Persistence of *Salmonella* and *E. Coli* on the Surface of Restaurant Menus

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Abstract **To the authors' knowledge, the role of restaurant menus as a vehicle for pathogens has not been explored. Menus, however, can pose as a vector for bacterial contamination and transfer. Sampling menus from two restaurants in the Houston, Texas, area showed the presence of up to 100 CFU/cm² aerobic bacteria. Follow-up studies designed to investigate the ability of *Salmonella* and *E. coli* to persist on paper and laminated menus at various time points (0, 6, 24, 48, and 72 hours) demonstrated that bacteria persist more efficiently on laminated menus as compared to paper menus. Transfer studies performed to quantitatively determine the ability of bacteria to transfer from menus to fingertips and from fingertips to clean menus showed that bacteria can be transferred for up to 24 hours. The study described here showed that restaurant menus may serve as vehicles for pathogens and hence present a public health issue within the retail food environment.**

Introduction

The Centers for Disease Control and Prevention (CDC) estimate that each year 48 million Americans get sick, 128,000 are hospitalized, and 3,000 die of foodborne disease (CDC, 2011). Nontyphoidal *Salmonella* and pathogenic *E. coli* (O157:H7) are responsible for 35% and 4% of the hospitalizations, respectively. Changes in lifestyle and socioeconomic status have contributed to the increase in the number of American consumers eating foods prepared in restaurants. Consuming food prepared away from home has implications for public health (Creel, Sharkey, McIntosh, Anding, & Huber, 2008).

According to CDC, 50% of foodborne outbreaks occurred in restaurants from 1998 to 2002 (CDC, 2006). These data show that the risk of foodborne illness increases when food is not prepared domestically. A *Salmonella* outbreak associated with a restaurant in Atlantic City, New Jersey, was reported in 2007. A total of 30 confirmed cases occurred and investigators traced the source of contamination to fruit salad and an ill restaurant worker (CDC, 2008). In 2007, two ill delicatessen workers were found to be responsible for a *Salmonella* outbreak in a Minnesota grocery store (Hedican et al., 2010). It has been estimated that the average foodborne outbreak costs an operation $100,000 and a 30% reduction in sales (Grover & Dausch, 2000).

The possible causes of contamination in restaurants include contaminated food, temperature abuse of foods, personnel coming to work sick, contaminated equipment, improper cooking, or poor personal hygiene including bare hand contact with foods. Poor employee hygiene is considered an important contributor to foodborne illness outbreaks associated with restaurants (Olsen, MacKinnon, Goulding, Bean, & Slutsker, 2000). Approximately 20% of foodborne illnesses caused by bacteria have been shown to come from infected employees who reported sick to work (Greig, Todd, Bartleson, & Michaels, 2007). This factor may affect contact surfaces outside the kitchen such as furniture, equipment, eating utensils, and possibly menus.

Previous studies investigating the cleanliness of cutting boards, faucet handles on sinks, refrigerator door handles, microwave oven controls, and bin lids showed that the majority of visually clean surfaces failed to meet hygiene requirements based on adenosine triphosphate swabs and traditional microbiological testing (Tebbutt, Bell, & Aislabie, 2007). It was suggested that pathogens may

multiply on these surfaces, which results in cross contamination of foods that leads to foodborne disease outbreaks (Holtby, Tebbutt, Grunert, Lyle, & Stenson, 1997). Food service employees primarily come in contact with these surfaces and their hands could be the vehicle for pathogen transfer to the menu.

Previous studies have demonstrated the ability of Gram-positive and Gram-negative pathogens to survive over time (several hours to days) on surfaces such as plastic, wood, stainless steel (e.g., countertops or deli slicers), and kitchen floors. These include *E. coli* and *Salmonella enterica*, which are members of the Enterobacteriaceae family. *S. enterica* causes gastroenteritis in humans (Neidhart, 1996). *E. coli* is a commensal of the intestine in mammals; however, some variants of *E. coli*, most notably Shiga-toxin-producing strains, are pathogenic to humans and may cause death in extreme cases (Neidhart, 1996). Since *Salmonella* and *E. coli* are transmitted via the fecal-oral route, customers or restaurant employees who are sick (or carriers of these pathogens) and lacking appropriate hand washing may contaminate the menus with their hands. Unlike other high contact surfaces in a restaurant environment, no standard protocols are currently in place for sanitizing menus or methods to determine if the menu is hygienic. Menus are often inspected simply by touch or sight (Choi & Almanza, 2011).

To the best of our knowledge, the role of restaurant menus as a source of microbial contamination has not been investigated. This is an important question, however, since restaurant customers are diverse and may include young children, elderly, and immunocompromised individuals (e.g., transplant patients or individuals undergoing chemotherapy). If pathogens are present on the menus, they may be transferred to consumers' hands and from hands to the mouth. Hence, unhygienic and improperly cleaned menus may pose a public health risk to restaurant customers.

The objective of our study was threefold: 1) sample restaurant menus for the presence of microorganisms; 2) demonstrate the ability of microorganisms (specifically *Salmonella* and *E. coli*) to survive on restaurant menus at 0, 6, 24, 48, and 72 hours; and 3) investigate the transfer of *Salmonella* and *E. coli* from inoculated menus to fingertips and fingertips to noninoculated menus at 0, 6, 24, and 48 hours.

Materials and Methods

Sampling Restaurant Menus

Six laminated menus from two restaurants in the Houston, Texas, area were sampled for aerobic microorganisms. The menus were handled aseptically using sterile gloves and brought to the laboratory in sterile stomacher bags. A 5-cm^2 template was prepared using aluminum foil. The aluminum foil templates were sterilized by autoclaving and stored at room temperature. The template was used to sample a 5-cm^2 area (on the sides of the menus that are associated with most contact) with a sterile cotton swab. The swabs were placed in 3.5 mL sterile phosphate buffered saline and mixed thoroughly using a vortex. Appropriate serial dilutions were performed and 1 mL of each dilution was placed on aerobic count petrifilms. The petrifilms were incubated at 37°C for 24 hours and colonies were enumerated. Three biological replicates were performed for the sampling experiment.

Bacterial Growth Conditions

Salmonella Typhimurium ATCC 53647, *E. coli* ATCC 25922, and *E. coli* ATCC 10798 (*E. coli* K12) used in our study were obtained from American Type Culture Collection and stored at -80°C in glycerol. The cultures were streaked on brain heart infusion (BHI) agar and incubated at 37°C overnight. Single colonies were used to inoculate BHI broth, grown at 37°C for 18 hours, and used to make a cocktail prior to the experiment.

Restaurant Menus

Menus (matte paper, 65-pound weight) were obtained from an independent restaurant in Houston and cut into 10 × 10 cm coupons. The coupons were laminated using HeatSeal laminating pouches to simulate laminated restaurant menus. The paper and laminated menu coupons were wrapped in aluminum foil and autoclaved at 121°C at 15 pounds per square inch for 15 minutes. The coupons were stored at room temperature (23°C) until the experiment was performed.

Inoculation of Bacteria on Menu Coupons

A microbial cocktail containing *Salmonella* Typhimurium 53647, *E. coli* 25922, and *E. coli* 10798 (K12) was prepared by adding 1 mL of each overnight (18 hours) bacterial culture to 47 mL 0.1% peptone water. The laminated and paper menu coupons were placed on sterile Petri dishes (145 mm) and inoculated with 0.5 mL of the bacterial cocktail. This is approximately 10^6 CFU/cm^2 of each surrogate on the menu coupon. The inoculum was uniformly distributed over the surface of the coupon using a sterile cell spreader. The bacteria were enumerated at 0, 6, 24, 48, and 72 hours after inoculation on the coupons. For enumeration, the coupon was first rinsed with 10 mL sterile water to remove loosely attached bacterial cells and placed in a sterile stomacher bag with 90 mL 0.1% peptone water. The bag was stomached for 120 seconds, diluted, and samples plated on eosin methylene blue (EMB) agar. The EMB media enables differentiation between *Salmonella* (appear light pink) and *E. coli* (appear metallic green) colonies. A total of three biological replicates were performed for this experiment.

Determination of Bacterial Detection Limit on Menu Coupons

The detection limit for *Salmonella* and *E. coli* was determined to be approximately 1 log CFU/cm^2 coupon based on our analysis for enumeration of *Salmonella* and *E. coli* on menu coupons. For this, the 10-cm^2 menu coupon was placed in 90 mL sterile 0.1% peptone water. This results in a 1:10 dilution. Appropriate dilutions were prepared and 0.1 mL of each dilution was spread plated on EMB agar. The detection limit was determined to be 1 log CFU/cm^2 (i.e., 10 CFU/cm^2) to avoid the introduction of error into the results.

Volunteers

Permission was obtained from the University of Houston Committee for the Protection of Human Subjects for the use of volunteers (between ages 18 to 65) for our study. Any individuals with cuts and abrasions on their hands were excluded. Individuals on medication for acute health issues and immunocompromised individuals were also excluded. Each volunteer was briefed on the experimental protocol and risks before being asked to sign the consent form.

Fingertip Transfer Study

Using the methods described by Lingaas and Fagernes (2009) with some modifications, a cocktail containing bacterial surrogates of *Salmonella* Typhimurium 53647, *E. coli* 25922, and *E. coli* 10798 (K12) was prepared

as described in the previous section and inoculated on laminated and paper menus. The participants were asked to wash their hands for 30 seconds with antibacterial soap and warm water (approximately 45°C). The subjects used disposable paper towels to dry their hands and sprayed their hands with 70% ethanol (approximately 3 mL). The subjects were asked to touch the contaminated menu coupon with the index finger (primary transfer) followed by touching a sterile menu (secondary transfer). Following each transfer the subject was asked to wear a glove containing 1 mL buffer in the index finger section of the glove and vortex their finger for 20 seconds. A sterile pipette was used to pipette the buffer from the index finger region of the glove. The buffer was diluted and plated on EMB media to quantify viable *Salmonella* and *E. coli* colonies. The transfer experiments were performed on both paper and laminated menu coupons (slightly moistened and dry) at 0, 6, 24, and 48 hours.

Transfer of bacteria from wet laminated menus was studied for several reasons. On some occasions the restaurant worker may wipe down the table for the customer. Hence, when the customer is seated, the table is not completely dry. Menus could become moistened when they come into contact with the table. Another possibility is that water spills and comes into contact with the menus. In rare cases, the menus may be slightly moistened when given to the customer since they may not have been stored appropriately. Wet menus were prepared by moistening the coupon with a spray (approximately 1 mL) of sterile distilled water. The experiment was repeated three times.

Statistical Analysis

Statistics were done to analyze the standard deviations using Microsoft Excel (2007).

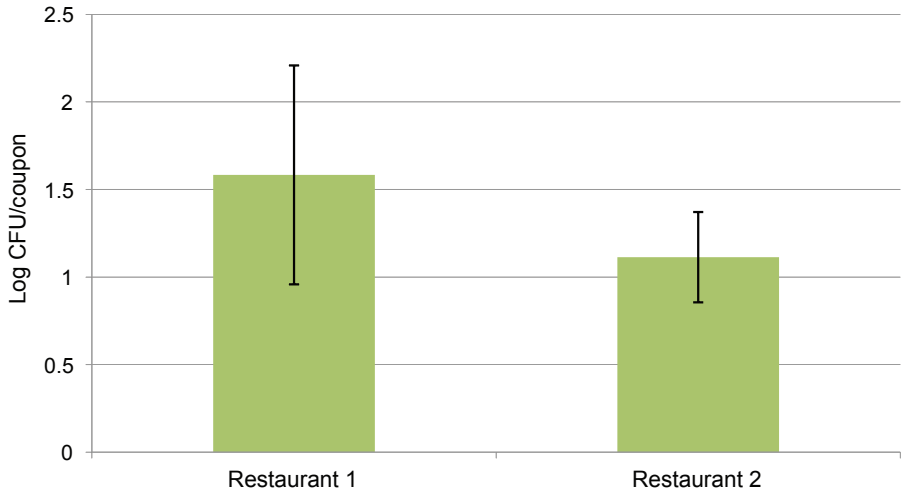
Results

Aerobic Plate Counts on Restaurant Menus

Laminated restaurant menus from two restaurants in the Houston area were sampled for the presence of aerobic microorganisms. Both of these restaurants had laminated menus. A 5-cm^2 area was selected in three different sections on the menus. We sampled a total of six menus for this experiment. As seen in Figure 1, the results demonstrated the presence of 1 to 2 log CFU/cm^2 aerobic microorganisms on the restaurant menus.

FIGURE **1**

Presence of Aerobic Microorganisms on Restaurant Menus (*N* = 6)



Survival of Microorganisms on Menus

The ability of microorganisms (*Salmonella* and *E. coli*) to survive or persist on menus was investigated by inoculating approximately 10^7 CFU total of each bacterial cocktail on paper and laminated menu coupons. The coupons were incubated at room temperature (23°C) and sampled at 0, 6, 24, 48, and 72 hours. As seen in Figure 2, the microorganisms did not survive on the paper menu coupons beyond 0 hours. Both *E. coli* and *Salmonella* demonstrated the ability to persist on the laminated menu coupons, however. Approximately 2.7 log CFU/cm^2 *E. coli* survived on the laminated coupons for 24 hours. *Salmonella* survived on the laminated menu coupons from 0 hours (~4 log CFU/cm^2) to 72 hours (~2.7 log CFU/cm^2).

Transfer of Microorganisms From Menus

To further understand the implications of the possible presence of pathogenic microorganisms on restaurant menus, follow-up studies were designed to quantify the transfer of microorganisms from contaminated menus to fingertips and from potentially contaminated fingertips to clean menus. The analysis was performed on both dry and wet menu coupons. Figure 3 demonstrates the transfer of microorganisms at the various time points. The transfer for *Salmonella* and *E. coli* from inoculated menus to fingertips at 0 hours was approximately 6 log CFU/cm^2. The transfer of microorganisms from fingertips to clean menus was approximately 4 log CFU/cm^2 at 0 hours.

At six hours, the transfer of microorganisms from the wet menus to fingertips and fingertips to clean menus was 6 log CFU/cm^2 and 5 log CFU/cm^2, respectively. The dry menus at six hours showed a transfer of approximately 6 log CFU/cm^2 from menu to fingertips and 4.5 to 5.5 log CFU/cm^2 from fingertips to clean menus. The data demonstrate a high transfer of bacteria from dry menus at six hours since the inoculated microorganisms on the laminated menus did not dry completely. At 24 hours the transfer decreased to approximately 2 to 2.5 log CFU/cm^2 for all three surrogates from inoculated wet menus to fingertips. No detectable microorganism counts were observed at 24 hours from fingertips to clean menus for wet laminated menu coupons. No transfer was detected for dry laminated menu coupons at 24 hours and wet or dry menu coupons at 48 hours.

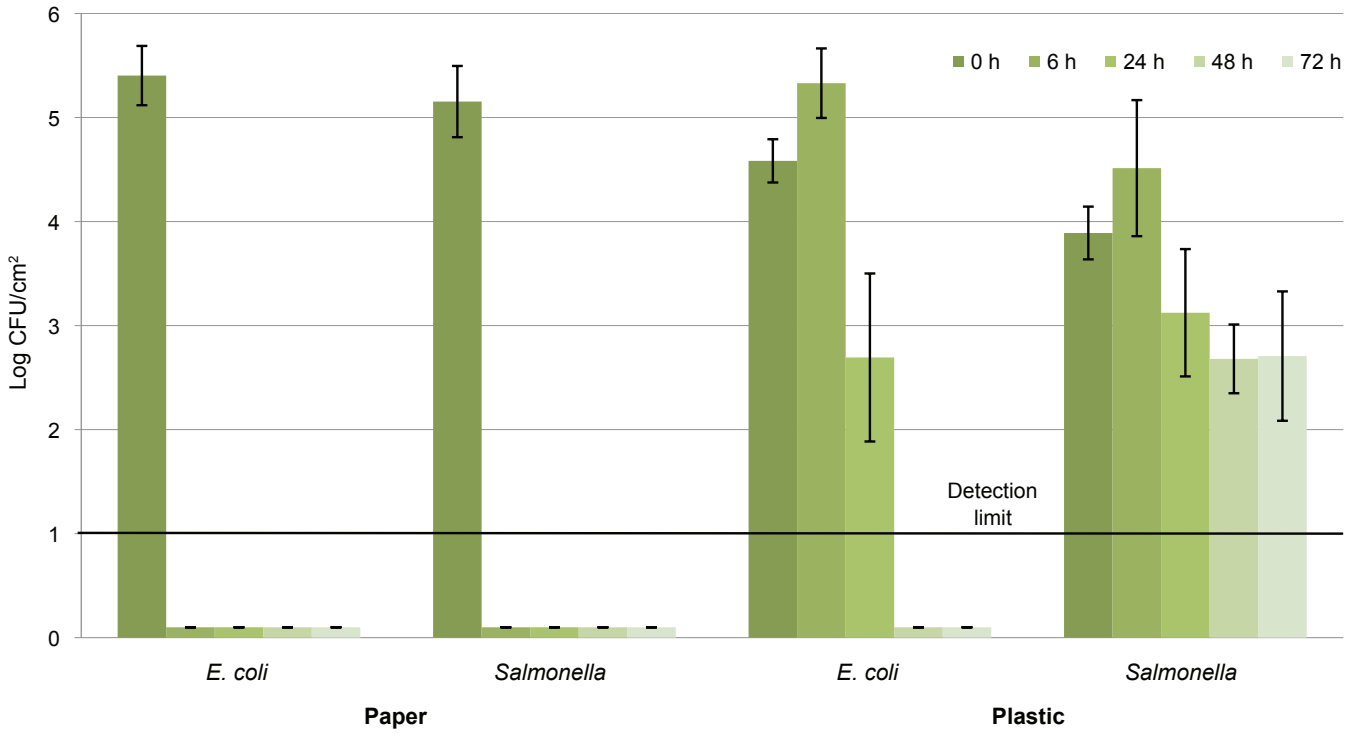
Discussion

Microorganisms on Restaurant Menus

Restaurants target a diverse clientele that may include young children, elderly, and immu-

FIGURE **2**

Survival of Microorganisms (*E. coli* and *Salmonella* Typhimurium) on Paper and Laminated Menu Coupons at 0, 6, 24, 48, and 72 Hours



Three biological replicates were performed.

nocompromised populations. To the best of our knowledge, the implication of unhygienic restaurant menus has not been documented in the scientific literature. The role of menus as a source of contamination cannot be ruled out due to possible risks to the customer. Our results demonstrate that up to 2 log CFU/cm^2 aerobic microorganisms may be present on restaurant menus, including laminated menus. Hence, we designed follow-up studies to investigate the persistence of microorganisms on menus and the transfer rates of these microorganisms from menus to fingertips and fingertips to clean menus.

Persistence of *Salmonella* and *E. coli* on Laminated Menus

Our results showed that both *Salmonella* and *E. coli* survived on laminated menu coupons up to 72 hours and 48 hours, respectively. Neither organism survived on paper menu coupons. This could be because the paper menus lost water (due to evaporation) over time. This may have led to a loss of available nutrients to the pathogenic surrogates. An independent study demonstrated that the survival of Gram-negative organisms on paper (used for food packaging) is extremely rare (Ansari, Springthorpe, Sattar, Tostowaryk, & Wells, 1991).

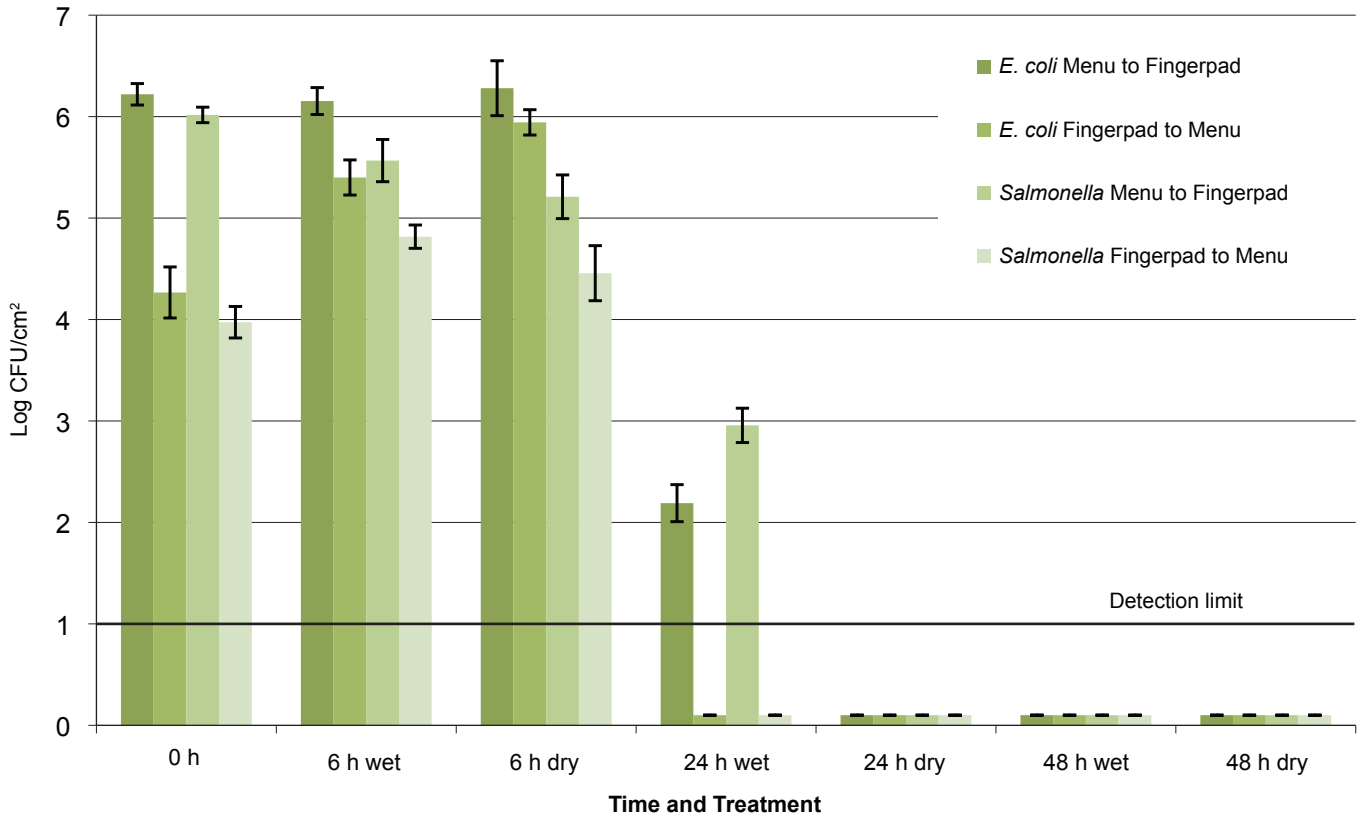
Previous studies screened several Gram-negative bacteria (including *E. coli*, *Salmonella*, and *Pseudomonas*) for their ability to survive on polyethylene plastic sheets. The results showed that microorganisms could survive on plastic for up to six hours (Taylor, 1979). Researchers have also investigated the survival of Gram-positive organisms on various surfaces that were pertinent to hospitals and the results demonstrated that *Staphylococcus aureus* survives on polypropylene plastic for 22 to 90 days (Neely & Maley, 2000). Additionally, Milling and co-authors (2005) demonstrated that *E. coli* survived on plastic for up to 168 hours.

Our data show that *E. coli* survives up to 24 hours, whereas *Salmonella* persists until 72 hours on the laminated menu coupons (Figure 2). This is consistent with previous findings in the literature that show that *Salmonella* is more efficient at surviving in secondary habitats (i.e., outside the animal host) as compared to *E. coli*. Survival of *E. coli* in secondary habitats requires the ability to overcome low nutrient availability and temperature fluctuations (Lim & Flint, 1989). Studies have shown that a lack of nutrients and harsh environmental conditions prevent *E. coli* from dividing outside the animal host. Conversely, *Salmonella* can survive in its secondary habitat for extended periods of time ensuring passage into the next host (Winfield & Groisman, 2003).

E. coli and *Salmonella* are released into the environment from infected humans and animals. Studies have shown that even after sanitization, *Salmonella* can survive up to 15

FIGURE **3**

Transfer of Microorganisms (*E. coli* and *Salmonella* Surrogates) From Inoculated Menus to Fingertips and From Fingertips to Clean Menus at 0, 6, 24, and 48 Hours



Three biological replicates were performed. Dry menu coupons were sampled by placing coupons in the stomacher bag and adding sterile buffer. Wet menus were simulated by moistening menu coupons with a sterile water spray (approximately 1 mL).

days (Parker & Mee, 1982) whereas *E. coli* has a negative growth rate (Gordon, Bauer, & Johnson, 2002). Similarly, *Salmonella* has the ability to outlive *S. aureus* and *Vibrio cholera* in groundwater (Chao, Ding, & Chen, 1987). *Salmonella* may have the ability to employ additional genetic mechanisms to respond to environmental stressors and hence have the ability to survive stressors more effectively than other microorganisms (Munro, Flatau, Clement, & Gauthier, 1995).

Cross Contamination

Cross contamination could occur if an ill (or carrier) customer or restaurant employee touches the menus or if menus come in contact with contaminated fomites. Previous studies have investigated the presence of heterotrophic plate count (HPC) bacteria on a variety of fomites in airports, bus stations, restaurants, malls, and children's playgrounds. The results showed that most of the sites were positive for some level of HPC bacteria ranging from below detection levels to 6 log CFU/cm^2 (Reynolds, Watt, Boone, & Gerba, 2005). Food contact surfaces and other fomites have also been shown to be a possible source of cross contamination. For example, Kusumaningrum and co-authors (2003) showed that *Salmonella* can survive on stainless steel for at least four days.

Transfer studies were designed to further understand the ability of microorganisms to transfer from menus to fingertips of customers and from fingertips to clean menus. Our results (Figure 3) demonstrate that approximately 6 log CFU/cm^2 microorganisms were transferred from menus to fingertips at 0 hours and 6 hours. The transfer from fingertips to clean menus was approximately 4 log CFU/cm^2 at 0 hours. At 24 hours the transfer from contaminated menus to fingertips was 2 to 2.5 log CFU/cm^2. No detectable transfer was observed for dry menus at 24 hours or for wet and dry menus at 48 hours.

Previous studies have shown that *E. coli* and *Salmonella* serotypes can survive on fingertips of volunteers for up to three hours (Pether & Gilbert, 1971). Additionally, the researchers were able to recover *Salmonella* from fingertips even after a 15-second hand wash. Exposure to contaminated fomites (such as menus) is particularly important with young children who have not yet developed proper sanitary

habits (i.e., use of toilet facilities, hand washing, and frequent hand-to-mouth or fomite-to-mouth contact) (Springthorpe & Sattar, 1990). Additionally, elderly and immunocompromised individuals should also be protected from pathogenic microorganisms since they have weakened immune systems and are not able to fight off infections effectively.

Conclusion

The results from our study have demonstrated that microorganisms can survive more efficiently on laminated versus paper menus. Moreover, the cross contamination results suggest that microorganisms could be transferred from damp menus to fingertips for up to 24 hours. Hence, a possibility of cross contamination exists from menus to the hands of the customer leading to illness. From a hygiene standpoint, laminated menus are easier to sanitize. It is crucial for restaurant managers to schedule routine cleaning and sanitizing of menus. The menus should be sanitized and cleaned daily by spraying an all-purpose cleaning solution and dried completely with a disposable cleaning cloth. The menus should be stored in a cool dry place and must be dry when given to the customer.

Acknowledgements: The authors thank Ms. Katie Kirsch for her help with the experiments. The authors also acknowledge Dr. Kristen E. Gibson for her critical review of this article.

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DAVIS CALVIN WAGNER SANITARIAN AWARD

The American Academy of Sanitarians announces the annual Davis Calvin Wagner Award. The award will be presented by the academy during the Annual Educational Conference of the National Environmental Health Association. The award consists of a plaque and a $500 honorarium.

Nominations for this award are open to all diplomates of the academy who:

1. Exhibit resourcefulness and dedication in promoting the improvement of the public's health through the application of environmental and public health practices.
2. Demonstrates professionalism, administrative and technical skill, and competence in applying such skills to raise the level of environmental health.
3. Continues to improve oneself through involvement in continuing education type programs to keep abreast of new developments in environmental and public health.
4. Is of such excellence to merit academy recognition.

The nomination for the award may be made by a colleague or a supervisor and must include the following:

1. Name, title, grade, and current place of employment of the nominee.
2. A description of the nominee's educational background and professional experience.
3. A description of the nominee's employment history, including the scope of responsibilities.
4. A narrative statement of specific accomplishments and contributions on which the nomination is based, including professional association activities, publications, and community/civic activities.
5. Three endorsements (an immediate supervisor and two other members of the professional staff or other person as appropriate).

NOMINATIONS MUST BE RECEIVED BY APRIL 15, 2013. THREE COPIES OF THE NOMINATION DOCUMENT MUST BE SUBMITTED TO:

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