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Bacterial transfer from mouth to different utensils and from utensils to food

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**BACTERIAL TRANSFER FROM MOUTH TO UTENSILS AND
FROM UTENSILS TO FOOD**

**A Thesis
Presented to
the Graduate School of
Clemson University**

**In Partial Fulfillment
of the Requirements of the Degree
Masters of Food Science, Nutrition & Culinary Science**

**by
Chaitali Purohit
August 2009**

**Accepted by:
Dr. Paul L. Dawson, Committee Chair
Dr. Julie Northcutt
Dr. Xiuping Jiang**

ABSTRACT

This study examined transfer of bacteria from mouth to different surfaces (spoon, chopstick, hand) and from surfaces to food (chicken broth, rice). Three different sets of experiments were conducted. In the first experiment, bacterial transfer from mouth to utensils (spoon or chopstick) was determined. The second experiment measured bacterial transfer from mouth to broth and included scooping and stirring with a spoon. In the third experiment, bacterial transfer from mouth to food rice was tested using either spoon or hand. Ten or seven subjects were used for each of the three experiments. Results indicated that there was a transfer of approximately $5 \log_{10}\text{cfu}$ of total bacteria to the spoon or chopstick when either was placed in the mouth with or without food. Between 4 and 5 log cycles of bacteria were transferred to broth when the spoon was placed in the mouth six times while eating. Approximately 1 million bacteria were transferred each time from mouth to rice by using hands. More than 5 log cycles were transferred to the rice when the spoon was used to consume the rice then placed back in the bowl for 5 cycles. There were high numbers of bacteria transferred to the common bowl of rice when a spoon was used however much lower number than when the hand was used. The overall conclusion was that significant bacterial transfer from mouth to utensils (spoon or chopstick) and from mouth to food occurred when utensils or hands were placed back into food after consumption.

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Chapter 1 Introduction

Cross contamination is the transfer of bacteria from one surface or medium to another.(www.pittwater.nsw.gov.au). The most factors in bacterial transfer from one surface to another are moisture, contact time and pressure which can result in higher transfer between surfaces. Bacterial transfer studies can be divided into two groups based on the degree of experimental control. The first category includes studies with a great degree of control and is typically conducted in a laboratory. Control of some factors while varying others allows the determination of certain environmental factors on bacterial transfer rate. For example, longer contact time leads to greater coalescence and more interactions on recipient surfaces and that leads to higher transfer of bacteria (Dawson et al., 2006). A second type of experiments is studies performed outside the laboratory in food environments. Bacterial transfer studies are useful in indentifying contamination routes in processing environment such as factories, food service operations, and domestic, kitchen, etc. (Perz-Rodriguez, 2007).

Factors affecting bacterial transfer can be divided into two groups: environmental and intrinsic factors. The first group includes surface material properties, presence of bio-fouling layers, moisture availability, contact pressure and contact time. The second group includes factors unique to bacterial species such as exopolysaccharide layers, biofilm forming ability, clump formation and the presence of extracellular structures. Environmental factors include adherence of bacteria enhanced by surface structural hydrophilic and hydrophobic properties. Bacterial attachment depends upon the degree of surface roughness with rough surfaces having a lower level of bacterial transfer

initially but a greater degree of adhesion. When bacteria colonize on a rough surface, they are no longer in a direct contact with the transfer surface and thus are not easily transferred (Perez-Rodriguez, 2007). The concentration of bacteria on the surface or in an inoculum can also affect bacterial transfer (Montville and Schaffner, 2003). Montville and Schaffner (2003) reported that the higher the inoculum size on the source surfaces the lower the transfer rate.

Hands can be a surface upon which bacteria reside for cross contamination to another medium. Contaminated hands are a major source of bacterial transfer in food processing and preparation. Microbial flora found on hands has been categorized in to two types: resident and transient. The resident microflora consists of organisms that normally are always present on the skin. These are mainly found on the surface of the skin under the superficial cells of the stratum corneum. They are not typically considered pathogens but may cause infections in body cavities such as the eyes. Resident bacteria can survive longer on intact skin than do gram negative transient species. The transient skin flora consists of bacteria, fungi and viruses that may sometimes be found on skin. Usually they do not multiply on the skin but they can survive and cause disease. The transmissibility of transient bacteria depends on the species, number of cells on the hand, their survival on the skin and the dermal moisture content. Temporary resident microflora flora multiplies and persists for a limited period on the skin (Kampf and Kramer, 2004). Good personal hygiene and scrupulous hand washing can reduce the transfer of fecal microorganisms from hand to mouth and may prevent the spread of potentially transient

microorganisms (Shojaei et al., 2006; Allowed et al., 2004; Daniels et al., 2002; Fry et al., 2005; Sneed et al., 2004).

Cross contamination by microbial pathogens in the kitchen environment play an important role in sporadic and epidemic food borne illnesses. Hands are potentially a critical control point for reducing or preventing bacterial cross contamination from ill and asymptomatic food workers who might shed high levels of pathogens particularly those originating from the nasal cavity. Sharing of food may also be responsible for cross contamination and may lead to a higher number of food borne illness outbreaks. Sharing food from the same utensils is traditional in many Asian cultures (India, Japan, China, and Pakistan) and utensils used for the ethnic cuisine may also support cross contamination. The present study focuses on the bacterial transfer from mouth to utensils (spoon, chopstick) and from utensils to food (chicken broth, rice). This research also addresses bacterial transfer from mouth to food (rice) via hand and spoon. Results from this study may play a role in documenting the potential for cross contamination where sharing food is practiced.

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Chapter 2 Review of Literature

Consumption of contaminated food may result in illness or even death and is considered a serious worldwide public health problem. Microbiological cross contamination from improper personal hygiene is a significant factor in food borne illness incidence. Factors that cause foodborne illness events include an agent (microorganisms), source (surface), mode of transmission (contact) and host (human). Pathogenic microorganisms can contaminate food from food prepared by an infected person (person to person spread), through the air, or by insects, pests, rodent or pets. In some cases the disease causing microorganisms can remain with the person after recovery. A person with this condition is known as carrier. One of the most infamous cases of a ‘carrier’ occurred in the early part of twentieth century by Mary Mallon (Typhoid Mary) whom was later identified as a chronic carrier for transmission of the typhoid fever bacteria. (Marriot, Roberts & Gravari, 2006). ‘Typhoid Mary’ was responsible for the transmission of typhoid fever due to her poor personal sanitary habits. Fingers can transfer bacteria through touching equipment, contaminated food, clothing or other areas of the body. Finger nails, jewelry, nose, eyes, ears can also lead to bacterial transfer since these can be reservoirs for pathogens. Recovery of typhoid bacteria in Mallon’s stool verified the important role personal hygiene plays in the transmission of disease. Handling of food (person to person spread) by a colonized person is a frequently identified factor that contributing to typhoid fever, shigellosis and staphylococcal food poisoning. Twenty percent of reported salmonellosis and 22% of *Vibrio parahaemolyticus* gastroenteritis are due to cross contamination (WHO, 2001). Cross contamination during preparation of raw

chicken has contributed towards outbreaks of salmonellosis and infections related to *Campylobacter* and *Staphylococcus aureus*. Factors such as pH, oxidation-reduction potential, and lack of inhibitors contribute to the survival and growth of microorganisms. Humans are a major source of food cross contamination. Hands, breath, hair and perspiration contaminate food. Coughing and sneezing are also responsible for the transmission of bacteria. Illnesses such as respiratory tract infections, the common cold, fever, sore throat, intestinal disorders also occur due to cross contamination. The mouth plays an important role in the transfer of bacteria. During sneezing, bacteria are transferred to the air and may land on hands or on food. Spitting may also be a mode of bacterial transmission and product contamination.

Improper handling and sanitation practices lead to person-to-person, person to food and utensils to food cross contamination that ultimately results into 27% of reported outbreaks and infection from food borne pathogens (WHO, 2001).

After preparation of artificially contaminated chicken, target organisms were found on utensils and surfaces in contact with the contaminate food (Wit et al., 1979). Surfaces play very important role in the transmission of bacteria from hand or cloth to other surfaces (Scott and Bloomfield, 1990). *Campylobacter* and *Salmonella* were recovered from hands and contact surfaces following food preparation of meat and chicken (Cogan et al., 1999).

A primary line of defense against any kind of microorganisms is good hand hygiene. Approximately 38% of food contamination is related to inadequate hand

washing. Hand washing for 15 sec with soap and water will remove transient bacteria. Hand washing and drying efficacy against resident micro flora ranges from 35 to 60%. Drying of hands with paper towels makes this process more effective in removing pathogenic bacteria from hands compared to only manual hand washing (Marriot, 2006). A 10 to 20 sec massage cycle has been clinically proven to be 60% more effective than a non massage washing (Marriot, 2006). Combination of antimicrobial soap followed by sanitizer leads to significant reduction of bacteria on the hand. Systemic evaluation of the risk associated with different hand washing techniques indicates that proper hand washing can reduce the risk of bacterial contamination on hands. Experimental data on quantitative risk assessment suggest the primary factors influencing final bacterial counts on hands are sanitizer, soap and drying method (Montville, 2002). Gloves which are not changed frequently can become a source of cross contamination. Hand washing is still essential even after wearing gloves as the microorganisms on skin can multiply and can lead to cross contamination similar to unwashed hand (Lues and Tonder, 2007). Improper hand washing techniques can lead to transfer of bacteria and hand transmitted nosocomial infection. Hand drying is a critical step in the hand washing process and needs to be implemented in a correct manner to reduce the chance of cross contamination. Drying should be effective so that contamination of hands does not take place.

Recovery of organisms from surfaces is influenced by numerous factors such as surface type, transfer medium, temperature, relative humidity, degree of drying, light ,the presence of disinfectants and/or competing organisms (Harrison et al.,2003).

The importance of contaminated surfaces in potential transfer of pathogens to food is apparent in food processing, catering and the domestic food preparation /handling environment. Exposure of pathogens takes place by direct contact with contaminated objects or indirectly through airborne particles. The risk of food borne infection is associated with cross contamination which mainly depends on two factors; the level of contamination on surfaces and the likelihood that the contaminating bacteria will be consumed (Kusumaningrum et al., 2003) . *S. Enteritidis*, *S. aureus* and *C.jejuni* may be viable on dry stainless surfaces for hours (*C.jejuni*) or days after contamination (*S. Enteritidis* and *S. aureus*) with survival being dependent on the initial number of the contaminating microorganisms. These bacteria may be easily transferred from kitchen surfaces or utensils to food. The factors affecting the survival of *Campylobacter*, *Salmonella* and *E.coli* O157:H7 during a typical hand washing process influence the potential for transfer of bacteria to sites in the kitchen (Kusumaningrum et al., 2003). Survival of *Campylobacter* was poor in comparison to *Salmonella* and *E. coli* when dirty dishes were left to air dry. There is a relatively small risk of viable bacteria surviving the washing and drying process on washed surfaces but bacteria are capable of contaminating tea towels or sponges having implications for domestic hygiene.

Infectious diseases in the home and community are a serious public health problem in the developed and developing country. Good hygiene is a key component for reducing the burden of infectious diseases. The impact of personal hygiene in reducing infectious diseases can be increased by convincing people to practice appropriate hand hygiene procedures. For the optimization of health benefits, determining proper hand

hygiene practices must be accompanied with hygiene education along with promotion of other aspects of hygiene like surface and cloth hygiene (Bloomfield and Aiello, 2007). Improvement in the hand hygiene practices of health care workers has significantly reduced the transmission of disease over the past few years. These reductions in bacterial transfer from health workers to patients are related to the use of an alcohol based sanitation method (Sax, 2007). Alcohol based hand rubs are preferred over standard hand washing with soap and water they serve as primary mode of hand disinfection in dermatology offices due to their broad spectrum antimicrobial coverage (including *S. aureus*, *P. aeruginosa*, *Klebisella* spp. and Rotavirus), rapid activity, good spreadability, convenience (lack of a sink being required for their use) and patient bedside availability. Alcohol based hand rubs are as effective as other hand hygiene products (chlorhexidine and triclosan) with better tolerance, reasonable cost and fewer adverse side effects on the skin. Hand washing with antiseptic soaps is still preferred when hands are visibly or highly soiled (Messina, 2008).

In the home, a major concern is the transmission of food borne pathogens by cross contamination of food via food contact surfaces. These surfaces include cutting boards which play an important role in food cross contamination and are considered to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Messina, 2008). Large numbers of bacteria can be transferred to cutting boards after use with raw chicken. These bacteria can survive for more than four hours on cutting boards and can be transferred to other foods if the cutting board is not properly cleaned (Zhao et al., 1998). Many antimicrobial products have been developed to provide fast and effective

sanitation in food preparation areas and have replaced the traditional two step detergent and rinse cleaning method. Antimicrobial dishwashing was found effective in reducing pathogens in laboratory suspensions test, but was not found to be effective on sponges. Antimicrobial products are effective in disinfecting food preparation surfaces only if products instructions are carefully followed (DeVerre, 2007).

Dental caries are a complex disease involving microbial plaque communities on tooth surfaces. This disease includes destruction of susceptible dental hard tissue by acidic bacterial by products. The disease process is initiated within the bacterial biofilms (dental plaque) that covers the tooth surface. It is a multifactorial disease that starts with a biological shift within the complex biofilms and that is affected by salivary flow, saliva composition, exposure to fluoride, composition of dietary sugars and preventive measures such as teeth cleaning (Selwitz, 2007). Bacteria and viruses are key etiologic factors in the development of periodontal disease, with local, systemic, genetic and environmental susceptibility factors playing important roles (Kamma et al.,2004). There is a direct correlation between carious lesions and *S.mutans*, *Lactobacilli* in saliva. *Lactobacilli* are cariogenic bacteria (Koll Klais et al., 2005) and these cariogenic microorganisms are indicators of caries. *Lactobacilli* and *S.mutans* are responsible for the tooth decay and they are present on the all teeth with carious lesions (Coogan, 2008). *Lactobacilli* make up approximately 1 percent of the cultivable oral microflora with the most common species being hetrofermentative. *Lactobacilli* such as *Lactobacillus casei* and *Lactobacillus fermentum* and the homofermentative *Lactobacillus salivarius* are the major types found (Koll Klais et al., 2005). *A.israeli* has been identified as producing

the most carcinogenic biofilms which produces the most demineralization in incipient carious lesions in the tooth cementum (Yip, 2007).

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Chapter 3 Manuscript

Title: Transfer of bacteria from mouth to different utensils and from utensils to food.

Authors: Chaitali Purohit, Paul Dawson, Inyee Han

Key Words: Cross contamination, bacterial transfer, food borne pathogens

Abstract

Three different sets of experiments were conducted to determine the transfer of bacteria from mouth to different utensils (spoon, chopstick, hand) and from utensils to food (chicken broth, rice). In the first experiment set, bacterial transfer from mouth to utensils (spoon or chopstick) was tested. In second experiment set, bacterial transfer from mouth to broth was determined when a spoon was used for scooping and stirring. In the third experiment set, bacterial transfer from the mouth to rice was measured when either a spoon or hand was used to consume the rice. Over 5 logs of total aerobic bacteria were transferred to the spoon and chopstick when the utensil was placed in the mouth with or without food. Between 10^4 and 10^5 bacteria were transferred to broth when the spoon was placed in the mouth six times. 1 million bacteria were transferred to the rice each time hands were used to pick up rice. Over 10^5 bacteria were transferred to the rice when the spoon was used to consume the rice then placed back in the bowl for 5 cycles. There were high numbers of bacteria transferred to the common bowl of rice when a spoon was used

although there were even more bacteria transferred when the hand was used to consume rice. Overall there was significant bacterial transfer from mouth to utensils (spoon or chopstick) and from mouth to food when utensils or hands were placed back into food after food consumption.

Introduction

Cross contamination is the transfer of bacteria from raw food, unclean utensils or unclean surfaces to ready to eat food, clean utensils or clean surfaces (www.pittwater.nsw.gov.au). Factors affecting the bacterial transfer include pressure, contact area, moisture level, temperature, contact time and personal hygiene. Longer contact time between contaminated and non-contaminated surfaces will lead to higher transfer to the recipient surface (Dawson et al., 2006).

Factors affecting bacterial transfer can be divided into two groups: environmental and intrinsic factors. The first group includes surface material properties, presence of bio-fouling layers, moisture availability, contact pressure and contact time. The second group includes factors unique to bacterial species such as exopolysaccharide layers, biofilm forming ability, clump formation and the presence of extracellular structures. Environmental factors include adherence of bacteria enhanced by surface structural hydrophilic and hydrophobic properties. Bacterial attachment depends upon the degree of surface roughness with rough surfaces having a lower level of bacterial transfer initially but a greater degree of adhesion. When bacteria colonize on a rough surface, they are no

longer in a direct contact with the transfer surface and thus are not easily transferred (Perez-Rodriguez et al., 2008). The concentration of bacteria on the surface or in an inoculum can also affect bacterial transfer (Montville and Schaffner, 2003).

Contaminated hands are a major source of bacterial transfer in food processing and preparation. Microbial flora found on hands has been categorized in to two types: resident and transient. The resident microflora consists of organisms that normally are always present on the skin. These are mainly found on the surface of the skin under the superficial cells of the stratum corneum. They are not typically considered pathogens but may cause infections in body cavities such as the eyes. Resident bacteria can survive longer on intact skin than do gram negative transient species. The transient skin flora consists of bacteria, fungi and viruses that may sometimes be found on skin. Usually they do not multiply on the skin but they can survive and cause disease. The transmissibility of transient bacteria depends on the species, number of cells on the hand, their survival on the skin and the dermal moisture content. Temporary resident microflora flora multiplies and persists for a limited period on the skin (Kampf and Kramer, 2004). Good personal hygiene and scrupulous hand washing can reduce the transfer of fecal microorganisms from hand to mouth and may prevent the spread of potentially transient microorganisms (Shojaei et al., 2006; Allowed et al., 2004; Daniels et al., 2002; Fry et al., 2005; Sneed et al., 2004).

Cross contamination by microbial pathogens in the kitchen environment play an important role in sporadic and epidemic food borne illnesses. Hands are potentially a

critical control point for reducing or preventing bacterial cross contamination from ill and asymptomatic food handlers who might shed high levels of pathogens particularly those originating from the nasal or oral cavity. One recent study (Women's hands home to More Types of Gems-healthfinder.gov) identified that hands harboured an average of 150 bacterial species. Left and right hands of the same individual shared only about 17 percent of the same bacteria types. Differences between men and women might be related to different hormone production, slight variation in pH and skin dryness (Women's hands home to More Types of Gems-healthfinder.gov). Sharing of food may also be responsible for cross contamination and higher number of food borne pathogen related outbreaks. Sharing food from the same utensils in traditional Asian cultures (India, Japan, China, and Pakistan) and utensils used for the ethnic cuisine supports cross contamination. The overall objective of this study was to determine bacterial transfer from mouth to different utensils and to food. The oral cavity is very diverse ecosystem with up to 600 different microbial species. Transfer of oral bacteria to food using different utensils presents a unique cross contamination phenomenon (Papaioannou et al., 2009). Bacterial transfer studies are useful in identifying contamination routes in factories, food service operations, and domestic kitchen (Perez-Rodriguez et al., 2008). The present study of cross contamination and bacterial transfer may open new insights on the safety of sharing food. The specific objectives of the current study were: 1) to determine the amount of transfer of bacteria from the mouth to utensils (spoon or chopstick) and 2) to determine the transfer of bacteria from the mouth to food (broth or rice) using spoon or hand to consume the food.

Material and Methods

Three different sets of experiments were conducted to determine the cross contamination from mouth to utensils (spoon, chopstick) and from mouth to food (broth, rice) using the spoon or hand. Each experiment was replicated 3 times on different days. Utica fine quality 18/0 stainless steel spoons (Utica Fine Quality Cutlery Company, Utica, New York USA) and disposable wooden chopsticks (Home Plus, Flushing, New York USA) were used as utensils. Utensils were sterilized before use. Swanson chicken broth (Campbell Soup Company, Camden New Jersey, USA) and Mahatma extra long grain enriched rice (Riviana Foods Inc., Houston Texas) was used for food samples. Salt concentration of the broth was 4 mg/ml. To reduce the salt concentration, 20 ml of chicken broth was diluted 1:4 with sterile water resulting in a salt concentration in the diluted broth of 1mg/ml and a final volume of 80 ml. Mahatma extra long grain enriched rice was cooked in microwave oven (Magic Chef, USA) for 15 minutes using a 1:2 rice to water ratio.

For experiments 1 and 2, ten subjects (five non smoker males and five non smoker females) and for the experiment 3 seven subjects (four non smoker males and three non smoker females) were used. Subjects were trained to use similar techniques when conducting the experiment.

Experiment set 1 comprised three experiments to determine the transfer of bacteria from mouth to spoon or chopsticks: 1.1. Transfer from mouth to spoon while consuming broth.

1.2. Transfer from mouth to spoon while consuming rice and 1.3. Transfer from mouth to chopstick while consuming rice.

1.1. Transfer from mouth to spoon while consuming broth: this experiment used four treatments: a. spoon control, b. broth control, c. mouth to spoon only, and d. mouth to spoon with broth.

a. Spoon Control (spooncon): A sterile spoon (Utica fine quality 18/0 stainless steel spoon) was placed in a sterile bag containing 20 ml of 0.1% peptone water (Difco laboratories, Detroit, MI, U.S.A) then rinsed and manually scrubbed for 30 sec. A 1 ml aliquot was taken from the rinse solution and was serially diluted and pour-plated in duplicate using standard plate count (SPC) agar (Difco laboratories, Detroit, MI, U.S.A) then incubated at $37\pm 2^{\circ}\text{C}$ for 48 hrs. Dilutions with 25-250 colonies were counted and then converted to log cfu/20ml rinse.

b. Broth Control (brothcon): Sterile chicken broth was placed on a sterile spoon then the broth was discarded and that spoon was placed in 20 ml of 0.1% peptone water (Bacto peptone, Difco laboratories, Detroit, MI, U.S.A.) and rinsed for 30 sec. A 1 ml aliquot was taken from the rinse solution and enumerated as described for the spoon control.

c. Mouth to spoon only (spoonmou): A sterile spoon was swiped once in the mouth without contact with broth. The spoon was placed in 20 ml of 0.1% peptone water (Bacto peptone, Difco laboratories, Detroit, MI, U.S.A.) and

rinsed for 30 sec. A 1 ml aliquot was taken from the rinse solution and enumerated as described for the spoon control.

d. Mouth to spoon with broth (brothspoonmou): Sterile diluted chicken broth was placed on a sterile spoon and spoon was swiped once in mouth and then the spoon was placed in 20 ml of 0.1% peptone water (Bacto peptone, Difco laboratories, Detroit, MI, U.S.A.) and rinsed for 30 sec. A 1 ml aliquot was taken from the rinse solution and enumerated as described for the spoon control.

1.2. Transfer from mouth to spoon while consuming rice: this experiment used four treatments: a. spoon control (spooncon), b. rice control (ricecon), c. mouth to spoon only (spoonmou), and d. mouth to spoon with rice (ricespoonmou). These treatments were handled exactly the same as experiment 1.1 (Transfer from mouth to spoon while consuming broth) except that instead of broth, rice was placed on the spoon.

1.3. Transfer from mouth to chopstick while consuming rice: this experiment used four treatments: a. chopstick control (chopcon), b. rice control (ricecon), c. mouth to chopstick only (chopmou), and d. mouth to chopstick with rice (ricechopmou). These treatments were handled exactly the same as experiment 1.2 (Transfer from mouth to spoon while consuming rice) except that instead of using a spoon, a chopstick was used.

Experiment set 2 comprised one experiment with five treatments to measure the transfer of bacteria from the mouth to sterile diluted broth using stirring and scooping treatments. To simulate consuming broth, scooping with a spoon to remove a broth from a bowl and

stirring before scooping was used as treatments. Each treatment started with 80 ml of diluted chicken broth. The five treatments were: a. control, b. scoop c. scoopstirr d. scoopmouth e. scoopstirmouth. After each treatment was imposed, 1 ml of the diluted broth was directly pour-plated and serially diluted then pour-plated using SPC agar (Difco laboratories, Detroit, MI, U.S.A.) Plates were incubated at $37\pm 2^{\circ}\text{C}$ for 48 hrs. Dilutions with 25-250 colonies were counted then converted to log cfu/20 ml rinse.

a. control: One ml of the diluted broth was sampled without placing the spoon in the broth and enumerated as described above for experiment 2.

b. scoop: 80 ml of diluted broth was scooped six times with a sterile spoon without putting the spoon in the mouth. The average amount of broth remaining in the bowl after six scoops was 28.17 ml and 1 ml of broth was sampled and enumerated as described above for experiment 2.

c. scoopstir: 80 ml of broth was stirred three times before each of six scoops with a sterile spoon without placing the spoon in the mouth. The average amount of broth remaining after six cycles of stirring and scooping was 36.14 ml and 1 ml of broth was sampled and enumerated as described above for experiment 2.

d. scoopmouth: 80 ml of broth was scooped six times with a sterile spoon placing the spoon in the mouth after each scoop. The average amount of broth remaining after six scoops was 33.02 ml and 1 ml of broth was sampled and enumerated as described above for experiment 2.

e. scoopstirmouth: 80 ml of broth was stirred 3 times before each of six scoops placing the spoon in the mouth after each scoop. The average amount of broth remaining after stirring and scooping was 26.84 ml and 1 ml of broth was sampled and enumerated as described above for experiment 2.

Experiment set 3 comprised two experiments; 3.1. Transfer of bacteria from mouth to rice using hand 3.2. Transfer of bacteria from mouth to rice using spoon. Each subject began with 100 g of cooked rice.

Experiment 3.1. Transfer from mouth to rice using hand had three treatments, a. ricecontrol, b. ricehand, c. rice mouth.

a. ricecontrol: the average weight (30 g) of rice remaining after treatments 3.1.b and 3.1.c and used as rice control. Thirty g of rice was placed in 100 ml of 0.1% peptone solution (BD Bacto peptone, Difco laboratories, Detroit, MI, U.S.A.) without contact with hand or mouth then stomached (Seward Stomacher 400 Circulator, Seward, Inc, UK) for 1 min at 230 rpm. One ml of the sample diluents was directly pour-plated and also serially diluted and pour-plated in duplicate using SPC agar (Difco plate agar Difco laboratories, Detroit, MI, U.S.A.) then incubated at $37\pm 2^{\circ}\text{C}$ for 48 hrs. Plates from dilutions having 25-250 colony forming units were counted then converted to log cfu/100ml rinse and log cfu/g.

b. ricehand: Subjects washed their hands with antibacterial soap for 20 sec with warm water then rinsed hands with warm water for 10 sec then towel-dried

using sterile paper towels. Subjects took cooked rice with their hands from a sterile plate six times without placing their hands in their mouth. Bacterial enumeration of the rice was performed as described for the ricecontrol. Each time a handful of rice was taken an average 10.57g of rice was removed leaving about 36g after six cycles.

c. Ricemouth: Subjects washed their hands with antibacterial soap for 20 sec with warm water then rinsed hands with warm water for 10 sec then towel-dried using sterile paper towels. Subjects took cooked rice with their hands from a sterile plate six times placing their hands in their mouth. Bacterial enumeration of the rice was performed as described for the ricecontrol. Each time a handful of rice was taken an average 11.61g of rice was removed leaving about 30g after six cycles.

Experiment 3.2. Transfer from mouth to rice using spoon had three treatments; a) ricecont b) ricespoon c) ricemouth

a. ricecont: An average weight of 40.75 g of rice was sampled based on the amount of rice remaining from preliminary tests using treatment 3.2.b and 3.2.c. Bacterial enumeration was performed as described as in experiment 3.1.

b. ricespoon: Subjects removed an average of 10.59g of rice in each spoonful using a sterile spoon without putting spoon in the mouth. This was repeated six times leaving about 35.90g in the plate after six cycles. Bacterial enumeration was performed as described as in experiment 3.1.

c. ricemouth: Subjects removed an average of 8.98g of rice in each spoonful using a sterile spoon without putting spoon in the mouth. This was repeated six times leaving about 45.72g in the plate after six cycles. Bacterial enumeration was performed as described as in experiment 3.1

Bacterial Recovery

Recovery rate of bacteria from diluted (1:4, as described in experiment 2) Swanson chicken broth (Campbell Soup Company, Camden New Jersey, U.S.A.) and rice (Mahatma extra long grain enriched rice Riviana Foods Inc., Houston Texas) was determined by inoculating each food with an ampicillin-resistant strain of *E. coli*. Rice was cooked in microwave oven (Magic Chef, U.S.A.) for 15 minutes (1:2 rice to water ratio for cooked rice). All recovery treatments were repeated three times and averaged.

Recovery control: A culture of ampicillin-resistant *E. coli* was grown overnight at $37\pm 2^{\circ}\text{C}$ in 10 ml of tryptic soy broth (Difco Laboratories, Detroit, MI, USA) containing 100 ppm of ampicillin. The 10 ml culture was centrifuged (International Equipment Company, USA) at 1,000 x g for 15 min then the spent broth was discarded and the pellet resuspended in 10 ml of sterile 0.1% peptone water. 1 ml of this suspensions was serially diluted and 0.1 ml of the dilutions were surface plated on tryptic soy agar (Difco Laboratories, Detroit, MI, USA) then incubated at $37\text{ C}\pm 2\text{ C}$ for 48 hr then plates from dilutions having from 25-250 colonies were counted and converted to log cfu/ml.

Recovery from broth: One ml of the washed suspension from the overnight ampicillin-resistant *E. coli* culture (prepared as described for recovery control) was inoculated into 80 ml of sterile diluted (1:4) chicken broth. The inoculated broth was mixed by gentle shaking for 20 sec then 1 ml of the mixture was removed and serially diluted then enumerated as described for the recovery control samples. The percentage of recovery was calculated from:

$$\text{Recovery} = (\# \text{ of cells recovered from broth} / \# \text{ of cells recovered from control}) \times 100$$

Recovery from rice: One ml of the washed suspension from the overnight ampicillin-resistant *E. coli* culture (prepared as described for recovery control) was inoculated into 30g of cooked rice. The inoculated rice was placed in 100 ml of 0.1% peptone solution (BD Bacto peptone, Difco laboratories, Detroit, MI, U.S.A.) then stomached (Seward Stomacher 400 Circulator, Seward, Inc, UK) for 1 min at 230 rpm then 1 ml of the stomached mixture was sampled and enumerated as described for the recovery control samples. The percentage of recovery was calculated from:

$$\text{Recovery} = (\# \text{ of cells recovered from rice} / \# \text{ of cells recovered from control}) \times 100$$

Water Activity of Rice

Water activity was measured in triplicate on a sample of cooked rice by Rotronic Hygroscopic DT (Rotronic Instrument Corp, Huntington, NY) at the temperature of 25.2 °C. The rice samples were kept in small dishes and inserted into the water activity

chamber. After 30 minutes, when equilibrium was reached, relative humidity was recorded from the meter and converted to water activity.

$$\text{Water activity} = \text{Relative humidity} / 100$$

Statistical Analysis

Each experiment was replicated three times. Experiments 1 and 2 had 10 subjects and experiment 3 used 7 subjects with all plating performed in duplicate. All treatments were subjected to analysis of variance using SAS (2006) to determine if there was a significant ($p \leq 0.05$) overall affect due to treatments. For all three experiments means and significant differences between means were calculated using the proc glm, stderr, and pdiff commands.

Recovery and water activity experiments were replicated three times and all plating was in duplicate. All observations were averaged.

Results

Recovery of bacteria inoculated into diluted chicken broth and rice was 98.64 and 89.66%, respectively. The higher recovery rate in diluted broth compared to rice was likely due to the compositional differences in the foods. Chicken provides an enriched medium for the growth of the microorganism (Perez-Rodriguez, et al., 2008) while rice had water activity (a_w) of 0.94 (compared to an a_w of near 1.00 for broth) which may have restricted bacterial growth, survival and recovery in rice.

Experiments 1.1. Determining the transfer of bacteria from mouth to spoon while consuming broth. 1.2. Determining the transfer from mouth to spoon while consuming rice and 1.3. Determining the transfer from mouth to chopstick while consuming rice.

There was a transfer of over 10^5 bacterial cells to the spoon and chopstick when placed in the mouth with or without food (Table 1). There was no difference in bacterial transfer whether the utensil was first placed in food or not before placing the utensil in the mouth or just placing the utensil in the mouth without food (Table 1). The population of bacteria recovered from utensils after being placed into the food was less than 10 cells for both the spoon in broth or rice and for the chopstick in rice and did not differ from the population recovered from the control utensils which were neither placed in the food or the mouth (Table 1).

Experiment 2. Determining the transfer of bacteria from the mouth to sterile broth while using stirring and/or scooping.

The practice of scooping and stirring is very common while eating soup or broth. No published studies have reported the rate of bacterial transfer from the mouth to broth while consuming broth with a spoon. Each time the spoon is placed in the mouth before scooping and stirring there is potential for bacterial transfer. In fact, between 7.0×10^4 and 9.0×10^4 bacteria were transferred to broth when the spoon was placed in the mouth between scooping six times (Table 2). It was estimated that 10^4 bacteria were transferred into the broth each time subjects placed the spoon in their mouths prior to placing the spoon back in the broth. The control treatments (i.e. when the spoon was not placed into the mouth) the number of bacteria transferred to the broth was near the sensitivity levels of recovery (10-20 cells) and did not significantly differ.

Experiment 3. Determining the transfer of bacteria from mouth to rice using hands (3.1) or a spoon (3.2).

Sharing of food in traditional Asian cultures (India, Japan, China and Pakistan) is common and supports cross contamination. The transfer of bacteria from mouth to rice using the hand to carry the rice resulted in an increase in bacterial population in the rice compared to treatments (Table 3). Even use of the hand to remove rice from the common bowl without placing the hand in the mouth resulted in an increase in total bacteria of about 3.0×10^3 bacteria in the rice while placing the hand in the mouth on each transfer resulted in nearly 1 million bacteria (Table 3). This resulted into about 10^4 bacteria per gram of rice after 6 cycles of using the hand to consume rice. Thus, someone eating from a common bowl after the previous person used the hand to consume rice would be

exposed to a significant number of bacteria originating from the previous consumer's mouth. In many cultures, people still eat food with their hands. Eating of rice and Nan with hand is very common in Indian cultures. Contaminated hands are a major source of cross contamination in any food service area (Hui, 2006). The hand is potentially a critical control point for cross contamination that can result in an increase the number of food borne pathogen related outbreaks. Bacterial transfer is likely to occur from contaminated hands to different surfaces following food preparation (Hui, 2006). Improper handling and sanitation practices lead to cross contamination from person to person, person to food and ultimately results into 27% of reported outbreaks and infection of food borne pathogens (WHO, 2001). The first line of defense against cross contamination is hand washing and (Montville, 2003) suggested that hand washing for 15 sec with soap and water can remove transient bacteria. Eating with hands from a common bowl or plate is common in many settings including popcorn and nuts at movies and bars.

The spoon was also tested for bacterial transfer when used to consume rice from a common bowl. Over 10^4 bacteria were transferred to rice when the spoon was used to consume the rice then placed back in the rice bowl for 6 cycles (Table 4). This calculated to about 8.0×10^3 bacteria per gram of rice remaining in the rice after 6 cycles. There were less than 20 bacteria recovered from the rice when the spoon was used to remove rice without placing the spoon in the mouth. Thus, there were a significantly higher number of bacteria transferred to the common bowl of rice when a spoon was used however much lower number than when the hand was used.

Discussion

The term cross contamination is used to describe the transfer of pathogens from a contaminated food or surface (usually raw items such as meat, poultry and vegetables) to other foods whether it occurs directly or indirectly. Direct contamination describes when a contaminated source touches food while indirect contamination occurs when transfer requires an intermediate surface. For example, direct contamination occurs when people touch sandwiches with dirty hands while indirect contamination can occur when raw meat is placed on a cutting board after which a cooked product is placed on the contaminated cutting board. Indirect contaminations would also occur when raw meat juices are left on a knife which is later be used for slicing ham (<http://archive.food.gov.uk/hea/teachers/english/part4.html>). Some studies have reported on the transfer of bacteria from surfaces to food while other studies can be found on transfer of bacteria from food to other surfaces (Scott and Bloomfield 1990; Zhao et al.1998; Chen et al. 2001; Montville et al. 2001).Bacterial transfer from stainless steel to cucumber were reported by Kusumaningrum et al., (2003) and Chen et al., (2001) while Moore et al., (2003) studied bacterial transfer to lettuce from cutting board and stainless steel. Food contamination can result from variety of surfaces including hands, other foods and utensils contaminated with different bacterial loads and also bacteria carried in various media.

The oral cavity can also be a source of contamination and of pathogenic microorganisms. Bacterial cells in the mouth are attached to teeth surfaces through

dynamic microbial communities called biofilms (Kolenbrander et al., 2002). Most oral bacterial communities ultimately result in plaque formation. Previous studies have shown that number of bacterial species found in the mouth range from 500 to 700. Sneezing and coughing can cause oral bacteria to become airborne (Micik et al., 1969). At least five infectious diseases that can be transferred through oral saliva droplets and aerosols, including pneumonic plague, tuberculosis, influenzas, Legionnaires ' disease and severe respiratory syndrome. If bacterial transfer from mouth through the air is possible then it is likely that bacteria can also be transferred between humans by exposure to food that have become contaminated with saliva (Haral and Molinari, 2004). The Centers for Disease control (<http://www.cdc.gov/flu/protect/covercough.htm>) recommends covering the mouth to prevent spreading “serious respiratory illnesses like influenza, respiratory syncytial virus (RSV), whooping cough and severe acute respiratory syndrome (SARS)”while sneezing, coughing and touching contaminated surfaces are vectors for spreading diseases., as the disease agents originate from the mucus of infected persons (<http://www.health.state.ny.us/publications/7110/>). Orally contaminated foods may also be a transfer vector. The length of time that infectious agents can survive outside the body on environment surfaces varies greatly. The suspected range is from a few seconds up to 48 hrs depending on the specific agent and the type of surface. It is generally believed that cold and flu viruses survive longer on non porous surfaces such as plastic, metal or wood than they do on porous surfaces such as fabric or paper (<http://www.point-sourceaudio.com/microphone-health.pdf>). Although infectious diseases primarily spread from person to person contact they can also spread from contact with contaminated

objects or surfaces. Microorganisms can survive in food longer than on most surfaces and can even multiply in food to promote transfer from person to person when food is shared.

Table 1. Estimate of bacterial transfer from mouth to spoon and chopstick.

Treatment	Logcfu/utensil ¹	Standard Error
spooncon ²	0.35 ^b	0.142
brothcon	0.07 ^b	0.142
spoonmou	5.52 ^a	0.142
brothspomou	5.17 ^a	0.142
ricecon ³	0.00 ^a	0.10
spooncon	0.19 ^b	0.10
spoonmou	5.67 ^a	0.10
ricespoonmou	5.11 ^a	0.10
chopcon ⁴	0.06 ^b	0.072
ricecon	0.09 ^b	0.072
chopmou	5.94 ^a	0.072
ricechopmou	5.56 ^a	0.072

^{a,b} means within sub experiments with different superscripts are significantly different (p≤0.05)

¹ Logcfu recovered from utensil

² sub experiment testing bacterial transfer from mouth to spoon with broth.

spooncon= bacteria recovered from spoon with no contact with mouth or broth

brothcon=bacteria recovered from the spoon with contact with broth without placing in the mouth

spoonmou=bacteria recovered from the spoon placing in the mouth without broth

brothspoonmou =bacteria recovered from the spoon placing in the mouth with broth

³ sub experiment testing bacterial transfer from mouth to spoon with rice.

ricecon = bacteria recovered from spoon and rice without placing in the mouth

spooncon= bacteria recovered from spoon with no contact with mouth or rice

spoonmou= bacteria recovered from spoon placing in the mouth without rice

ricespoonmou= bacteria recovered from spoon placing in the mouth with rice

⁴ sub experiment testing bacterial transfer from mouth to chopstick with rice.

chopcon= bacteria recovered from chopstick no contact with mouth or rice

ricecon= bacteria recovered from chopstick and rice without placing in the mouth

chopmou=bacteria recovered from chopstick placing in the mouth without rice

ricechopmou= bacteria recovered using the chopstick placing in the mouth with rice

Table 2. Estimate of bacterial transfer from mouth to broth by performing scooping and stirring treatments with spoon.

Treatment	Log total cfu ¹	Std.error	Log cfu/cont²	Std.error
scoopmouth	4.86 ^a	0.16	4.00 ^a	0.13
scoopstirmouth	4.86 ^a	0.16	4.11 ^a	0.13
scoop	0.65 ^c	0.16	0.37 ^c	0.13
scoopstir	0.462 ^c	0.16	0.25 ^c	0.13
cont	1.12 ^b	0.16	0.76 ^b	0.13

^{a,b,c} means within same experiment set with different superscripts are significantly different ($p \leq 0.05$)

¹logtotalcfu=total number of bacteria recovered from 80 ml broth

²logcfuconta=estimate of bacterial transfer per contamination

scoopmouth =spoon scooped six times with spoon placed in the mouth after each scoop

scoopstirmouth =spoon was stirred three times before each of six scoops after which spoon was placed in the mouth

scoop =scooping was performed six times in the broth without placing spoon in the mouth

scoopstir =stirring was performed three times before six times scooping was performed with spoon placed in mouth after each scoop

cont =No treatment was performed only broth was used for the dilutions

Table 3. Bacterial transfer from mouth to rice using the hand to carry the rice.

Treatment	Logtotalcfu ¹	SE	Logcfugm ²	SE	Cfu/cont ³	SE	Logcfucont ⁴	SE
ricecont	0.00 ^c	0.13	0.00 ^c	0.13	0 ^b	675	0.00 ^c	0.13
ricehand	3.47 ^b	0.13	1.95 ^b	0.13	39 ^b	675	1.25 ^b	0.13
ricemout	5.88 ^a	0.13	4.42 ^a	0.13	8550 ^a	675	3.71 ^a	0.13

^{a,b,c} means within same experiment set with different superscripts are significantly different ($p \leq 0.05$)

¹logtotalcfu presents total bacteria recovered from rice

²logcfugm represents total no of bacteria recovered per gm of rice

³ cfu conta represents bacterial transfer after each contamination

⁴logcfu cont represents the estimation of bacterial transfer after each contamination

ricecont= control treatment with no hand or mouth contact with the rice.

ricehand= Rice was taken out from plate five times without putting hand in the mouth.

ricemouth=Rice was eaten for five times each time hand was put in the mouth.

Table 4. Estimate of bacterial transfer from mouth to rice using spoon.

Treatment	Logtotalcfu ¹	SE	Logcfugm ²	Std E	Cfuconta ³	Std E	Logcfucon ta ⁴	Std E
Ricemout	5.53 ^a	0.21	3.91 ^a	0.084	2323.34 ^a	269.48	3.20 ^a	0.057
Ricespoo	1.25 ^b	0.21	0.52 ^b	0.084	1.36 ^b	269.48	0.17 ^b	0.057
Ricecont	0.43 ^c	0.21	0.064 ^c	0.084	0.09 ^b	269.48	0.00 ^c	0.057

^{a,b,c} means within same experiment set with different superscripts are significantly different

¹logtotalcfu presents total no of bacteria recovered from rice

²logcfugm presents total no of bacteria recovered per gm of rice

³cfuconta represents the bacterial transfer after each contamination

⁴logcfucon represents the total no of bacteria recovered after each contamination

Ricemout=rice was eaten with the spoon each time spoon was put in the mouth

Ricespoo= five times rice was taken out from the plate with spoon no contact with the mouth

Ricecont=average amount of remaining rice was measured from the ricemout and ricespoo treatments

Conclusions

There was a transfer of over 5 log cycles of bacteria to the spoon when placed in the mouth with or without food. The population of bacteria recovered from the utensil after being placed into the food was less than 10 cells for both the spoon in broth or rice and for the chopstick in rice. Nearly 5 log cycles of bacteria were transferred to broth when the spoon was placed in the mouth between scooping six times. About 4 logs of bacteria were estimated into the broth each time subjects placed the spoon in their mouth prior to placing the spoon back in the broth. The transfer of bacteria from the mouth to the rice using the hand to carry the rice resulted in higher number of bacterial transfer in the rice than when a spoon was used. Using of hands to remove rice several times from the common bowl without placing the hand in the mouth resulted in an increase in total bacteria of over 3.0×10^3 bacteria in the remaining rice. Placing the hand in the mouth on each of 6 transfers resulted in nearly 1 million bacteria in the rice. This way 2.6×10^4 bacteria were calculated per gram of rice after 6 cycles of using the hand to consume rice. Therefore it can be concluded that someone eating from the common bowl after the previous person used the hand to consume rice would be exposed to significant numbers of bacteria originating from the previous consumer's mouth. This way sharing of food from the same bowl could lead to significant amount of bacterial transfer. Similarly, 3.0×10^4 bacteria were transferred to the rice when the spoon was placed back in the bowl for 6 cycles. This calculated to about 8.0×10^3 bacteria per gram of rice remaining in the rice after 6 cycles with placing spoon in the mouth each cycle. Recovered bacteria were less than 20 from the rice when the spoon was used to remove rice without placing the

spoon in the mouth. Therefore it can be stated that there was a significant number of bacteria transferred to the common bowl of rice when a spoon was used but transfer was comparatively lower than when the hand was used.

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