Appl. Environ. Microbiol. doi:10.1128/AEM.01838-16

1 Title: **Longer Contact Times Increase Cross-Contamination of *Enterobacter***

2 ***aerogenes* from Surfaces to Food**

3

4 Running title**: Is the five-second rule real?**

5

6 Robyn C. Miranda and Donald W. Schaffner#

7 Rutgers, The State University of New Jersey

8 65 Dudley Road, New Brunswick, NJ, 08901

9

10 #: Corresponding author email: schaffner@aesop.rutgers.edu

11 Key words: cross-contamination, contact time, surface, 5-second rule

12

13 To Be Submitted To: Applied and Environmental Microbiology

14

Abstract (250)

Bacterial cross-contamination from surfaces to food can contribute to foodborne disease. The cross-contamination rate of *Enterobacter aerogenes* was evaluated on household surfaces 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 16 cm^2 contact area) were dropped on the surfaces from a height of 12.5 cm and left to rest as appropriate. Post transfer 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 % transfer from the surface to the food. Contact time, food and surface type all had a highly significant effect ($P<0.000001$) on log % transfer of bacteria. The inoculum matrix (TSB or peptone buffer) also had a significant effect on transfer ($P = 0.013$), and most interaction terms were significant. More bacteria transferred to watermelon (~0.2-97%) relative to other foods, while fewer bacteria transferred to gummy candy (~0.1-62%). Transfer of bacteria to bread (~0.02-94%) and bread with butter (~0.02-82%) were similar, and transfer rates under a given set of condition were more variable compared with watermelon and gummy candy.

Importance (150)

The popular notion of the "five second rule" states food dropped on the floor for less than five seconds 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 release, but remain unpublished. We explore this topic 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 show that longer contact times result in more transfer, we also show 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 <1 s, disproving the "five second rule".

Introduction

The Centers for Disease Control and Prevention (CDC) estimates that each year there are more than 9 million episodes of foodborne illness, over 55 thousand hospitalizations and at least 1,351 deaths that can be attributed to foods consumed in the US (1). The CDC regularly publishes reports that summarize data on surveillance for foodborne disease outbreaks in the US (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 3 categories related to contamination, proliferation or 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 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 a focus of numerous cross-contamination studies; 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 due to its resistance to corrosion, mechanical strength, ease of cleaning and its resistance to chemical degradation (17, 18), although stainless steel may have higher bacterial transfer rates when compared to 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 plastic cutting boards (23, 24), and the studies have come to contradictory conclusions in part due to differences in the methods. The United States Department of Agriculture (USDA) recommends one cutting board for 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 using 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" states food dropped on the floor for less than five seconds 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” use different surfaces, foods, organisms, contact times and number 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 less than 5 seconds (27). The popular television show MythBusters aired an episode on the “five second rule” in 2005, and found no conclusive difference when comparing contact times of 2 and 6 seconds (28). In the only peer-reviewed research on the topic, researchers from Clemson University concluded that longer contact times (5, 30 and 60 s) did increase the transfer of *Salmonella* 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 showing that contact time significantly affected transfer of both *E. coli* and *S. aureus* contaminated surface (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 up cookies to strangers in a park – after dropping them onto the ground. The shows narrator stated "Moist foods left longer than 30 seconds collect 10 times the bacteria than those snapped up after only three" but offered no data in support of this statement (30).

This research seeks to quantify cross-contamination between a variety of foods and common kitchen surfaces varying time and bacterial matrix, and to do so in an extensive and comprehensive manner. The results described below 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 *Salmonella,* was used for all experiments (Vivolac Cultures, Indianapolis, Ind.) (14). The *E. aerogenes* strain is resistant to nalidixic acid, which allows it to be enumerated in the presence of other microorganisms on the food samples or surfaces. Control experiments (by sampling and plating onto TSA-na) showed that nalidixic acid-resistant *E. aerogenes* cells were not initially present on any of the foods or surfaces at levels > 2 log CFU/surface or food.

119 Cultures were prepared based on prior work in our lab (13) and by others (14). A frozen
120 stock of *E. aerogenes* in 80% sterile glycerol was streaked onto tryptic soy agar, (Difco,
121 BD, Sparks, MD) with 50 ug/ml nalidixic acid (Sigma Chemical Co., St. Louis, Mo.)
122 (TSA-na). One colony from each plate was transferred to 10 ml of tryptic soy broth
123 (Bacto, BD, Sparks, MD) with 50 ug/ml nalidixic acid (TSB-na) and incubated at 37°C
124 for 24 h. Inoculum matrices were of two types; using cells harvested by centrifugation at
125 5,000 × g for 10 min and washed twice in 10 ml of 0.1% peptone (Difco, BD) or using
126 cells taken directly from inoculated, overnight TSB-na culture. A final concentration of
127 ~10^8 CFU/ml was verified by enumeration on TSA-na.

128 ***Preparation of domestic surfaces***

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

139 ***Food types***

140 Four foods (watermelon, white bread (ShopRite, Wakefern Food Corp., Elizabeth, NJ),
141 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 crust) were cut into pieces (approximately 4 by 4 cm). Unsalted butter was brought to ambient temperature (~24°C) prior to spreading onto bread. All foods had equivalent contact areas (~16 cm^2). The pH and water activity of samples were measured in triplicate using a surface pH probe (Accumet Basic AB15 pH Meter, Fisher Scientific) and water activity 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 using eight to ten drops spread over the 5 x 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 surface was 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 on the respective surfaces using gloved hands from a height of 12.5 cm and left to rest for four different times (<1, 5, 30 and 300 s). The height of 12.5 cm was selected because it was the greatest height possible that still ensured that the entire food 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 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.* Plates were incubated at 37°C for 24 h. Colonies were counted and population levels were expressed as CFU per food or surface sample.

Data analysis

Percent transfer was calculated as:

[[Total CFU food] / [Total CFU food + Total CFU surface]] × 100

Percent transfer rates from surface to food were log transformed using Microsoft Excel (Microsoft, Redmond, WA) and Sigma Plot (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 when $P < 0.05$. Multiple linear regression analysis was performed using StatPlus for Microsoft Excel (AnalystSoft, Inc., Walnut, CA). Quantitative values were given to surfaces - tile (0), stainless steel (1), wood (2) and carpet (3), foods - bread (0), bread with butter (1), gummy (2) and watermelon (3) and matrices – TSB (0), buffer (1) for regression analysis.

Results

187 ***pH and Water Activity (a_W) Measurements***

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

197 ***Statistical analysis of transfer rates***

198 The contact time, food, surface and the food*time interaction was shown to significantly
199 ($P < 0.000001$) influence log % transfer. The surface*time ($P = .0019$), surface*food ($P =$
200 0.00019) and surface*matrix ($P = 0.00005$) effect on log % transfer were also significant.
201 The inoculum matrix, i.e. TSB or buffer ($P = 0.013$) and food*matrix interaction ($P =$
202 0.045) were statistically significant, although less so than the other factors. The
203 time*matrix interaction did not have a statistically significant effect on log % transfer (P
204 $= 0.49$) (Table 2).
205 Transfer of bacteria from inoculated surfaces to watermelon, bread, bread with butter and
206 gummy candies, is summarized in Tables 1S, 2S, 3S and 4S respectively. Each table
207 shows six different statistical parameters that were used to characterize the log % transfer
208 rate: mean ($\bar{x}$), median (M), standard deviation (σ), minimum (min), maximum (max)

and range. The tables will be referenced as needed to supplement the discussion of the figures below.

Bacteria transfer from 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 Figure 1 and 2, respectively. Error bars in Figures 1 and 2 indicate the standard deviation 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 watermelon occurred (Figure 1). Log % transfer of bacteria from tile to watermelon for cells contained within the TSB inoculum was highest at 5 s with 1.99 mean log % transfer (97%) (Figure 1M). Transfer of bacteria from stainless steel was between 1.96 (90%) and 1.97 mean log % transfer (93%) (Figure 1N). Overall, there was no significant difference in bacterial transfer from any surface to watermelon at different contact times (Figure 1 MNOP).

Bacterial transfer from buffer-inoculated surfaces to watermelon was more variable than the TSB inoculum matrix (Figure 2 MNOP). Transfer of bacteria from tile was between 1.17 (15%) to 1.96 mean log % transfer (91%) (Figure 2M). Greater transfer at <1 s was observed from stainless steel and wood (Figure 2NO) with transfer of 1.96 (91%) and 1.93 mean log % transfer (86%) to watermelon, respectively (Figure 2NO). Transfer from carpet ranged from -0.75 (0.2%) to 0.14 mean log % transfer (1%) (Figure 2P).

The mean transfer rates and standard deviations associated with the means are similar for stainless steel, tile and wood to watermelon. However for 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 on TSB inoculated tile, stainless steel, wood or carpet, the highest transfer rate was observed at 30 s from wood (Figure 1C), although a significant difference between transfer at 30 and 300 s was not observed from wood. Transfer of bacteria from stainless steel was between -0.56 (0.3%) and 1.97 mean log % transfer (93%) (Figure 1B). For bread dropped on tile, the transfer ranged from -0.95 (0.1%) to 1.96 mean log % transfer (92%) (Figure 1A), and transfer from wood ranged from -0.64 (0.2%) to 1.97 mean log % transfer (94%) (Figure 1C). Transfer from carpet ranged from -0.87 (0.1%) to 0.58 mean log % transfer (4%), was less in comparison to the other three contact surfaces (Figure 1D). At <1 s, 18/20 and 19/20 replicates were below the detection limit for TSB and buffer-inoculated carpet, respectively.

Bread dropped on the surfaces behaved similarly regardless of TSB or buffer-inoculated matrix. The transfer of bacteria from buffer-inoculated surfaces was highest at 300 s for all surfaces. Transfer of bacteria from tile to bread was between -0.68 (0.2%) and 1.79 mean log % transfer (62%) (Figure 2A). Stainless steel had the highest transfer of bacteria to bread after 300 s at 1.91 mean log % transfer (80%) (Figure 2B). Transfer of bacteria from wood over time was between -0.91 (0.1%) and 1.89 mean log % transfer (78%) (Figure 2C) and transfer of bacteria from carpet was -1.68 (0.02%) and -0.79 mean log % transfer (0.2%) (Figure 2D). The standard deviation of stainless steel, tile and

wood was greatest at <1 s, while the standard deviation of carpet to bread was similar regardless of time.

Inoculated surface to bread with butter

Bacteria transfer from all surfaces to bread with butter at <1 s was low; on average, 10/20 replicates were below the detection limit for TSB inoculated surfaces where the detection limit was 2 log % transfer based on the protocols used in our experiments (Figure 1). When buttered bread was in contact with inoculated tile, transfer of bacteria increased from <1 to 300 s between -1.08 (0.08%) and 1.81 mean log % transfer (65%) (Figure 1E). The transfer of bacteria from stainless steel to buttered bread was between -1.63 (0.02%) and 1.91 mean log % transfer (82%) (Figure 1F) and transfer from wood to buttered bread was between -1.18 (0.07%) and 1.81 mean log % transfer (65%) (Figure 1G). Carpet transferred fewer bacteria in comparison to the other contact surfaces; yet transfer still increased over time from -1.15 (0.07%) to 0.9 mean log % transfer (8%) (Figure 1H). Transfer of *E. aerogenes* from buffer-inoculated surfaces to bread with butter is shown in Figure 2 . There was an increase in bacterial transfer for all surfaces as contact time increased. Tile inoculated with cells contained in buffer transferred more bacteria to buttered bread than any other surface (Figure 2). When bread with butter contacted tile, transfer of bacteria ranged from -0.86 (0.1%) to 1.67 mean log % transfer (47%) (Figure 2E). Stainless steel and wood transferred a similar fraction of cells contained in buffer to bread with butter. Stainless steel transferred -0.86 (0.1%) and 1.42 mean log % transfer (26%) at <1 to 300 s (Figure 2F) respectively, while wood transfer rates ranged from -0.29 (0.5%) to 1.48 mean log % transfer (30%) (Figure 2G). Carpet again showed the

lowest transfer rates ranging from -0.56 (0.3%) to 0.19 mean log % transfer (2%) (Figure 2H).

Inoculated surface to gummy candy

The transfer rate to gummy candy increased with time from tile, ranging from -0.88 (0.1%) to 0.28 mean log % transfer (2%) (Figure 1I). Transfer was lowest at 300 s from carpet to gummy candies with a -0.51 mean log % transfer (0.3%) (Figure 1L). 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 (Figure 1J). The highest transfer observed for any surface to gummy candy occurred at 300 s from stainless steel to gummy with 1.80 mean log % transfer (63%) (Figure 1J).

When gummy candies were dropped on all surfaces containing the inoculum in buffer, the mean log % transfer was low, regardless of time. On average, 19/20 replicates for gummy to all surfaces at <1 s were below the detection limit and an average of 8/20 were below the detection limit at 300 s. The highest transfer was observed at 300 s from tile with bacterial transfer of -0.89 mean log % transfer (0.1%) (Figure 2I).

Discussion

Our study shows that bacterial transfer is dependent on the surface, food type, contact time and inoculum matrix. Studies involving transfer of similar surfaces to foods have come to varying conclusions (7, 8). These differences may be due to the range of experimental procedures among published studies. Differences include the contact time between surfaces (7, 8, 11), organism used (7, 8, 11, 33) and food and contact surfaces used (7, 8, 11, 33) each of which can result in differing 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 literature. Studies reporting on bacterial adhesion to surfaces use 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 transfer rate by recipient surface/source surface (13), whereas in our study, transfer rate was analyzed by 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 use very small numbers of replicates and/or fail to statistically transform the percent transfer rates, and may 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 based on the food.

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 was applied (20 g/cm^2), 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 is applied) (37). Research by D'Souza et al. 2006 showed that pressure changes from ~1 to 100 g/cm^2 had no effect on virus transfer (38). Later research from the same laboratory showed more transfer at higher pressures (~100 g/cm^2) compared with lower pressures (~10 g/cm^2), especially where the inoculum was drier (39).

Applied and Environmental Microbiology

323 Our data clearly showed that contact time does influence bacterial transfer, with more
324 bacteria transferred at longer times. Peer reviewed research by Dawson et al. reported
325 that longer food contact times (5, 30 or 60 s) did result in greater transfer but only at
326 longer drying times (≥ 8 h) (8) roughly equivalent to our drying time of 5 h. Non-peer
327 reviewed research from the University of Illinois on bacterial transfer from tile inoculated
328 with generic *E. coli* to cookies and gummy bears found that bacterial transfer was
329 observed in less than 5 seconds (27) (consistent with our <1 s observations) although
330 other contact times were not studied. The popular television show MythBusters (28) aired
331 an episode on the "five second rule" and found no conclusive difference when pastrami
332 and crackers were exposed to contaminated tile with contact times of 2 and 6 seconds. It
333 is unclear from viewing the episode what was used to contaminate the tile surface,
334 although the inoculated tile was left for 5 days before beginning the experiment.
335 Mythbusters also used less than 10 replicates per scenario. A press release by Aston
336 University, in the United Kingdom, showed that time significantly affected transfer
337 depending on the contaminated surface and food (29). The Aston University study
338 observed the transfer of *E. coli* and *S. aureus* from carpet, wood and tile to toast, pasta,
339 biscuit and a sticky sweet with 3 and 30 s contact time. Moist foods that contacted
340 contaminated wood and tile showed higher transfer rates, and longer times increased
341 transfer for these foods and surfaces. The Aston University study shows that transfer
342 from carpet was not affected by the food composition or the contact time (29).
343 Our data show that the rate of bacterial transfer was greatest for tile, stainless steel and
344 wood surfaces at 300 s. The food with the highest transfer rate was watermelon,
345 regardless of 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 compared to bread or gummy candies. Jensen et al. also found that transfer from stainless steel and tile to watermelon had the highest transfer in comparison to the other produce types used in that study (7). Kusumaningrum et al. measured the transfer rates to cut cucumber from stainless steel, and observed that almost all 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 or infiltration into absorbent carpet fibers. Dawson et al. also found that transfer from carpet to bologna was very low (<0.5%) in comparison to the transfer from wood and tile to bologna (5-68%) (8).

The starting concentration of all surfaces in our experiments were ~7 log CFU/surface. Although this was not a variable explicitly considered, the starting concentration may have an affect on how much bacterial transfer occurs to the recipient surface. 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 transfer rate was statistically significant ($P < 0.0001$) for all transfer rate data, and that greater inoculum size resulted in lower transfer rates (41).

Transfer of bacteria from surfaces to food appear to be most affected by the moisture of the food as show by transfer of *E. aerogenes* from tile, stainless steel, wood and carpet to watermelon. Longer food contact times usually resulted in transfer of more bacteria from

each surface to food. Carpet has very low transfer rates, compared with tile and stainless steel, whereas transfer from wood was 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 will depend on factors including prevalence, concentration and type of organism, the nature of the food (especially moisture), 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 5-second rule is "real" in the sense that longer contact time result 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 5-second rule is a significant oversimplification of what actually happens when bacteria transfer from a surface to food.

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.
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.
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.
4. **Centers for Disease Control and Prevention (CDC).** 2013. Surveillance for Foodborne Disease Outbreaks United States, 2011: Annual Report. 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. 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. 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.
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.
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:**258-63.
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.
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.
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.
19. **Kuhn PJ.** 1983. Doorknobs: a source of nosocomial infection. Diagnost Med. 62-63.
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:**225-34.
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.
22. **Berto AM.** 2007. Ceramic tiles: Above and beyond traditional applications. J Eur Ceram Soc. **27:**1607-1613.
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. 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. PhD 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. http://news.aces.illinois.edu/news/if-you-drop-it-should-you-eat-it-scientists-weigh-5-second-rule.
28. **Anonymous.** 2005. 5 Second Rule With Food On Floor. http://www.discovery.com/tv-shows/mythbusters/mythbusters-database/5-second-rule-with-food/.
29. **Garbett J.** 2014. Researchers prove the five-second rule is real. 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. http://www.huffingtonpost.com/entry/5-second-rule-science-explainer_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.
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.
34. **Donlan RM.** 2002. Biofilms: microbial life on surfaces. Emerg Infect Dis. **8:**881-890.
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.
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:**757-63.
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.
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:**927-35.
40. **Pérez-Rodríguez F, Valero A, Carrasco E, García RM, Zurera G.** 2008. Understanding and modelling bacterial transfer to foods: a review. Trend Food Sci Tech. **19:**131-144.
41. **Montville R, Schaffner DW.** 2003. Inoculum size influences bacterial cross contamination between surfaces. Appl Environ Microbiol. **69:**7188-7193.

504 ***Figure Legends***

505 ***Figure 1***. The effect of contact time on Log % transfer of *Enterobacter aerogenes*

506 inoculated onto four household surfaces in a tryptic soy broth matrix to four foods.

507

508 ***Figure 2***. The effect of contact time on Log % transfer of *Enterobacter aerogenes*

509 inoculated onto four household surfaces in a peptone buffer matrix to four foods

Table 1 - pH and Water Activity measurements of four foods to which *Enterobacter aerogenes* are transferred from common household surfaces.

Food type	Water Activity	pH
Bread	0.95 ± 0.01	5.80 ± 0.02
Butter	0.97 ± 0.01	6.25 ± 0.03
Gummy	0.72 ± 0.01	2.80 ± 0.03
Watermelon	0.99 ± 0.01	5.43 ± 0.01

Table 2 - Multiple Linear Regression analysis results for the effects of contact time, inoculum matrix, food type, surface type, and their interactions on the transfer of *Enterobacter aerogenes* from common household surfaces to foods.

	Coefficient	Standard Error	LCL	UCL	t Stat	p-level
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

[1]Quantitative values given to variables: Surface – tile (0), stainless steel (1), wood (2), carpet (3), Food – bread (0), bread with butter (1), gummy (2), watermelon (3); Inoculum matrix – TSB (0), Buffer (1)