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RECOVERY, SURVIVAL AND TRANSFER OF BACTERIA ON RESTAURANT MENUS

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ABSTRACT

The objectives of this study were to detect bacteria on restaurant menus, to determine the bacterial transfer from menus to consumers' hands and to determine the survival of bacteria on menu surfaces. Local restaurant menus were sampled at different periods of operation. The average total plate count (TPC) was 28 (0–210) cfu/15 cm^2 menu sampling area during "busy" periods and 15 (0–85) cfu/15 cm^2 menu sampling area during "less busy" periods. The staphylococcal count averaged 6 (0–83) cfu/15 cm^2 during busy periods and 2 (0–25) cfu/15 cm^2 menu sampling area during less busy periods. *Escherichia coli* was transferred to menus at 11.17% of the hand population with a high variability between subjects (10.45% standard deviation). Survival of bacteria in menus was 1.40% after 24 h and 1.34% after 48 h, respectively. Bacterial populations found on randomly sampled menus were low; however, bacteria survived and were transferred from menus to a consumer's hands.

PRACTICAL APPLICATIONS

While many food-contact surfaces are routinely cleaned and sanitized to minimize the presence of contamination, some surfaces such as restaurant menus are not and thus may be a potential contamination risk. The current study revealed that in the relatively small sample size of menus examined, there were some that were contaminated. Furthermore, there was measurable transfer of bacteria present on menus to consumer's hands and bacteria survived on menus at least 2 days. Therefore, to reduce the chance of illness from menus that are touched by many different people, especially food service workers, menus may be considered to be included as part of a standard sanitation operating procedure.

INTRODUCTION

Nonfood and food-contact surfaces are routinely sanitized and menus should be included as both restaurant employees and customers handle menus. Seventy percent of consumers will not return to an establishment at which they contracted food poisoning (Food Safety Agency 2009), thus cleanliness is paramount from an economic and moral standpoint (Roberts *et al.* 2003).

In 2005, it was estimated that restaurants served 170 billion meals in the U.S.A. (Angulo and Jones 2006) and 47% of all money spent on food in the U.S.A. was spent at restaurants. Food industry employees account for about 19% of the nation's workforce income. It is also estimated that 4 in 10 Americans dine at a restaurant sometime each day and 1 in 6 consumes 15 meals in a restaurant each week (Angulo and Jones 2006).

Routine restaurant inspections are intended to prevent foodborne illness by promoting safe food handling (Cruz *et al.* 2001). Although different jurisdictions enforce different standards of sanitation and cleanliness, inspections are required by food sanitation guidelines in the U.S.A (Buzby *et al.* 2001). Food safety is the basis of these restaurant inspections and abiding by the food safety rules is a necessity to obtain a good inspection grade (Meng and Doyle 2002).

Of all food-related settings, restaurants are the third most often reported high-risk settings at 14.1% for foodborne illnesses (Hedberg *et al.* 2006). Restaurant settings are targeted by public health interventions as the public perceives that restaurants offer wholesome and nutritious food (Lee and Middleton 2003). Case-control studies have found that people with foodborne illnesses traditionally consume more of their food outside the household compared with nonill controls (Green *et al.* 2005), and that foodborne illness occurred more often in restaurants with low food safety ratings (Simon *et al.* 2005). Observation records indicate that an important fraction of known foodborne outbreaks are related to restaurants (Olsen *et al.* 2000).

Moore and Griffith (2002) concluded that visual evaluation of food underestimates the level of surface contamination on restaurant surfaces. Furthermore, prevention of contamination is the most effective technique to fight the respiratory and gastrointestinal diseases that cause over 6 million annual deaths worldwide (Reynolds *et al.* 2005). Illnesses that result from foodborne pathogens have become one of the most prevalent public health issues in the world today (Reynolds *et al.* 2005). Foodborne illnesses related to microbes, biotoxins and chemicals in food have been found to be the most serious threats to the health of millions of consumers (Reynolds *et al.* 2005).

Cross-contamination between hands and food or food-contact surfaces has been studied extensively. Effective interventions to decrease cross-contamination include adequate hygiene of hands and the environment surrounding food-contact surfaces (Moore and Griffith 2002). Public dining places such as restaurants, cafeterias and bars are locations most often cited for foodborne illnesses and food-related diseases (Redmond and Griffith 2003). These frequently identified establishments were responsible for 54% of outbreaks in the United Kingdom between 1993 and 1998 (WHO 2000) and were associated with 45% of outbreaks in the U.S.A (Olsen *et al.* 2000). When a particular foodborne outbreak occurs and a restaurant is identified as the responsible setting, financial losses (reduced number of customers, lawsuits, etc.) may occur and these may lead to fines or bankruptcy (Clayton and Griffith 2004; Marler Clark 2006).

Henson *et al.* (2006) found that hygiene was the most often mentioned characteristic used by customers to define food safety in restaurants. Other characteristics used by consumers to assess food safety at restaurants included: general excellence of the restaurant, density of customers and outside data, such as restaurant reviews, different views of visitors such as friends and family and inspection grading cards. Even though restaurants in the U.S.A. undergo inspections by their local health departments, studies have constantly shown that large proportions (60% restaurants) regularly have insufficient food hygiene practices (Knight *et al.* 2007). Even though health departments inspect restaurants on a routine basis, little data are accessible in regard with the effectiveness of the hygiene standards in preventing foodborne illness (Knight *et al.* 2007).

A growing number of meals in the U.S.A. are consumed in or bought from restaurants. As part of the prevention of restaurant-related foodborne incidents (FBIs), the local health departments regularly review restaurants. In Los Angeles County, approximately $10 million per year is devoted to these reviews. Counties promote plans to target restaurants that are likely to have these FBIs and these restaurants receive extra recurrent assessments. Modifications in inspection protocols could more strongly weight particular features of the setting that are related with foodborne illness outbreaks reports (Buchholz *et al.* 2002).

In some cases, a previous consumer may have transferred bacteria to a menu that was then transferred to another consumer (Aycicek *et al.* 2006). The surface type affects bacterial transfer and a nonporous surface increases the possibility of bacterial transfer to skin more than a porous surface (Julian 2010). Allwood *et al.* (2004) found that only 52% of the individuals responsible for retail food establishments were able to elucidate hand-washing processes as defined in the Minnesota Food Code and only 48% of foodservice employees were able to exhibit code-obedient hand washing. A study by the Food Standards Agency in the United Kingdom found that 55% of foodservice employees did not wash their hands before the handling and preparation of food, and about 33% had not received basic hygiene certification (Rudder 2006). Personnel are advised to wash their hands after handling each meal; however, the overall compliance with this Food Code recommendation for frequency during restaurant service is 5%. Thus, restaurant workers are washing their hands less often than the Food Code recommendation. This may lead to bacterial transfer from restaurant staff onto menus as workers handle menus more often than a single customer (Meyer *et al.* 2008).

Griffith *et al.* (2000) indicated that hand-contact surfaces in restaurants were contaminated and do not meet food industry standards for preventing foodborne illnesses. Taku *et al.* (2002) reported that restaurants may be harboring hepatitis A virus and that repetitive hand contact with tainted surfaces increase the spread of hepatitis A virus from consumer to consumer, including restaurant staff. In November 2003, 601 patrons contracted hepatitis A due to an outbreak at a single restaurant in Pennsylvania, resulting in 124 hospitalizations and three deaths (Wheeler *et al.* 2005).

Research has shown that certain types of surfaces harbor bacteria, including plastic. It is commonly known that bacteria are present on nearly all public surfaces and pathogens present can potentially pose health risks (Aycicek *et al.* 2006). Menus are often laminated with plastic to prolong their use. Plastic also protects menus from food and drink.

Thus, plastic surfaces can harbor bacteria, especially when these plastic surfaces are tainted with food residue that supports bacterial growth. Food residue such as droplets of juice, food particles and moisture will support bacterial growth and survival.

Environmental conditions affect bacterial populations on menus and posing risk to consumers (Teixeira *et al.* 2007). Some bacteria have the ability to attach to plastic but exactly how this occurs is not entirely understood. *Listeria monocytogenes* can adhere to surfaces and adherence depends on the type of surface. Because *L. monocytogenes* can adhere to the surface of plastics, there is a possibility that this pathogen can be present on the plastic that covers the menus (Araujo *et al.* 2007). Biofilm bacteria (known as sessile) display unique gene expression not seen in free-flowing cells (known as planktonic cells) when forming biofilms on a solid surface/fluid interface. They form in a stepwise manner with single cells adhering to the surface with extracellular polymeric substance acting as the glue (Neu *et al.* 2001; Flemming 2002). The attachment of bacteria to a surface initiates changes in the cell resulting in a new type of cell. A biofilm can develop in three ways: through redistribution of cells on the surface, through division of attached cells on the surface or by addition of free-flowing cells from the surrounding bulk fluid (Kuchma and O'Toole 2000; Stoodley *et al.* 2002). Thus, examining the possible role of menus as a vehicle to harbor and transmit bacteria is important to examine. Therefore, the objectives of the present study were to sample menus from local restaurants for total and staphylococcal bacterial populations and to determine the survival and transfer of bacteria from menus inoculated with *Escherichia coli* to hands.

MATERIALS AND METHODS

Experiment 1. Presence of Bacteria on Restaurant Menus

Pretest. To develop menu sampling methodology, a preliminary test was conducted to identify the areas most often touched by consumers when handling menus. A study at Purdue University found that most customers often touch the two far sides of the menu (Choi *et al.* 2011). In the current experiment, six participants covered their hands with a luminous cream (Glo germ gel lotion, Science Bob Store, Newton, MA) and then held an A4-sized paper in their hands as if they were holding a restaurant menu. They handled the paper as if they were handling a menu at a restaurant. The paper size was chosen because it was similar to the size used by most local restaurants. The paper was then observed under ultraviolet (UV) light (UVP, 8 W hand-held model, Upland, CA) to determine the locations having Glo-gel present. The pretest supported the Purdue University findings that test subjects most often touched the left and right edges of the menu while some subjects touched the middle of the menu when pointing at a meal choice.

Restaurant Menu Bacterial Counts. Eighteen different local restaurants (designated A–R) categorized into six different types were chosen to create a cross-section menu sources. A total of 216 samples were collected over a period of 8 months throughout the fall and spring. The high-traffic period (busy hours) was during the lunch and dinner (lunch = 11:30 a.m.–3:00 p.m.; dinner = 5:00 p.m.–8:00 p.m.), while the low-traffic period (nonbusy hours) was during times other than these. Day of the week of sampling was also recorded. These different times were chosen to determine if the consumer traffic affected the presence of bacteria on menus.

Swabs (3M Swabs, 3M Company, St. Paul, MN) were chosen because they are simple to use, affordable and rapid for retrieving environmental samples (Clemons 2010). Swabs were kept cool prior to use and also after the menus had been swabbed. Swabs were taken to restaurants in a cooler bag (Everest cooler bag, Wal-Mart, Central, SC) and the swabs were kept cool at all times until plating was completed. The sterile swab was used as per manufacturer's directions. The swab's cotton tip was then rubbed slowly and thoroughly over the menu surface. The swabbing technique utilized a zigzag pattern of a total of five lines from left to right, from top to bottom, and from the top left corner to the bottom right corner and also from the top right corner to the bottom left corner (for a total of 20 lines). The sizes of the restaurant menus samples fell into three general sizes of either ~603, 768 or 1207 cm^2. The average linear distance covered was 57 cm and the average area covered was 11 cm^2.

After the surface was swabbed, the swab was placed back in the tube, the cap was screwed on tightly and the tubes were transported to laboratory in a cooler bag with frozen cold packs. Upon returning to the laboratory, tubes were vigorously shaken by hand for 10 s under a biosafety hood (Labconco Purifier 36208-02 Class II/A Laminar Flow Biohazard Hood, LABEQUIP LTD, Markham, Ontario, Canada) to release bacteria from the swab. The swab cotton tip was then squeezed with fingers inside the tube to gather all the solution in the tube.

Staphylococcus spp. and total plate count (TPC) Petrifilm plates (3M Company) were placed on a flat surface. The top sheet of the film was lifted, then the sample tube was placed perpendicular to the Petrifilm plate and 1 mL of the sampling solution was placed (entire 1 mL of Letheen broth solution) onto the center of the film. Petrifilm plates were placed in an incubator (VWR symphony Gravity Convection Incubator, Radnor Corporate Center, Radnor, PA) for 24 h for the *Staphylococcus* spp. Petrifilm and 48 h for the

TPC Petrifilm at 37C. After incubation, bacteria were counted using a colony counter [Quebec Darkfield Manual Colony Counter (220V/50 Hz), Reichert Technologies World Headquarters and North American Service Center, Depew, NY]. Bacterial populations were reported as colony forming unit (cfu) per 15 cm^2 sampling area on a menu (cfu/15cm^2 sampling area).

Experiment 2. Bacteria Transfer from the Menu to Hands

Bacterial growth, cultivation and growth medium. An *E. coli* ampicillin-resistant strain with a fluorescent gene was used for the bacterial transfer and survival studies. A non-pathogenic *E. coli* strain JM109 was labeled with jellyfish green fluorescent protein according to the following protocol as described previously (Jiang *et al.* 2002). The competent bacterial cells were electroporated in a Gene Pulser II (Bio-Rad, Hercules, CA, USA) with plasmid vector pGFPuv (ClonTech, Palo Alto, CA). Transformants were selected from isolated colonies grown on Luria-Bertani agar plates containing 100 g of ampicillin/mL. The resulting ampicillin-resistant transformants emitted bright green fluorescence under UV light. The stability of green fluorescent protein (GFP) label in the *E. coli* strain was determined by streaking on trypticase soy agar (TSA) plates containing 100 g ampicillin/mL for several generations. The *E. coli* JM 109 culture was held in a –80C freezer in vials containing tryptic soy broth (Becto Tryptic Soy Broth, Becton Dickinson and Company, Sparks, MD) supplemented with 20% (v/v) glycerol (Sigma, St. Louis, MO). 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 tryptic soy broth (TSB) (Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing 0.5% ampicillin (Sigma-Aldrich, St. Louis, MO, USA in two loosely screw-capped tubes and then the tubes were incubated for 16–18 h at 37C 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 37C with shaking.

After incubation, the cells were harvested by centrifugation at 1,200× *g* (IEC HN-SII Centrifuge, International Equipment Co., Inc., Needham Heights, MA), then the pellet was 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, franklin Lakes, NJ, USA) and incubated at 37C for 24 h.

Menu Sampling. Test menus were created from 12.7 × 20.32 cm index cards (Staples index cards, Staples, Charlotte, NC) that were laminated using an office-laminating machine (Xyron Ezlaminator, Staples, Henderson, NC; polyethylene terephthalate) (European Patent 2012). The menus were covered in paper towels, foil (Kitchen cooking foil, Kirkland, Costco, Greenville, SC) and autoclaved and kept sealed until use. Menus were inoculated with the fluorescent *E. coli* by submersing test menus in 160 mL of bacterial inoculum solution containing approximately 5–6 log cfu/mL. The inoculum solution was made from resuspended bacteria pellet in 160 mL of sterile 0.1% peptone. The menus were placed into a stainless steel sterile tray (31.12 × 19.69 × 5.72 cm instrument tray, Polar Ware Company, Kiel, WI) with the depth of 5.72 cm and the width of 19.69 cm and the length of 31.12 cm. Sterile forceps were used to transfer inoculated menus onto a sterile test tube rack (Endicott-Seymour, Labsource Incorporated, Romeoville, IL) and then into the subject's hands. The test tube support was a sterilized and the menus were allowed to dry at room temperature for 30 min.

Instructions were presented so that all the subjects followed a similar menu handling technique. Each subject was handed a menu and was instructed to hold the menu as if in a restaurant setting and making menu choices. Each subject held and manipulated the menu for 1 min. Menus were placed into a stomacher bag (Stomacher 400 Classic Bags, 177 × 305 mm, 80–400 mL, Seward, Dominion House, Worthing, West Sussex, UK) with 40 mL sterile peptone solution (0.1%) and massaged for 30 s. Each hand singly (left and right) was massaged in a stomacher bag (Stomacher 400 Classic Bags, 177 × 305 mm, 80–400 mL, Seward, Dominion House) with 20 mL sterile peptone (0.1%) for 30 s prior to plating.

Nine milliliters test tubes of sterile peptone solution (0.1%) were used for serial dilution of samples (Clark *et al.* 1958). About 0.1 mL from sample dilutions were pipetted and spread onto TSA plates containing 100 g ampicillin/mL. Plates were held for 5–10 min and were then inverted and placed in an incubator at 37C for 24 h. The next day, the plates were inspected under UV light and appropriate Petri dishes were chosen for counting. The plates with a number ranging from 25 to 250 cfu/plate were counted and then these were multiplied by the dilution number that was used in the plating process. Plates were examined under the UV light and only the fluorescent bacteria were counted.

Experiment 3. Bacterial Survival on Menus

For testing the survival of bacteria on a menu after 24 and 48 h, nine menus per replication were inoculated with 0.1 mL of the 5–6 log of *E. coli* JM109 inoculum that was

prepared prior to the experiment as described in Experiment 2. Menus were allowed to dry for 30 min then placed in an incubator at room temperature for 24 and 48 h. Three menus were rinsed using peptone solution (0.1%) and after 30 min were sampled immediately after drying and recorded as the initial bacterial population on menus. After 24 or 48 h, the three menus for each time period were washed with the peptone solution (0.1%) in stomacher bags (Stomacher 400 Classic Bags, 177 × 305 mm, 80–400 mL, Seward, Dominion House) with 40 mL of sterile peptone solution (0.1%) and massaged for 30 s, 10 s for each side and an additional 10 s prior to plating. The recovery solution was serially diluted and surface plated as previously described in Experiment 2. Plates were counted after 24 h at 37C. The experiment was replicated three times.

Statistical Analysis

Experiment 1. Menus from 18 different restaurants were sampled during high (during lunch and dinner hours) and low (during other hours of the day) traffic periods. Six different menus [two per visit (replication)] were sampled at the high- and low-traffic periods each for a total of 12 samplings per restaurant. Restaurants were grouped into the following types for analysis: Mexican (4), bar (3), pizza (2), steakhouse (2), upscale (4) and other (3). The data were analyzed as a completely randomized design with a factorial arrangement using SAS (SAS, 2010, Version 9.2, Cary, NC) examining the main effects (high/low traffic, restaurant type, replication, day of week) and two-way interactions of main effects for significance at the $P \leq 0.05$ level. When main effects or interactions were significant, the pdiff option of SAS was used to determine statistical differences between means and to generate the standard error of the mean.

Experiment 2. Eight subjects completed the study participating in three replications on different days. The mean and standard deviation were calculated using SAS (SAS, 2010, Version 9.2) for all measurements overall, based on gender and the predominant hand (right or left handedness). A *t*-test was also conducted paired by subject to determine if the average transfer to right hand (log cfu right hand) differed from transfer to left hand. The paired *t*-test was conducted for transfer overall, for left-handed subjects and for right-handed subjects. The transfer was calculated according to the equation N/NH × 100 = transfer, where *NH* is the cfu/mL recovered from hands + N and *N* is the cfu/mL recovered from menus.

Experiment 3. Menus were inoculated then held for 48 h under ambient conditions (~27C, ~ 5% relative humidity) and sampled for *E. coli* populations at 0, 24 and 48 h. Three replications using three menus per sampling time were utilized for a total of 27 menus. Data were analyzed as a completely randomized design with a factorial arrangement using SAS (SAS, 2010, Version 9.2) examining the main effects (menu and holding time) and two-way interactions for significance at the 5% level. The survival was calculated according to the equation N/N0 × 100 = survival, where N0 is the cfu/mL at zero time and N is the cfu/mL in the samples after they had been kept at room temperature for 24 and 48 h.

RESULTS

Experiment 1. Presence of Bacteria on Restaurant Menus

Of the effects tested (replication, restaurant, traffic, day, restaurant by traffic and traffic by day), the only effects having a significant effect on TPC and *Staphylococcus* spp. (Staph) were restaurant, traffic and the restaurant by traffic interaction. Restaurants were grouped into types, and of the effects tested (replication, restaurant type, traffic, day, traffic by restaurant type and traffic by day), only restaurant type had a significant effect on TPC with no significant effects on Staph population. The minimum aerobic bacteria count was below detection levels at some restaurants during both busy hours and nonbusy hours. The minimum Staph count was also below detection levels at certain restaurants during both busy and nonbusy hours.

The maximum TPC was 210 cfu/15 m^2 sampling area on a menu during busy hours and 85 cfu/15 cm^2 sampling area on a menu during nonbusy hours, indicating the presence of bacteria on menus with high variation. The maximum Staph count was 83 cfu/15 cm^2 sampling area on a menu during busy hours and 23 cfu/cm^2 sampling area on a menu during nonbusy hours. For both TPC and Staph count, the minimum during both traffic periods was below detection levels. *Staphylococcus* spp. are often associated with human skin and can be a potential health threat. The mean TPC and mean Staph for each restaurant separately can be seen on Figs. 1 and 2 for both high- and low-traffic periods.

The restaurant type, sampling period (busy versus nonbusy) and restaurant type by sampling period interaction all had a significant effect on menu TPC and Staph populations (Table 1). Restaurants were categorized into groups based on type (bar, Mexican, pizzeria, steakhouse, upscale and other restaurants other than the other categories). The mean TPC and standard deviation of each type of restaurant can be seen in Table 2.

Extrapolating the number of bacteria from the 15 cm^2 area to the total area of the menus sampled (603,768 and 1207 cm^2) would be 6,030, 7,680 and 12,070 total aerobic bacteria, respectively. Extrapolated Staph populations for 603, 768 and 1207 cm^2 sizes were 1,648, 2,100 and 3,300.

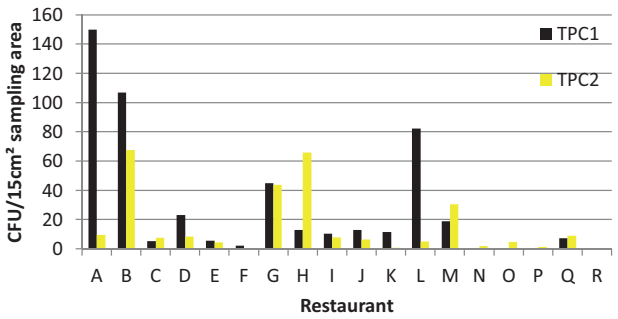

FIG. 1. MEAN AEROBIC BACTERIA POPULATIONS (TOTAL PLATE COUNT, TPC) ON MENUS FROM LOCAL RESTAURANTS DURING HIGH-TRAFFIC PERIODS (TPC1) AND LOW-TRAFFIC PERIODS (TPC2)

Extrapolating these populations is probably an overestimation but gives some estimate of bacterial numbers on the menus sampled.

Experiment 2. Transfer of Bacteria to Hands from Menus

Bacteria transferred from the menu to the hands of the subjects with a large variation in transfer. This may be due to differences in hand size, touch technique or capability of bacteria to attach to menus and skin. Transfer of microorganisms, even in small numbers, can result in foodborne illness.

The mean transfer of bacteria from a menu to a subject's hands was 8% for the right hand and 3.15% for the left hand and 11.17% on both hands combined. The standard deviation of the transfer of bacteria from a menu to the subject's hands was 9.58% for the right hand, 4.83 % for the left hand and 10.45% for both hands combined.

Different contact techniques were observed during the handling of the menus and this may also have contributed to the variation in the transfer s of subjects, as some touched the menus gently and fewer than other subjects. Transfer among genders was similar and was higher for the right hand than the left hand for both genders (Table 3).

The number of samples from right-handed subjects was three times that of left- handed samples (N of R = 36, N of L = 12). The transfer of bacteria from the menu to the right hand and total bacterial population was significantly different ($P = 0.030$) from that of the left hand (Table 4). The transfer of bacteria from the menu to the right hand was compared with that of left hand. Transfer to right hand versus transfer to left hand was significantly different for

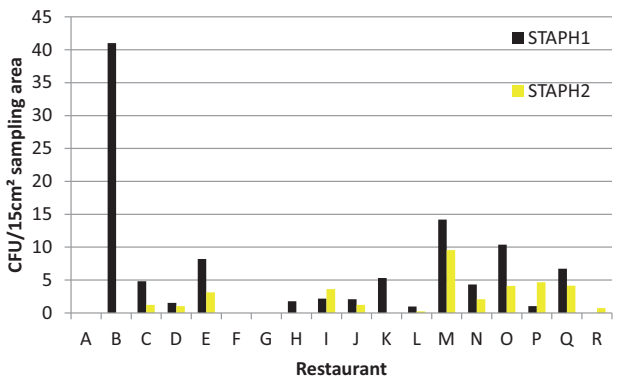

FIG. 2. MEAN *STAPHYLOCOCCUS SPP.* POPULATIONS ON MENUS FROM LOCAL RESTAURANTS DURING HIGH-TRAFFIC PERIODS (STAPH1) AND LOW-TRAFFIC PERIODS (STAPH2)

TABLE 1. *P* VALUES FOR TOTAL AEROBIC PLATE COUNT AND *STAPHYLOCOCCUS* SPP. (STAPH) FOR MENUS SAMPLED FROM DIFFERENT RESTAURANT TYPES AND BUSINESS HOURS

	p value	
Effect	TPC	Staph
Restaurant type	0.0001	0.0054
Sampling period	0.0099	0.0279
Rest × Period	0.0001	0.0212

TABLE 2. FREQUENCY OF SAMPLING AND MEAN TOTAL PLATE COUNT FOR DIFFERENT TYPES OF RESTAURANTS

Restaurant type	Frequency	Mean total plate count
Bar (3)	36	7.1[b]
Mexican (4)	48	41.0[a]
Pizzeria (2)	24	9.8[b]
Steakhouse (2)	24	2.2[b]
Upscale (4)	48	9.9[b]
Other (3)	36	3.5[b]

Means with different superscript letters are significantly different ($P \leq 0.05$).

TABLE 3. GENDER EFFECT ON TRANSFER OF BACTERIA FROM MENU TO HANDS AND POPULATION OF BACTERIA ON HANDS AFTER CONTACT WITH CONTAMINATED MENUS

Variable	Male transfer (%)	Female transfer (%)
Right hand	7.8 ± 7.9	8.2 ± 11.2
Left hand	3.9 ± 5.7	2.4 ± 3.7
Both hands	11.7 ± 8.9	10.6 ± 12
	Male log cfu for hand sampling	Female log cfu for hand sampling
Right hand	4.5 ± 0.9	4.9 ± 0.4
Left hand	3.9 ± 0.9	2.9 ± 0.9
Total bacterial count	5.8 ± 0.68	6.3 ± 0.53

$n = 24$ for both males and females.

TABLE 4. HANDEDNESS EFFECT ON TRANSFER OF BACTERIA FROM MENU TO HANDS

Handedness	Hand	Transfer (%)	Log cfu
Right handedness	Right	9.9 ± 10.3	4.8 ± 0.6
	Left	2.0 ± 2.4	3.9 ± 0.9
	Both	12.0 ± 11.0	3.9 ± 0.9
Left handedness	Right	2.1 ± 1.2	4.2 ± 0.8
	Left	6.6 ± 8.0	6.1 ± 0.6
	Both	11.7 ± 8.9	5.9 ± 0.9

Right hand, $n = 36$ observations; left hand, $n = 12$ observations.

total bacteria recovered from both hands ($P = 0.030$) and from right hand only ($P = 0.001$); but not from left hand only ($P = 0.059$; Table 5).

Experiment 3. Survival of Bacteria on Restaurant Menus

The survival of *E. coli* JM109 was tested on plastic laminated menus at after 0, 24 and 48 h at room temperature (20 ± 3C; Table 6).

Testing the survival of bacteria yielded 1.39%, 2.06% after 24 h as the mean survival and the survival standard deviation, respectively, and 1.34%, 1.89% after 48 h as the mean survival and the survival standard deviation, respectively.

DISCUSSION

The possible role of pathogens on menus in the transmission of foodborne illnesses may warrant further research as this study found that bacteria is present on menus and does transfer from restaurant menus to the consumers' hands. Foodborne illnesses resulting from a restaurant are often assumed to be due to foods and food-contact surfaces and nonfood-contact surfaces such as menus may be overlooked. A primary step that could help assess the risk of transfer from contaminated menus is to evaluate the transfer of different types of foodborne pathogens from menu to hands. This study is one of the first to examine the hygiene of restaurant menus, assess the transfer of bacteria from a menu to a consumer's hands and to test the survival of bacteria after 24–48 h at room temperature. The findings of this study can be used as a basis for such research ideas as well as determining menu hygiene and safety sanitation protocols that effectively minimize menu contamination with foodborne pathogens.

TABLE 6. SURVIVAL (LOG CFU/MENU) OF BACTERIA ON MENUS AFTER DIFFERENT TIMES

Time (h)	log cfu/menu	Standard deviation
0	5.5	0.4
24	3.7	0.3
48	3.6	0.3

Staphylococcus aureus and Staphylococcal Food Poisoning

S. aureus is a pathogen that is sometimes toxin-mediated, invasive and antibiotic resistance (Jones *et al.* 2002; Gaebler and De Souza 2010). This bacterium causes nosocomial illnesses and illnesses spread by the community. The infections that are caused by this bacterium can be foodborne illnesses as well as nonfood illnesses that are caused by mediators other than food. The symptoms that result from staphylococcal infections of nonfood mediators can be from a simple pimple to furuncles, toxic shock syndrome and sepsis (Todor 2008).

Staphylococcal enterotoxins are produced by some of strains of *Staphylococcus* that cause staphylococcal food poisonings. Staph can be found in warm-blooded animals' nostrils, skin and hair. Approximately 30–50% of the human population carries *Staphylococcus* spp. *Staphylococcus* spp. can live and thrive at temperatures that range from 7 to 48.5C with an optimum of 30 to 37C. *S. aureus* can also survive between the pH of 4.2 and 9.3, with an optimum of 7 to 7.5 and a sodium chloride concentration of up to 15%. The ability to survive in a wide range of environmental conditions enables *Staphylococcus* spp. to grow in a variety of settings (Baron *et al.* 2003).

Restaurant menus may harbor *S. aureus* which is a potential health risk. The presence of these pathogens may be especially dangerous for the immunocompromised. The presence of pathogens on restaurant menus and other restaurant surfaces could cause be a financial risk for the restaurant business which can result in fines and loss of

TABLE 5. T-TEST RESULTS FOR DETERMINING IF TRANSFER AND LOG POPULATION DIFFERED FOR TRANSFER TO RIGHT HAND VERSUS TRANSFER TO LEFT HAND

		P value for the test statistic	
Variable	*n*	Transfer to right hand versus transfer to left hand (%)	Log cfu/right hand versus log cfu/left hand
Total bacteria	48	0.030	0.0001
Left-handed	12	0.059	0.4545
Right-handed	36	0.001	0.0001

business should an outbreak occur. The infective dose of *S. aureus* toxin is <1.0 μg. This level of toxicity is achieved when *S. aureus* populations reach more than 100,000 cells per gram (Bad Bug Book 2013). The numbers found by this study vary from restaurant to restaurant and the highest numbers are less than the infective dose that was established by the Food and Drug Administration.

The data from the present study showed that *Staphylococcus* spp. was present on laminated menus. These results are supported by Neely and Maley (2000) who found that *S. aureus* is capable of adhering to plastic and also surviving on plastic for at least 1 day. Some were capable of surviving up to 56 days on certain plastic materials such as polyester and from 22 to 90 days on polyethylene. Restaurant menu coatings have been cited in the literature as a polyethylene-base laminate (Kavasch and Rivlin 2005) which is the same type used in the current study (polyethylene terephthalate; European Patent 2012).

The sampling of restaurant menus could be useful in determining the overall cleanliness of restaurants and may be useful for restaurant personnel and managers to maintain hygienic standards (Todd *et al.* 2010). The general public may also be interested in the hygiene of restaurant menus prompting them to wash their hands after touching menus (Jin and Leslie 2003). TPC and *Staphylococcus* spp. counts were present in higher levels during busy hours than they were at nonbusy hours. Given that health regulations emphasize the importance of a clean restaurant surface and utensils, there may be a concern about the cleanliness of the restaurant menus. As consumers and restaurant workers handle menus, perhaps more emphasis should be placed on menu sanitation as part of restaurant sanitation protocol (Snyder 2005).

Transfer of Bacteria from Menus to Consumer Hands

The main factors targeted by the Centers for Disease Control in reducing retail foodborne illness include food worker handwashing, food preparation surfaces and food temperature control, while menus are rarely considered a factor. Transfer of bacteria is affected by the type of surface, such as nonporous surfaces (menus laminated with plastic, polyethylene terephthalate), and these surfaces increase the possibility of bacteria transferring to the skin more than if the surface was porous (Julian 2010). The current study found that bacteria transfer from plastic-laminated menus to human hands with differences due to gender and handedness.

E. coli Adherence to Plastic

In both studies, *E. coli* JM109 was recovered from plastic-laminated menus, which coincided with Torres *et al.* (2005), showing that *E. coli* was capable of adhering to plastic. The survival of *E. coli* has been tested before on different surfaces with different textures. A study performed by Milling *et al.* (2005), on the survival of *E. coli* on plastic particles, showed that after 24 h there was 10^6 cfu/g with an initial of 10^8 cfu/g of bacterial colonies. This indicates that the bacteria only decreased 2 logs after 24 h at 37C. Similar results were observed at 21C after 24 h, but at lower temperatures such as 4C; there was no decline in bacterial populations even after 48 h. It was reported that after 6 days, populations decreased by 1 log. These research results by Milling *et al.* (2005) were in accordance with the results from the present study showing that *E. coli* survives on plastic.

Survival of Bacteria on Plastic-Laminated Menus

Menus are often laminated with plastic for extended life and to prevent the paper from absorbing spilled water and food. Research has shown that plastic can harbor bacteria and bacteria have the ability to attach to plastic but the mechanism is still not entirely understood. *L. monocytogenes* is one of these bacteria and adherence depends on surface type. As *L. monocytogenes* has the ability to adhere to the surface of plastics, it is possible that bacteria can adhere to menus that are covered in plastic (Araujo *et al.* 2007). In conclusion, the data collected indicates the possibility of bacterial transfer from contaminated menus to hands. While there was variation between subjects, transfer from menus to subjects occurred from contaminated menus. Bacteria were found to survive on menus after 48 h at room temperature, indicating the importance of cleaning menus daily. Thus, menu hygiene may be considered as a standard sanitation operating procedure for restaurants as this may be a critical control point in food retail establishments (Aycicek *et al.* 2006).

Future research may include testing adherence and survival of bacteria to different types of menus. The testing of bacterial survival on menus that have media on the surface to simulate food juices/particles on menus may be another possible study. Testing the transfer of different types of foodborne pathogens and their adherence to menus could also be determined as well as the effect of menu material to determine how the different porosity of materials affects bacterial transfer and survival.

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