

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

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

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

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15 **Abstract (250)**

16

17 Bacterial cross-contamination from surfaces to food can contribute to foodborne disease.

18 The cross-contamination rate of *Enterobacter aerogenes* was evaluated on household

19 surfaces using scenarios that differed by surface type, food type, contact time (<1, 5, 30

20 and 300 s), and inoculum matrix (tryptic soy broth or peptone buffer). The surfaces used

21 were stainless steel, tile, wood and carpet. The food types were watermelon, bread, bread

22 with butter and gummy candy. Surfaces (25 cm²) were spot inoculated with 1 ml of

23 inoculum and allowed to dry for 5 h, yielding an approximate concentration of 10⁷

24 CFU/surface. Foods (with 16 cm² contact area) were dropped on the surfaces from a

25 height of 12.5 cm and left to rest as appropriate. Post transfer surfaces and foods were

26 placed in sterile filter bags and homogenized or massaged, diluted and plated on tryptic

27 soy agar. The transfer rate was quantified as the log % transfer from the surface to the

28 food. Contact time, food and surface type all had a highly significant effect (P<0.000001)

29 on log % transfer of bacteria. The inoculum matrix (TSB or peptone buffer) also had a

30 significant effect on transfer (P = 0.013), and most interaction terms were significant.

31 More bacteria transferred to watermelon (~0.2-97%) relative to other foods, while fewer

32 bacteria transferred to gummy candy (~0.1-62%). Transfer of bacteria to bread (~0.02-

33 94%) and bread with butter (~0.02-82%) were similar, and transfer rates under a given set

34 of condition were more variable compared with watermelon and gummy candy.

35

36 **Importance (150)**

37 The popular notion of the "five second rule" states food dropped on the floor for less than

38 five seconds is "safe", because bacteria need time to transfer. The rule has been explored

39 by a single study in the published literature and on at least two television shows. Results
40 from two academic laboratories have been shared through press release, but remain
41 unpublished. We explore this topic using four different surfaces (stainless steel, ceramic
42 tile, wood and carpet), four different foods (watermelon, bread, bread with butter and
43 gummy candy), four different contact times (<1, 5, 30 and 300 s), and two bacterial
44 preparation methods. Although we show that longer contact times result in more transfer,
45 we also show that other factors including the nature of the food and the surface are of
46 equal or greater importance. Some transfer takes place “instantaneously” at times <1 s,
47 disproving the “five second rule”.
48

49 **Introduction**

50
51 The Centers for Disease Control and Prevention (CDC) estimates that each year there are
52 more than 9 million episodes of foodborne illness, over 55 thousand hospitalizations and
53 at least 1,351 deaths that can be attributed to foods consumed in the US (1). The CDC
54 regularly publishes reports that summarize data on surveillance for foodborne disease
55 outbreaks in the US (2-6). Those reports list more than 30 contributing factors linked to
56 foodborne disease outbreaks in the year or years summarized in the reporting period.
57 Factors are grouped into 3 categories related to contamination, proliferation or survival of
58 foodborne pathogens. Food handlers or others suspected to be infectious are linked to
59 several contamination factors. One factor is specifically related to cross-contamination
60 from surfaces and not ill individuals. When those surface cross-contamination data are
61 summarized from 1998 to present, about 12% of all outbreaks reported to the CDC are
62 linked in some way to this type of surface cross-contamination. This is the 6th most
63 common contributing factor (out of 32) (2-6).
64 Household and other surface types have been a focus of numerous cross-contamination
65 studies; surfaces studied include ceramic tile (7-9), stainless steel (7, 9-12), wood (8),
66 glass (7), plastic (7, 13, 14) and carpet (8, 15, 16). Stainless steel has often been
67 considered the optimal material choice for kitchen sinks and commercial food preparation
68 surfaces due to its resistance to corrosion, mechanical strength, ease of cleaning and its
69 resistance to chemical degradation (17, 18), although stainless steel may have higher
70 bacterial transfer rates when compared to other surfaces (19-21). Tile is also a common
71 surface found in homes; the variations of tile (unglazed versus glazed) may have an effect
72 on the bacterial transfer rate because of varying surface topography (22). Wood surfaces

73 are commonly found in households, either as flooring or as cutting board surfaces. The
74 sanitary properties of wood cutting boards have been compared to plastic cutting boards
75 (23, 24), and the studies have come to contradictory conclusions in part due to differences
76 in the methods. The United States Department of Agriculture (USDA) recommends one
77 cutting board for produce and bread and a separate cutting board for raw meat, poultry
78 and seafood (25). Carpet is a likely site of contamination in the household and
79 inactivating or removing bacteria using conventional cleaning methods is difficult once
80 the carpet is contaminated (16). Microorganisms on carpet can be controlled by specific
81 chemical treatments of the fibers or the materials used in constructing the carpet (26).
82 The popular culture notion of the "five second rule" states food dropped on the floor for
83 less than five seconds is "safe", because bacteria need time to transfer. The rule has been
84 explored to a limited degree in the published literature and popular culture. Previous
85 studies on the "five second rule" use different surfaces, foods, organisms, contact times
86 and number of replicates, making comparisons and conclusions difficult. The first known
87 research recorded on this topic was performed at the University of Illinois, but was never
88 published in the peer-reviewed literature (27). These researchers used tile inoculated with
89 *Escherichia coli* and studied transfer to cookies and gummy bears and found that
90 bacterial transfer was observed in less than 5 seconds (27). The popular television show
91 MythBusters aired an episode on the "five second rule" in 2005, and found no conclusive
92 difference when comparing contact times of 2 and 6 seconds (28). In the only peer-
93 reviewed research on the topic, researchers from Clemson University concluded that
94 longer contact times (5, 30 and 60 s) did increase the transfer of *Salmonella*
95 Typhimurium from wood, tile or carpet to bologna or bread but only ≥ 8 h after the

96 surface was inoculated (8). Researchers at Aston University in the United Kingdom,
97 published a press release in 2014 showing that contact time significantly affected transfer
98 of both *E. coli* and *S. aureus* contaminated surface (carpet, laminate and tile) to food
99 (toast, pasta, biscuit and a sticky sweet) (29). Discovery Science Channel's "The Quick
100 and the Curious" television show aired a short segment offering up cookies to strangers in
101 a park – after dropping them onto the ground. The shows narrator stated "Moist foods
102 left longer than 30 seconds collect 10 times the bacteria than those snapped up after only
103 three" but offered no data in support of this statement (30).

104 This research seeks to quantify cross-contamination between a variety of foods and
105 common kitchen surfaces varying time and bacterial matrix, and to do so in an extensive
106 and comprehensive manner. The results described below advance our understanding of
107 cross-contamination and the factors that influence it. This research informs the popular
108 culture, and enhances our scientific understanding of cross-contamination and the factors
109 that influence it.

110 **Materials and Methods**

111 *Bacterial strain and preparation of culture*

112 A nonpathogenic, food-grade microorganism, *Enterobacter aerogenes* B199A, with
113 attachment characteristics similar to *Salmonella*, was used for all experiments (Vivolac
114 Cultures, Indianapolis, Ind.) (14). The *E. aerogenes* strain is resistant to nalidixic acid,
115 which allows it to be enumerated in the presence of other microorganisms on the food
116 samples or surfaces. Control experiments (by sampling and plating onto TSA-na) showed
117 that nalidixic acid-resistant *E. aerogenes* cells were not initially present on any of the
118 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 ~10⁸ 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

142 (Haribo, Strawberries)) were purchased online or from a local supermarket. Whole
143 watermelon was stored at 4°C prior to use. The watermelon (flesh only) and bread
144 (excluding crust) were cut into pieces (approximately 4 by 4 cm). Unsalted butter was
145 brought to ambient temperature (~24°C) prior to spreading onto bread. All foods had
146 equivalent contact areas (~16 cm²). The pH and water activity of samples were measured
147 in triplicate using a surface pH probe (Accumet Basic AB15 pH Meter, Fisher Scientific)
148 and water activity meter (Rotronic Instrument Corp., Hauppauge, NY) respectively.

149 *Transfer between food and surfaces*

150 Transfer scenarios were evaluated for each contact surface type (4), each food type (4),
151 four contact times and two inoculum matrices, totaling 128 scenarios. Each scenario was
152 replicated 20 times, totaling 2,560 measurements. Each contact surface type was spot
153 inoculated with 1 ml of inoculum using eight to ten drops spread over the 5 x 5 cm
154 surface. The surfaces were placed in a biosafety cabinet (SterilGARD Hood, The Baker
155 Company, Inc., Sanford, ME) for 5 h, after which the surface was visibly dry. Prior to 5
156 h, surfaces were still wet and at times longer than 5 h, the difference in recovery rate
157 between the inoculum matrices increased. Both the peptone buffer and TSB-na inoculum
158 matrices yielded an approximate concentration of 10⁷ CFU/surface after drying. Foods
159 were dropped on the respective surfaces using gloved hands from a height of 12.5 cm and
160 left to rest for four different times (<1, 5, 30 and 300 s). The height of 12.5 cm was
161 selected because it was the greatest height possible that still ensured that the entire food
162 would reliably contact the entire surface.
163 Surfaces were placed into a sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI), 20
164 ml of peptone buffer was added, and hand massaged for 2 min. Foods were placed into a

165 sterile filter bag (Fisherbrand, Lab Blender Bags) with 50 ml of peptone buffer and the
166 samples were homogenized (Stomacher, Cooke Laboratory Products, Alexandria, VA)
167 for 3 min. Surfaces and food samples were serially diluted in 0.1% peptone buffer and
168 surface plated (0.1 ml) onto TSA-na for enumeration of *E. aerogenes*. Plates were
169 incubated at 37°C for 24 h. Colonies were counted and population levels were expressed
170 as CFU per food or surface sample.

171 *Data analysis*

172 Percent transfer was calculated as:

$$173 \left[\frac{\text{Total CFU food}}{\text{Total CFU food} + \text{Total CFU surface}} \right] \times 100$$

174 Percent transfer rates from surface to food were log transformed using Microsoft Excel
175 (Microsoft, Redmond, WA) and Sigma Plot (Systat Software Inc., San Jose, CA), as prior
176 research has shown that untransformed transfer rates are highly skewed, and log
177 transformed transfer rates are approximately normally distributed (13, 31). When foods
178 contained less than the detection limit (2 log CFU), transfer rates were calculated as if the
179 concentration on the foods was at the detection limit. Variables and the interactions
180 between variables were considered significant when $P < 0.05$. Multiple linear regression
181 analysis was performed using StatPlus for Microsoft Excel (AnalystSoft, Inc., Walnut,
182 CA). Quantitative values were given to surfaces - tile (0), stainless steel (1), wood (2) and
183 carpet (3), foods - bread (0), bread with butter (1), gummy (2) and watermelon (3) and
184 matrices – TSB (0), buffer (1) for regression analysis.

185 **Results**

186

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)

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

211 ***Bacteria transfer from inoculated surface to food***

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

217 ***Inoculated surface to watermelon***

218 When all TSB inoculated surfaces contacted watermelon, a high degree of transfer of
219 bacteria to watermelon occurred (Figure 1). Log % transfer of bacteria from tile to
220 watermelon for cells contained within the TSB inoculum was highest at 5 s with 1.99
221 mean log % transfer (97%) (Figure 1M). Transfer of bacteria from stainless steel was
222 between 1.96 (90%) and 1.97 mean log % transfer (93%) (Figure 1N). Overall, there was
223 no significant difference in bacterial transfer from any surface to watermelon at different
224 contact times (Figure 1 MNOP).

225 Bacterial transfer from buffer-inoculated surfaces to watermelon was more variable than
226 the TSB inoculum matrix (Figure 2 MNOP). Transfer of bacteria from tile was between
227 1.17 (15%) to 1.96 mean log % transfer (91%) (Figure 2M). Greater transfer at <1 s was
228 observed from stainless steel and wood (Figure 2NO) with transfer of 1.96 (91%) and
229 1.93 mean log % transfer (86%) to watermelon, respectively (Figure 2NO). Transfer from
230 carpet ranged from -0.75 (0.2%) to 0.14 mean log % transfer (1%) (Figure 2P).

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

235 *Inoculated surface to bread*

236 When bread was dropped on TSB inoculated tile, stainless steel, wood or carpet, the
237 highest transfer rate was observed at 30 s from wood (Figure 1C), although a significant
238 difference between transfer at 30 and 300 s was not observed from wood. Transfer of
239 bacteria from stainless steel was between -0.56 (0.3%) and 1.97 mean log % transfer
240 (93%) (Figure 1B). For bread dropped on tile, the transfer ranged from -0.95 (0.1%) to
241 1.96 mean log % transfer (92%) (Figure 1A), and transfer from wood ranged from -0.64
242 (0.2%) to 1.97 mean log % transfer (94%) (Figure 1C). Transfer from carpet ranged from
243 -0.87 (0.1%) to 0.58 mean log % transfer (4%), was less in comparison to the other three
244 contact surfaces (Figure 1D). At <1 s, 18/20 and 19/20 replicates were below the
245 detection limit for TSB and buffer-inoculated carpet, respectively.

246 Bread dropped on the surfaces behaved similarly regardless of TSB or buffer-inoculated
247 matrix. The transfer of bacteria from buffer-inoculated surfaces was highest at 300 s for
248 all surfaces. Transfer of bacteria from tile to bread was between -0.68 (0.2%) and 1.79
249 mean log % transfer (62%) (Figure 2A). Stainless steel had the highest transfer of
250 bacteria to bread after 300 s at 1.91 mean log % transfer (80%) (Figure 2B). Transfer of
251 bacteria from wood over time was between -0.91 (0.1%) and 1.89 mean log % transfer
252 (78%) (Figure 2C) and transfer of bacteria from carpet was -1.68 (0.02%) and -0.79 mean
253 log % transfer (0.2%) (Figure 2D). The standard deviation of stainless steel, tile and

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

256 ***Inoculated surface to bread with butter***

257 Bacteria transfer from all surfaces to bread with butter at <1 s was low; on average, 10/20
258 replicates were below the detection limit for TSB inoculated surfaces where the detection
259 limit was 2 log % transfer based on the protocols used in our experiments (Figure 1).

260 When buttered bread was in contact with inoculated tile, transfer of bacteria increased
261 from <1 to 300 s between -1.08 (0.08%) and 1.81 mean log % transfer (65%) (Figure 1E).

262 The transfer of bacteria from stainless steel to buttered bread was between -1.63 (0.02%)
263 and 1.91 mean log % transfer (82%) (Figure 1F) and transfer from wood to buttered
264 bread was between -1.18 (0.07%) and 1.81 mean log % transfer (65%) (Figure 1G).

265 Carpet transferred fewer bacteria in comparison to the other contact surfaces; yet transfer
266 still increased over time from -1.15 (0.07%) to 0.9 mean log % transfer (8%) (Figure 1H).

267 Transfer of *E. aerogenes* from buffer-inoculated surfaces to bread with butter is shown in
268 Figure 2. There was an increase in bacterial transfer for all surfaces as contact time
269 increased. Tile inoculated with cells contained in buffer transferred more bacteria to

270 buttered bread than any other surface (Figure 2). When bread with butter contacted tile,
271 transfer of bacteria ranged from -0.86 (0.1%) to 1.67 mean log % transfer (47%) (Figure

272 2E). Stainless steel and wood transferred a similar fraction of cells contained in buffer to
273 bread with butter. Stainless steel transferred -0.86 (0.1%) and 1.42 mean log % transfer
274 (26%) at <1 to 300 s (Figure 2F) respectively, while wood transfer rates ranged from -

275 0.29 (0.5%) to 1.48 mean log % transfer (30%) (Figure 2G). Carpet again showed the

276 lowest transfer rates ranging from -0.56 (0.3%) to 0.19 mean log % transfer (2%) (Figure
277 2H).

278 *Inoculated surface to gummy candy*

279 The transfer rate to gummy candy increased with time from tile, ranging from -0.88
280 (0.1%) to 0.28 mean log % transfer (2%) (Figure 1I). Transfer was lowest at 300 s from
281 carpet to gummy candies with a -0.51 mean log % transfer (0.3%) (Figure 1L). The
282 transfer from stainless steel increased over time from <1 to 300 s, although, at <1, 5 and
283 30 s, on average 16/20 replicates were below the detection limit (Figure 1J). The highest
284 transfer observed for any surface to gummy candy occurred at 300 s from stainless steel
285 to gummy with 1.80 mean log % transfer (63%) (Figure 1J).

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

291 **Discussion**

292 Our study shows that bacterial transfer is dependent on the surface, food type, contact
293 time and inoculum matrix. Studies involving transfer of similar surfaces to foods have
294 come to varying conclusions (7, 8). These differences may be due to the range of
295 experimental procedures among published studies. Differences include the contact time
296 between surfaces (7, 8, 11), organism used (7, 8, 11, 33) and food and contact surfaces
297 used (7, 8, 11, 33) each of which can result in differing outcomes. Our research also
298 shows that the nature of the matrix containing the cells inoculated onto the surface can
299

300 play an important role, even when all other experimental variables are the same, an
301 observation we have seldom seen reported in literature. Studies reporting on bacterial
302 adhesion to surfaces use a variety of drying times, in comparison to the 5 h drying time
303 used in this study (7, 8, 34, 35). Additionally, there is a difference in data analysis
304 regarding transfer rates. Some studies determined transfer rate by recipient surface/source
305 surface (13), whereas in our study, transfer rate was analyzed by recipient surface/(source
306 surface + recipient surface) (7, 8, 11), which can lead to slight differences when the
307 number of bacteria transferred to the recipient surface is high. More importantly, some
308 studies use very small numbers of replicates and/or fail to statistically transform the
309 percent transfer rates, and may come to erroneous conclusions (31, 36). Although not
310 always reported in studies, standard deviation is a good indication of the degree of
311 variability (13). In our study, the standard deviation varied considerably based on the
312 food.

313 Although pressure was not a variable in our study, it may play a role in facilitating
314 bacterial transfer. Kusumaningrum et al. found that more transfer occurred when light
315 pressure was applied (20 g/cm²), although differences were slight (~0.3-log percent
316 transfer difference) (33). Mbithi et al. used pressures of 200 and 1,000 g/cm², with and
317 without friction and found that differences in transfer rates were also small (a ~0.5-log
318 percent transfer difference when pressure is applied) (37). Research by D'Souza et al.
319 2006 showed that pressure changes from ~1 to 100 g/cm² had no effect on virus transfer
320 (38). Later research from the same laboratory showed more transfer at higher pressures
321 (~100 g/cm²) compared with lower pressures (~10 g/cm²), especially where the inoculum
322 was drier (39).

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,

346 it is very moist, and moisture is known to facilitate transfer (40), regardless of whether
347 the contact surface is dry or wet. Watermelon may also present a flatter, more uniform
348 surface at the microscopic level compared to bread or gummy candies. Jensen et al. also
349 found that transfer from stainless steel and tile to watermelon had the highest transfer in
350 comparison to the other produce types used in that study (7). Kusumaningrum et al.
351 measured the transfer rates to cut cucumber from stainless steel, and observed that almost
352 all bacteria (~100%) transferred to the cucumber regardless of pressure (33). Cut
353 cucumbers also have a moist, uniform surface, which may facilitate bacterial transfer. We
354 observed lower transfer rates (~0.2%) when transfer was from carpet to food. Carpet may
355 promote less bacterial transfer because of bacterial attachment or infiltration into
356 absorbent carpet fibers. Dawson et al. also found that transfer from carpet to bologna was
357 very low (<0.5%) in comparison to the transfer from wood and tile to bologna (5-68%)
358 (8).

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

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

369 each surface to food. Carpet has very low transfer rates, compared with tile and stainless
370 steel, whereas transfer from wood was more variable. The topography of the surface and
371 food seems to play an important role in bacterial transfer. The risk of illness resulting
372 from deciding to consume food that has fallen on the floor will depend on factors
373 including prevalence, concentration and type of organism, the nature of the food
374 (especially moisture), the nature of the surface topology as well as the length of time the
375 food is in contact with the surface. Although this research shows that the 5-second rule is
376 “real” in the sense that longer contact time result in more transfer, it also shows that other
377 factors including the nature of the food and the surface are of equal or greater
378 importance. The 5-second rule is a significant oversimplification of what actually happens
379 when bacteria transfer from a surface to food.
380

381 **References**

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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.

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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

¹Quantitative 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)



