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Transfer of *Escherichia coli* while using Salad Tongs

Bartz M, Buyukyavuz A, Dawson E, Diener M, Gates R, Han I, Johnson L, Marvin P, Musselwhite C, Nicholson A, Randar D, Ritterpusch M, Zazzara M and Dawson P*

Department of Food, Nutrition and Packaging Science, Clemson University, Clemson, SC, USA

***Corresponding author:** Paul Dawson, Department of Food, Nutrition and Packaging Science, Clemson University, Clemson, S.C, USA, Tel: 864-656-1138; E-mail: pdawson@clemson.edu

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Abstract

The transmission of infectious disease often involves the touching of surfaces by multiple people which can include public and institutional eating facilities. In the current study, the transfer of *Escherichia coli* from inoculated hands to salad bar tongs (experiment 1) and from inoculated tongs to hands (experiment 2) was determined in separate experiments. Transfer of *E. coli* averaged approximately 10% from hands to tongs and around 5% from tongs to hands. However, the transfer was as high as over 50% from both hands to tongs and tongs to hands. Handling of food bar tongs by multiple individuals could result in the transfer of bacteria and viruses between individuals and the spread of infectious agents.

Keywords: Cross contamination; Salad bars; Tongs; Bacteria; *E. coli*

Introduction

The U.S. Centers for Disease Control (CDC) estimates that each year roughly 1 in 6 Americans (or 48 million people) gets sick. Out of these sick Americans, 128,000 are hospitalized, and 3,000 die of foodborne diseases (CDC, 2011) [1]. According to the Economic Research Service (ERS) of the USDA, $6.9 billion in costs are spent each year in response to five different bacterial pathogens: *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7, and Shiga-toxin producing *E. coli* non-O157:H7 (STEC). These costs are associated with medical expenses, lost productivity, and death (USDA, 2014) [2]. With these losses in both money and lives, there is much concern over the cleanliness and sanitation of restaurants, cafeterias, and buffets. Pathogenic bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant enterococci (VRE) were found to survive on various fabrics generally for days but up to 90 days 100% of the time on cotton, polyester and polypropylene [3], making these materials potential carriers of pathogens in food establishments. Furthermore, da Silva et al. [4], reported that *S. aureus* was transferred to stainless steel and polyethylene immediately upon contact with meat cubes and that populations increased from 3 to 8 log cycles within 24 hours at 20°C. Restaurant nonfood contact surfaces including tables, chairs, highchairs, booth seats and booster seats were found to have over 100 colony forming units per 100 cm^2 indicating the need for more information on their possible role in food cross contamination [5].

Cross contamination in food service is highlighted as a preventive measure by the National Restaurant Association (NRA, 2015) [6]. Sources of cross contamination during the preparation of food are well known and easily prevented, but sources after a food is prepared and served might be overlooked, specifically by consumers. For example, Lynch et al. [7] reported that 9.6 and 4.4 % of tortillas carried coliform bacteria when handled by food service workers using gloves and bare hands, respectively. Overall these researchers found no significant difference in bacterial contamination due to wearing gloves. At buffets, dining halls, and cafeterias, multiple people handle the same utensils, which could lead to the spread of disease in utensils. *E. coli* O157:H7 was found to be able to attach to stainless steel, pure titanium, and glass [8]. In a study of surfaces in cafeterias in public schools, the bacteria count was determined and an ATP bioluminescence test was performed for each surface. Both of these tests showed that bacteria were present on surfaces like countertops, cutting boards, blenders, dishes, and refrigerators de Oliveira et al. [9].

Previous studies have focused on food service workers and food contact services as points for cross contamination but no studies were found examining cross contamination between consumers at eating establishments. This research attempts to fill this gap in information concerning bacterial transfer at self-serve eating stations such as salad bars. In this study, the rate of bacterial transfer from contaminated hands to stainless steel serving tongs and from tongs to hands was determined by inoculating hands (or tongs) and then handling tongs similar to those used at eating establishments. The overall goal of the research was to determine if the amount of bacteria that transferred from hands to tongs and from tongs to hands was a cause of concern for the consumers.

Materials and Methods

In this study a non-pathogenic *Escherichia coli* strain JM109 labeled with jellyfish green fluorescent protein Jiang et al. [10] was used as the bacteria for transfer. The competent bacterial cells were electroporated in a Gene Pulser II (Bio-Rad) with plasmid vector pGFPuv (ClonTech, Palo Alto, CA). Transformants were selected from isolated colonies grown on Luria-Bertani agar (LB) plates containing 100 g of ampicillin/mL. The resulting ampicillin-resistant transformants emitted bright green fluorescence under a handheld UV light. The stability of GFP label in *E. coli* was determined by streaking on trypticase soy agar (TSA) plates containing 100g ampicillin/mL for several generations. The *Escherichia coli* JM 109 culture was held in a -80°C freezer in vials containing tryptic soy broth (Becto™ Tryptic Soy Broth, Becton Dickinson and company Sparks, MD, USA) supplemented with 20% (v/v) glycerol (Sigma, St. Louis, MO, USA). The frozen vial was thawed at room temperature prior to culturing. From this thawed vial, 0.1 ml of culture was transferred to 10 ml TSB

(DIFCO) containing 0.5% ampicillin (Sigma, St Louis, MO, USA) in 2 loosely screw-capped tubes and then the tubes were incubated for 16-18 h at 37°C with vigorous shaking (Thermolyne Maxi-Mix III type 65800, Barnstead/ Thermolyne, Dubuque, IA). The second transfer was prepared from this first transfer culture by adding 0.1 ml from the first transfer tube to another fresh 10 ml TSB (DIFCO) with 0.5% ampicillin (Sigma), and again incubated for 16-18 h at 37°C with shaking.

After incubation, the cells were harvested by centrifugation at 3000 rpm (1200 g) (IEC HN-SII Centrifuge, International Equipment CO., Inc., Needham Heights, MA, USA), then the pellet resuspended in 10 mL of sterile peptone solution (0.1%) (Bacto peptone, Becton Dickinson) to obtain a population of approximately 6-7 log CFU/ml. Initial cell populations were verified by enumeration of the cells following surface plating in TSA containing 0.5% ampicillin (DIFCO™ Tryptic Soy Agar, Becton Dickinson and company Sparks, MD, USA) and incubating at 37°C for 24 h. The approximate number of bacteria used per inoculation of tongs and hands were 10^6 CFU/ml.

Experiment 1: Bacterial Transfer from Hands to Tongs

Subjects' hands were washed with warm water and soap, dried and then 1mL of the *E. coli* inoculum was deposited in the center of their dominate hand. The *E. coli* was spread onto both hands by rubbing hands together for 30 seconds than allowed to air dry for 30 seconds. Each subject then picked up tongs, one in each hand, squeezed the tongs, and then placed the tongs onto a sterile surface. This step is then repeated five times and then the tongs are placed into separate stomacher bags, each with 20 mL of sterile 0.1% peptone. The tongs and peptone were mixed for 30 seconds in the bag. Both the right and the left hands of each subject were then placed into the sterile stomacher bag with 20 mL of sterile 0.1% peptone. Hands were massaged for 30 seconds with the peptone solution being swirled around the inoculated hand making contact with all fingers, palm, and back of the hand. Then the tong and hand rinsates were serially diluted then plated in duplicate on TSA agar, and evenly spread on the agar surface. These plates were incubated at 35°C for 24 h then duplicate plates from dilutions having between 25-250 colonies were counted under UV light and converted to and reported as colony forming units (CFU) per tong/hand. Initial bacterial population on hands was calculated by adding the bacterial population found on the tongs and the population found on hands together.

The same protocol described above was also conducted for control samples except no *E. coli* inoculum was included.

Experiment 2: Bacterial Transfer from Tongs to Hands

Subjects' hands were washed with warm water and soap, dried. A sterile tong was placed in a sterile bag containing 20 mL of the *E. coli* inoculum then the tong was inoculated by shaking the bag for 30 sec and allowed to air dry for 30 seconds. Each subject then picked up tongs, one in each hand, squeezed the tongs, and then placed the tongs onto a sterile surface. This step is then repeated five times and then the tongs are placed into separate stomacher bags, each with 20 mL of sterile 0.1% peptone. The tongs and peptone were mixed for 30 seconds in the bag. Both the right and the left hands of each subject were then placed into the sterile stomacher bag with 20 mL of sterile 0.1% peptone. Hands were massaged for 30 seconds with the peptone solution being swirled around the inoculated hand making contact with all fingers, palm, and back of the hand. Then the tong and hand rinsates were serially diluted then plated in duplicate on TSA agar, as described for Experiment 1.

Bacterial enumeration

The bacteria were counted 24 hours after plating by identifying CFUs under a UV light. The fluorescence in the *E. coli* was used to identify the colonies that were derived from the initial hand or tong inoculation. The percentage of bacteria transferred was calculated using the following formulae:

Experiment 1

$$\%\ \text{transfer from hands to tongs} = \frac{(\text{hand population} + \text{tong population}) - \text{tong population}}{\text{tong population}} \times 100$$

Experiment 2

$$\%\ \text{transfer from tongs to hands} = \frac{(\text{hand population} + \text{tong population}) - \text{hand population}}{\text{hand population}} \times 100$$

Statistical analysis

The bacterial transfer from hands to tongs experiment was replicated three times for each hand by 12 different subjects for each treatment yielding 38 total observations. The bacterial transfer from tongs hands was replicated 8 times using the dominant hand by 8 subjects yielding 74 total observations. Simple means and standard deviations were determined for each treatment using the Statistical Analysis System.

Results and Discussion

Experiment 1: Bacterial transfer from hands to tongs

For the total of 76 observations (38 left and 38 right hand), there was transfer of bacteria 100% of the time. The *E. coli* inoculum carried approximately 10^6-10^7 cells/ml thus of the 1 ml placed on the subjects hands, an average of between 10^5 and 10^6 cells were recovered per hand Table 1. After handling tongs with inoculated hands an average of between 10^4 -10^5 cells were recovered from tongs being transferred from hands to tongs at about a 10-14% rate. There was significant variation in bacterial counts on hands and bacteria transferred from hands to tongs. Viral diseases are particularly contagious with only one and one hundred noroviruses capable of causing illness Wang et al. [11]. In a study published in 2012, the transfer of the human norovirus from produce to utensils was tested and found that between 0.9 and 5.1 log PFU (plaque-forming unit, synonymous with a colony-forming unit for bacteria) was transferred to the knives Wang et al. [11]. The bacteria that was recovered on the hands from this study was much higher than the viruses that were recovered from the knives in Wang's study Wang et al. [11], while tongs recovered slightly fewer CFU than the PFU reported in Wang's study, on average 4.37 log CFU on the left tong and 4.19 log CFU on the right tong Table 1.

	Inoculation left hand[1]		Inoculation right hand[2]		Recovery left tong[3]		Recovery right tong[4]		Transfer LT[5]	Transfer RT[6]
	cfu/hand[1]	log cfu/hand	cfu/hand	log cfu/hand	cfu/tong	log cfu/tong	cfu/tong	log cfu/tong	(%)	(%)
Mean	322877	5.51	234325	5.37	19349	4.29	12933	4.11	11.8	9
Stand dev	398100	5.6	264081	5.42	18381	4.26	10662	4.03	12.6	9.9
Median	168700	5.23	149300	5.17	16400	4.21	11900	4.08	9.9	6.2
Maximum	1340200	6.13	959400	5.98	67200	4.83	39400	4.6	66.4	51.6
Minimum	2120	3.33	2040	3.31	40	1.6	20	1.3	0.4	0.1

Table 1: Bacteria recovered and percentage of bacteria transferred from hands inoculated with *Escherichia coli* to tongs. [1]Inoculation left hand– bacteria recovered from inoculated left hands; [2]Inoculation right hand – bacteria recovered from inoculated right hands; [3]Recovery left tong – bacteria recovered from tongs handled with inoculated left hands; [4]Recovery right tong – bacteria recovered from tongs handled with inoculated right hands; [5]transfer LT - % of bacteria transferred to tongs from left hands; [6]transfer RT - % of bacteria to tongs from right hands. N=38.

Experiment 2: Bacterial transfer from tongs to hands

Since there was no significant effect of right and left hand on bacterial transfer, these treatments were combined. In 6 out of the 76 observations there was no detectable transfer of bacteria from tongs to hands thus there was transfer 92% of the time. With an average starting population of over 5 logs on tongs, an average of nearly 4 log cycles were transferred to hands (4.6%) after tongs were handled (Table 2).

	Tong Inoculation CFU		Hand CFU		Percent transfer[5]
	cfu/tong[1]	log cfu/tong[2]	cfu/hand[3]	log cfu/hand[4]	
Mean	187083	5.27	9461	3.98	4.6
Stand deviation	138247	5.14	17569	4.24	7.8
Median	146000	5.16	4000	3.6	1.9
Maximum	580000	5.76	106000	5.03	56.1
Minimum	28000	4.45	0	0	0

Table 2: Bacterial recovered and percentage of bacteria transferred from tongs inoculated with *Escherichia coli* to hands. [1]cfu/tong – colony forming units of bacteria recovered per inoculated tong; [2]log cfu/tong - log10 of colony forming units of bacteria recovered per inoculated tong; [3]cfu/hand – colony forming units of bacteria recovered per hand after handling inoculated tongs; [4]log cfu/hand - log10 of colony forming units of bacteria recovered per hand after handling inoculated tongs; [5]Percent transfer- % of bacteria transferred to hands from inoculated tongs. N=76.

The sanitation of communal utensils and dishware being used by large groups of people can impact the threat of foodborne outbreaks within a community [12-14]. The need for proper sanitation of utensils, especially in public eating areas, should be obvious since diseases associated with sputum include but are not limited to influenza, tuberculosis, pneumonia, scarlet fever, diphtheria, whooping cough, trench mouth, typhoid, dysentery, human noroviruses and hepatitis A virus. The infective agents causing these diseases are transmitted by direct and indirect contact from an infected case or carrier among the patrons or personnel of the establishment Ronnqvist et al. [15], found that human noroviruses were transferred from hands and gloved hands to cucumbers and utensils (knives) 10/12 times during sandwich preparation. These researchers also found that noroviruses were transferred from cucumbers and knives back to hands. In the current experiment it was found that the transfer of bacteria from hands to tongs and from tongs to hands was significant enough to raise concern about the possibility of transferring harmful bacteria among a population. When microorganisms, not causing harm to the individual carrier, comes into contact with others, a microbe may become infectious, so the need to sanitize or prevent the spread of disease is important in areas of high traffic or on substances that come into contact with large groups of people like eating utensils in restaurants, more specifically college campus dining halls. The current study tested the transferability of *E. coli* from hands to stainless steel tongs and from tongs to the hands and found that tongs and hands can contract a significant amount of bacteria in both scenarios. In Experiment 1, tongs accrued on average about twenty-three thousand colony forming units for the left tong and about fifteen thousand colony forming units for the right tong, or about 10% of the bacteria from inoculated hands. In Experiment 2, an average of 5% of the bacteria on inoculated tongs were transferred to hands. However, a maximum of over 50% of bacteria on hands were transferred to tongs and also from tongs to hands.

According to Tsuji and Yokoigawa, out of multiple abiotic surfaces stainless steel, the material the tongs in this experiment consisted of, was the easiest surface to which the bacteria could attach [8]. Therefore, though stainless steel was the material with the most bacteria due to its highly habitable surface, the hands still accumulated more bacteria, improving the likelihood of bacterial transfer.

Conclusions

There is a lack of control on hand sanitation of customers in public eating locations and those that have self-serve stations such as salad bars create possible scenarios for cross contamination between customers. Based on the levels of bacterial transfer found in this study, it is evident that the need for controlling cross-contamination in food handling is necessary to reduce the risk of foodborne illness. The current study was a controlled laboratory study thus did not survey levels of bacteria found on serving utensils in public eating areas.

Studies examining the levels of and types of bacteria found on serving utensils used by multiple consumers as eating establishments would increase the knowledge of potential risk of food borne illness.

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