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Longer Contact Times Increase Cross-Contamination of *Enterobacter aerogenes* from Surfaces to Food

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ABSTRACT

Bacterial cross-contamination from surfaces to food can contribute to foodborne disease. The cross-contamination rate of *Enterobacter aerogenes* on household surfaces was evaluated by using scenarios that differed by surface type, food type, contact time (<1, 5, 30, and 300 s), and inoculum matrix (tryptic soy broth or peptone buffer). The surfaces used were stainless steel, tile, wood, and carpet. The food types were watermelon, bread, bread with butter, and gummy candy. Surfaces (25 cm^2) were spot inoculated with 1 ml of inoculum and allowed to dry for 5 h, yielding an approximate concentration of 10^7 CFU/surface. Foods (with a 16-cm^2 contact area) were dropped onto the surfaces from a height of 12.5 cm and left to rest as appropriate. Posttransfer, surfaces and foods were placed in sterile filter bags and homogenized or massaged, diluted, and plated on tryptic soy agar. The transfer rate was quantified as the log percent transfer from the surface to the food. Contact time, food, and surface type all had highly significant effects ($P < 0.000001$) on the log percent transfer of bacteria. The inoculum matrix (tryptic soy broth or peptone buffer) also had a significant effect on transfer ($P = 0.013$), and most interaction terms were significant. More bacteria transferred to watermelon (~0.2 to 97%) than to any other food, while the least bacteria transferred to gummy candy (~0.1 to 62%). Transfer of bacteria to bread (~0.02 to 94%) was similar to transfer of bacteria to bread with butter (~0.02 to 82%), and these transfer rates under a given set of conditions were more variable than with watermelon and gummy candy.

IMPORTANCE

The popular notion of the "five-second rule" is that food dropped on the floor and left there for <5 s is "safe" because bacteria need time to transfer. The rule has been explored by a single study in the published literature and on at least two television shows. Results from two academic laboratories have been shared through press releases but remain unpublished. We explored this topic by using four different surfaces (stainless steel, ceramic tile, wood, and carpet), four different foods (watermelon, bread, bread with butter, and gummy candy), four different contact times (<1, 5, 30, and 300 s), and two bacterial preparation methods. Although we found that longer contact times result in more transfer, we also found that other factors, including the nature of the food and the surface, are of equal or greater importance. Some transfer takes place "instantaneously," at times of <1 s, disproving the five-second rule.

The Centers for Disease Control and Prevention (CDC) estimates that each year there are more than 9 million episodes of foodborne illness, over 55,000 hospitalizations, and at least 1,351 deaths that can be attributed to foods consumed in the United States (1). The CDC regularly publishes reports that summarize data on surveillance for foodborne disease outbreaks in the United States (2–6). Those reports list more than 30 contributing factors linked to foodborne disease outbreaks in the year or years summarized in the reporting period. Factors are grouped into three categories related to contamination with and proliferation and survival of foodborne pathogens. Food handlers or others suspected to be infectious are linked to several contamination factors. One factor is specifically related to cross-contamination from surfaces and not ill individuals. When those surface cross-contamination data are summarized from 1998 to the present, about 12% of all outbreaks reported to the CDC are linked in some way to this type of surface cross-contamination. This is the 6th most common contributing factor (out of 32) (2–6).

Household and other surface types have been the focus of numerous cross-contamination studies; the surfaces studied include ceramic tile (7–9), stainless steel (7, 9–12), wood (8), glass (7), plastic (7, 13, 14), and carpet (8, 15, 16). Stainless steel has often been considered the optimal material choice for kitchen sinks and commercial food preparation surfaces because of its resistance to corrosion, mechanical strength, ease of cleaning, and resistance to chemical degradation (17, 18), although stainless steel may have higher bacterial transfer rates than other surfaces (19–21). Tile is also a common surface found in homes; the variations of tile (unglazed versus glazed) may have an effect on the bacterial transfer rate because of varying surface topography (22). Wood surfaces are commonly found in households, either as flooring or as cutting board surfaces. The sanitary properties of wood cutting boards have been compared to those of plastic cutting boards (23, 24), and the studies have come to contradictory conclusions, in part because of differences in the methods used. The United States Department of Agriculture recommends one cutting board for

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produce and bread and a separate cutting board for raw meat, poultry, and seafood (25). Carpet is a likely site of contamination in the household, and inactivating or removing bacteria by conventional cleaning methods is difficult once the carpet is contaminated (16). Microorganisms on carpet can be controlled by specific chemical treatments of the fibers or the materials used in constructing the carpet (26).

The popular-culture notion of the "five-second rule" is that food dropped on the floor for <5 s is "safe" because bacteria need time to transfer. The rule has been explored to a limited degree in the published literature and popular culture. Previous studies on the five-second rule used different surfaces, foods, organisms, contact times, and numbers of replicates, making comparisons and conclusions difficult. The first known research recorded on this topic was performed at the University of Illinois but was never published in the peer-reviewed literature (27). These researchers used tile inoculated with *Escherichia coli* and studied transfer to cookies and gummy bears and found that bacterial transfer was observed in <5 s (27). The popular television show MythBusters aired an episode on the five-second rule in 2005 and found no conclusive difference between contact times of 2 and 6 s (28). In the only peer-reviewed research on the topic, researchers at Clemson University concluded that longer contact times (5, 30, and 60 s) did increase the transfer of *Salmonella enterica* serovar Typhimurium from wood, tile, or carpet to bologna or bread, but only ≥8 h after the surface was inoculated (8). Researchers at Aston University in the United Kingdom published a press release in 2014 reporting that contact time significantly affected the transfer of both *E. coli*- and *Staphylococcus aureus*-contaminated surfaces (carpet, laminate, and tile) to food (toast, pasta, biscuit, and a sticky sweet) (29). Discovery Science Channel's The Quick and the Curious television show aired a short segment offering cookies to strangers in a park—after dropping them onto the ground. The show's narrator stated that "moist foods left longer than 30 seconds collect 10 times the bacteria than [sic] those snapped up after only three" but offered no data in support of this statement (30).

This research sought to quantify cross-contamination between a variety of foods and common kitchen surfaces while varying the contact time and bacterial matrix and to do so in an extensive and comprehensive manner. The results described here advance our understanding of cross-contamination and the factors that influence it. This research informs the popular culture and enhances our scientific understanding of cross-contamination and the factors that influence it.

MATERIALS AND METHODS

Bacterial strain and preparation of culture. A nonpathogenic, food-grade microorganism, *Enterobacter aerogenes* B199A, with attachment characteristics similar to those of *Salmonella*, was used for all experiments (Vivolac Cultures, Indianapolis, IN) (14). The *E. aerogenes* strain used here is resistant to nalidixic acid, which allows it to be enumerated in the presence of other microorganisms on food samples or surfaces. Control experiments (done by sampling and plating onto tryptic soy agar [Difco, BD, Sparks, MD] with 50 μg/ml nalidixic acid [Sigma Chemical Co., St. Louis, MO] [TSA-na]) showed that nalidixic acid-resistant *E. aerogenes* cells were not initially present on any of the foods or surfaces at levels of >2 log CFU/surface or food.

Cultures were prepared on the basis of prior work done in our lab (13) and by others (14). A frozen stock of *E. aerogenes* in 80% sterile glycerol was streaked onto TSA-na. One colony from each plate was transferred to 10 ml of tryptic soy broth (Bacto, BD, Sparks, MD) with 50 μg/ml nalidixic acid (TSB-na) and incubated at 37°C for 24 h. Inoculum matrices were of two types, one using cells harvested by centrifugation at 5,000 × *g* for 10 min and washed twice in 10 ml of 0.1% peptone (Difco, BD) and one using cells taken directly from an inoculated overnight TSB-na culture. A final concentration of ~10^8 CFU/ml was verified by enumeration on TSA-na.

Preparation of domestic surfaces. Four different surfaces typical of those found in domestic environments were used: stainless steel (type 304, 0.018-in. thickness, 16 gauge; OnlineMetals, Seattle, WA), ceramic glazed tile (Brancacci Windrift Beige; Daltile, Dallas, TX), maple laminate wood (Northern Maple; Mohawk, Calhoun, GA), and indoor-outdoor carpet (Morella; Foss Manufacturing, Hampton, NH). They were ordered online or purchased from a local home improvement store. Surface materials were cut into coupons (5 by 5 cm). The stainless steel and ceramic tile coupons were disinfected prior to inoculation by soaking in 70% ethanol for 1 h, removed, air dried, and autoclaved. Disinfection of wood and carpet coupons caused structural changes, so these were discarded after autoclaving following a single use.

Food types. Four foods (watermelon, white bread [ShopRite, Wakefern Food Corp., Elizabeth, NJ], unsalted butter [ShopRite, Wakefern Food Corp., Elizabeth, NJ], and gummy candy [Haribo Strawberries]) were purchased online or from a local supermarket. Whole watermelon was stored at 4°C prior to use. The watermelon (flesh only) and bread (excluding the crust) were cut into pieces (approximately 4 by 4 cm). Unsalted butter was brought to ambient temperature (~24°C) prior to being spread onto bread. All of the foods had equivalent contact areas (~16 cm^2). The pH and water activity (a_W) of samples were measured in triplicate with a surface pH probe (Accumet Basic AB15 pH meter; Fisher Scientific) and an a_W meter (Rotronic Instrument Corp., Hauppauge, NY), respectively.

Transfer between food and surfaces. Transfer scenarios were evaluated for each contact surface type (4), each food type (4), four contact times, and two inoculum matrices, totaling 128 scenarios. Each scenario was replicated 20 times, totaling 2,560 measurements. Each contact surface type was spot inoculated with 1 ml of inoculum by using 8 to 10 drops spread over the 5- by 5-cm surface. The surfaces were placed in a biosafety cabinet (SterilGARD Hood; The Baker Company, Inc., Sanford, ME) for 5 h, after which the surfaces were visibly dry. Prior to 5 h, surfaces were still wet and at times longer than 5 h, the difference in recovery rate between the inoculum matrices increased. Both the peptone buffer and TSB-na inoculum matrices yielded an approximate concentration of 10^7 CFU/surface after drying. Foods were dropped onto the respective surfaces by using gloved hands from a height of 12.5 cm and left to rest for four different times (<1, 5, 30, and 300 s). A height of 12.5 cm was selected because it was the greatest height possible that still ensured that the entire food sample would reliably contact the entire surface.

Surfaces were placed into a sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI), 20 ml of peptone buffer was added, and the mixture was hand massaged for 2 min. Foods were placed into a sterile filter bag (Fisherbrand Lab Blender Bags) with 50 ml of peptone buffer, and the samples were homogenized (Stomacher; Cooke Laboratory Products, Alexandria, VA) for 3 min. Surfaces and food samples were serially diluted in 0.1% peptone buffer and surface plated (0.1 ml) onto TSA-na for enumeration of *E. aerogenes* colonies. Plates were incubated at 37°C for 24 h. Colonies were counted, and population levels were expressed in numbers of CFU per food or surface sample.

Data analysis. Percent transfer was calculated as follows: [total CFU food/(total CFU food + total CFU surface)] × 100. Percent rates of transfer from surface to food were log transformed with Microsoft Excel (Microsoft, Redmond, WA) and SigmaPlot (Systat Software Inc., San Jose, CA), as prior research has shown that untransformed transfer rates are highly skewed and log-transformed transfer rates are approximately normally distributed (13, 31). When foods contained less than the detection limit (2 log CFU), transfer rates were calculated as if the concentration on the foods was at the detection limit. Variables and the interactions between variables were considered significant at a *P* value of <0.05. Multiple

TABLE 1 pH and a_W measurements of four foods to which *E. aerogenes* bacteria are transferred from common household surfaces

Food type	Mean a_W ± SD	Mean pH ± SD
Bread	0.95 ± 0.01	5.80 ± 0.02
Butter	0.97 ± 0.01	6.25 ± 0.03
Gummy candy	0.72 ± 0.01	2.80 ± 0.03
Watermelon	0.99 ± 0.01	5.43 ± 0.01

linear regression analysis was performed with StatPlus for Microsoft Excel (AnalystSoft, Inc., Walnut, CA). Quantitative values were given to the surfaces tile (0), stainless steel (1), wood (2), and carpet (3); the foods bread (0), bread with butter (1), gummy candy (2), and watermelon (3); and the matrices TSB (0) and buffer (1) for regression analysis.

RESULTS

pH and a_W measurements. The pH and a_W measurements for all food types are shown in Table 1. Watermelon had the highest a_W of the foods studied. Bread and butter had measured a_W values close to that of watermelon. The a_W of the gummy candy was considerably lower than that of the other foods measured (0.72 versus ≥0.95). Butter had the highest pH (6.25) of any of the foods measured, and gummy candy had the lowest (2.80). Although a low pH is known to cause stress injury to microorganisms, it is unlikely, given the short contact time in this study, that this would have occurred in the gummy candy experiments (32). The measured pH values of bread and watermelon were intermediate (5.80 and 5.43, respectively).

Statistical analysis of transfer rates. The contact time, food, and surface and the food × time interaction were shown to significantly ($P < 0.000001$) influence log percent transfer. The surface × time ($P = 0.0019$), surface × food ($P = 0.00019$), and surface × matrix ($P = 0.00005$) effects on log percent transfer were also significant. The inoculum matrix, i.e., TSB or buffer ($P = 0.013$) and the food × matrix interaction ($P = 0.045$) were statistically significant, although less so than the other factors. The time × matrix interaction did not have a statistically significant effect on log percent transfer ($P = 0.49$) (Table 2).

Transfer of bacteria from inoculated surfaces to watermelon, bread, bread with butter, and gummy candies is summarized in Tables S1 to S4 in the supplemental material, respectively. Each table shows six different statistical parameters that were used to characterize the log percent transfer rate: mean ($\bar{x}$), median (M), standard deviation (σ), minimum (min), maximum (max), and range. The tables are referenced as needed to supplement the discussion of the figures below.

Bacterial transfer from an inoculated surface to food. The transfer of *E. aerogenes* from TSB and buffer-inoculated surfaces (tile, stainless steel, wood, and carpet) to food (watermelon, bread, bread with butter, and gummy candy) over time (<1, 5, 30, and 300 s) is shown in Fig. 1 and 2, respectively. Error bars in Fig. 1 and 2 indicate the standard deviations of the recorded observations. Since many scenario results were similar, not all observations will be specifically discussed below.

Inoculated surface to watermelon. When all TSB-inoculated surfaces contacted watermelon, a high degree of transfer of bacteria to the watermelon occurred (Fig. 1). The mean log percent transfer of bacterial cells contained within the TSB inoculum from tile to watermelon was highest at 5 s, i.e., 1.99 (97%) (Fig. 1M; see Table S1 in the supplemental material). The mean log percent transfer of bacteria from stainless steel was between 1.96 (90%) and 1.97 (93%) (Fig. 1N; Table S1). Overall, there was no significant difference in bacterial transfer from any surface to watermelon at different contact times (Fig. 1M to P).

TABLE 2 Multiple linear regression analysis[a] results for the effects of contact time, inoculum matrix, food type, and surface type and their interactions on the transfer of *E. aerogenes* from common household surfaces to foods

Variable or interaction	Coefficient	SE	LCL[b]	UCL[c]	*t* statistic	*P* value
Intercept	0.38	0.09	0.20	0.56	4.18	0.000030
Time	0.01	0.00	0.01	0.01	13.40	<0.000001
Matrix	−0.26	0.11	−0.47	−0.06	−2.49	0.012944
Food	0.23	0.04	0.15	0.32	5.36	<0.000001
Surface	−0.25	0.04	−0.33	−0.16	−5.78	<0.000001
Time × matrix	0.00	0.00	0.00	0.00	−0.68	0.494994
Time × food	0.00	0.00	0.00	0.00	−7.90	<0.000001
Time × surface	0.00	0.00	0.00	0.00	−3.11	0.001896
Matrix × food	−0.08	0.04	−0.17	0.00	−2.01	0.044589
Matrix × surface	−0.17	0.04	−0.25	−0.09	−4.06	0.000050
Food × surface	0.07	0.02	0.03	0.11	3.74	0.000190

[a] Quantitative values were given to the following variables: surface (tile [0], stainless steel [1], wood [2], and carpet [3]), food (bread [0], bread with butter [1], gummy candy [2], and watermelon [3]), and inoculum matrix (TSB [0] and buffer [1]).
[b] LCL, lower confidence limit.
[c] UCL, upper confidence limit.

Bacterial transfer from buffer-inoculated surfaces to watermelon was more variable than with the TSB inoculum matrix (Fig. 2M to P). The mean log percent transfer of bacteria from tile was between 1.17 (15%) and 1.96 (91%) (Fig. 2M; see Table S1 in the supplemental material). Greater transfer from stainless steel and wood at <1 s was observed (Fig. 2N and O), with mean log percent transfers of 1.96 (91%) and 1.93 (86%) to watermelon, respectively (Fig. 2N and O; Table S1). The mean log percent transfer from carpet ranged from −0.75 (0.2%) to 0.14 (1%) (Fig. 2P; Table S1).

The mean rates of transfer to watermelon and the standard deviations associated with the means are similar for stainless steel, tile, and wood. However, for transfer from carpet to watermelon, the mean transfer rates and standard deviations differ considerably from one inoculum to another.

Inoculated surface to bread. When bread was dropped onto TSB-inoculated tile, stainless steel, wood, or carpet, the highest rate of transfer from wood was observed at 30 s (Fig. 1C), although a significant difference between transfers from wood at 30 and 300 s was not observed. The mean log percent transfer of bacteria from stainless steel was between −0.56 (0.3%) and 1.97 (93%) (Fig. 1B; see Table S2 in the supplemental material). For bread dropped on tile, the mean log percent transfer ranged from −0.95 (0.1%) to 1.96 (92%) (Fig. 1A; Table S2), and the mean log percent transfer from wood ranged from −0.64 (0.2%) to 1.97 (94%) (Fig. 1C; Table S2). The mean log percent transfer from carpet ranged from −0.87 (0.1%) to 0.58 (4%), which was lower than that from the other three contact surfaces (Fig. 1D; Table S2). At <1 s, 18/20 and 19/20 replicates were below the detection limit for TSB- and buffer-inoculated carpet samples, respectively (Table S2).

Bread dropped onto the surfaces behaved similarly regardless of whether a TSB- or buffer-inoculated matrix was used. The transfer of bacteria from buffer-inoculated surfaces was highest at 300 s for all surfaces. The mean log percent transfer of bacteria from tile to bread was between −0.68 (0.2%) and 1.79 (62%)

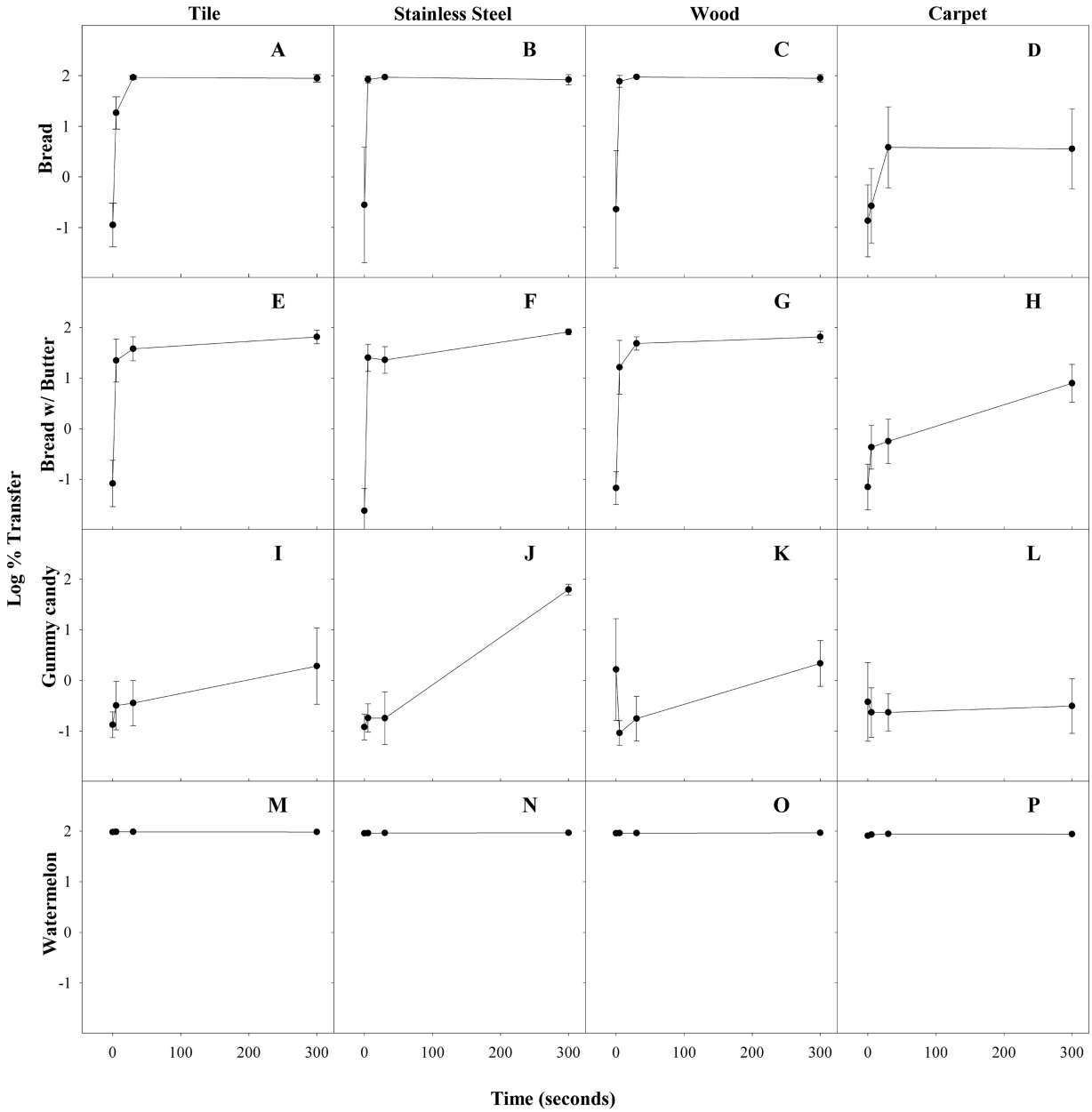

FIG 1 Effect of contact time on log percent transfer of *E. aerogenes* inoculated onto four household surfaces in a TSB matrix to four foods.

(Fig. 2A; see Table S2 in the supplemental material). Stainless steel had the highest mean log percent transfer of bacteria to bread after 300 s, at 1.91 (80%) (Fig. 2B; Table S2). The mean log percent transfer of bacteria from wood over time was between −0.91 (0.1%) and 1.89 (78%) (Fig. 2C; Table S2), and that of bacteria from carpet was between −1.68 (0.02%) and −0.79 (0.2%) (Fig. 2D). The standard deviation of stainless steel, tile, and wood was greatest at <1 s, while the standard deviation of carpet-to-bread transfer was similar at all time points (Table S2).

Inoculated surface to bread with butter. Bacterial transfer from all surfaces to bread with butter at <1 s was low; on average, 10/20 replicates were below the detection limit on TSB-inoculated surfaces (see Table S3 in the supplemental material), where the detection limit was 2 log percent transfer based on the protocols used in our experiments (Fig. 1). When buttered bread was in contact with inoculated tile, the mean log percent transfer of bacteria increased between −1.08 (0.08%) and 1.81 (65%) from <1 to 300 s (Fig. 1E; Table S3). The mean log percent transfer of bacteria from stainless steel to buttered bread was between −1.63 (0.02%) and 1.91 (82%) (Fig. 1F; Table S3), and the mean log percent transfer from wood to buttered bread was between −1.18 (0.07%) and 1.81 (65%) (Fig. 1G; Table S3). Carpet transferred fewer bacteria than the other contact surfaces, yet the mean log percent transfer still increased over time from −1.15 (0.07%) to 0.9 (8%) (Fig. 1H; Table S3).

Transfer of *E. aerogenes* from buffer-inoculated surfaces to bread with butter is shown in Fig. 2. There was an increase in bacterial transfer from all of the surfaces as the contact time in-

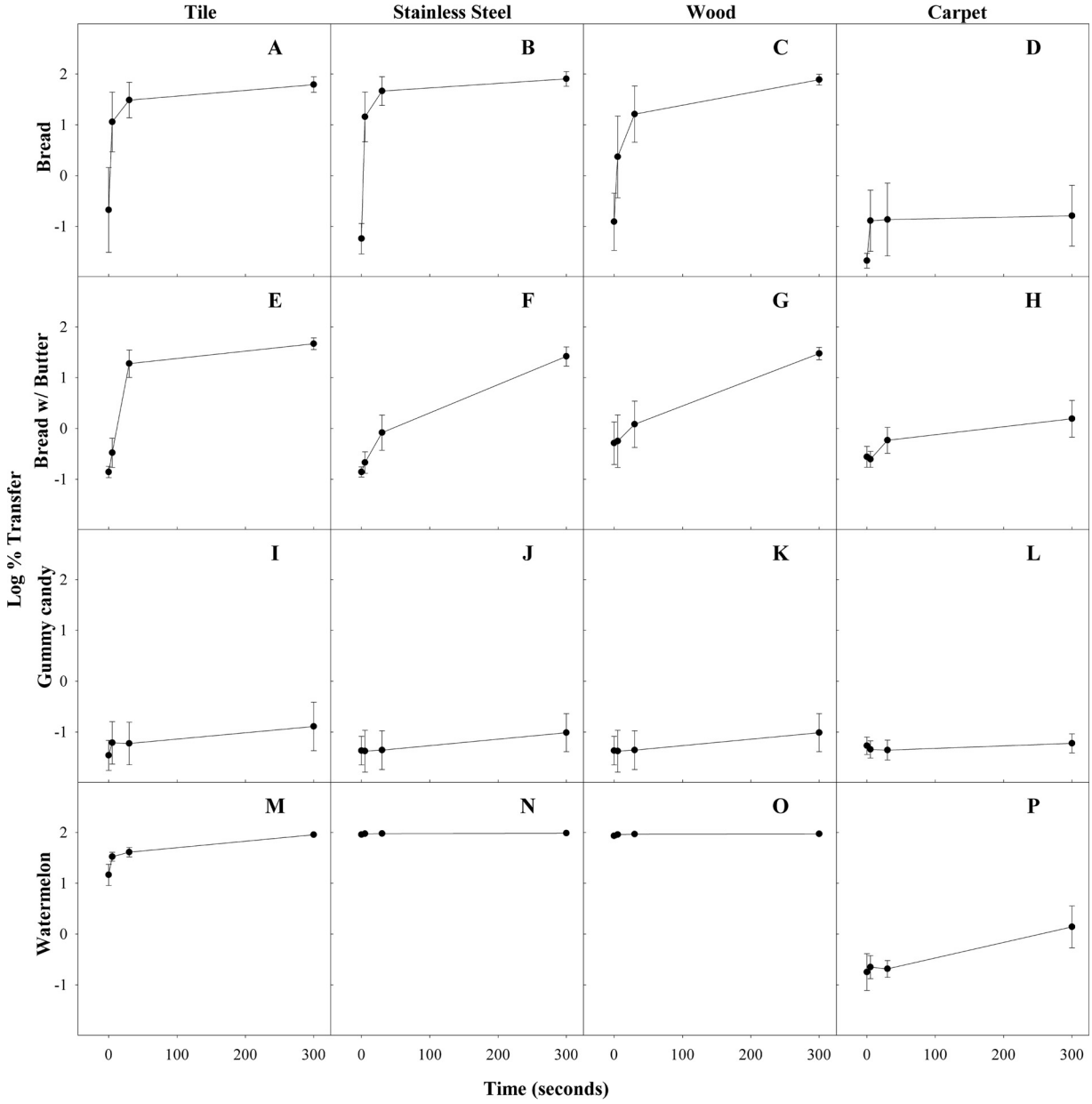

FIG 2 Effect of contact time on log percent transfer of *E. aerogenes* inoculated onto four household surfaces in a peptone buffer matrix to four foods.

creased. Tile inoculated with cells contained in buffer transferred more bacteria to buttered bread than any other surface (Fig. 2). When bread with butter contacted tile, the mean log percent transfer of bacteria ranged from −0.86 (0.1%) to 1.67 (47%) (Fig. 2E; see Table S3 in the supplemental material). Stainless steel and wood transferred similar fractions of cells contained in buffer to bread with butter. With stainless steel, the mean log percent transfer was between −0.86 (0.1%) and 1.42 (26%) at <1 to 300 s (Fig. 2F; Table S3), respectively, while with wood the mean log percent transfer rates ranged from −0.29 (0.5%) to 1.48 (30%) (Fig. 2G; Table S3). Carpet again showed the lowest mean log percent transfer rates, ranging from −0.56 (0.3%) to 0.19 (2%) (Fig. 2H; Table S3).

Inoculated surface to gummy candy. The mean log percent rate of transfer from tile to gummy candy increased with time, ranging from −0.88 (0.1%) to 0.28 (2%) (Fig. 1I; see Table S4 in the supplemental material). The mean log percent transfer from carpet to gummy candy was lowest at 300 s, at −0.51 (0.3%) (Fig. 1L; Table S4). The transfer from stainless steel increased over time from <1 to 300 s, although at <1, 5, and 30 s, on average, 16/20 replicates were below the detection limit (Fig. 1J; Table S4). The highest mean log percent transfer observed for any surface to gummy candy was from stainless steel at 300 s, i.e., 1.80 (63%) (Fig. 1J; Table S4).

When gummy candies were dropped on any surface containing the inoculum in buffer, the mean log percent transfer was low, regardless of the time. On average, 19/20 replicates of gummy candy to any surface at <1 s were below the detection limit and an

average of 8/20 were below the detection limit at 300 s (see Table S4 in the supplemental material). The highest mean log percentage of bacterial transfer observed was from tile at 300 s, i.e., −0.89 (0.1%) (Fig. 2I; Table S4).

DISCUSSION

Our study shows that bacterial transfer is dependent on the surface, food type, contact time, and inoculum matrix. Studies involving transfer from similar surfaces to foods have come to various conclusions (7, 8). These differences may be due to the range of experimental procedures among published studies. Differences include the times of contact between surfaces (7, 8, 11), the organisms used (7, 8, 11, 33), and the foods and contact surfaces used (7, 8, 11, 33), each of which can result in different outcomes. Our research also shows that the nature of the matrix containing the cells inoculated onto the surface can play an important role, even when all other experimental variables are the same, an observation we have seldom seen reported in the literature. Studies of bacterial adhesion to surfaces used a variety of drying times, in comparison to the 5-h drying time used in this study (7, 8, 34, 35). Additionally, there is a difference in data analysis regarding transfer rates. Some studies determined the transfer rate by calculating the recipient surface/source surface (13), whereas in our study, the transfer rate was analyzed by calculating the recipient surface/(source surface + recipient surface) (7, 8, 11), which can lead to slight differences when the number of bacteria transferred to the recipient surface is high. More importantly, some studies used very small numbers of replicates and/or failed to statistically transform the percent transfer rates and may have come to erroneous conclusions (31, 36). Although not always reported in studies, standard deviation is a good indication of the degree of variability (13). In our study, the standard deviation varied considerably with the food tested.

Although pressure was not a variable in our study, it may play a role in facilitating bacterial transfer. Kusumaningrum et al. found that more transfer occurred when light pressure (20 g/cm^2) was applied, although differences were slight (~0.3-log percent transfer difference) (33). Mbithi et al. used pressures of 200 and 1,000 g/cm^2, with and without friction, and found that differences in transfer rates were also small (a ~0.5-log percent transfer difference when pressure was applied) (37). Research by D'Souza et al. showed that pressure changes ranging from ~1 to 100 g/cm^2 had no effect on virus transfer (38). Later research in the same laboratory showed more transfer at higher pressures (~100 g/cm^2) than at lower pressures (~10 g/cm^2), especially when the inoculum was drier (39).

Our data clearly show that contact time does influence bacterial transfer, with more bacteria transferred at longer times. Peer-reviewed research by Dawson et al. reported that a longer food contact time (5, 30, or 60 s) did result in greater transfer but only at longer drying times (≥8 h) (8) roughly equivalent to our drying time of 5 h. Non-peer-reviewed research at the University of Illinois on bacterial transfer from tile inoculated with generic *E. coli* to cookies and gummy bears found that bacterial transfer was observed in <5 s (27) (consistent with our <1-s observations), although other contact times were not studied. The popular television show MythBusters (28) aired an episode on the five-second rule and found no conclusive difference when pastrami and crackers were exposed to contaminated tile with contact times of 2 and 6 s. It is unclear from viewing the episode what was used to contaminate the tile surface, although the inoculated tile was left for 5 days before the experiment was begun. MythBusters also used <10 replicates per scenario. A press release by Aston University, in the United Kingdom, showed that time significantly affected transfer, depending on the contaminated surface and the food (29). The Aston University study observed the transfer of *E. coli* and *S. aureus* from carpet, wood, and tile to toast, pasta, biscuit, and a sticky sweet at 3- and 30-s contact times. Moist foods that contacted contaminated wood and tile showed higher transfer rates, and longer times increased the transfer between these foods and surfaces. The Aston University study shows that transfer from carpet was not affected by the food composition or the contact time (29).

Our data show that the rate of bacterial transfer was greatest for tile, stainless steel, and wood surfaces at 300 s. The food with the highest transfer rate was watermelon, regardless of the contact time, which may be due to several factors. When watermelon is cut, it is very moist, and moisture is known to facilitate transfer (40), regardless of whether the contact surface is dry or wet. Watermelon may also present a flatter, more uniform surface at the microscopic level than bread or gummy candies. Jensen et al. also found that transfer from stainless steel or tile to watermelon was the highest of any produce type used in their study (7). Kusumaningrum et al. measured the rates of transfer to cut cucumber from stainless steel and observed that almost all of the bacteria (~100%) transferred to the cucumber, regardless of pressure (33). Cut cucumbers also have a moist, uniform surface, which may facilitate bacterial transfer. We observed lower transfer rates (~0.2%) when transfer was from carpet to food. Carpet may promote less bacterial transfer because of bacterial attachment to or infiltration of absorbent carpet fibers. Dawson et al. also found that transfer from carpet to bologna was very low (<0.5%) in comparison to transfer from wood and tile to bologna (5 to 68%) (8).

The starting concentration of all of the surfaces in our experiments was ~7 log CFU/surface. Although this was not a variable explicitly considered, the starting concentration may have an effect on how much bacterial transfer to the recipient surface occurs. Montville and Schaffner reported on the influence of inoculum size on bacterial cross-contamination between surfaces. Their results showed that the effect of inoculum size on the transfer rate was statistically significant ($P < 0.0001$) for all transfer rate data and that a greater inoculum size resulted in a lower transfer rate (41).

Transfer of bacteria from surfaces to food appears to be affected most by the moisture of the food, as shown by the transfer of *E. aerogenes* from tile, stainless steel, wood, and carpet to watermelon. Longer food contact times usually resulted in the transfer of more bacteria from each surface to food. Carpet has very low transfer rates, compared with those of tile and stainless steel, whereas transfer from wood is more variable. The topography of the surface and food seems to play an important role in bacterial transfer. The risk of illness resulting from deciding to consume food that has fallen on the floor depends on factors including the prevalence, concentration, and type of organism; the nature of the food (especially moisture); and the nature of the surface topology; as well as the length of time the food is in contact with the surface. Although this research shows that the five-second rule is "real" in the sense that longer contact time resulted in more transfer, it also shows that other factors, including the nature of the food and the surface, are of equal or greater importance. The five-second rule is a significant oversimplification of what actually happens when bacteria transfer from a surface to food.

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REFERENCES

1. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States–major pathogens. Emerg Infect Dis **17:**7–15. http://dx.doi.org/10.3201/eid1701.P11101.
2. **Gould LH, Walsh KA, Vieira AR, Herman K, Williams IT, Hall AJ, Cole D.** 2013. Surveillance for foodborne disease outbreaks—United States, 1998–2008. MMWR Surveill Summ **62:**1–34. http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6202a1.htm.
3. **Centers for Disease Control and Prevention (CDC).** 2013. Surveillance for foodborne disease outbreaks—United States, 2009–2010. Morb Mortal Wkly Rep **62:**41–47. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6203a1.htm.
4. **Centers for Disease Control and Prevention (CDC).** 2013. Surveillance for foodborne disease outbreaks—United States, 2011: annual report. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2011-508c.pdf.
5. **Centers for Disease Control and Prevention (CDC).** 2014. Surveillance for foodborne disease outbreaks—United States, 2012: annual report. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2012-508c.pdf.
6. **Centers for Disease Control and Prevention (CDC).** 2015. Surveillance for foodborne disease outbreaks—United States, 2013: annual report. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2013-508c.pdf.
7. **Jensen DA, Friedrich LM, Harris LJ, Danyluk MD, Schaffner DW.** 2013. Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. J Food Prot **76:**1530–1538. http://dx.doi.org/10.4315/0362-028X.JFP-13-098.
8. **Dawson P, Han I, Cox M, Black C, Simmons L.** 2007. Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood, and carpet: testing the five-second rule. J Appl Microbiol **102:**945–953.
9. **Wendt C, Dietze B, Dietz E, Rüden H.** 1997. Survival of *Acinetobacter baumannii* on dry surfaces. J Clin Microbiol **35:**1394–1397.
10. **Kusumaningrum HD, vanAsselt ED, Beumer RR, Zwietering MH.** 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. J Food Prot **67:**1892–1903.
11. **Moore CM, Sheldon BW, Jaykus LA.** 2003. Transfer of *Salmonella* and *Campylobacter* from stainless steel to romaine lettuce. J Food Prot **66:**2231–2236.
12. **Midelet G, Carpentier B.** 2002. Transfer of microorganisms, including *Listeria monocytogenes*, from various materials to beef. Appl Environ Microbiol **68:**4015–4024. http://dx.doi.org/10.1128/AEM.68.8.4015-4024.2002.
13. **Chen Y, Jackson KM, Chea FP, Schaffner DW.** 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. J Food Prot **64:**72–80.
14. **Zhao P, Zhao T, Doyle MP, Rubino JR, Meng J.** 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. J Food Prot **61:**960–963.
15. **Lankford MG, Collins S, Youngberg L, Rooney DM, Warren JR, Noskin GA.** 2006. Assessment of materials commonly utilized in health care: implications for bacterial survival and transmission. Am J Infect Control **34:**258–263. http://dx.doi.org/10.1016/j.ajic.2005.10.008.
16. **Rice DH, Hancock DD, Szymanski MH, Scheenstra BC, Cady KM, Besser TE, Chudek PA.** 2003. Household contamination with *Salmonella enterica*. Emerg Infect Dis **9:**120–122. http://dx.doi.org/10.3201/eid0901.020214.
17. **Holah JT, Thorpe RH.** 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. J Appl Bacteriol **69:**599–608. http://dx.doi.org/10.1111/j.1365-2672.1990.tb01554.x.
18. **Wilks SA, Michels HT, Keevil CW.** 2006. Survival of *Listeria monocytogenes* Scott A on metal surfaces: implications for cross-contamination. Int J Food Microbiol **111:**93–98. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.04.037.
19. **Kuhn PJ.** 1983. Doorknobs: a source of nosocomial infection. Diagn Med **November-December:**62–63. http://www.antimicrobialcopper.org/sites/default/files/upload/Media-library/Files/PDFs/UK/Scientific_literature/kuhn-doorknob.pdf.
20. **Robine E, Boulangé-Petermann L, Derangère D.** 2002. Assessing bactericidal properties of materials: the case of metallic surfaces in contact with air. J Microbiol Methods **49:**225–234. http://dx.doi.org/10.1016/S0167-7012(01)00371-2.
21. **Wilks SA, Michels H, Keevil CW.** 2005. The survival of *Escherichia coli* O157 on a range of metal surfaces. Int J Food Microbiol **105:**445–454. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.021.
22. **Berto AM.** 2007. Ceramic tiles: above and beyond traditional applications. J Eur Ceram Soc **27:**1607–1613. http://dx.doi.org/10.1016/j.jeurceramsoc.2006.04.146.
23. **Ak NO, Cliver DO, Kaspar CW.** 1994. Cutting boards of plastic and wood contaminated experimentally with bacteria. J Food Prot **57:**16–22.
24. **Welker C, Faiola N, Davis S, Maffatore I, Batt CA.** 1997. Bacterial retention and cleanability of plastic and wood cutting boards with commercial food service maintenance practices. J Food Prot **60:**407–413.
25. **United States Department of Agriculture (USDA).** 2013. Cutting boards and food safety. United States Department of Agriculture, Washington, DC. http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/cutting-boards-and-food-safety.
26. **Yu H.** 2007. The effect of chemical finishing on the microbial transfer from carpets to human skin and selected fabrics. Ph.D. dissertation. University of Georgia, Athens, GA.
27. **Anonymous.** 2003. If you drop it, should you eat it? Scientists weigh in on the 5-second rule. University of Illinois at Urbana-Champaign, Urbana, IL. http://news.aces.illinois.edu/news/if-you-drop-it-should-you-eat-it-scientists-weigh-5-second-rule.
28. **MythBusters.** 2005. 5 second rule with food on floor. Discovery Communications, Silver Spring, MD.http://www.discovery.com/tv-shows/mythbusters/mythbusters-database/5-second-rule-with-food/.
29. **Garbett J.** 10 March 2014. Researchers prove the five-second rule is real. Aston University, Birmingham, United Kingdom. http://www.aston.ac.uk/about/news/releases/2014/march/five-second-food-rule-does-exist/.
30. **Moran L.** 2016. Science explains why the 5-second rule is actually true. The Huffington Post, New York, NY. http://www.huffingtonpost.com/entry/5-second-rule-scienceexplainer_us_56b07205e4b057d7d7c809a8.
31. **Schaffner DW.** 2003. Challenges in cross contamination modelling in home and food service settings. Food Aust **55:**583–586.
32. **Sharma M, Taormina PJ, Beuchat LR.** 2003. Habituation of foodborne pathogens exposed to extreme pH conditions: genetic basis and implications in foods and food processing environments. Food Sci Technol Res **9:**115–127. http://dx.doi.org/10.3136/fstr.9.115.
33. **Kusumaningrum HD, Riboldi G, Hazeleger WC, Beumer RR.** 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int J Food Microbiol **85:**227–236. http://dx.doi.org/10.1016/S0168-1605(02)00540-8.
34. **Donlan RM.** 2002. Biofilms: microbial life on surfaces. Emerg Infect Dis **8:**881–890. http://dx.doi.org/10.3201/eid0809.020063.
35. **Ryu JH, Beuchat LR.** 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. Appl Environ Microbiol **71:**247–254. http://dx.doi.org/10.1128/AEM.71.1.247-254.2005.
36. **Wachtel MR, Charkowski AO.** 2002. Cross-contamination of lettuce with *Escherichia coli* O157:H7. J Food Prot **65:**465–470.
37. **Mbithi JN, Springthorpe VS, Boulet JR, Sattar SA.** 1992. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. J Clin Microbiol **30:**757–763.
38. **D'Souza DH, Sair A, Williams K, Papafragkou E, Jean J, Moore C, Jaykus L.** 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. Int J Food Microbiol **108:**84–91. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.10.024.
39. **Escudero BI, Rawsthorne H, Gensel C, Jaykus LA.** 2012. Persistence and transferability of noroviruses on and between common surfaces and foods. J Food Prot **75:**927–935. http://dx.doi.org/10.4315/0362-028X.JFP-11-460.
40. **Pérez-Rodríguez F, Valero A, Carrasco E, García RM, Zurera G.** 2008. Understanding and modelling bacterial transfer to foods: a review. Trends Food Sci Technol **19:**131–144. http://dx.doi.org/10.1016/j.tifs.2007.08.003.
41. **Montville R, Schaffner DW.** 2003. Inoculum size influences bacterial cross contamination between surfaces. Appl Environ Microbiol **69:**7188–7193. http://dx.doi.org/10.1128/AEM.69.12.7188-7193.2003.