Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule

P. Dawson[1], I. Han[1], M. Cox[1], C. Black[2] and L. Simmons[1]

1 Department of Food Science and Human Nutrition, Clemson University, Clemson, SC, USA
2 South Carolina Governor's School for Science and Mathematics summer research internship at Clemson University

Keywords
bacterial transfer, cross-contamination, five-second rule, food contact surface, residence time, *Salmonella*.

Correspondence
P.L. Dawson, Department of Food Science and Human Nutrition, 204 Poole Hall, Clemson University, Clemson, SC 29634-0316, USA. E-mail: pdawson@clemson.edu

2006/0018: received 6 January 2006, revised and accepted 28 July 2006

doi:10.1111/j.1365-2672.2006.03171.x

Abstract

Aims: Three experiments were conducted to determine the survival and transfer of *Salmonella* Typhimurium from wood, tile or carpet to bologna (sausage) and bread.

Methods and Results: *Experiment 1*. After 28 days, 1·5 to 2·5 $\log_{10}$ CFU cm^{-2} remained on tile from and the more concentrated media facilitated the survival of *S*. Typhimurium compared with the more dilute solutions. *Experiments 2 and 3*. The bacterial transfer rate to food decreased as the bacterial residence time on the surface increased from 2, 4, 8 to 24 h with transfers of 6·5, 4·8, 4·6 and 3·9 log CFU ml^{-1} in the rinse solutions, respectively. Over 99% of bacterial cells were transferred from the tile to the bologna after 5 s of bologna exposure to tile. Transfer from carpet to bologna was very low (<0·5%) when compared with the transfer from wood and tile (5–68%).

Conclusions: (i) *Salmonella* Typhimurium can survive for up to 4 weeks on dry surfaces in high-enough populations to be transferred to foods and (ii) *S*. Typhimurium can be transferred to the foods tested almost immediately on contact.

Significance and Impact of the Study: This study demonstrated the ability of bacteria to survive and cross-contaminate other foods even after long periods of time on dry surfaces, thus reinforcing the importance of sanitation on food contact to minimize the risk of foodborne illness.

Introduction

It is usual practice in food production and service industries that food for human consumption that has been dropped onto unsanitary surfaces should be discarded. However, there is a perception by the general population that if food is dropped and picked up very quickly from an unsanitary surface, the food may not be too contaminated to consume (Sefton 2003; Wikipedia 2005). Food contact surfaces have the potential to act as reservoirs for bacteria over extended time periods, and they have been shown to transfer pathogenic bacteria to food (De Wit *et al.* 1979; Humphrey *et al.* 1994, 2001; Chen *et al.* 2001; Gorman *et al.* 2002; De Cesare *et al.* 2003; Kusumaningrum *et al.* 2003; Moore *et al.* 2003; Rayner *et al.* 2004). An estimated 76 million cases of foodborne illnesses occur annually in the United States, of which 5200 are fatal (Mead *et al.* 1999) with *Campylobacter* spp. and *Salmonella* spp. accounting for 2·4 million and 1·4 million of these cases, respectively. Poultry and poultry products have been implicated as a major source of *Campylobacter* spp. and *Salmonella* spp. infection in humans (Bailey 1998; Corry and Atabay 2001). A recent study revealed that 70·7% of the poultry carcasses and 91% of the retail chicken products in the United States examined were contaminated with *Campylobacter* spp. (Zhao *et al.* 2001). Based on U.S. Department of Agriculture (USDA)-Food Safety and Inspection Service surveillance, the prevalence of *Salmonella* spp. contamination of freshly processed poultry carcasses was reported to be

11·4% in 1999 and 9·1% in 2000 (http://www.usda.gov). Incidence of pathogens on fresh poultry was reported by Simmons *et al.* (2003). They found the percentages of carcasses testing positive for *Salmonella* spp. ranged from 0 (for 1 week) to >60% (for 3 weeks) over a 20-week sampling period. For only 4 of the 20 weeks was the number of *Salmonella* spp. positive carcasses less than 20%. For the entire 20-week study, 85 (33·9%) of the 251 carcasses tested were found to be *Salmonella* spp. positive. For those processing plants from which >10 carcasses were obtained, the percentages of carcasses testing positive for *Salmonella* spp. ranged from <20 (two plants) to >40% (four plants). Raw poultry products have been cited as a frequent source of bacterial cross-contamination of food preparation surfaces, partially owing to the presence of *Salmonella* spp. (Humphrey *et al.* 2001; Gorman *et al.* 2002).

While spores are known to survive in nature for millions of years, vegetative bacterial cells can also survive for long times. Humphrey *et al.* (1994) found that *Salmonella* spp. could persist on Formica for at least 24 h. *Escherichia coli* and *Salmonella* spp. were also found to survive on clothes, hands and utensils for several days. The term biofilm has been used to describe the micro-surface environment in which bacteria exist on surfaces. These biofilms are often comprised of food purge that may contain protein, carbohydrates and fats in solution that protect the bacteria from dehydration and cleaning. In a review of biofilms, Mattila-Sandhom and Wirtanen (1992) stated that biofilms are layers consisting mainly of polysaccharides that protect microbes from hostile environments and trap nutrients. Joseph *et al.* (2001) showed that biofilms protected *E. coli* and *Salmonella* spp. from sanitizers on both cement and stainless steel allowing bacteria to survive multiple days. In their study, *Salmonella* spp. populations on surfaces after 48 h at 28°C were 7·21, 6·12 and 5·4 log CFU cm^{-2} for high-density polyethylene (HDPE), cement and stainless steel, respectively. These authors also recorded that 5·6, 3·1 and 2·6 log CFU cm^{-2} *Salmonella* spp. were still present on HDPE, cement and stainless steel, respectively, after 10-min exposure to 50-ppm chlorine. Rayner *et al.* (2004) verified the presence of bacterial biofilms on a variety of surfaces (including salad vegetables, cutting boards, sponges and towels) using scanning electron microscopy.

As bacteria can survive on food contact surfaces, the opportunity for food cross-contamination upon contact also exists. Cross-contamination has been studied in several settings, for example, Patrick *et al.* (1997) found that washing contaminated hands did not prevent the transfer of bacteria to liquorice, skin and utensils. However, drying of hands significantly reduced this transfer. Thus, moisture might play an important role in the transfer of bacteria from surfaces to food. 'Wet sites', such as sinks, dishcloths and sponges have been cited as sources of contamination (Scott and Bloomfield 1990), although high numbers of viable bacteria have been recovered from dry surfaces up to 2 weeks after inoculation (Dawson *et al.* 2003). In 200 households surveyed, high numbers of aerobic bacteria were isolated with 49% of the food contact surfaces being estimated as potential pathogen reservoirs (Scott *et al.* 1982). De Wit *et al.* (1979) reported that artificially inoculated chicken carcasses contaminated kitchen sinks, cutting boards, tables and dishcloths during normal food preparation. Previous cross-contamination studies have used surrogate strains, such as *Enterobacter aerogenes*. For example, Zhao *et al.* (1998) supported the findings of De Wit *et al.* (1979), reporting that 10^5 CFU cm^{-2} of *Enterobacter aerogenes* were transferred to cutting boards, hands and vegetables from chicken skin inoculated with 10^6 CFU g^{-1}. In another study using *Enterobacter* spp. as a surrogate, Chen *et al.* (2001) reported that bacterial transfer between hands, foods and kitchen surfaces ranged from <0·1% to 100%.

Many factors may contribute to the rate of bacterial transfer from surfaces to food, including food composition, surface type, residence time of bacteria on the surface and contact time of the food with the surface. Moore *et al.* (2003) reported differences in the number of *Salmonella* Typhimurium and *Campylobacter jejuni* from stainless steel coupons to dry lettuce compared with wet lettuce. These researchers reported an approximate 2 $\log_{10}$ loss in *S.* Typhimurium and *C. jejuni* on stainless steel after 2 h and a transfer of 1·6 to 6·45 log CFU cm^{-2} from surfaces having 2·55 to 6·96 $\log_{10}$ CFU cm^{-2}. A percentage of transfer was calculated and found to be higher (65%) for dry lettuce compared with wet lettuce (30%) when bacteria were left for 60 min before contact, but similar for wet (22%) and dry lettuce (20%) when bacteria were left for 120 min before contact.

A common phrase related to the cleanliness of food dropped on surfaces is the 'five second rule', the implication being that if dropped food is picked up quickly enough, it is not contaminated. A study by undergraduates at the University of Maine concluded that food waste could be reduced if dropped food were consumed by practising the five-second rule, and that this might improve children's immune systems (Strout 2001). Thus, one objective of this research was to determine the effects of food contact time with contaminated surfaces on the transfer of *S.* Typhimurium to bologna and bread. Another objective was to determine the residence time effects on the survival of bacteria on surfaces and the transfer of *S.* Typhimurium to food.

Materials and Methods

Salmonella Typhimurium inoculum

An *S.* Typhimurium environmental isolate from the laboratory of Dr B.W. Sheldon (Poultry Science Department, North Carolina State University) was used for testing and maintained in a −70°C freezer. The *S.* Typhimurium isolate was resistant to 1000 ppm of nalidixic acid (^{NAR}) (Sigma Chemical Co., St. Louis, MO, USA). No background flora were recovered when bacteria from bologna or bread were enumerated on 1000-ppm naladixic media. The naladixic acid media was made by adding 1000 ppm of naladixic acid to tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI, USA). *Salmonella* Typhimurium ^{NAR} was cultured in screw-capped tubes containing 9·9 ml of tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA), and aerobically incubated at 37°C for 16 h with agitation at 200 rev min^{-1}. A separate inoculation culture was grown for each replicate of each experiment. Cells were harvested from the 9·9-ml culture by centrifugation at 10 000 ***g*** for 15 min. The supernatant was discarded and the pellet was resuspended in 100-ml 0·1% peptone water to obtain an approximate concentration of 10^{7-8} CFU ml^{-1}.

Experiment 1: residence time and media concentration effects on *Salmonella* Typhimurium recovery from tile

Autoclaved tile (finished ceramic tile by American Olean, Mexico) surfaces (10 × 10 cm) were inoculated with 1 ml of resuspended pellet from an approximate 7–8 log CFU ml^{-1} overnight culture of *S.* Typhimurium, then sampled immediately (after 5-min drying) and after 2, 4, 8, 24, 48, 72 (3 days), 120(5 days), 168(7 days), 336 h (14 days), 504 (21 days) and 672 h (28 days), following incubation at 21°C and 50% relative humidity (RH) in a temperature- and humidity-controlled chamber. Three different media concentrations were used to carry the inoculants, 0·1% peptone water, TSB at a 1% concentration and TSB at a 10% concentration. The TSB concentrations were percentages of the normal media strength used for the broth, which is 30%. Thus, the actual TSB concentrations were 0·3% for the 1% level and 3·0% for the 10% level. Cells were recovered from each tile by rinsing the surface with 10 ml of 0·1% peptone water in a sterile stomacher bag followed by 30 s of massaging within the bag. Enumeration of bacteria for all experiments was accomplished by decimal dilution followed by pour plating using TSA (Difco Laboratories) containing 1000 ppm naladixic acid. Plates were incubated for 48 h at 37°C, after which, plates containing 25–250 cells per plate were counted and the counts converted to CFU cm^{-2} and log CFU cm^{-2}. When the $\log_{10}$ CFU ml^{-1} *vs* residence time graph was examined, two phases of bacterial death were observed. Based on this observation, *D*-values for each medium (0·1% peptone water, 1% TSB and 10% TSB) were calculated using the 0-, 2-, 4- and 8-hour $\log_{10}$ survival populations and *D*-value for each medium was calculated using the 8-, 24-, 48-, 72-, 120-, 168-, 336-, 504- and 672-h $\log_{10}$ cell count values. The correlation coefficient of the fitted line to each of the time phase relationships to log CFU was calculated using SAS (Statistical Analysis System, Cary, NC, 2000).

Experiment 2: bacterial residence time and surface contact time effects on transfer of *Salmonella* Typhimurium to bologna

All surfaces were autoclaved to sterilize the surface, then handled aseptically prior to inoculation. One millilitre of the 10^{7--8}-CFU ml^{-1} *S.* Typhimurium cell suspension was spread onto each of three 10 × 10-cm surfaces (Fig. 1: tile, wood, carpet) in a circular motion using a sterile glass rod. After drying for 5 min, inoculated tile (finished ceramic tile by American Olean, Mexico), carpet (ST103 Stratos, sold by Lowes, Inc.), and wood (Bruce hardwood Floors, Dura-luster plus finish, Armstrong CO. Addison, TX, USA) samples were each held in an environmental chamber (Hotpack Corporation, Philadelphia, PA, USA) at 21°C with 50% relative humidity for residence times of 0, 2, 4, 8 and 24 h prior to sampling. 10 × 10 cm square precut bologna slices (29·14-g average weight), containing approximately 21% fat, 11% protein, 7% carbohydrate and 60% water, were placed onto each inoculated surface for 5, 30 and 60-s bologna exposure times at each residence time. No additional pressure was applied to each slice during contact with each surface; thus, the only contact pressure resulted from the weight of each slice. The transfer of *S.* Typhimurium to bologna was measured by stomaching the slices 10 ml of 0·1% peptone water at 230 rev min^{-1} (Seward stomacher, Seward Limited, London, UK), prior to plating. Cells were enumerated by decimal dilution followed by pour plating using TS agar containing 1000 ppm naladixic acid. The remaining inoculum cells were then recovered from each surface by rinsing the surface, and enumerated as described in experiment 1. As bologna surface area was 100 cm^2, and the whole surface was rinsed and stomached with 10-ml peptone water, the CFU ml^{-1} rinse values would be equivalent to 0·1 CFU cm^{-2} or 10 CFU per slice. The percentage of bacterial transfer was calculated, as described by Moore *et al.* (2003) using:

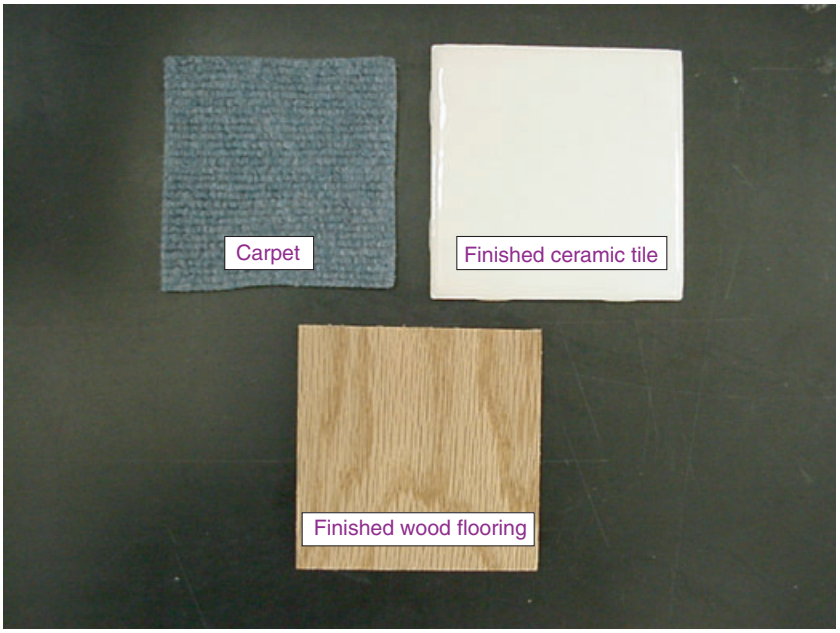

Figure 1 Pictures of the three contact surfaces (tile, wood, carpet) used in the experiments.

$$\% \text{ transfer} = \frac{\text{CFU destination}_{(\text{bologna or bread})}}{\text{CFU destination} + \text{CFU source}_{(\text{tile, wood, carpet})}} \times 100$$

Experiment 3: bacterial residence time and surface contact time effects transfer of *Salmonella* Typhimurium to bread

One ml of the 10^{7-8}-CFU ml^{-1} *S.* Typhimurium cell suspension was spread onto 10 × 10-cm squares of tile using a sterile glass rod. The inoculated tiles were held in an environmental chamber at 21°C with 50% relative humidity for residence times of 0, 2, 4, 8 and 24 h prior to sampling. 10 × 10-cm squares of precut bread slices (28·13-g average weight), containing approximately 4% fat, 52% carbohydrate, 8% protein and 36% water were placed on each inoculated surface for 5-, 30- and 60-s bread exposure times at each residence time. The transfer of *S.* Typhimurium to bread slices was measured by stomaching the slices in 10 ml of 0·1% peptone water at 230 rev min^{-1}, prior to plating. Cells were enumerated by decimal dilution, followed by pour plating on TSA containing 1000-ppm naladixic acid. The plates were incubated for 48 h at 37°C after which dilutions with 25–250 cells per plate were counted and numbers were converted to CFU cm^{-2} and $\log_{10}$ CFU cm^{-2}. The percentage of cells transferred from tile to bread was calculated as described previously.

Statistical analysis

Each surface was treated independently in the statistical analyses, and each was replicated three times on different days (analysed in duplicate per replication) using different materials and bacterial cultures. The experiments were analysed using a randomized complete block design in general linear model. Experiment 1 model included replication, residence time (0–672 h) and media concentration (0·1% peptone, 1·0% and 10% TSB) and residence time by media concentration interaction. The model for experiments 2 and 3 included replication, residence time (0, 20, 4, 8, 24 h), bologna (or bread) exposure time (5, 30, 60 s) and residence time by bologna (or bread) exposure time treatment interaction effects. The residual interaction effects were used as the error terms. As there were no significant effects caused by replication, replications were pooled. Where residence time and bologna (or bread) exposure effects or their interactions were significant ($P \leqslant 0{\cdot}05$), means were separated using the pdiff command of SAS (Statistical Analysis System, Cary, NC, 2000).

Results

Experiment 1: residence time and media concentration effects on *Salmonella* Typhimurium recovery from tile

The differences in bacterial survival or inactivation rate were found to be the result of surface type and inocula-

Table 1 *D*-values and correlation coefficients for *Salmonella* Typhimurium inoculated on to tile at different media concentrations

	Residence time period	
	0–8 h	8–672 h
Media concentration	*D*-value in hours (correlation coefficient)	
0·1% peptone	3·95 (−0·951)	277·8 (−0·956)
1% of normal TSB media (0·3% TSB concentration)	3·08 (−0·983)	288·1 (−0·985)
10% TSB of normal TSB media (3·0% TSB concentration)	5·32 (−0·958)	264·2 (−0·954)

TSB, tryptic soy broth.

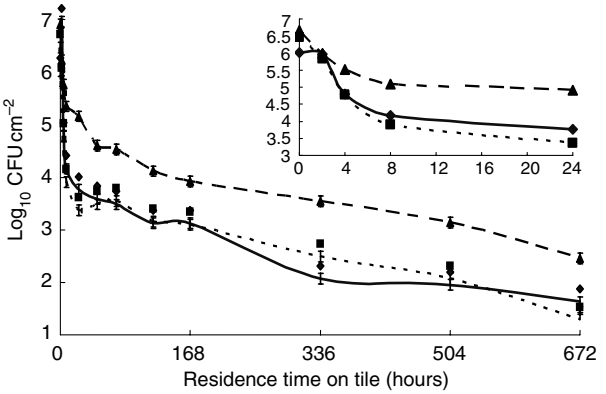

Figure 2 Effect of residence time (0–672 h) on the survival *of Salmonella* Typhimurium on tile for cells inoculated on to the tile surface in 0·1% peptone water (◆), 1% tryptic soy broth (■) and 10% tryptic soy broth (▲). 2a (inset). 0 to24-h residence times.

tion medium. There was a significant effect ($P < 0{\cdot}001$) of both residence time and media type on the presence of *S.* Typhimurium (Table 1). After 28 days, 1·5 to 2·5 $\log_{10}$ CFU cm^{-2} remained on tile from an initial 10^8-CFU ml^{-1} inoculation level. This converts to 3·5 to 4·5 $\log_{10}$ CFU for the total 10 × 10-cm tile surface. There was a decrease in *S.* Typhimurium cells over the residence times; however, significant bacterial numbers remained to allow cross-contamination (Fig. 2). The more concentrated media (10% TSB) facilitated the survival of approximately 1 $\log_{10}$ greater *S.* Typhimurium compared with the more dilute solutions after 8 h through 28 days of bacterial residence time.

Experiments 2 and 3: bacterial residence time and surface contact time effects on transfer of *Salmonella* Typhimurium to bologna or bread

Three contact surfaces (wood, tile, carpet) were evaluated for the transfer of *S.* Typhimurium to bologna. Residence time ($P < 0{\cdot}0001$) and the residence time by bologna exposure time interaction ($P < 0{\cdot}0001$) on wood were found to have a significant effect on the transfer of *S.* Typhimurium to bologna, while bologna exposure time ($P = 0{\cdot}24$) did not. Residence time ($P < 0{\cdot}0001$), bologna exposure time ($P = 0{\cdot}0047$) and the residence time by bologna exposure time interaction ($P = 0{\cdot}038$) on carpet, all had significant effects on the transfer of *S.* Typhimurium to bologna. For tile, residence time ($P < 0{\cdot}0001$), bologna exposure time ($P < 0{\cdot}0001$), and the residence time by bologna exposure time interaction ($P < 0{\cdot}0001$) on tile, all had significant effects on the transfer of *S.* Typhimurium to bologna.

At each incremental increase in residence time, there was a decrease ($P < 0{\cdot}05$) in the number of *S.* Typhimurium cells recovered from bologna, in contact with wood and tile (Figs 3 and 4). This trend did not hold for carpet, where initial *S.* Typhimurium levels recovered from

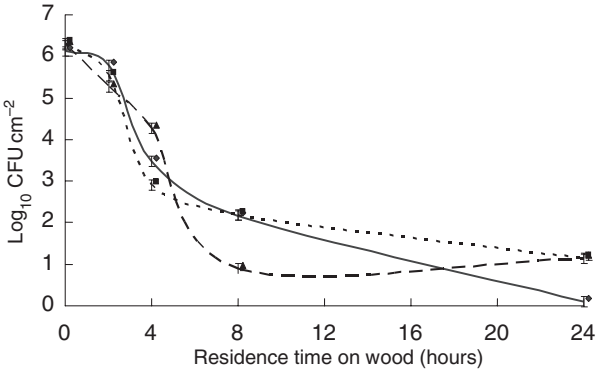

Figure 3 Transfer of *Salmonella* Typhimurium to bologna exposed to wood for 5 (◆), 30 (■) or 60 s (▲) for surfaces held 0, 2, 4, 8 and 24 h after inoculation.

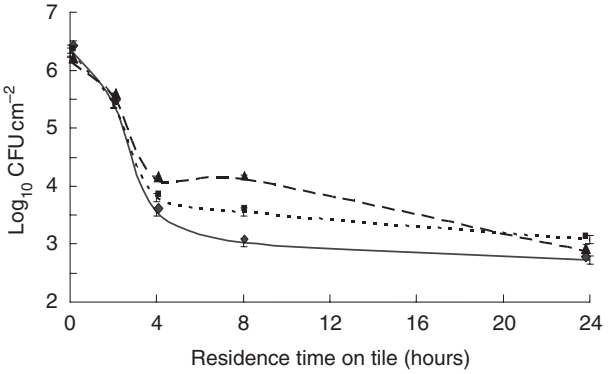

Figure 4 Transfer of *Salmonella* Typhimurium to bologna exposed to tile for 5 (◆), 30 (■) or 60 s (▲) for surfaces held 0, 2, 4, 8 and 24 h after inoculation.

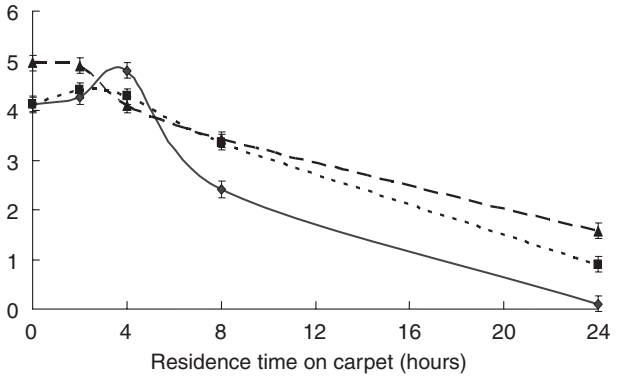

Figure 5 Transfer of *Salmonella* Typhimurium to bologna exposed to carpet for5 (◆), 30 (■) or 60 s (▲) for surfaces held 0, 2, 4, 8 and 24 h after inoculation.

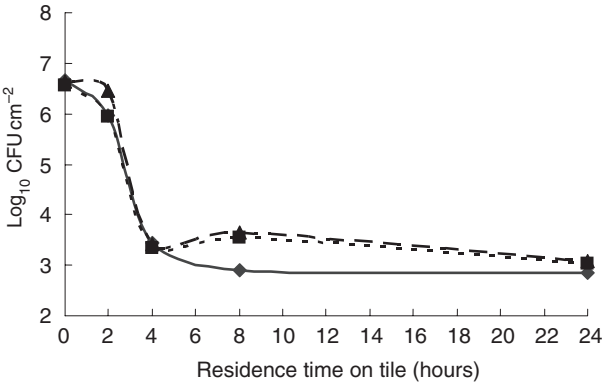

Figure 6 Transfer of *Salmonella* Typhimurium to white bread exposed to tile for 5 (◆), 30 (■) or 60 s (▲) for surfaces held 0, 2, 4, 8 and 24 h after inoculation.

bologna after contact at time 0 were lower than that for tile and wood (Fig. 5). At 0, 2 and 4 h, there was no difference in the number of bacteria transferred to bologna from carpet; however at 8-h residence time, the carpet transferred about 1·5 $\log_{10}$ CFU cm^{-2} (2·5 CFU per slice) more than wood. There was a similar trend for the transfer of bacteria from tile and wood to bologna, in that there was a rapid decrease in the transfer rate between 0, 2 and 4 h, after which the transfer rates were constant at 4- and 24-h residence time. Carpet displayed a different transfer rate curve over time with very little change between 0 and 4 h of residence time, followed by a steady decrease in transfer rate through 24 h.

One contact surface (tile) was evaluated for the transfer of *S.* Typhimurium to white bread. The residence time of *S.* Typhimurium on tile was the only factor that had a significant effect ($P < 0{\cdot}0001$) on the number of cells transferred, and the percent of cells transferred to bread. On the whole, the contact time did not have an effect on cell transfer to bread except at the 8-h residence time, where the number of bacteria recovered at the 5-s contact was more than 0·5 $\log_{10}$ CFU cm^{-2} (1·5 CFU per slice) less than the 30 and 60-s contact times (Fig. 6). A contact time effect ($P < 0{\cdot}01$) was found at the 8-h residence time for all surfaces (bologna and bread). This was not the case for any other residence time. In addition, the transfer rate curves for bread and bologna, exposed to inoculated tile, were remarkably similar, despite obvious differences in surface properties and surface moisture levels.

While food contact time had no effect on the percentage of *S.* Typhimurium transferred, bacterial residence time prior to food contact did affect the percentage of cells transferred to the food, and exhibited a similar trend for all surfaces. The percentage of bacterial cells transferred to the food decreased from initial rates as the intermediate residence times were reached, and subsequently, the rates increased at the latter residence times (Table 2). The 4-h residence time had the lowest percentage transfer of available cells for tile and wood for both bologna and bread. Carpet displayed a similar trend; however, the percentage transfer was so low for carpet that the resulting values were not different from a practical standpoint. All percentage transfer rates for carpet at different residence times were below 0·5%. However, this did reveal that while carpet did not transfer a high percentage of bacterial cells present, actual numbers transferred were in the same range as other surfaces, and that carpet retained bacteria for a longer period of time. This is particularly evident in the first 8 h, when over 7·4 $\log_{10}$ CFU cm^{-2} were recovered from carpet compared with tile and wood, for which cell recovery decreased from ~5·5 to 3·5 log CFU cm^{-2} between 0 and 8 h (Fig. 7). Therefore, while the 'wicking' effect of carpet may have reduced the percentage of cells transferred upon food contact, this effect also created an environment that allowed bacteria to survive, resulting in total levels 10 to 100 times greater than wood and tile after 24 h (Table 2).

Discussion

Few published papers have reported on the transfer of bacteria from surfaces to food, while several have studied the transfer of bacteria from food to other surfaces (Scott and Bloomfield 1990; Zhao *et al.* 1998; Chen *et al.* 2001; Montville *et al.* 2001). Kusumaningrum *et al.* (2003) reported on bacterial transfer from stainless steel to cucumbers, while Moore *et al.* (2003) and Chen *et al.* (2001) studied bacterial transfer to lettuce from stainless steel and cutting boards, respectively. Moore *et al.* (2003) further discussed the disparity in how bacterial transfer data are reported and statistically analysed. The methods varied from reporting bacterial numbers recovered from

Table 2 The percentage of *Salmonella* Typhimurium transferred and total bacteria transferred to bologna and bread from various surfaces at different bacterial residence times prior to food contact

Residence time* (h)	Bologna on wood		Bologna on tile		Bologna on carpet		Bread on tile	
	% transfer†	CFU cm^{-2} transfer‡	% transfer	CFU cm^{-2} transfer	% transfer	CFU cm^{-2} transfer	% transfer	CFU cm^{-2} transfer
0	47·87 a	$1{\cdot}8 \times 10^{6}$ A	68·55 a	$2{\cdot}3 \times 10^{6}$ a	0·12 b, c	$5{\cdot}3 \times 10^{4}$ a	48·65 a	$5{\cdot}3 \times 10^{6}$ a
2	30·67 b	$4{\cdot}5 \times 10^{5}$ b	39·96 b	$3{\cdot}1 \times 10^{5}$ b	0·37 a	$7{\cdot}3 \times 10^{4}$ a	44·79 a	$9{\cdot}6 \times 10^{5}$ b
4	5·47 c	$2{\cdot}0 \times 10^{4}$ c	14·72 d	$9{\cdot}9 \times 10^{3}$ b	0·13 c	$3{\cdot}9 \times 10^{4}$ a, b	5·72 d	$3{\cdot}7 \times 10^{3}$ c
8	13·08 c	$1{\cdot}5 \times 10^{2}$ c	48·60 a	$7{\cdot}3 \times 10^{3}$ b	0·02 c	$8{\cdot}2 \times 10^{3}$ b, c	16·11 c	$3{\cdot}2 \times 10^{3}$ c
24	9·72 c	$1{\cdot}3 \times 10^{1}$ c	25·93 c	$9{\cdot}8 \times 10^{2}$ b	0·24 b	$1{\cdot}8 \times 10^{1}$ c	32·70 b	$3{\cdot}0 \times 10^{3}$ c

*Residence time is the time from inoculation of the surface before food contact.

†% transfer = percentage of *S.* Typhimurium transferred from the surface to the food using:

$$\%\ \text{transfer} = \frac{\text{CFU destination (bologna or bread)}}{\text{CFU destination} + \text{CFU source (tile, wood, carpet)}} \times 100$$

‡CFU cm^{-2} transfer = the number of *S.* Typhimurium per millilitre recovered from the 10-ml rinse solution from a 10 × 10-cm food sample.

a–d: different letters shown below the means within columns indicate means that significantly differ ($P \leqslant 0{\cdot}05$).

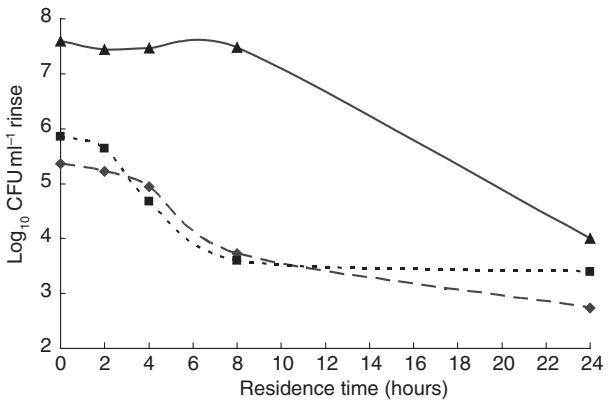

Figure 7 Survival of *Salmonella* Typhimurium on wood (◆), tile (■) and carpet (▲) during 24-h residence time.

the 'destination' surface to reporting a percentage of transferred bacteria. The log CFU and actual cell numbers have been reported, and the transfer percentage has been calculated using at least two different methods: (i) (bacteria on destination/bacteria on source) × 100 and (ii) (bacteria on destination/ bacteria on destination + bacteria on source) × 100. One flaw in using method (i) is that accurate measurement of bacteria on the source surface is a challenge. One cannot assume that all bacteria in the inoculum will be present on the source surface, and experimental enumeration of the source surface must be conducted on a control surface, which may not match the actual contact surface. Thus, the second percentage transfer calculation method, which includes the bacteria recovered from the surface and from the food as the total bacteria available from transfer, has begun to be favoured by some researchers.

Salmonella spp. are common foodborne pathogens associated with raw foods and implicated in cross-contamination scenarios. The nutrient concentration of 'media', carrying bacteria to a surface will affect its survival on that surface. In the present study, the more nutrient-dense medium did retain more bacteria than the less-dense media; however, the greater number retained was attributed to a slower initial death rate, after which surviving bacteria died at similar rates, regardless of media. From a food safety standpoint, it is important to note that 3·5 to 4·5 $\log_{10}$ CFU 10 × 10-cm surface was present on tile after 4 weeks.

Food contamination can result from contact with a variety of surfaces, including hands, other foods and utensils contaminated with different bacterial loads and bacteria carried in various 'media'. The contaminating vector may maintain a bacterial presence by means of a biofilm. Biofilms are microscopic layers that form when bacteria excrete exogenous compounds that allow them to attach to nearly any type of surface. These biofilms allow surface bacteria to survive and sometimes grow at a different rate than planktonic cells (which are not attached to a surface) depending upon the presence of nutrients and moisture. The attached or sessile bacteria actually behave much differently and are often more problematic than planktonic cells of the same type (Center for Biofilm Engineering 2006). Rayner *et al.* (2004) used scanning electron microscopy to visualize biofilms on various foods and food preparation surfaces, including cutting boards, which showed a significant presence of biofilms. Owing to these bacterial micro-environments, pathogens survive on what appear to be clean surfaces. Persistence and survival of pathogens on food contact surfaces is an important factor to consider when evaluating cross-contamination from surfaces to food. Humphrey *et al.* (1994) demonstrated that bacteria could survive on surfaces, reporting that *Salmonella* Enteriditis was recovered

through 24 h from Formica®, previously exposed to contaminated egg. Surface drying would be expected to cause a reduction in bacterial populations, as was shown by Moore *et al.* (2003), who reported a decrease from 5·5 to 4·0 log CFU per 5 × 5-cm stainless steel coupon after 2-h drying. In the current study, three different media were evaluated for their effect on *S.* Typhimurium survival on ceramic tile. Media effects on survival were also evaluated by De Cesare *et al.* (2003), who used *C. jejuni* and a five-strain *Salmonella* spp. cocktail suspended in either a phosphate-saline buffer (PSB) solution or a full strength TSB as the inoculating media on to ceramic tile, Formica® or 100% cotton cloth.

De Cesare *et al.* (2003) reported that *Salmonella* spp. were 1·7 to 3·3 times more persistent (based on *D*-values), when suspended in the more nutrient-dense TSB medium, compared with PBS prior to inoculation on to surfaces. These researchers reported *D*-values of 17·1, 426·6, 118·6 and 41·9 min for *Salmonella* spp. suspended in PBS and 48·2, 136·2, 481·8 and 154·2 min for *Salmonella* spp. suspended in TSB before inoculation on to cotton, Formica®, stainless steel and ceramic tile, respectively. In the current study, the bacterial death rate data on surfaces was biphasic, with one linear phase from 0 to 8 h and another linear phase from 8 to 672 h (Fig. 2). *Salmonella* Typhimurium *D*-values were 3·95, 3·08 and 5·32 h between 0 and 8 h for the 0·1% peptone water, 1·0% TSB and 10% TSB inoculation media, respectively (Table 1). From 8 through 672-h residence, *S.* Typhimurium *D*-values were 277·8, 288·1 and 264·2 h for 0·1% peptone water, 1·0% TSB and 10% TSB, respectively. Thus, during the first 8 h *S.* Typhimurium suspended in the more nutrient-dense medium (10% TSB) had a lower (1·4 to over 2 h) decimal reduction rate compared with the less nutrient-dense media (0·1% peptone and 1·0% TSB), when placed on ceramic tile. After 8 h, the death rate slowed 50 to 80 times for each media type with less relative variation in *D*-value owing to media type during that phase compared with the first 8 h. Similar numbers were reported by De Cesare *et al.* (2003), but more importantly, the same trend was found during shorter residence times (De Cesare *et al.* measured from 0 to 4 h), that is, the more nutrient-dense medium facilitated greater survival. Thus, the greater survival of*S.* Typhimurium carried in the more nutrient-dense media in the present study was primarily the result of the protective effect during the first 8 h.

Both bologna and bread exposed to tile displayed two straight line phases between residence times of 0 and 4 h and between 4 and 24 h. This implies that bacterial transfer from surfaces to food is more dependent on the 'source' surface (bacterial levels present and surface properties) and less dependent on the characteristics of the 'destination' food properties. Moore *et al.* (2003) also reported differences in bacterial transfer rates from surfaces to food as a result of the source surface residence time and an effect attributed to 'wet' *vs* 'dry' lettuce. However, the 'dry' lettuce had a higher transfer rate when the contact with the contaminated surface was at bacterial residence times of 1 h or less, while 'wet' lettuce had higher transfer rates at 2-h residence times. Thus, residence time and the source surface properties had an overriding effect on transfer rate. Differences between the current study and that of Moore *et al.* (2003) include the food materials used (bologna/bread and lettuce, respectively) and bacterial residence times (0–24 h and 0–2 h, respectively).

The transfer of bacteria from surfaces to food was most affected by bacterial residence time, and was the most influential factor in the transfer of *S.* Typhimurium from wood, tile and carpet to bologna. Longer food contact times (5, 30 or 60 s) did result in transfer of more bacteria for each surface and food tested, but only when the food was placed in contact with the surface 8 h after the surface had been inoculated. As no residence times between 4 and 8 h and between 8 and 24 h were tested, it was not determined if this food contact effect lasted for several hours or not. Another interesting result was that carpet had very low transfer rates (<0·5%) compared with tile and wood (5–69%); however, owing to survival rate of greater than 2 logs during the first 8 h after inoculation, contact with carpet resulted in the transfer of similar numbers of *S.* Typhimurium through 8 h and greater numbers at 24 h compared with tile and wood. While not directly compared, similar transfer patterns of *S.* Typhimurium from tile were found for both bologna and bread. Consequently, this study concludes that proper and diligent sanitation of food contact surfaces is needed to reduce cross-contamination to food, because even very short contact times result in the transfer of large numbers of bacteria.

References

Bailey, J.S. (1998) Detection of *Salmonella* cells within 24 to 26 hours in poultry samples with the polymerase chain reaction BAX system. *J Food Prot* **61**, 792–795.

Center for Biofilm Engineering (2006) *A National Science Foundation Engineering Research Center*. Montana State University, http://www.erc.montana.edu, website accessed 7-07-06.

De Cesare, A., Sheldon, B.W., Smith, K.S. and Jaykus, L.-A. (2003) Survival and persistence of *Campylobacter* and *Salmonella* species under various organic loads on food contact surfaces. *J Food Prot* **66**, 1587–1594.

Chen, Y., Jackson, M., Chen, F. and Schaffner, D. (2001) Quantification and variability analysis of bacterial cross-

contamination rates in common food service tasks. *J Food Prot* **64**, 72–80.

Corry, J.E. and Atabay, H.I. (2001) Poultry as a source of *Campylobacter* and related organisms. *Sym Series Soc Appl Micro* **30**, 96S–114S.

Dawson, P., Han, I., Cox, E., Black, C. and Simmons, L. (2003) Food contact time effects on pick-up of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule. *Proceedings of the 2nd Global Congress Dedicated to Hygeinic Coatings Surfaces*. Orlando, FL, 26–28 January, pp. 1–10.

Gorman, R., Bloomfield, S. and Adley, C. (2002) A study of cross-contamination of foodborne pathogens in the domestic kitchen in the Republic of Ireland. *Int J Food Micro* **76**, 143–150.

Humphrey, T.J., Martin, K.W. and Whitehead, A. (1994) Contamination of hands and work surfaces with *Salmonella* Enteriditis PT4 during the preparation of egg dishes. *Epid Infect* **113**, 403–409.

Humphrey, T.J., Martin, W., Slader, J. and Durham, K. (2001) *Campylobacter* spp. in the kitchen: spread and persistence. *J Appl Micro* **90**, 115S–120S.

Joseph, B., Otta, S.K. and Karunasagar, I. (2001) Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *Int J Food Micro* **64**, 367–372.

Kusumaningrum, H.D., Riboldi, G., Hazelberger, W.C. and Beumer, R.R. (2003) Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *Int J Food Micro* **85**, 227–236.

Mattila-Sandhom, T. and Wirtanen, G. (1992) Biofilm formation in the industry: a review. *Food Rev Int* **8**, 573–603.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. (1999) Food-related illness and death in the United States. *Emerg. Infect Dis* **5**, 607–625.

Moore, C.M., Sheldon, B.W. and Jaykus, L.-A. (2003) Transfer of *Salmonella* and *Campylobacter* from stainless steel to Romaine lettuce. *J Food Prot* **66**, 2231–2236.

Montville, R., Chen, Y. and Schaffner, D.W. (2001) Glove barriers to bacterial cross-contamination between hands and food. *J Food Prot* **64**, 845–849.

Patrick, D.R., Findon, G. and Miller, T.E. (1997) Residual moisture determines the level of touch-associated bacterial transfer following hand washing. *Epid Infect* **119**, 319–325.

Rayner, J., Veeh, R. and Flood, J. (2004) Prevalence of microbial biofilms on selected fresh produce and household surfaces. *Int J Food Micro* **95**, 29–39.

Scott, E. and Bloomfield, S. (1990) The survival and transfer of microbial contamination via cloth, hands, and utensils. *J Appl Bact* **68**, 271–278.

Scott, E., Bloomfield, S. and Barlow, C.G. (1982) An investigation of microbial contamination in the home. *J Hyg Camb Univ* **89**, 279–293.

Sefton, D. (2003) *Intern puts science behind the five-second rule*. Newhouse News Service. http://www.newhousenews.com/archive/sefton092903.html.

Simmons, M., Fletcher, D., Cason, J. and Berrang, M. (2003) Recovery of *Salmonella* from retail broilers by a whole-carcass enrichment procedure. *J Food Prot* **66**, 446–450.

Strout, S. (2001). *Does the five second rule exist*? http://student-orgs.umf.maine.edu/%7Emainestream/010502/fivesecond.html.

Wikipedia (2005) *Wikipedia, the free encyclopedia*. Five_second_rule.html.

De Wit, J., Broekhuizen, G. and Kampelmacher, E. (1979) Cross contamination during preparation of frozen chicken in the kitchen. *J Hyg Camb Univ* **83**, 27–32.

Zhao, C., Ge, B., de Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. *et al.* (2001) Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. *Appl Environ Micro* **67**, 5431–5436.

Zhao, P., Zhao, T., Doyle, M., Rubino, J. and Meng, J. (1998) Development of a model for evaluation of microbial cross-contamination in the kitchen. *J Food Prot* **61**, 960–963.