3		
T-1	4.9±0.5	4.8±0.5	0.22±0.2	0.01-0.69
T-2	4.8±0.4	4.9±0.4	0.16±0.1	0.10-0.39
T-3	4.5±0.5	3.8±1.0	0.09±0.1	0.02-0.29
T-4	4.5±0.2	4.1±0.1	0.07±0.0	0.03-0.15
T-5	4.0±0.5	3.4±0.7	0.03±0.0	0.00-0.05
T-6	4.1±0.3	<3.3±0.9[b]	0.03±0.0	0.00-0.06
T-7	3.4±1.1	<2.8±2.0[b]	0.02±0.0	0.00-0.10
T-8	3.7±0.3	3.4±0.7	0.02±0.0	0.00-0.05
T-9	<3.1±0.9[a]	<2.8±1.1[b]	<0.01±0.02[b]	0.00-0.07
T-10	<2.7±1.1[a]	<2.4±1.0[b]	8/9	

[a] one replication was negative upon enrichment
[b] one replication was below the limit of detection

Table 4-18: *Salmonella* transfer from inoculated dirty reusable gloves to ten tomatoes touched subsequently with an hour dried inoculum (n=9).

Tomato no.	Tomato TSAR	BSAR	TC Average	Range
Glove	4.3±0.3	<1.6±0.5[a]		
T-1	4/9	4/9	4/9	
T-2	3/9	3/9	3/9	
T-3	3/9	3/9	3/9	
T-4	2/9	2/9	2/9	
T-5	3/9	3/9	3/9	
T-6	3/9	3/9	3/9	
T-7	5/9	5/9	5/9	
T-8	2/9	2/9	2/9	
T-9	3/9	3/9	3/9	
T-10	3/9	3/9	3/9	

[a] four replications were below the limit of detection

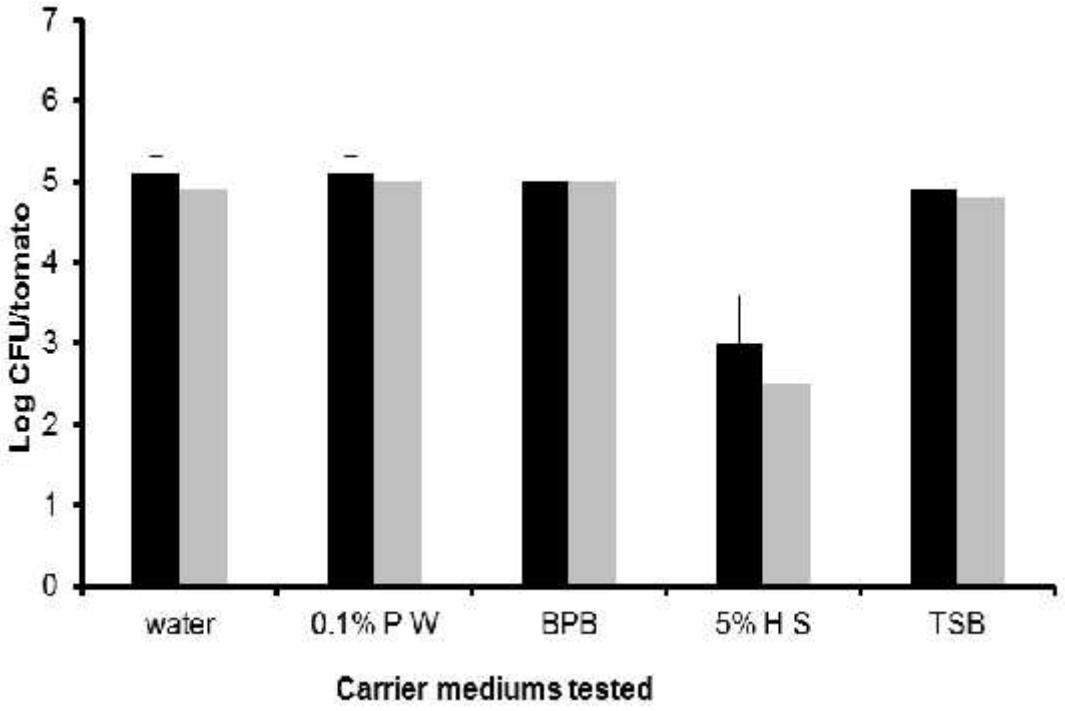

Figure 4-1: Comparison of different carrier mediums for transfer from inoculated glove (single-use) to tomatoes with it after 0 h (black bar) and 1 h (light-grey bar) drying time (n=3-12).

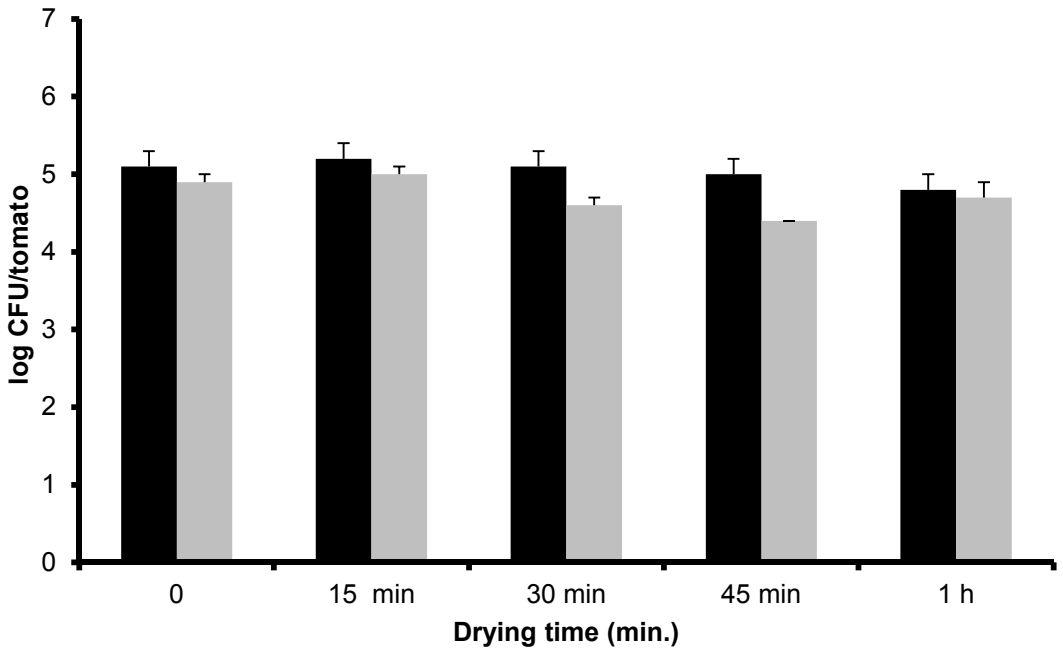

Figure 4-2: Comparison of transfer after different drying times of inocula using 0.1% peptone water as carrier medium by performing enumerations on TSAR (black bar) and BSAR (light-grey bar) media (n=6-12).

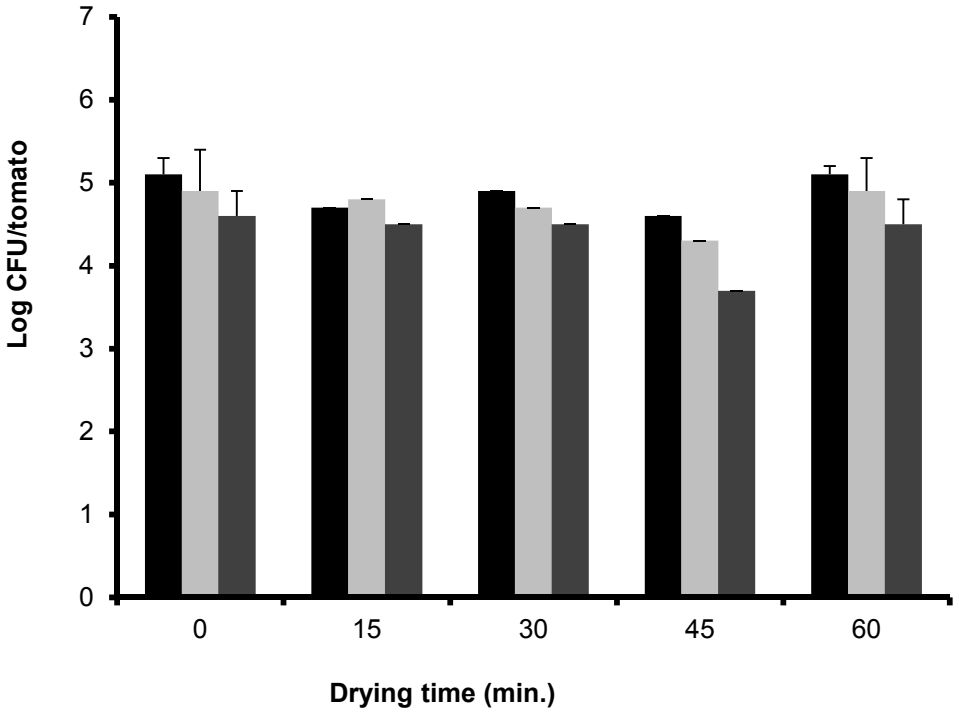

Figure 4-3: Comparison of different culture media; TSAR (black bar), BSAR (light-grey bar) and XLDR (dark grey bar) for recovery of Salmonella from tomatoes. Water was used as carrier medium for inocula (n=3).

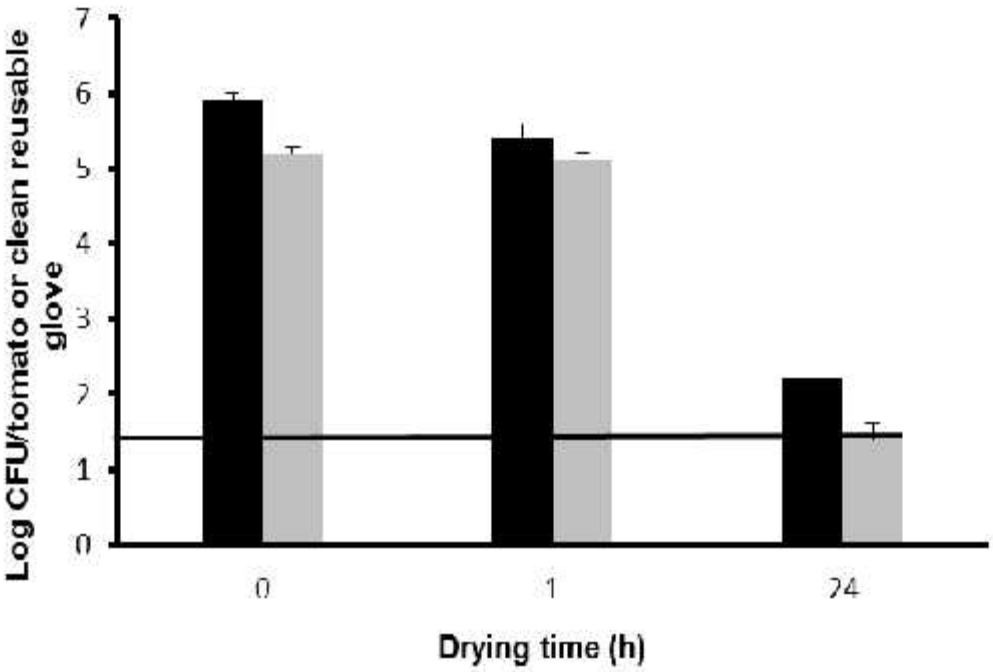

Figure 4-4: Population of *Salmonella* inoculated onto clean reusable glove (black color bar) and transferred to a tomato (light-grey color bar) following a 5 s touch (n=9-18). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

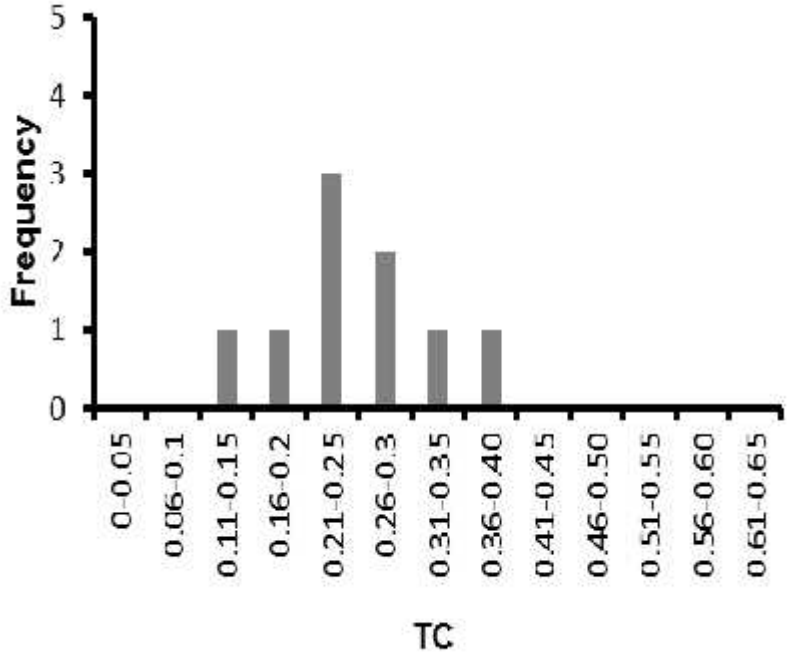

Figure 4-5. Distribution of *Salmonella* transfer coefficients (TCs) from reusable gloves to tomatoes with 0 h dried inoculum using TSAR media (n=9).

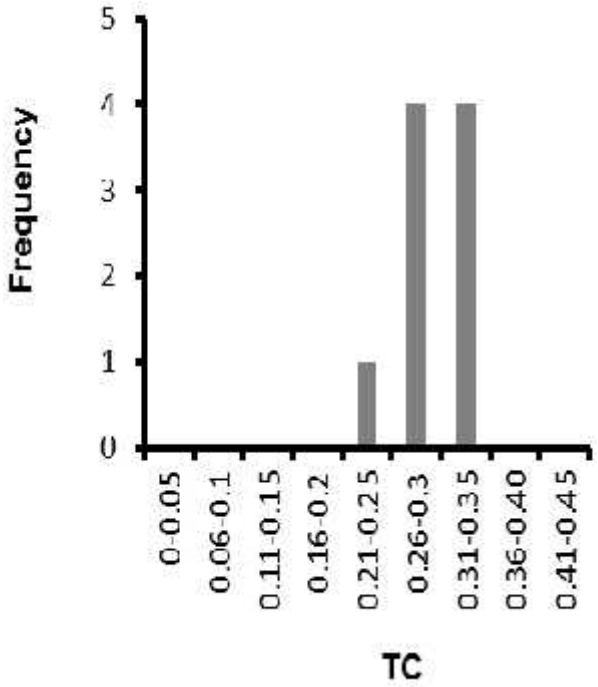

Figure 4-6. Distribution of *Salmonella* transfer coefficients (TCs) from reusable gloves to tomatoes with 0 h dried inoculum using BSAR media (n=9).

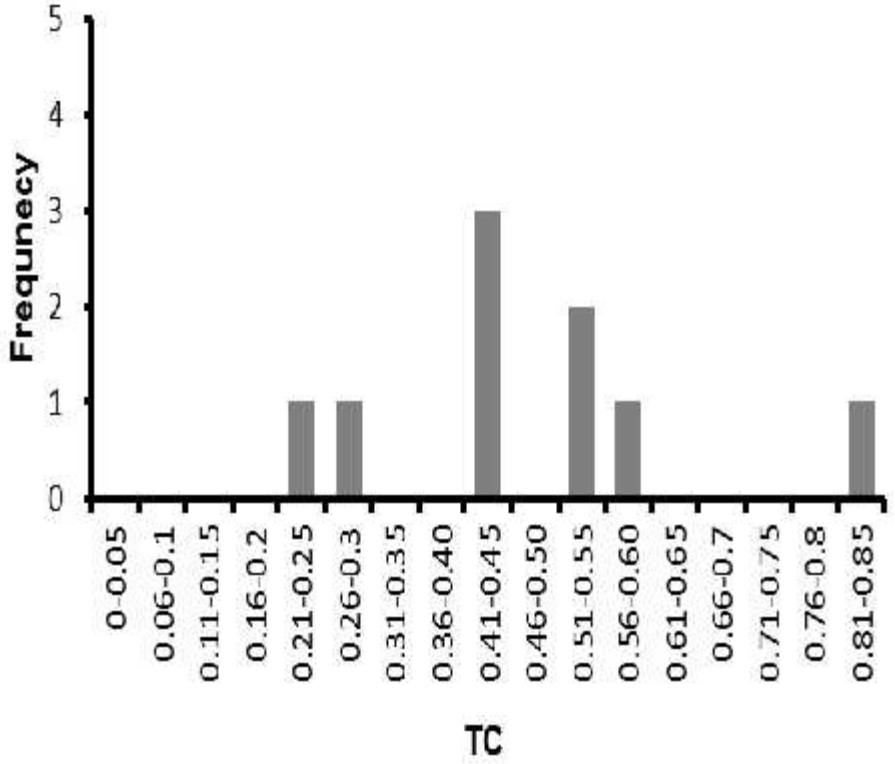

Figure 4-7. Distribution of *Salmonella* transfer coefficients (TCs) from reusable gloves to tomatoes with 1 h dried inoculum using TSAR media (n=9).

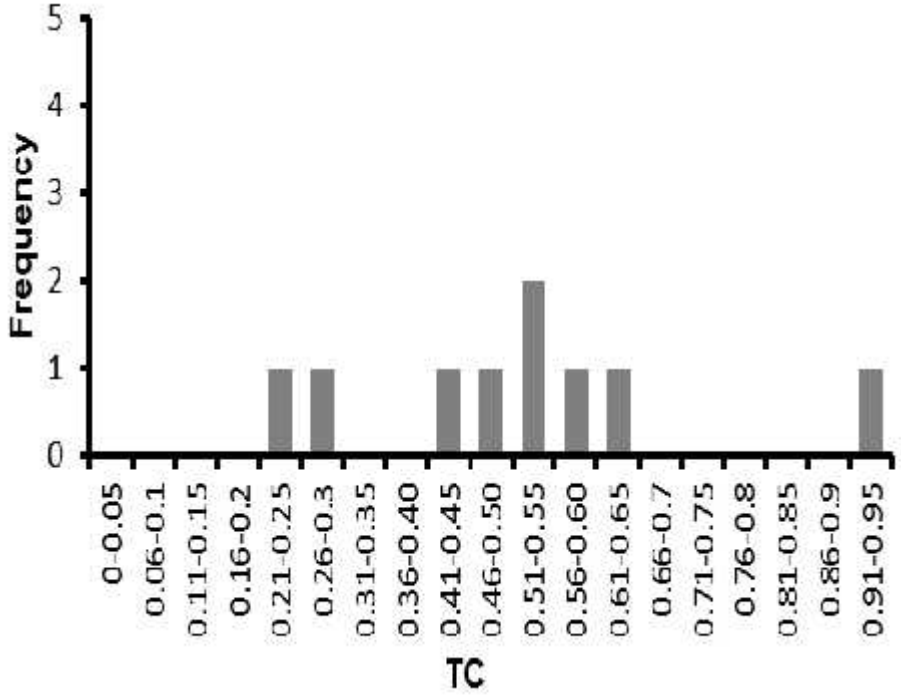

Figure 4-8. Distribution of *Salmonella* transfer coefficients (TCs) from reusable gloves to tomatoes with 1 h dried inoculum using BSAR media (n=9).

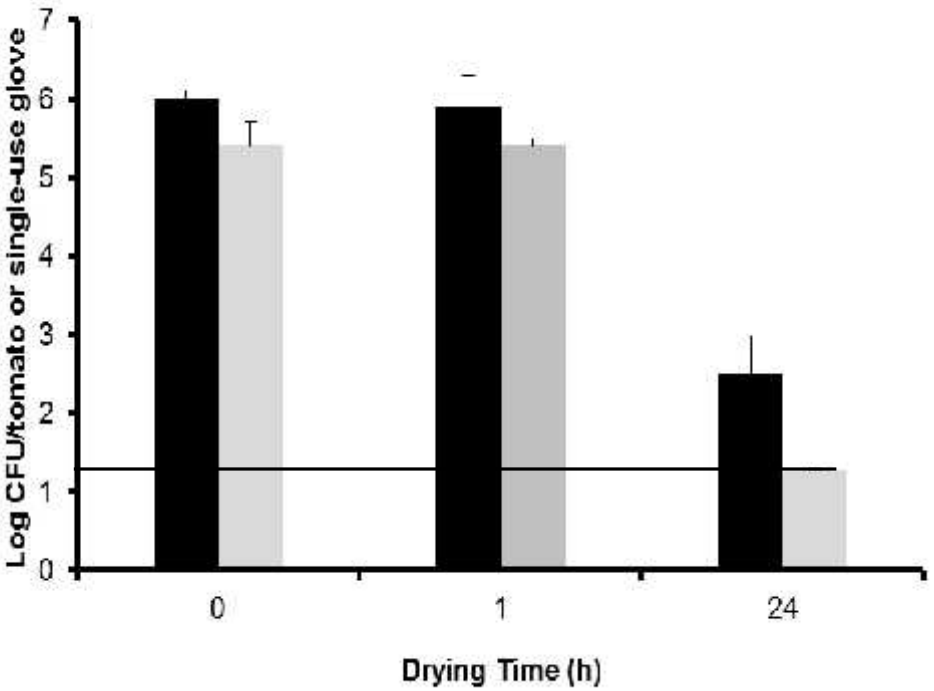

Figure 4-9: Population of *Salmonella* inoculated onto clean single-use glove (black color bar) and transferred to a tomato (light-grey color bar) following a 5 s touch (n=9-18). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

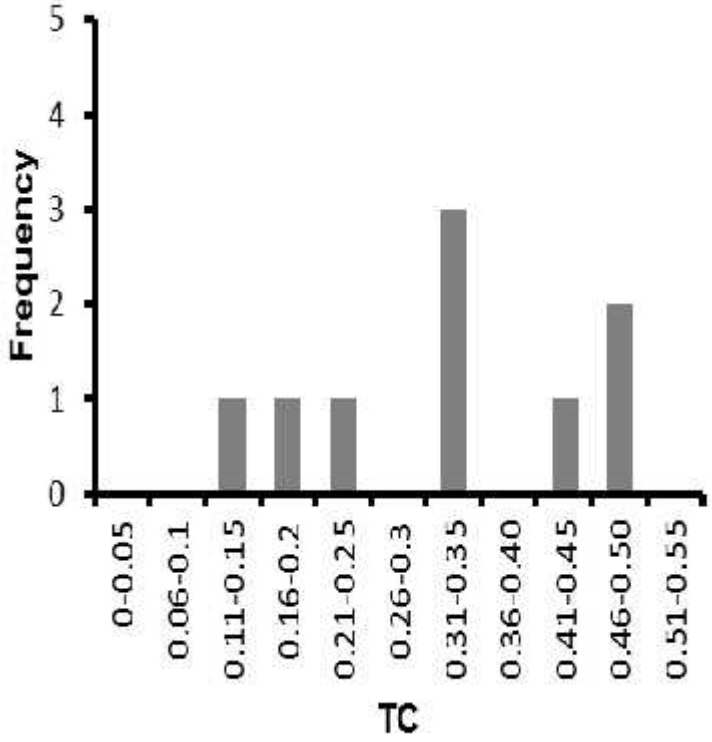

Figure 4-10. Distribution of *Salmonella* transfer coefficients (TCs) from single-use gloves to tomatoes with 0 h dried inoculum using TSAR media (n=9).

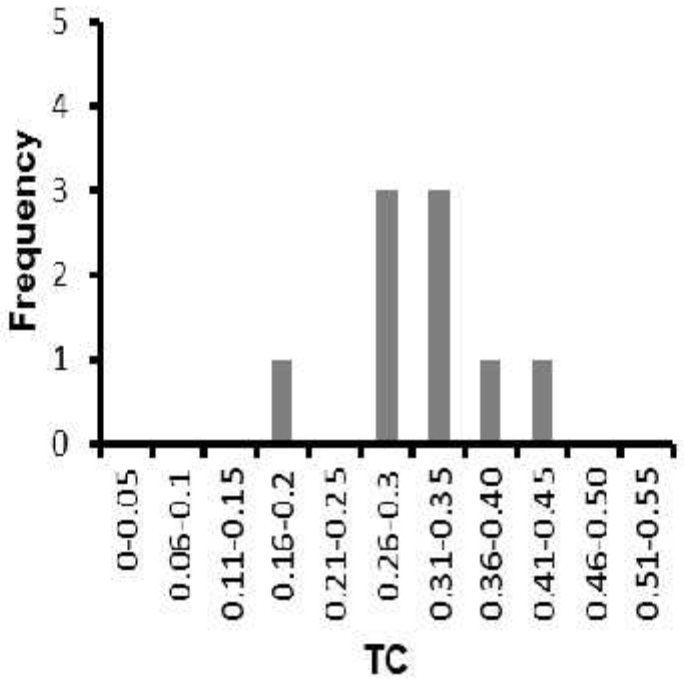

Figure 4-11. Distribution of *Salmonella* transfer coefficients (TCs) from single-use gloves to tomatoes with 0 h dried inoculum using BSAR media (n=9).

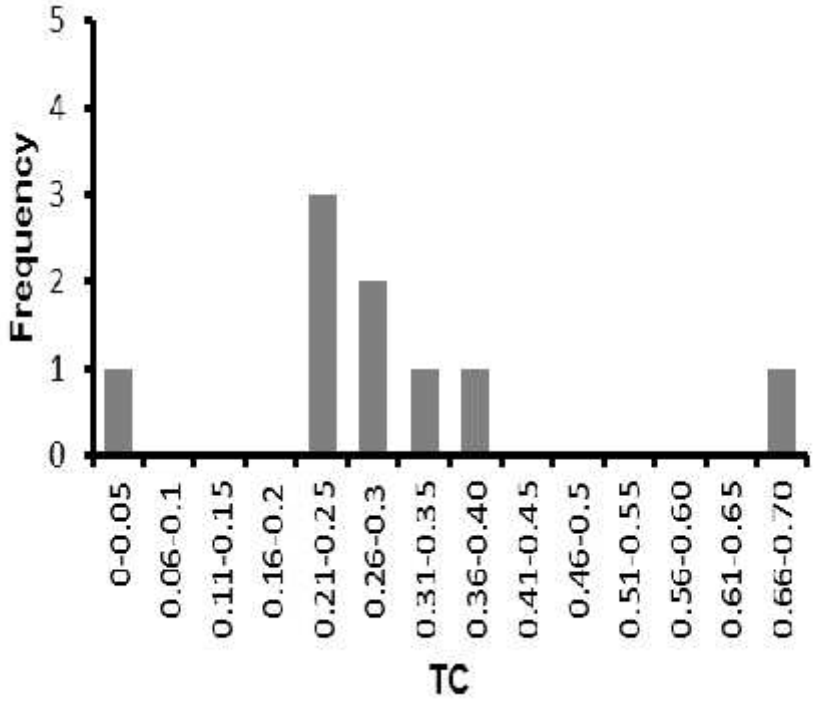

Figure 4-12. Distribution of *Salmonella* transfer coefficients (TCs) from single-use gloves to tomatoes with 1 h dried inoculum using TSAR media (n=9).

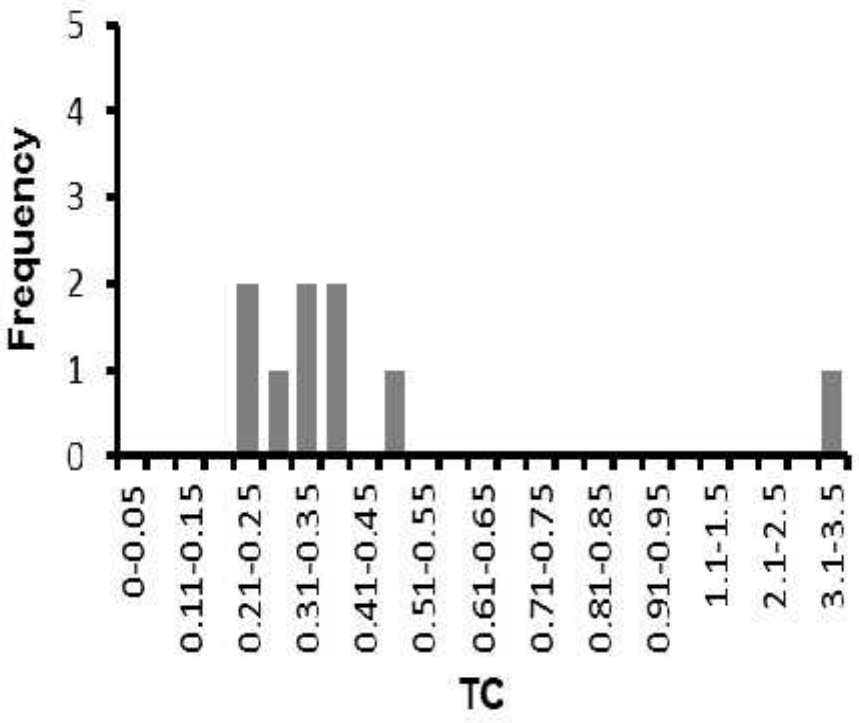

Figure 4-13. Distribution of *Salmonella* transfer coefficients (TCs) from single-use gloves to tomatoes with 1 h dried inoculum using BSAR media (n=9).

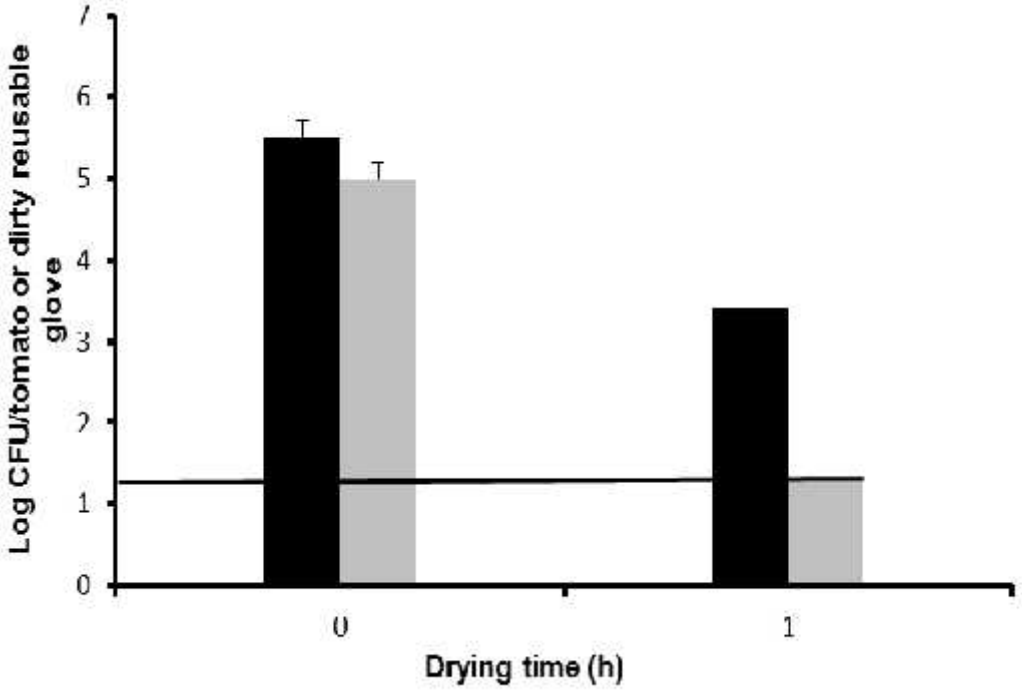

Figure 4-14: Population of *Salmonella* inoculated onto dirty gloves (black color bar) and transferred to tomatoes (light-grey color bar) following a 5 s touch (n=9). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

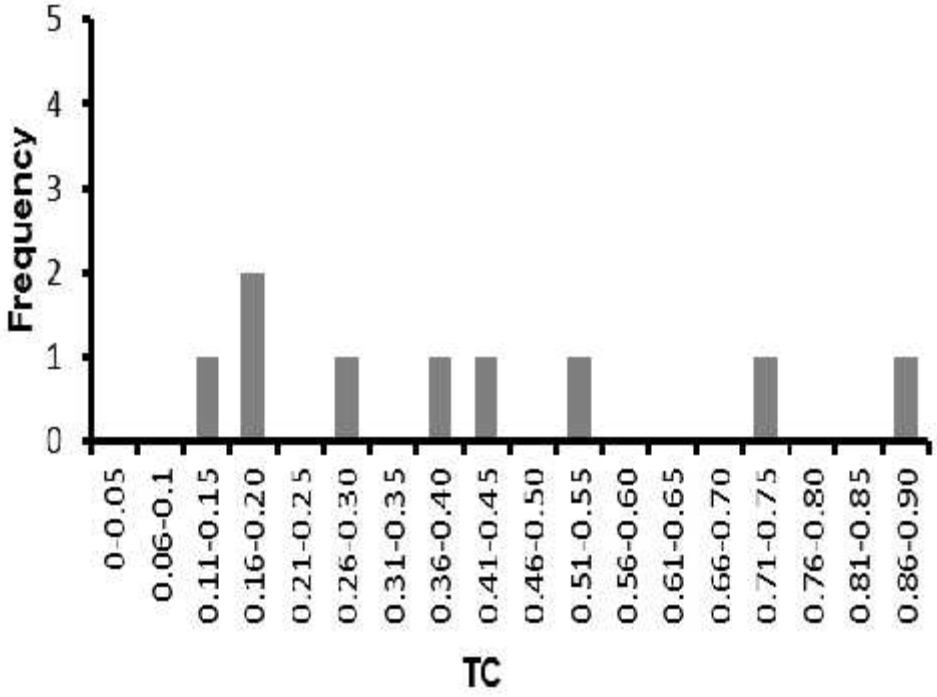

Figure 4-15. Distribution of *Salmonella* transfer coefficients (TCs) from dirty reusbale gloves to tomatoes with 0 h dried inoculum using TSAR media (n=9).

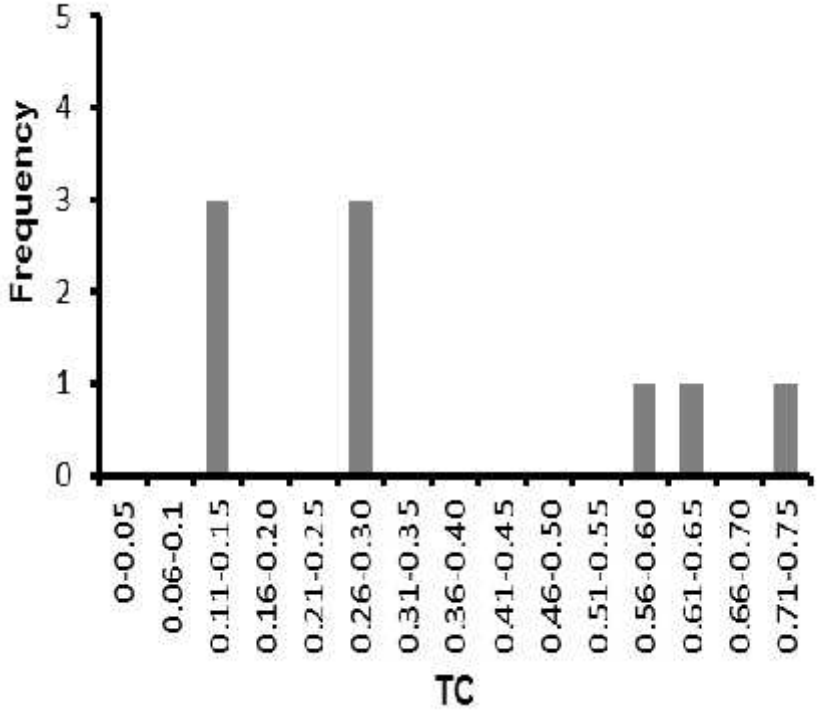

Figure 4-16. Distribution of *Salmonella* transfer coefficients (TCs) from dirty reusbale gloves to tomatoes with 0 h dried inoculum using BSAR media (n=9).

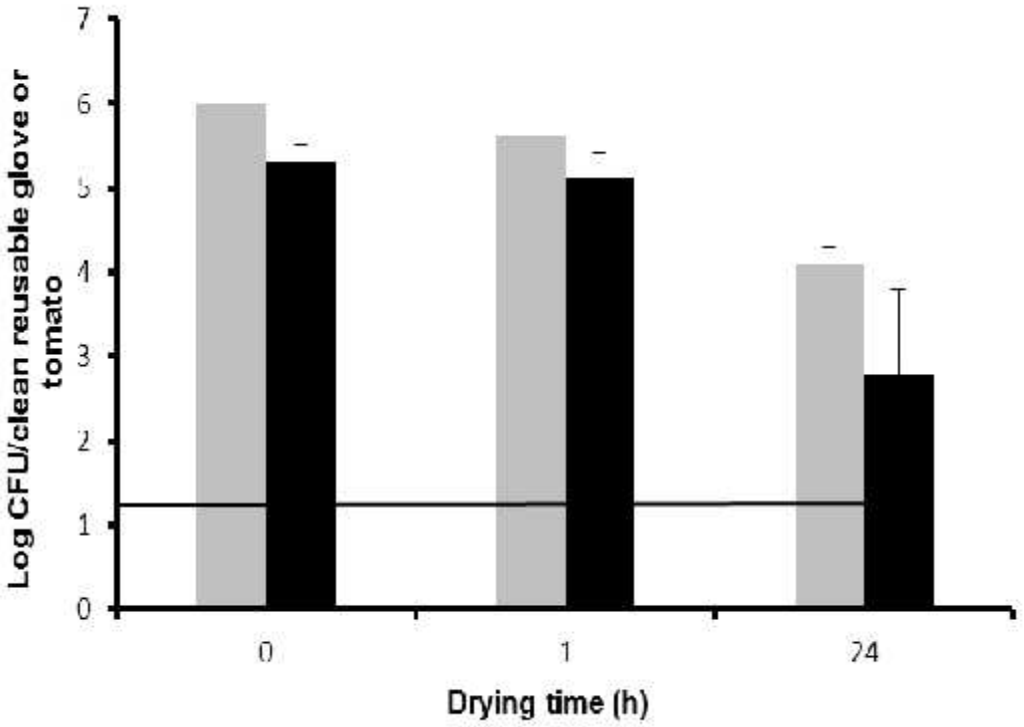

Figure 4-17: Population of *Salmonella* inoculated onto tomatoes (light-grey color bar) and transferred to a clean reusable glove (black color bar) following a 5 s touch (n=9-18). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

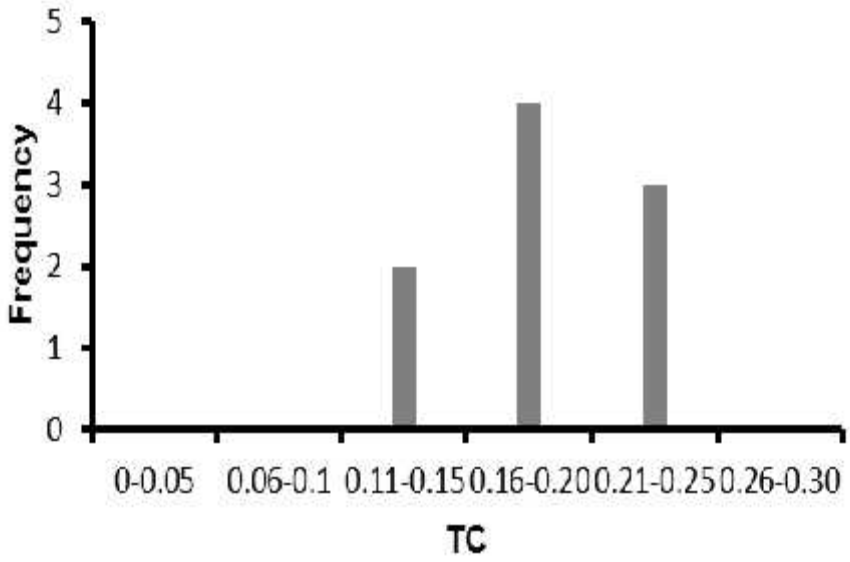

Figure 4-18. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to reusbale gloves with 0 h dried inoculum using TSAR media (n=9).

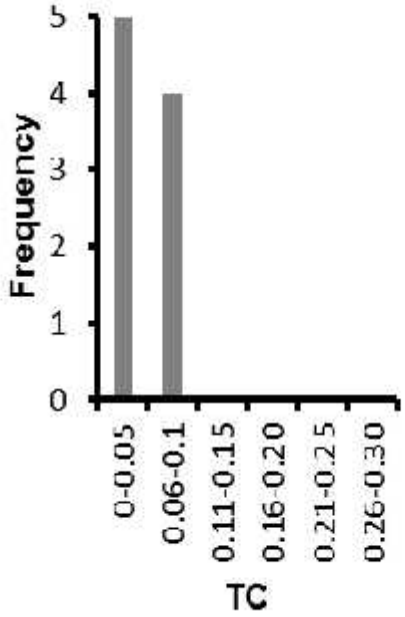

Figure 4-19. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to reusbale gloves with 0 h dried inoculum using BSAR media (n=9).

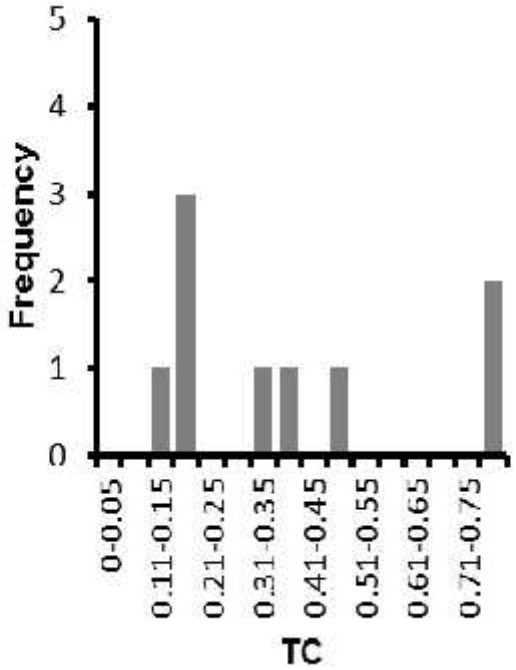

Figure 4-20. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to reusbale gloves with 1 h dried inoculum using TSAR media (n=9).

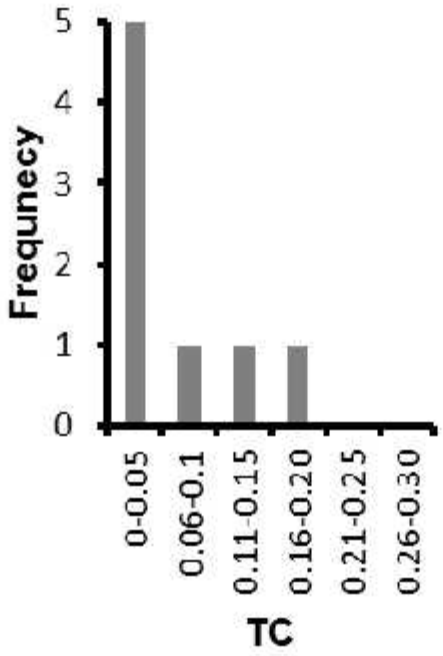

Figure 4-21. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to reusbale gloves with 1 h dried inoculum using BSAR media (n=9).

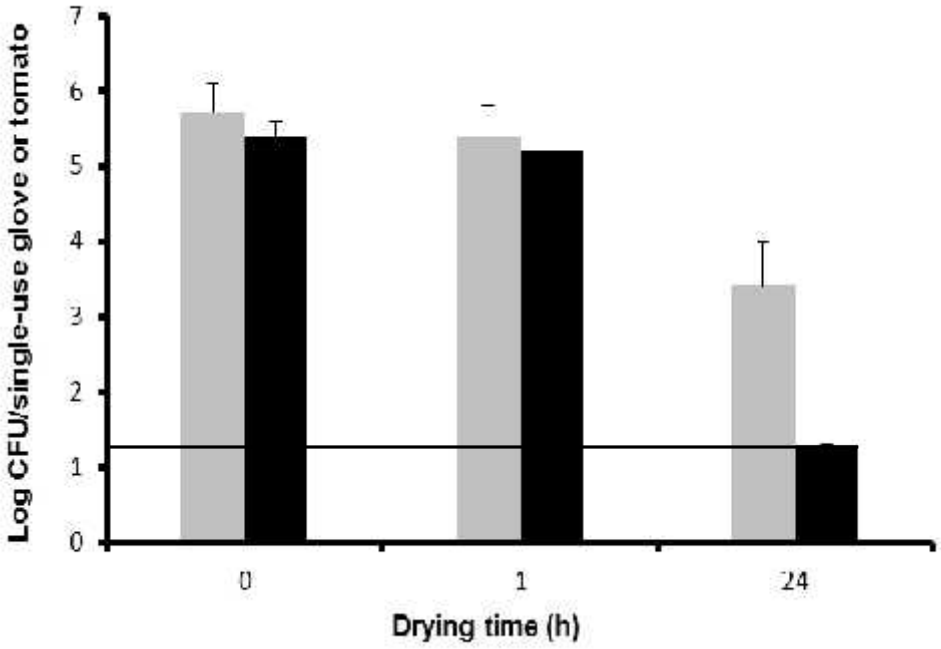

Figure 4-22: Population of *Salmonella* inoculated onto tomatoes (light-grey color bar) and transferred to a clean single-use glove (black color bar) following a 5 s touch (n=9-18). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

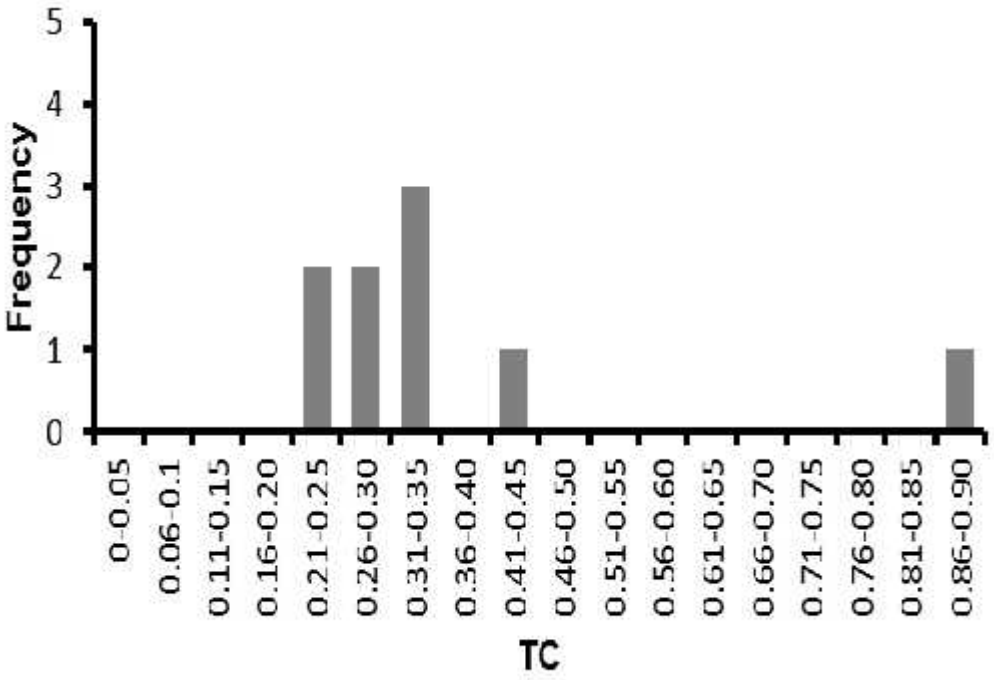

Figure 4-23. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to single-use gloves with 0 h dried inoculum using TSAR media (n=9).

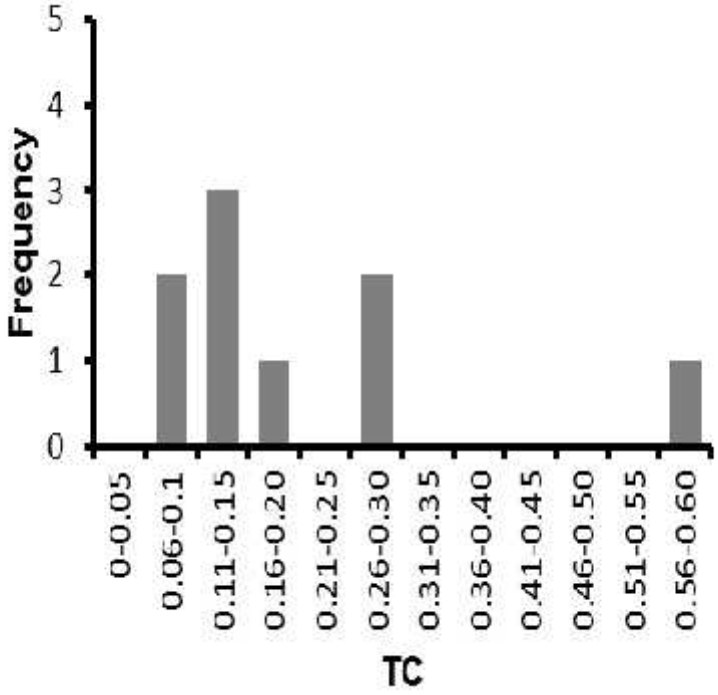

Figure 4-24. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to single-use gloves with 0 h dried inoculum using BSAR media (n=9).

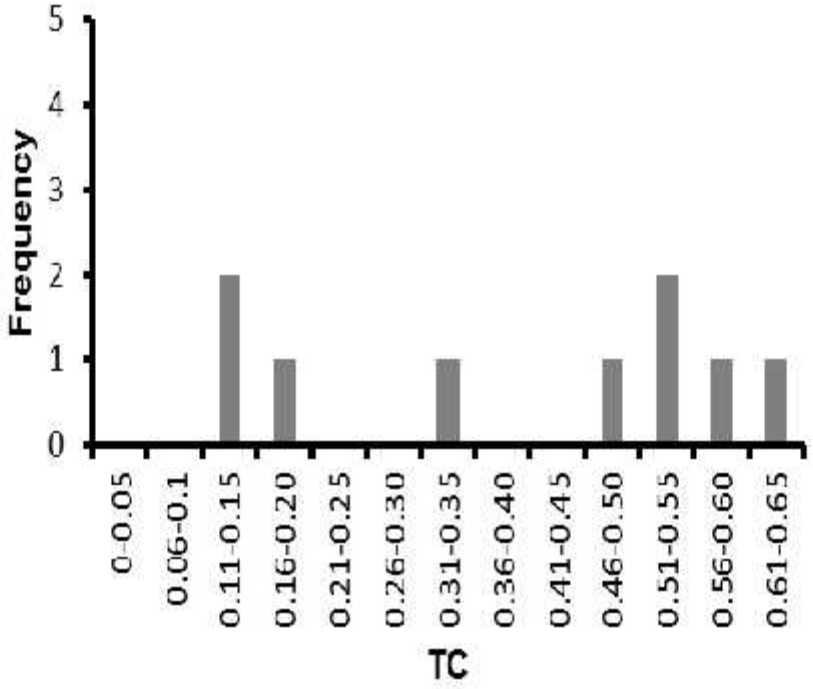

Figure 4-25. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to single-use gloves with 1 h dried inoculum using TSAR media (n=9).

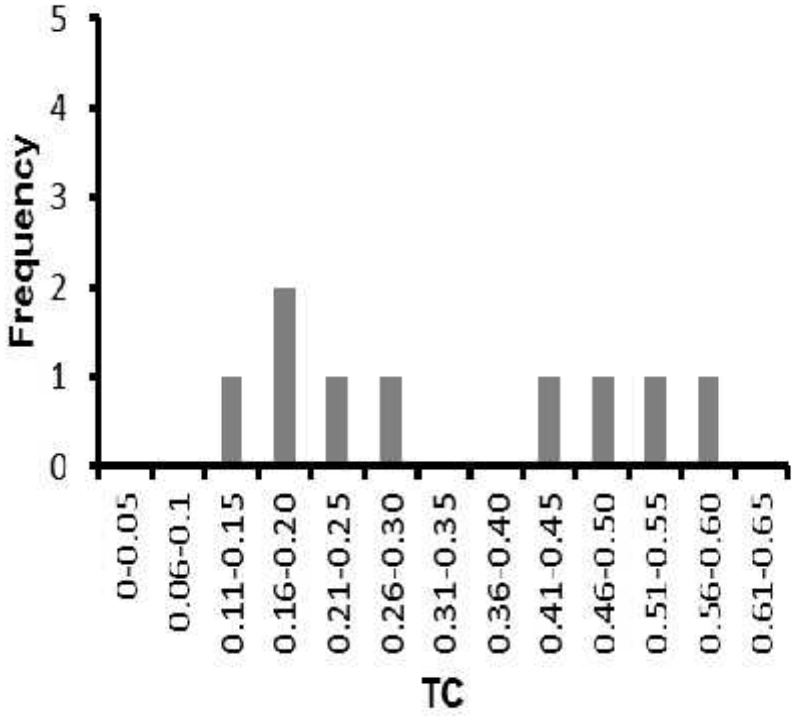

Figure 4-26. Distribution of *Salmonella* transfer coefficients (TCs) from tomatoes to single-use gloves with 1 h dried inoculum using BSAR media (n=9).

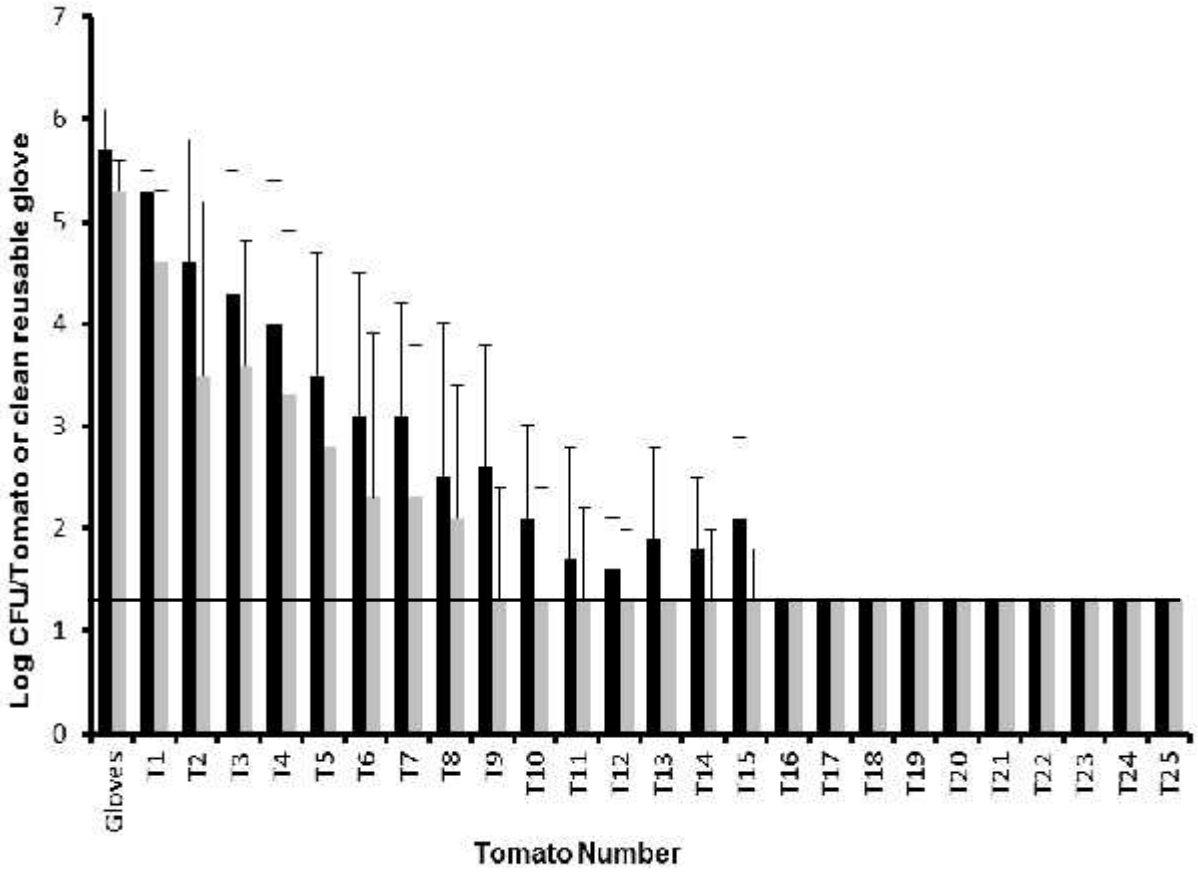

Figure 4-27: Population of *Salmonella* inoculated onto clean reusable gloves (first bars) and transferred to subsequently touched tomatoes (remaining bars) after 0 h (black color bars) and 1 h (light-grey color bars) of inoculums drying (n=9). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

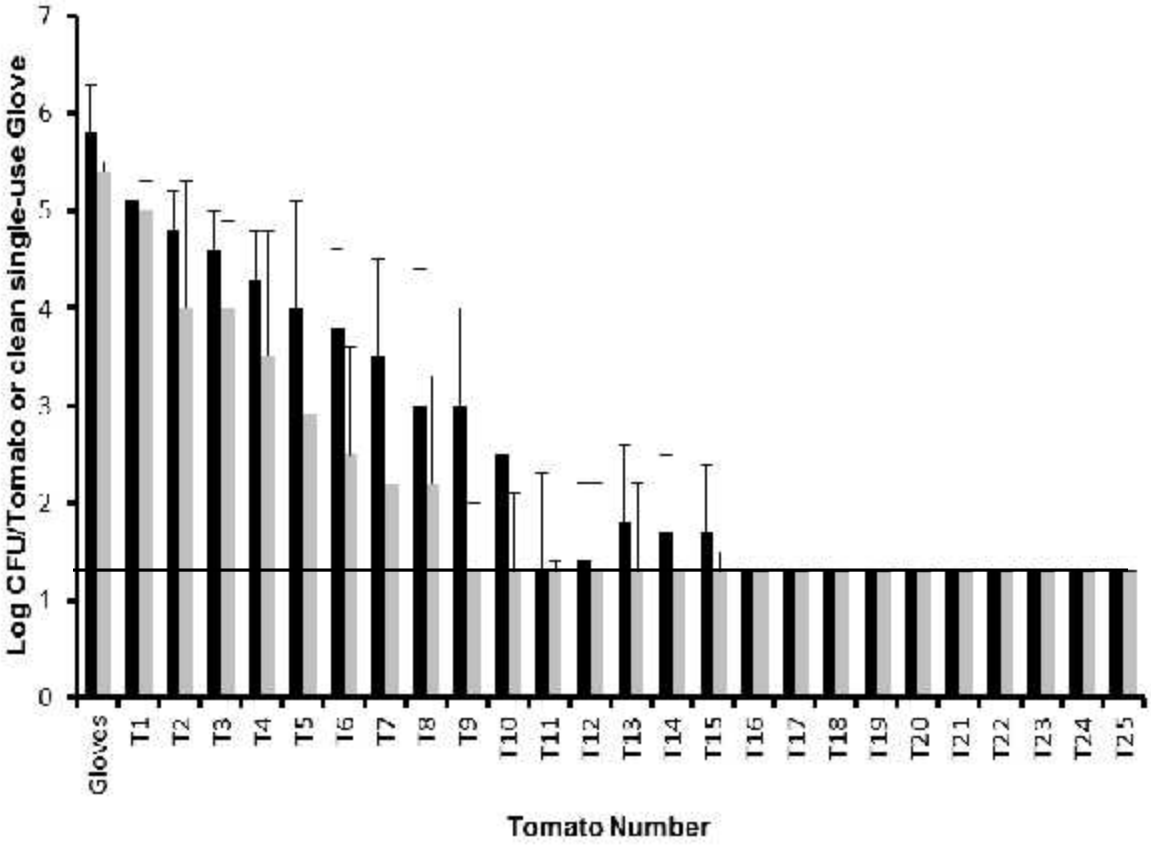

Figure 4-28: Population of *Salmonella* inoculated onto clean single-use gloves (first bars) and transferred to subsequently touched tomatoes (remaining bars) after 0 h (black color bars) and 1 h (light-grey color bars) of inoculums drying (n=9). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

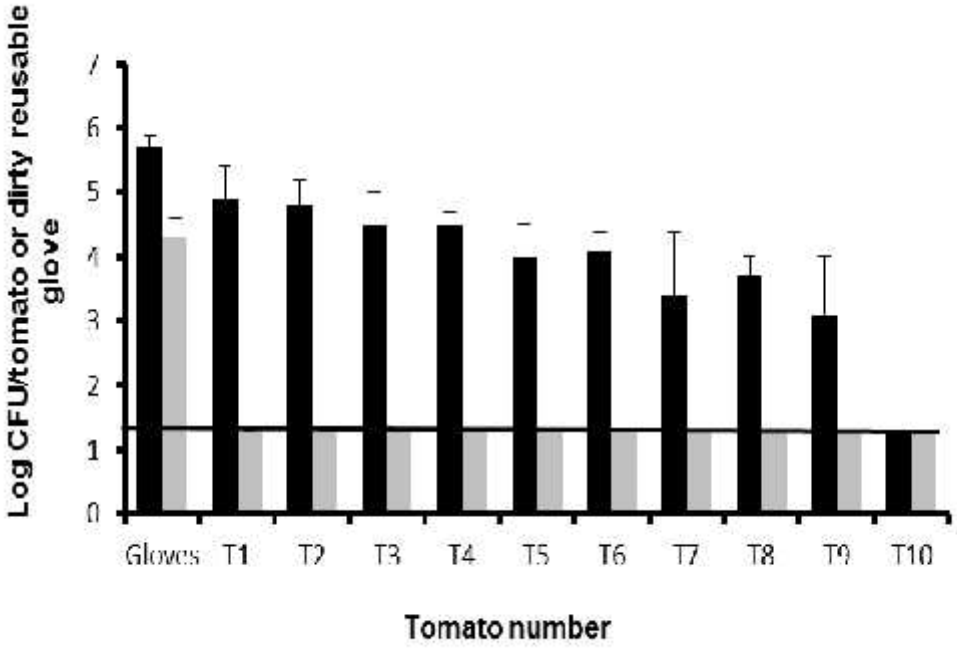

Figure 4-29: Population of *Salmonella* inoculated onto dirty reusable gloves (first bars) and transferred to subsequently touched tomatoes (remaining bars) after 0 h (black color bars) and 1 h (light-grey color bars) of inoculums drying (n=9). The solid line on the graph is the limit of detection (1.3 log CFU/glove or tomato).

CHAPTER 5
DISCUSSION

Tomato farming is the third largest industry in Florida (FASD, 2008). Florida tomato industry has suffered due to outbreaks of *Salmonella* illnesses associated with tomatoes. An outbreak of salmonellosis from jalapeno/serrano peppers which was first attributed to be linked to tomatoes, costs Florida tomato industry $100 million (CDC, 2008). All these incidences have raised the need of more research and inputs towards tomato safety. This research evaluates risks associated with cross-contamination of *Salmonella* between workers gloved hands and mature green, round tomatoes in simulated harvest and packinghouses upon single and sequential touches.

No significant differences were seen between results on selective on non-selective medias, and only results from non-selective media are discussed. Selective media promotes the growth of specific organism only and inhibit other organisms. For example, BSA, the selective media used in this study, inhibits the growth of gram-positive microorganisms. The other, TSA is non-selective media with casein and soybean meal the nutrient source. Addition of rifampicin as an antibiotic makes the rifampicin resistant *Salmonella* isolation, convenient. Rifampicin, an antibiotic, is used for experiments to eliminate the interference of any background flora, which could possibly affect the results.

Three different transfer scenarios were studied during experiments which were from (i) clean or dirty gloves to tomatoes (ii) tomato to clean gloves (iii) clean or dirty gloves to many tomatoes. Two different types of gloves (reusable or single use), two different hygienic conditions of gloves (clean and dirty) and three different drying time for inoculum (0 h, 1 h, 24 h) were studied, except doing experiments with dirty single-

use gloves and except drying inoculum for 24 h for gloves to many tomato transfer scenario. First transfer scenario simulates the transfer that could take place from gloves used by workers to the tomatoes touched with those gloved hands, and to see whether the transfer can take place from contaminated gloves of workers to the tomatoes. Second transfer scenario was selected to simulate transfer from contaminated tomatoes to worker's gloves. When the gloves are contaminated and used during harvesting, the number of tomatoes it can potentially contaminate was estimated through third transfer scenario. As single-use gloves are changed very frequently in tomato packinghouses, no experiments were performed with dirty single-use gloves.

Single Touch

Transfer coefficients obtained on touching green tomatoes with inoculated reusable gloves and single-use gloves were 25% and 32%, and transfer coefficients from inoculated green tomatoes to reusable and single-use gloves were ca. 18% and 37%, when the inoculum was wet. Similar results obtained from experiments performed from glove to tomato and tomato to glove with wet inoculum may be due to the smooth surface of gloves and tomatoes. Jimenez et al. (2007) also obtained high transference of *Salmonella enterica* serovar Typhimurium from inoculated bell pepper to gloved hands and from gloved hands to bell pepper as compared to bare hands, which are rough in comparison to gloved hands or bell pepper surface. Similar to our results, Jimenez et al. (2007) did not observe significant differences in transfer from glove surface to bell pepper and bell pepper surface to gloves.

Salmonella transfer from inoculated gloves to tomatoes after 1 h was significantly higher than wet inoculum ($p=0.04$). The possible reason behind this can be the curved surface of tomato, which may have had varied contact with the 6 or 8 inoculum drops on

glove surface at one time, potentially leaving some of the inoculum untouched. All the visible drops on the surface of gloves were touched after 1 h inoculum drying, which might be the reason for no change in *Salmonella* population from un-inoculated surface of tomatoes between 0 h and 1 h drying. However, drying the inoculum on flat glove surface did reduce the population of *Salmonella* due to drying or desiccation effect by less than 0.5 log CFU/glove. Hence the numerator of transfer coefficients calculation equation was same for both cases but the denominator decreases, which led to higher TCs after 1 h of drying.

Salmonella transfer from inoculated tomatoes to gloves did not change significantly after 1 h drying which is in contrary with the previous study by Lang et al. (2004), who found significant decrease in population from spot inoculated tomatoes after drying in biosafety hood for 1 h. The difference between the two studies is the site of inoculation on tomato surface. Lang and colleagues inoculated *Salmonella* on tomatoes at blossom end, while in this study, inoculation was done at the equator region, which a very different surface from the blossom end of tomatoes. Previous research by Rusin et al. (2002) has shown that *Salmonella* attachment to rough or porous surface is more than non-porous or smooth surface, which affects its transfer as well. This concept can be used to understand the different transfer rates after 1 h drying between this study and study by Lang et al. (2004).

Drying the inoculum on glove surface for 24 h, reduce the *Salmonella* population by ca. 3.7 log CFU/reusable gloves and ca. 4.5 log CFU/single-use glove surface, likely due to desiccation stress. Enrichments performed when counts fell below the limit of detection gave three out of nine positive from reusable gloves, while none of the nine

samples were positives for single-use gloves. Drying the inoculum on tomato surface reduced the population by 1.9 log CFU/ tomato for the experiment performed with reusable gloves and by 2.3 log CFU/tomato for the experiment with single-use glove. Similar to this study, Lang et al. (2004) also observed reduction in *Salmonella* population by 2.2 log CFU/tomato from spot inoculated tomatoes after 24 h of drying. The enrichment results obtained from reusable and single-use gloves touched with inoculated tomatoes were 7/9 and 0/9, respectively. Reusable gloves showed more positives in both cases; where it was inoculated and where it was touched with the inoculum. This may be due to the difference in material of reusable glove and single-use gloves, affecting the rate of drying of inoculum or rate of transfer of *Salmonella*. More reduction in *Salmonella* populations on the tomato surface in experiments with single-use glove than in the experiments with reusable glove may be the reason for more transfer from tomatoes to reusable glove. The different drying rates of inoculum on tomato surface for experiments with clean reusable and single-use gloves may be due to the position under the biosafety cabinet or a variation in surface properties of two lots of tomatoes used during experiments.

Biofilm formation is beyond the scope of this study; however the concept of biofilm can be used to provide possible explanations for some of the results. Bacterial biofilms can be defined as ‘an assemblage of microorganisms adherent to each other and/or to a surface and embedded in a matric of exopolymers’ (Costerton et al., 1999). Bacterial adhesion to surface depends on factors like surface composition, roughness of contact surface, charge and hydrophobicity of both the contact surface and the cells (Costerton et al., 1999). Bacterial biofilms protect bacteria from harsh and hostile environments.

Ukuku and Sapers (2001) have observed the decreased efficacy of chlorine and hydrogen peroxide sanitizers, when *Salmonella* was allowed to stay on cantaloupe melon surface for 24 h. The reason they propose for the decreased efficacy is biofilm formation by *Salmonella* on the fruit surface, whose strength increases with time during storage (Ukuku and Fett, 2002). If this is the case, these findings can be used to explain the reason for less reduction during 24h of drying of *Salmonella* on a tomato surface as compared to glove surface. Since the produce surface is biotic in nature, possibly the attachment and biofilm formation of *Salmonella* on tomato surface is stronger than on glove surface. Stronger biofilm may have helped the bacterial cells survive desiccation on tomato surface. Glove surfaces are made up of rubber material and rubber material are hydrophobic in nature with contact angle of 108.2±1.0° (Sinde and Carballo, 2000). High hydrophobicity of the glove surface may prevent attachment of *Salmonella* cells. Lack of attachment implies the presence of loose cells on the surface, which may die more easily than the adhered cells. Additionally, glove pieces used in experiments were flat, while the tomato surface was curved; the flat surface may have been more exposed the dry air and the drying effect of *Salmonella* on glove surface might be more pronounced.

Reusable gloves were made dirty by using soil (0.5 g, 0.3 g, 0.1 g), internal tissue of tomato (1/8 TSP, ¼ TSP, 1 drop) and one tomato leaf; rubbed on glove pieces for 5 s to 30 s. Experiments were performed with different types of dirty gloves and no significant differences were observed. Hence, rubbing a tomato leaf on glove surface for 20 s was selected as the best representation of dirty gloves in tomato harvesting field. In all the dirty gloves experiments, 0.1% of Tween-20 was used as a surfactant.

Surfactant is a substance that helps in lowering the interfacial tension between solid and a liquid or between two liquids. The use of Tween-20 in experiments was expected to improve the recovery of *Salmonella* from inoculated dirty glove surfaces. Dirty glove results did not differ significantly from clean reusable glove results, when inoculum was wet. Under wet inoculum transfer scenario, dirty gloves were made dirty, inoculated and immediately samples. Lack of the time between inoculum and dirty glove surface might be the reason for similar results from clean and dirty reusable gloves. On drying the inoculum in biosafety cabinet for 1 h, clean reusable gloves generate more *Salmonella* positive tomato samples than dirty reusable gloves. The layer of tomato leaf extract on glove surface possibly restricts *Salmonella* and might not let it transfer to tomato upon touching. This behavior was observed after 1 h inoculum drying, but not during immediate sampling, where the contact time for *Salmonella* with leaf debris was less. More contact time may help in better attachment of bacterial cells to the rough surface as per the concept of biofilm formation. Since dirty gloves are similar to rough surface while clean gloves are similar to smooth surface, 1 h drying might cause more attachment of *Salmonella* cells to dirty surface and prevent its transfer to tomatoes. Our results obtained after 1 h inoculum drying on dirty gloves are similar with the results obtained by Flores et al. (2006). They demonstrated that TCs from cutting boards to beef and beef to cutting boards decreased with increased amounts of beef tissue present on high density polyethylene cutting boards surfaces. Transfer of *E. coli* O157:H7 from contaminated board surfaces to beef tissues was studied under both wet and dry (5 min) inoculum conditions. They concluded that the decrease in TCs is due to reduction of the number of cells left on the surface after each subsequent contact.

The results obtained from touching single tomato with inoculated clean gloves after 0 h, 1 h drying time showed that *Salmonella* has potential to transfer from inoculated worker's gloves to tomatoes. After 24 h, transfer was observed from clean reusable gloves but not from single-use gloves, which implies clean reusable gloves can transfer *Salmonella that* may not have been removed from the glove during the previous days cleaning and sanitizing. For dirty gloves, *Salmonella* transfer was similar to clean gloves, when inoculum was wet. As the time of contact between dirty gloves and inoculum increased to 1 h, we believe that *Salmonella* gets trapped in the layer of tomato leaf extract, thus decreasing its transfer. *Salmonella* also transferred from inoculated tomatoes to both types of clean gloves after 0 h and 1 h drying time. However, after 24 h, transfer occurred to clean reusable gloves only, while single-use gloves did not report any transfer.

Subsequent Touches

On touching tomatoes subsequently with inoculated clean reusable gloves, TCs decreased continuously until the tomatoes were enriched for *Salmonella*. In case of clean reusable gloves, significant differences in TCs were observed between T1 and T3 to T9, while for single-use gloves, significant reductions were obtained between T1 and T7 to T9. Statistical analysis showed that TCs obtained from first two tomatoes (T1 and T2) touched with clean inoculated reusable gloves were significantly higher than all the tomatoes touched with inoculated single-use gloves, expect first tomato. This implies that *Salmonella* transfer from clean reusable gloves to first two tomatoes was higher, which was similar to transfer from single-use gloves from third tomato. However, from single-use gloves same TCs were obtained up to 7^{th} tomato. Thus, single-use gloves transfer less *Salmonella* to a higher number of tomatoes touched, while clean reusable

gloves transferred more *Salmonella* to a smaller number of tomatoes. In general, the results from clean gloves to many tomatoes show that as more and more tomatoes are touched with inoculum, the *Salmonella* transfer decreases, which is in contrary with Fravalo et al. (2009) findings, who concluded that *Salmonella* transfer is inversely related to the initial load.

For dirty reusable gloves, TCs did not change significantly for the ten tomatoes touched. Even after drying the inoculum for 1 h, almost same number of *Salmonella* positive samples was obtained from all the ten tomatoes touched subsequently with dirty reusable gloves, replicated nine times. Similar to statistical analysis from single-use gloves and clean reusable gloves, first two TCs obtained from clean reusable gloves differ significantly from all the TCs obtained from dirty reusable gloves. Two possible reasons behind less transfer from dirty reusable gloves can be either death of *Salmonella* cells or trapping of *Salmonella* cells in the leaf extract. Based on the transfer of *Salmonella* to the ten tomatoes touched with inoculated gloves at 0 h and 1 h, it is more likely that *Salmonella* is getting stuck in the layer and transferring to tomatoes at slower rate.

Worker's gloves are potential source of *Salmonella* transfer during tomato harvesting. As the day progress and gloves become dirtier, transfer of *Salmonella* to tomatoes takes place at lower rate, and the risk of using dirty gloves is no greater than using clean gloves. Our results have shown *Salmonella* transfer between reusable gloves and tomatoes even after it has dried on the surface for one day. Although the transfer was less than what was seen with wet or 1h dried inoculum, it cannot be

neglected and the use of adequate cleaning and sanitizing for washing of gloves at the end of the shift is an important step to eliminate the chances of cross-contamination.

CHAPTER 6
FUTURE WORK

The use of gloves is considered as a preventive step during tomato harvesting. The results of this research illustrate that gloves can be the potential source of cross-contamination to tomatoes during harvesting and contaminated tomatoes can transfer *Salmonella* to gloves. As hypothized, dirty gloves did not transfer more *Salmonella* to tomatoes; rather the risk of *Salmonella* transfer was similar for clean and dirty gloves, under wet inoculum conditions. Results also showed that clean reusable gloves transfer more *Salmonella* to a fewer number of tomatoes touched, while single-use gloves and dirty reusable gloves transfer less *Salmonella* to a greater number of tomatoes. The decision of which is risky transfer scenario among above two is a hard task. The results from this research left us with some unanswered questions, which need further research and inputs to ease the risk management solution.

Determining the risk potential of different sources that may transfer *Salmonella* or other pathogenic organisms is an important concern for researchers. This research focused on the hand harvesting of mature green, round tomatoes only. The surface of all fruits and vegetables are not identical, thus the wide application of the results obtained here to other produce varieties without subsequent experiments to quantify the risk of glove use in bacterial transfer would be unwise. For example, the skin of Roma tomatoes is thinner as compared to round tomatoes; *Salmonella* transfer to Roma or grape tomatoes may be different in comparison with round tomatoes. Similar transfer coefficient experiments should be performed to determine the risk of *Salmonella* transfer from and to other tomato varieties and other fruits and vegetables, mimicking glove and harvest conditions unique to each.

The gloved hands of workers are not the only potential point of cross-contamination during round tomato harvest. The study of different equipment used during harvesting should be another area of future study. Transfer from different equipment, including buckets, utensils, worker's shirts, that may contact tomatoes during harvest should be consider to understand the overall risk of *Salmonella* transfer during harvesting. These future studies would help strengthen any future risk assessment models that attempt farm to fork modeling including the risk of bacterial cross-contamination during harvesting of different produce items.

Equipment used during harvest, utensils, gloves and other items, can be cleaned and sanitized at the end of the day and reused again and again. The effectiveness different sanitizers used on these harvesting tools should be another area of research. The different sanitizers can be tested for different equipment, including, dirty gloves, buckets, etc. against various pathogen and a standard method for sanitizing harvesting equipment could be developed. Additionally, since the use of sanitizers may damage the equipment, the effect of sanitizer use on glove surface properties is an interesting topic for future study. The use of sanitizers on same gloves again and again might have increasing their roughness, which in turn might enhance the bacterial attachment potential of glove surface.

This research evaluated transfer of only *Salmonella* during tomato harvesting. No research has been conducted to evaluate the transfer of other pathogenic microorganisms like *E. coli* O157:H7 or *Listeria monocytogenes* during harvesting of any produce items.

Fresh produce continues to emerge as an important field of research in Food Safety. Multistate outbreaks linked to fresh produce have raised the concern about their safe production, harvesting, handling, storage and distribution. No reduction steps are currently commonly used for fresh produce items and they are mainly consumed by people as raw. Continued research is required which can help us determine different sources of contamination, risk associated with those sources of contamination and the ways to reduce those risks effectively. This research focused on the harvesting of mature green, round tomatoes with gloved hands. Above mentioned future work will provide a broader view to look into different things, which will help in reducing risk associated with different fresh produce and help in providing a safe produce items to consumers.

LIST OF REFERENCES

Aarnisalo, K., Sheen, S., Raaska, L., Tamplin, M., 2007. Modeling transfer of *Listeria monocytogenes* during slicing of 'gravad' salmon. International Journal of Food Microbiology 118, 69-78.

Ahvenainen, 1999. New approaches in improving the shelf life of minimally processed fruits and vegetables. Trends in Food Science and Technology 7, 179-187.

Asplund, K., Nurmi, E., 1991. The growth of salmonellae in tomatoes. International Journal of Food Microbiology 13, 177-181.

Bialka, K.L., Demirci, A., 2008. Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on raspberries and strawberries. Journal of Food Science 73, M201-M207.

Baker, R.C., Qureshi, R.A., Hotchkiss, J.H., 1986. Effect of an elevated level of carbon dioxide containing atmosphere on the growth of spoilage and pathogenic bacteria at 2, 7, and 13 C. Poultry Science 65, 729-737.

Baloda, S.B., Christensen, L., Trajcevska, S., 2001. Persistence of a *Salmonella enterica* serovar *Typhimurium* DT2 clone in a piggery and in agricultural soil amended with *Salmonella*-contaminated slurry. Applied and Environmental Microbiology 67, 2859-2862.

Barak, J.D., Liang, A.S., 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. PloS ONE 3, e1657.

Barker, J., Bloomfield, S.F., 2000. Survival of *Salmonella* in bathrooms and toilets in domestic homes following salmonellosis. Journal of Applied Microbiology 89, 137-144.

Bartz, J.A., Showalter, R.K., 1981. Infiltration of tomatoes by bacteria in aqueous suspension. Phytopathology 7, 515-518.

Bean, N.H., Griffin, P.M., 1990. Foodborne disease outbreaks in the United States. 1973-1987: pathogens, vehicles, and trends. Journal of Food Protection 53, 801-817.

Bean, N.H., Goulding, J.S., Daniels, M.T., Angulo, F.J., 1997. Surveillance for foodborne disease outbreaks United States, 1988-1992. Journal of Food Protection 60, 1265-1286.

Bell, C., Kyriadides, A. (Eds.), 2000. Foodborne pathogens: hazards, risk analysis and control 11. *Salmonella*: Woodhead Publishing Limited, Abington, England, pp. 307-311.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Minireview: Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology 12, 2385-2397.

Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection 59, 204-216.

Beuchat, L.R., 1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. International Journal of Food Microbiology 34, 329-333.

Beuchat, L.R., 1998. Surface decontamination of fruits and vegetables eaten raw: a review. World Health Organization, Food Safety Unit WHO/FSF/FOF/98.2.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infections 4, 413-423.

Beuchat, L.R., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection 67, 2342-2353.

Beuchat, L.R., 2008. Difficulties in eliminating human pathogenic microorganisms on raw fruits and vegetables. Acta Horticulturae 642, 151-160.

Beuchat, L.R., Ryu, J.H., 1997. Produce handling and processing practices. Emerging Infectious Diseases 3, 459-465.

Boring, J.R., Martin, W.T., Elliott, L.M., 1971. Isolation of *Salmonella* Typhimurium from municipal water, Riverside, California, 1965. American Journal of Epidemiology 93, 49-54.

Brandl, M.T., Mandrell, R.E., 2002. Fitness of *Salmonella enterica* serovar Thompson in the Cilantro Phyllosphere. Applied and Environmental Microbiology 68, 3614-3621.

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R., Swaminathan, B., 2000. *Salmonella* Nomenclature. Journal of Clinical Microbiology 38, 2465-2467.

Burnett, S.L., Beuchat, L.R., 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology and Biotechnology 27, 104-110.

Centers for Disease Control and Prevention (CDC), 1991. A multistate outbreak of *Salmonella* Javiana infections associated with raw tomatoes. Morbidity and Mortality Weekly Report 56, 909-911.

Centers for Disease Control and Prevention (CDC), 2002. Outbreak *Salmonella* serotype Javiana infections-Orlando, Florida, June 2002. Morbidity and Mortality Weekly Report 56, 909-911.

Centers for Disease Control and Prevention (CDC), 2005. Outbreaks of *Salmonella* infections associated with eating roma tomatoes-United States and Canada, 2004. Morbidity and Mortality Weekly Report 54, 325-328.

Centers for Disease Control and Prevention (CDC), 2007. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants-United States, 2005-2006. Morbidity and Mortality Weekly Report 56, 909-911.

Centers for Disease Control and Prevention (CDC), 2008. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items, United States 2008. Morbidity and Mortality Weekly Report 57, 929-934.

Centers for Disease Control and Prevention (CDC), 2008a. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants-United states, 2005-2006. Morbidity and Mortality Weekly Report 56, 909-911.

Centers for Disease Control and Prevention (CDC), 2009. Outbreak of *Salmonella* serotype Saintpaul infections associated with eating alfalfa sprouts-United States, 2009. Morbidity and Mortality Weekly Report 58, 1-3.

Centers for Science Public Interest (CSPI), 2005. Outbreak Alert! Closing the gaps in our federal food safety net. Washington D.C.

Chao, W., Ching, R., Chen, R., 1987. Survival of pathogenic bacteria in environmental microcosms. Chinese Journal of Microbiology and Immunology 20, 339-348.

Chen Y., Jackson, K.M., Chea, F.P., Schaffner D.W., 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. Journal of Food Protection 64, 72-80.

Cherry, W.B., Hanks, J.B., Thomason, B.M., Murlin, A.M., Biddle, J.W., Croom, J.M., 1972. Salmonellae as an index of pollution of surface waters. Applied Microbiology 24, 334-340.

Clemens, R., 2004. The expanding U.S. market for fresh produce. CARD (Center for Agricultural and Rural Development)-Iowa Ag Review 10, 8-9. Accessed at http://www.card.iastate.edu/iowa_ag_review/winter_04/article4.aspx. Accessed on July 15th, 2011.

Costerton, J.W., Stewart, P.S. and Greenberg, E.P.,1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318-1322.

Cox, P., Griffith, M., Angles, M., Deere, D., Ferguson, C., 2005. Concentrations of pathogens and indicators in animal feces in the Sydney watershed. Applied and Environmental Microbiology 71, 5929-5934.

Cummings, K., Barrett, E., Mohle-Boetani, J.C., Brocks, J.T., Farrar, J., Hunt, T., Fiore A., Komatsu, K., Werner, S.B., Slutsker, L., 2001. A multistate outbreak of *Salmonella enterica* serotype Baildon associated with domestic raw tomatoes. Emerging Infectious Diseases 7, 1046-1048.

D' Aoust, J.-Y., Aris, B.J., Thisdele, P., Durante, A., Brisson, N., Dragon, D., 1975. *Salmonella* Eastbourne outbreak associated with chocolate. Canadian Institute of Food Science and Technology Journal 8, 181-184.

D'Aoust, J.-Y., 1989. Foodborne bacterial pathogens: Marcel Dekkar, Inc., pp. 328-413.

D'Aoust, J.-Y., 1994. *Salmonella* and the international food trade. International Journal of Food Microbiology 24, 11-31.

D'Aoust, J., 1977. *Salmonella* and the chocolate industry. Journal of Food Protection 40, 718 - 727.

Dalton, C.B., Haddix, A., Hoffman, R.E., Mast, E.E., 1996. The cost of a foodborne outbreak of hepatitis A in Denver, Colo. Achieves of Internal Medicine 156, 1013-1016.

Das, E., Gürakan, G.C., Bayindirh, A., 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella* Enteritidis on cherry tomatoes. Food Microbiology 23, 430-438.

Department of Agriculture and Consumer Services (DACS), 2007. Tomato best practices: A guide to Tomato Good Agricultural Practices (T-GAP) and Tomato Best Management Practices (T-BMP). Accessed at http://www.freshfromflorida.com/fs/TomatoBestPractices.pdf. Accessed on July 19th, 2011.

De Roever, C., 1998. Microbiological safety evaluations and recommendations on fresh produce. Food Control. 9, 321-347.

Doebbeling, B.N., Stanley, G.L., Sheetz, C.T., Pfaller, M.A., Houston, A.K., Annis, L., Li, N., Wenzel, R.P., 1992. Comparative efficacy of alternative hand-washing agents in reducing nosocomial infections in intensive care units. New England Journal of Medicine 327, 88-93.

Dowd, S.E., Thurston-Enriquez, J.A., Brashears, M., 2008. Environmental reservoirs and transmission of foodborne pathogens. In: Beier, R.C., Pillai, S.D., Phillips, T.D. (Eds.), Preharvest and Postharvest Food Safety: Contemporary issues and future directions, Blackwell Publishing, Ames, Iowa, USA, pp. 161-172.

Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007-the problems with fresh produce: an overview. Journal of Applied Microbiology 105, 317-330.

Droffner, M.L., Yamamoto, N., 1991. Procedure for isolation of *Escherichia*, *Salmonella*, and *Pseudomonas* mutants capable of growth at the refractory temperature of 54°C. Journal of Microbiological Methods 14, 201-206.

Ed, G., 1997. Japan prepares as 0157 strikes again. The Lancet 349, 1156.

Ehrenkranz, N.J., 1992. Bland Soap Handwash or Hand Antisepsis? The Pressing Need for Clarity. Infection Control and Hospital Epidemiology 13, 299-301.

Food and Drug Administration (FDA), 2007. Guidance for industry: guide to minimize microbial food safety hazards of fresh-cut fruits and vegetable. College Park, MD: US Department of Health and Human Services, Food and Drug Administration. Accessed at http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm064458.htm. Accessed on July 3rd, 2011.

Fedorka-Cray, P.J., Kelley, L.C., Stabel, T.J., Gray, J.T., Laufer, J.A., 1995. Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine. Infection and Immunology 63, 2658-2664.

Felkey, K., Archer, D.L., Bartz, J.A., Goodrich, R.M., Schneider, K.R., 2006. Chlorine disinfection of tomato surface wounds contaminated with *Salmonella* spp. Horttechnology 16, 253-256.

Fisher, I.S., De Jong, B., Van Pelt, W., Aramini, J., Berghold, C., Matthys, F., Aramini, J., Berghold, C., Matthys, F., Powling, J., Siitonen, A., 2002. International outbreak of *Salmonella* Oranienburg, October-December Part 3: other countries. Eurosurveillance Weekly 6.

Flores, R.A., Tamplin, M.L., Marmer, B.S., Phillips, J.G. and Cooke, P.H., 2006. Transfer coefficient models for *Escherichia coli* O157:H7 on contacts between beef tissue and high density polyethylene surfaces. Journal of Food Protection. 49, 1248-1255.

Florida Agricultural Statistical Directory (FASD), 2008. Florida Dept. of Agriculture and Consumer Services, Charles H. Bronson, Commissioner, DACS -P-01304, Maitland,FL. Accessed at http://www.florida-agriculture.com/agfacts.htm. Accessed on July 20th, 2011.

Florida Tomato Committee (FTC), 2004. Handling Regulations. Regulatory Bulletin No. 1. Sept. 2004. Accessed at http://www.floridatomatoes.org/. Accessed on July 20th, 2011.

Food and Drug Administration (FDA), 1998. Guidance for industry: guide to minimize microbial food safety hazards for fresh fruits and vegetables. Food and Drug Administration. Center for Food Safety and Applied Nutrition, Washington, D.C.

Food and Drug Administration (FDA), 2001. FDA survey of imported fresh produce: FY 1999 field assignment. Accessed at http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatoryInformation/ucm118891.htm. Accessed on July 19th, 2011.

Food and Drug Administration (FDA), 2003. FDA survey of domestic fresh produce: FY 2000/2001 field assignement. Accessed at http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatoryInformation/ucm118306.htm. Accessed on July 19th, 2011.

Food and Drug Administration (FDA), 2007. Bacteriological Analytical Manual (BAM). Accessed at http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm. Accessed on July 19th, 2011.

Francis, G.A., Thomas, C. and O'Beirne, D., 1999. The microbiological safety of minimally processed vegetables. International Journal of Food Science and Technology 34, 1-22.

Fravalo, P., Laisney, M., Gillard, M., Salvat, G., Chemaly, M., 2009. *Campylobacter* transfer from naturally contaminated chicken thighs to cutting boards is inversely related to initial load. Journal of Food Protection 72, 1836-1840.

Garg, N., Churey, J.J., Splittstoesser, D.F., 1990. Effect of processing conditions on the microflora of fresh cut vegetables. Journal of Food Protection 53, 701-703.

Garthright, W.E., 1998. Most probable number of serial dilutions. FDA Bacteriological Analytical Manual, pp.1-12.

Gayler, G.E., MacCready, R.A., Reardon, J.P., MacKernan, B.F., 1955. An outbreak of salmonellosis traced to watermelon. Public Health Reports 70, 311-313.

Gill, C.O., Jones, T., 2002. Effects of wearing knitted or rubber gloves on the transfer of *Escherichia coli* between hands and meat. Journal of Food Protection 65, 1045-1048.

Gill, O.N., Sockett, P.N., Bartlett, C.L., Vaile, M.S., Rowe, B., Gilbert, R.J., Dulake, C., Murrell, H.C., Salmaso, S., 1983. Outbreak of *Salmonella* Napoli infection caused by contaminated chocolate bars. The Lancet 1, 574-577.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F., Painter, J.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiology and Infection 136, 157-165.

Guo, X., Chen, J., Brackett, R.E., Beuchat, L.R., 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Applied and Environmental Microbiology 67, 4760-4764.

Guo, X., van Iersel, M.W., Chen, J., Brackett, R.E., Beuchat, L.R., 2002. Evidence of association of salmonellae with tomato plants grown hydroponically in inoculated nutrient solution. Applied and Environmental Microbiology 68, 3639-3643.

Guzewich, J., Ross, M.P., 1999. Evaluation of risks related to microbiological contamination of ready-to-eat food by food preparation workers and the effectiveness of interventions to minimize those risks. Food and Drug Administration. Center for Food Safety and Applied Nutrition, Washington, D.C.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety. 2, 78-141.

Hedberg, C.W., Angulo, F.J., White, K.E., Langkop, C.W., Schell, W.L., Stobierski, M.G., Schuchat, A., Besser, J.M., Dietrich, S., Helsel, L., Griffin, P.M., McFarland, J.W., Osterholm, M.T., 1999. Outbreaks of salmonellosis associated with eating uncooked tomatoes: implications for public health. Epidemiology and Infection 122, 385-393.

Hedberg, C.W., Korlath, J.A., D'Aoust, J.-Y., White, K.E., Schell, W.L., Miller, M.R., Cameron, D.N., MacDonald, K.L., Osterholm, M.T., 1992. A multistate outbreak of *Salmonella* Javiana and *Salmonella* Oranienburg infections due to consumption of contaminated cheese. Journal of American Medical Association 268, 3203-3207.

Hedberg, C.W., MacDonald, K. L., Osterholm, M.T., 1994. Changing epidemiology of foodborne disease: A Minnesota perspective. Clinical Infectious Diseases 18, 671-680.

Hirano, S.S., Upper, C.D., 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*---a pathogen, ice nucleus, and epiphyte. Microbiology and Molecular Biology Reviews 64, 624-653.

Holley, R.A., Arrus, K.M., Ominski, K.H., Tenuta, M., Blank, G., 2006. Survival in manure-treated soils during simulated seasonal temperature exposure. Journal of Environmental Quality 35, 1170-1180.

Holley, R.A., Proulx, M., 1986. Use of egg wash pH to prevent survival of *Salmonella* at moderate temperatures. Poultry Sciences 65, 922-928.

Hurd, H.S., McKean, J.D., Griffith, R.W., Wesley, I.V., Rostagno, M.H., 2002. *Salmonella* enterica infections in market swine with and without transport and holding. Applied and Environmental Microbiology 68, 2376-2381.

Imperial College London, 2005. Glove selection guidance. Occupational Health: Imperial college OH services. Accessed at http://www3.imperial.ac.uk/OCCHEALTH/guidanceandadvice/gloveinformationandguidance/gloveselectionguidance. Accessed on July 3rd, 2011.

Iturriaga, M.H., Escartin, E.F., 2003. Changes in the effectiveness of chlorine treatments during colonization of *Salmonella montevideo* on tomatoes. Journal of Food Safety 30, 300-306.

Jablasone J., Brovko, L.Y., Griffiths, M.W., 2004. A research note: the potential for transfer of *Salmonella* from irrigation water to tomatoes. Journal of the Science of Food Agriculture 84, 287-289.

Jaquette, C.B., Beuchat, L.R., Mahon, B.E., 1996. Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Applied and Environmental Microbiology 62, 2212-2215.

Jimenez, M., Siller, J., Valdez, J., Carrillo, A., Chaidez, C., 2007. Bidirectional *Salmonella enterica* serovar Typhimurium transfer between bare/glove hands and green bell pepper and its interruption. International Journal of Environmental Health Research 17, 381-388.

King, C.H., Shotts, E.B., Jr., Wooley, R.E., Porter, K.G., 1988. Survival of coliforms and bacterial pathogens within protozoa during chlorination. Applied and Environmental Microbiology 54, 3023-3033.

Kirkland, E., Green, L.R., Stone, C., Reimann, D., Nicholas, D., Mason, R., Frick, R., Coleman, S., Bushnell, L., Blade, H., Radke, V., Selman, C., 2009. Tomato Handling Practices in Restaurants. Journal of Food Protection 72, 1692-1698.

Klein, S., Witmer, J., Tian, A., Dewaal, C.S., 2009. The ten riskiest foods regulated by U.S. Food and Drug Administration, Center for Science in the Public Interest (CSPI), Washington, DC. Accessed at http://cspinet.org/new/pdf/cspi_top_10_fda.pdf. Accessed on July 1st, 2011.

Klontz, K.C., Klontz, J.C., Mody, R.K., Hoekstra, R.M., 2010. Analysis of tomato and jalapeno and serrano pepper imports into the United States from Mexico before and during a national outbreak of *Salmonella* serotype Saintpaul infections in 2008. Journal of Food Protection 73, 1967-1974.

Kusumaningrum, H.D., van Asselt, E.D., Beumer, R.R., Zwietering, M.H., 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Camphylobacter* spp. via domestic kitchen surfaces. Journal of Food Protection 67:1892-1903.

Lang, M.M., Harris, L.J., Beuchat, L.R., 2004. Evaluation of inoculation method and inoculum drying time for their effects on survival and efficiency of recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated on the surface of tomatoes. Journal of Food Protection 67, 732-741.

Larson, E., Mayur, K., Laughon, B.A., 1989. Influence of two handwashing frequencies on reduction in colonizing flora with three handwashing products used by health care personnel. American Journal of Infection Control 17, 83-88.

Lin, C.M., Wei, C.I., 1997. Transfer of *Salmonella* Montevideo onto the interior surfaces of tomatoes by cutting. Journal of Food Protection 60, 858-862.

Lindow, S.E., Brandl, M.T., 2003. Microbiology of the Phyllosphere. Applied and Environmental Microbiology 69, 1875-1883.

Lucier, G., Lin, B.-H., Allshouse, J., Kantor, L.S., 2000. Factors affecting tomato consumption in United States. Vegetable Specialties 282, 26-32.

MacKintosh, C.A., Hoffman, P.N., 1984. An extended model for transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection. Journal of Hygiene 92, 345-355.

Madden, J.M., 1992. Microbial pathogens in fresh produce-the regulatory perspective. Journal of Food Protection 55, 821-823.

Mahon, B.E., Ponka, A., Hall, W.N., Komatsu, K., Dietrich, S.E., Siitonen, A., Cage, G., Hayes, P.S., Lambert-Fair, M., Bean, N.H., Griffin, P.M., Slutsker, L., 1997. An International outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. Journal of Infectious Diseases 175, 876-882.

Marina, S., Odumeru, J., 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. Journal of Food Protection 67, 2839-2849.

Marriott, N.G., Gravani, R.B. (Eds.), 2006. Principles of food sanitation: Fifth Edition: Springer Science Buisness Media, Inc.

Mattson, T.E., Johny, A.K., Amalaradjou, M.A., More, K., Schreiber, D.T., Patel, J., Venkitanarayanan, K., 2010. Inactivation of *Salmonella* spp. on tomatoes by plant molecules. International Journal Food Microbiology 144, 464-468.

Maurer, J., D'Aoust, J.-Y., 2007. *Salmonella* Species. In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology-Fundamentals and Frontiers, ASM Press, Washington, DC, pp. 141-178.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerging Infectious Diseases 5, 607-625.

Miles, J.M., Sumner, S.S., Boyer, R.R., Williams, R.C., Latimer, J.G., McKinney, J.M., 2009. Internalization of *Salmonella enterica* serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. Journal of Food Protection 72, 849-852.

Millemann, Y., Gaubert, S., Remy, D., Colmin, C., 2000. Evaluation of IS200-PCR and comparison with other molecular markers to trace *Salmonella enterica* subsp. *enterica* serotype Typhimurium bovine isolates from farm to meat. Journal of Clinical Microbiology 38, 2204-2209.

Mohle-Boetani, J.C., Reporter, R., Werner, S.B., Abbott, S., Farrar, J., Waterman, S.H., Vugia, D.J., 1999. An outbreak of *Salmonella* serogroup Saphra due to cantaloupes from Mexico. Journal of Infectious Diseases 180, 1361-1364.

Montville, R., Chen, Y.H., Schaffner, D.W., 2001. Glove barriers to bacterial cross-contamination between hands to food. Journal of Food Protection 64, 845-849.

Montville, R., Schaffner, D.W., 2003. Inoculum size influences bacterial cross contamination between surfaces. Applied and Environmental Microbiology 69, 7188-7193.

National advisory committee on Microbiological criteria for Foods (NACMCF), 1999. Microbiological safety evaluations and recommendations on sprouted seeds. International Journal of Food microbiology 52, 123-153

National Cancer Institute, 2002. Eat five a day foe better health. Accessed at http://www.cancer.gov/newscenter/pressreleases/2002/nabj5aday. Accessed on July 20th, 2011.

Natvig, E.E., Ingham, S.C., Ingham, B.H., Cooperband, L.R., Roper, T.R., 2002. *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Applied and Environmental Microbiology 68, 2737-2744.

Ness, A.R., Powles, J.W., 1997. Fruit and vegetables, and cardiovascular disease: a review. International Journal of Epidemiology 26, 1-13.

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition 34, 371-401.

North American Tomato Trade Work Group to Further Adoption of Good Agricultural Practices throughout the Fresh Tomato Supply Chain, 2006. Commodity specific food guidelines for the fresh tomato supply chain. Accessed at http://www.nproduce.com/data/tomato.pdf. Accessed on July 20th, 2011.

Notermans, S., Hoogenboom-Verdegaal, A., 1992. Existing and emerging foodborne diseases. International Journal of Food Microbiology 15, 197-205.

Notermans, S., Hoogenboom-Verdegaal, A., 2002. Existing and emerging foodborne diseases. International Journal of Food Microbiology 15, 197-205.

Nyachuba, D.G., 2010. Foodborne illness: is it on rise? Nutrition Reviews 68, 257-269.

O'Brien, R.D., Lindow, S.E., 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. Phytopathology 79, 619-627.

Oliver, J.D., Dagher, M., Linden, K., 2005. Induction of *Escherichia coli* and *Salmonella typhimurium* into the viable and nonculturable state following chlorination of water. Journal of Water and Health 3, 249-257.

Olson, S.M., Stall, W.M., Vallad, G.E., Webb, S.E., Taylor, T.G., Smith, S.A., Simonne, E.H., McAvoy, E., Santos, B.M., 2009. Vegetable production handbook for Florida: Tomato production in Florida. University of Florida, Gainesville, 32611, pp. 291-312.

O'Mahony, M., Cowden, J., Smyth, B., 1990. An outbreak of *Salmonella* Saint-paul infection associated with beansprouts. Epidemiology and Infection 104, 229-235.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., Busta, F.F., 2003. Methods to reduce eliminate pathogens from fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety 2, 161-173.

Patil, S., Valdramidis, V.P., Cullen, P.J., Frias, J., Bourke, P., 2010. Inactivation of *Escherichia coli* by ozone treatment of apple juice at different pH levels. Food Microbiology 27, 835-840.

Paulson, D.S., 1996. To glove or to wash: A current controversy. Food Quality, pp. 60-64.

Penteado, A.L., Leitão, M.F.F., 2004. Growth of *Salmonella* Enteritidis in melon, watermelon and papaya pulp stored at different times and temperatures. Food Control 15, 369-373.

Perry, J.J., Rodriguez-Romo, L.A., Yousef, A.E., 2008. Inactivation of *Salmonella enterica* serovar Enteritidis in shell eggs by sequential application of heat and ozone. Letters in Applied Microbiology 46, 620-625.

Pether, J.V.S., Gilbert, R.J., 1971. The survival of salmonellas on finger-tips and transfer of the organisms to foods. Journal of Hygiene 69, 673-681.

Poirier, E., Watier, L., Espie, E., Weill, F.X., De Valk, H., Desenclos, J.C., 2008. Evaluation of the impact on human salmonellosis of control measures targeted to *Salmonella* Enteritidis and Typhimurium in poultry breeding using time-series analysis and intervention models in France. Epidemiology and Infection 136, 1217-1224.

Proctor, M.E., Hamacher, M., Tortorello, M.L., Archer, J.R., Davis, J.P., 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. Journal of Clinical Microbiology 39, 3461-3465.

Raffatellu, M., Wilson, R.P., Winter, S.E., Bäumler, A.J., 2008. Clinical pathogenesis of typhoid fever. Journal of Infection in Developing Countries 2, 260-266.

Raiden, R.M., Sumner, S.S., Eifert, J.D., Pierson, M.D., 2003. Efficacy of detergents in removing *Salmonella* and *Shigella* spp. from the surface of fresh produce. Journal of Food Protection. 66, 2210-2215.

Rathinasabapathi, B., 2004. Survival of *Salmonella* Montevideo on tomato leaves and mature green tomatoes. Journal of Food Protection 67, 2277-2279.

Restaino, L., Wind, C.E., 1990. Antimicrobial effectiveness of hand washing for food establishments. Dairy, Food and Environmental Sanitation 10, 136-141.

Restaino, L., Frampton, E.W., Hemphill, J.B., Palnikar, P., 1995. Efficacy of ozonated water against various food-related microorganisms. Applied and Environmental Microbiology 61, 3471-3475.

Ritenour, M.A., Sargent, S.A., Bartz, J.A., 2002. Chlorine use in produce packing lines. Publ. HS-761. Horticultural Sciences Department, Florida Cooperative Extension Service, University of Florida/IFAS, Gainesville.

Roberts, T.A., Pitt, J.I., Farkas, J., Grau, F.H., 2005. Microorganisms in food 6: Microbial Ecology of Food Commodities 2, 379-389.

Rodriguez-Romo, L.A., Yousef, A.E., 2004. Inactivation of *Salmonella enterica* serovar Enteritidis on shell eggs by ozone and UV radiation. Journal of Food Protection 68, 711-717.

Rusin, P., Maxwell, S. and Gerba, C., 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. Journal of Applied Microbiology. 93, 585-592.

Sanyal, D., Douglas, T., Roberts, R., 1997. *Salmonella* infection acquired from reptilian pets. Archives of Disease in Childhood 77, 345-346.

Sargent, S.A., Brecht J.K., Olczy T., 1989. Handling Florida Vegetables Series-Round and Roma Tomato Types SS-VEC-928/VH079. EDIS Horticultural Societies, University of Florida.

Saroj, S.D., Shashidhar, R., Pandey, M., Dhokane, V., Hajare, S., Sharma, A., Bandekar, J.R., 2006. Effectiveness of radiation processing in elimination of *Salmonella* Typhimurium and *Listeria monocytogenes* from sprouts. Journal of Food Protection 69, 1858-1864.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States - major pathogens. Emerging Infectious Diseases 17, 1-9.

Scharff, 2010. Health-related costs from foodborne illness in the United States. Produce safety project at Georgetown University. Accessed at: http://www.producesafetyproject.org/admin/assets/files/Health-Related-Foodborne-Illness-Costs-Report.pdf-1.pdf. Accessed on May 5th, 2010.

Schneider, K.R., Goodrich, R.M., 2008. Dealing with foodborne illness: typhoid fever, *Salmonella* Typhi. University of Florida IFAS extension.

Scott, E., Bloomfield, S.F., 1990. The survival and transfer of microbial contamination via cloths, hands and utensils. Journal of Applied Bacteriology 68, 271-278.

Senter, S.D., Cox, N.A., Bailey, J.S., Forbus, W.R., 1985. Microbiological changes in fresh market tomatoes during packing operations. Journal of Food Science 50, 254-255.

Sewell, A.M., Farber, J.M., 2001. Foodborne outbreaks in Canada linked to produce. Journal of Food Protection 64, 1863-1877.

Shere, L., Kelley, M.J., Richardson, J.H., 1962. Effect of bromide-hypochlorite bactericides on microorganisms. Applied and Environmental Microbiology 10, 538-541.

Shi, X., Namvar, A., Kostrzynska, M., Hora, R., Warriner, K., 2007. Persistence and growth of different *Salmonella* serovars on pre-and postharvest tomatoes. Journal of Food Protection 70, 2725-2731.

Sinde, E., Carballo, J., 2000. Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethlyene: the influence of free energy and the effect of commercial sanitizers. Food Microbiology 17, 439-447.

Solomon, E.B., Yaron, S., Matthew, K.R., 2002. Transmission of *Escherichia coli* 0157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied and Environmental Microbiology 68, 397-400.

Stenstrom, T.A., 1989. Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. Applied and Environmental Microbiology 55, 142-147.

Strawn, L.K., Danyluk, M.D., 2010. Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh and frozen cut mangoes and papayas. International Journal of Food Microbiology 138, 78-84.

Synder, O.P., 1997. A "safe hands" hand wash program for retail food operations. St. Paul, MN: Hospitality Institute of Technology and Management. Accessed at http://www.hi-tm.com/Documents/Handwash-FL99.html. Accessed on July 20th, 2010.

Taormina, P.J., Beuchat, L.R., Erickson, M.C., Ma, L., Zhang, G., Doyle, M.P., 2009. Transfer of *Escherichia coli* O157:H7 to iceberg lettuce via simulated field coring. Journal of Food Protection 72, 465-472.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., Wachsmuth K., 1997. Microbial hazards and emerging issues associated with produce: A preliminary report to the national advisory committee on microbiologic criteria for foods. Journal of Food Protection 60, 1400-1408.

Tauxe, R.V., 1996. An update on *Salmonella*. Health and Environment Digest 10, 1-4.

Tauxe, R.V., 1997. Emerging foodborne diseases: an evolving public health challenge. Emerging Infectious Diseases 3, 425-434.

Tauxe, R.V., Hughes, J.M., 1996. International investigation of outbreaks of foodborne disease. British Medical Journal 313, 1093-1094.

Tindall, B.J., Grimont, P.A.D., Garrity, G.M., Euzeby, J.P., 2005. Nomenclature and taxonomy of the genus *Salmonella*. International Journal of Systemic and Evolutionary Microbiology 55, 521-524.

Todd, E.C.D., Greig, J.D., Bartleson, C.A., Michaels, B.S., 2009. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. Journal of Food Protection 72, 202-219.

Ukuku, D.O. and Fett, W.F., 2002. Relationship of cell surface charge and hydrophobicity to strength of muskmelon fruit. Journal of the American Society for Horticultural Science. 101, 412-415.

Ukuku, D.O. and Sapers, G.M., 2001. Effect of sanitizer treatments on *Salmonella* Stanley attachment to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. Journal of Food Protection. 65, 1093-1099.

United States Department of Agriculture (USDA), Economic Research Services, 1997. Food safety concerns for US fruits and vegetables. Agricultural Outlook Achieves. Accessed at http://www.ers.usda.gov/Publications/AgOutlook/Archives/. Accessed on July 29th, 2011.

United States Department of Agriculture (USDA), Economics, Statistics, and Marketing Information System, 2008a. Accessed at http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1212%20(29%20October%202008). Accessed on April 29th, 2011.

United States Department of Agriculture (USDA), Economics, Statistics, and Marketing Information System (USDA). 2008b. Accessed at http://usda.mannlib.cornell.edu/usda/current/FTS-yearbook/FTS-yearbook-10-30-2008.pdf. Accessed on April 23rd, 2011.

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesus, J., Platt, D.J., Olsen, J.E., 2000. Host adapted serotypes of *Salmonella enterica*. Epidemiology and Infection 125, 229-255.

VanSickle, J.J., Hodges, A.W., 2008. U.S. production trends and the impact of the Florida fresh market tomato industry to the economy of Florida., in EDIS Food and Resource Economics, University of Florida.

Wade, W.N., Beuchat, L.R., 2003. Metabiosis of proteolytic moulds and *Salmonella* in raw, ripe tomatoes. Journal of Applied Microbiology 95, 437-450.

Wei, C.I., Huang, T.S., Kim, J.M., Lin, W.F., Tamplin, M.L., Bartz, J.A., 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. Journal of Food Protection 58, 829-836.

Weissinger, W.R., Chantarapanont, W., Beuchat, L.R., 2000. Survival and growth of *Salmonella* Baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. International Journal of Food Microbiology 62, 123-131.

Wells, J.M., Butterfield, J.E., 1999. Incidence of *Salmonella* on fresh fruits and vegetables affected by fungal rots and physical injury. Plant Diseases 83.

Werber, D., Dreesman, J., Feil, F., Van Treeck, U., Fell, G., Ethelberg, S., Hauri, A., Roggentin, P., Prager, R., Fisher, I., Behnke, S., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschape, H., Kramer, M., Ammon, A., 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infectious Diseases 5, 7.

Winfield, M.D., Groisman, E.A., 2003. Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*. Applied and Environmental Microbiology 69, 3687-94.

Zahara, M., Johnson, S., 1981. Cost comparison of hand and mechanical harvest of mature green tomatoes. California Agriculture 35, 7-9.

Zhuang, R.Y., Beuchat, L.R., 1996. Effectiveness of trisodium phosphate for killing *Salmonella* Montevideo on tomatoes. Letters in Applied Microbiology 22, 97-100.

Zhuang, R.Y., Beuchat, L.R., Angulo, F.J., 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. Applied and Environmental Microbiology 61, 2127-2131.

BIOGRAPHICAL SKETCH

Pardeepinder Kaur Brar was born in the city of Punjab, India. Her father is working as revenue officer of Punjab state and mother is a school teacher. She started her undergraduate degree program in agriculture at the Punjab Agricultural University Ludhiana, Punjab, India in 2004. Throughout her degree she was awarded with a University Merit Scholarship. She received an award in the last year of her degree for highest Overall Credit Point Average in, her major, food science and technology. All through her college years, she participated in many campus activities and gained a lot of honors in sports and folk dances.

In spring 2009, Pardeepinder started her Master of Science in the food science program under the instruction of Dr. Michelle Danyluk at the University of Florida. She worked in a short project in the beginning of master's degree to get familiarize with different skills. Her short project focused on 'Efficacy of aqueous and alcohol based quaternary ammonium sanitizers for reducing *Salmonella* in dusts generated in almond hulling and shelling facilities'. She has participated in various departmental activities at UF like College bowl and product development. Pardeepinder is planning on continuing her education in Food Safety through a PhD.