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International Journal of Food Microbiology 85 (2003) 227–236
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods

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Received 19 April 2002; received in revised form 9 September 2002; accepted 15 November 2002

Abstract

The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. However, some non-spore-forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. In this study the survival of *Salmonella enteritidis*, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces at different initial levels was determined at room temperature. The transfer rates of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods were also investigated. *Staph. aureus* was recovered from the surfaces for at least 4 days when the contamination level was high (10^5 CFU/cm^2) or moderate (10^3 CFU/cm^2). At low levels (10 CFU/cm^2), the surviving numbers decreased below the detection limit (4 CFU/100 cm^2) within 2 days. *S. enteritidis* was recovered from surfaces for at least 4 days at high contamination levels, but at moderate level, the numbers decreased to the detection limit within 24 h and at low level within 1 h. *C. jejuni* was the most susceptible to slow-air-drying on surfaces; at high contamination levels, the numbers decreased below the detection limit within 4 h. The test microorganisms were readily transmitted from the wet sponges to the stainless steel surfaces and from these surfaces to the cucumber and chicken fillet slices, with the transfer rates varied from 20% to 100%. This study has highlighted the fact that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which a model of risks assessment in domestic setting could lead.

Keywords: Pathogens; Survival; Cross-contamination; Stainless steel surface

1. Introduction

The importance of contaminated surfaces in relation to potential transmission of pathogens to food is apparent in food processing, catering and the domestic environment. Exposure of pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles. Some bacteria attach to surfaces as their predominant form of survival in nature and man-made ecosystems (Lindsay and Von Holy, 1999). Several studies indicated that various bacteria, including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp., survive

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doi:10.1016/S0168-1605(02)00540-8

on hands, sponges/cloths, utensils and currency for hours or days after initial contact with the microorganisms (Scott and Bloomfield, 1990; Jiang and Doyle, 1999; Kusumaningrum et al., 2002). In some other studies, the extent of bacterial survival and cross-contamination between hands and foods or various kitchen surfaces have been quantified (Zhao et al., 1998; Chen et al., 2001; Montville et al., 2001). Dufrenne et al. (2001) found that the most likely mode of infection of *Salmonella* or *Campylobacter* in The Netherlands was cross-contamination directly from raw poultry or indirectly via contaminated surfaces or niches in the household kitchen to ready-to-eat products. Early studies also indicated that cross-contamination, from raw products via hands, cleaning cloths or sponges and utensils to foods not subjected to further cooking, contributed to the occurrence of outbreaks of food-borne salmonellosis in the United States (Bryan, 1988). Quantifying the cross-contamination risk associated with various steps in the food preparation process can provide a scientific basis for risk management efforts in both home and food service kitchens (Chen et al., 2001).

The kitchen surfaces are a focal point in the kitchen for the preparation of food. Stainless steel has been the material of choice for work surfaces or kitchen sinks for many years because of its mechanical strength, corrosion resistance, longevity and ease of fabrication (Holah and Thorpe, 1990). Modern domestic sink and work top material such as polycarbonate, mineral resin and some enamelled steels would be as cleanable as stainless steel when new, but stainless steel, due to its resistance to abrasion or impact damage, is more likely to retain its hygienic properties throughout a domestic working life (Holah and Thorpe, 1990; Stevens and Holah, 1993). However, although food particles are usually cleaned from the surface when good hygienic practices are applied, bacteria attached to these surfaces are not visible to the eye and may therefore not be removed. In this study the survival of *Salmonella enteritidis*, *Staph. aureus* and *Campylobacter jejuni* on stainless steel surfaces was determined. The transfer of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods was studied as to provide cross-contamination data for quantitative microbial risk assessments in domestic setting. The effect of food residues on the survival on surfaces was also investigated. Prior to the survival experiments, the efficiency of the sampling technique using contact plates was examined, in which *Bacillus cereus* spores were used in addition to the test microorganisms described before. The recovery of the pathogens from the surfaces using a single contact plate, as described by the standard procedure, was compared to the total recovery using five consecutive contact plates on the same surface area.

2. Materials and methods

2.1. Surfaces

Stainless steel (AISI type 304 standard, ODS, Barendrecht, The Netherlands) surfaces were prepared in two sizes: 20×20 cm^2 for survival experiments and 50×80 cm^2 for cross-contamination studies. Before use, the surfaces were disinfected with approximately 800 ppm hypochlorite solution (Glorix, Lever, The Netherlands) for 15 min; surfaces of 20×20 cm^2 were soaked and surfaces of 50×80 cm^2 were wiped. The surfaces were then washed with hot water with anionic-active detergent and rinsed with hot water. Prior to the artificial contamination the surfaces were sprayed with 70% (v/v) ethanol.

2.2. Test suspensions and growth conditions

S. enteritidis (phage type 4, chicken product isolate) and *Staph. aureus* 196E (human isolate, enterotoxin A producer) were obtained from the National Institute of Public Health and the Environment, The Netherlands (Rijksinstituut voor Volksgezondheid en Milieu, RIVM). *C. jejuni* (NCTC 81116) was from our culture collection and *B. cereus* spores (Bacto cereus spore suspension) were obtained from Difco Laboratories (Detroit, MI, USA).

The stock cultures were maintained at -80 °C in cryo vials (Greiner Bio-one, Frickenhausen, Germany) containing a stationary-phase culture suspension in Brain Heart Infusion (BHI; Difco, Becton Dickinson, Maryland, USA) broth with 25% (v/v) glycerol (Fluka-Chemica, Buchs, Switzerland) and glass beads (Ø 2 mm, Emergo, Landsmeer, The Netherlands). Strains were cultured by transferring one glass bead to 10 ml of BHI broth followed by incubation for 20–22 h at 37 °C for *S. enteritidis* and

Staph. aureus, and for 40–48 h micro-aerobically (6% O_2) obtained by an Anoxomat-System (type WS9000, MART®, Lichtenvoorde, The Netherlands) at 42 °C for *C. jejuni*. *B. cereus* spores were used directly from the ampoules.

The test suspensions were prepared by making serial dilutions of the microorganisms in peptone saline solution (PSS: NaCl (Merck, Darmstadt, Germany) 8.5 g/l and Neutralised Bacteriological Peptone (Oxoid, Basingstoke, England) 1 g/l). For the final dilution, saline solution containing 0.1% Tween 80 (SS–0.1% Tween 80 (Merck, Hohenbrunn, Germany)) was used in order to obtain an equal spread of bacteria on the surface. Three different contamination levels were prepared: high contamination (approximately 10^7 colony forming units (CFU)/100 cm^2), moderate contamination (approximately 10^5 CFU/100 cm^2) and low contamination (approximately 10^3 CFU/100 cm^2), obtained by spreading 1 ml of an appropriate solution on a surface of 20×20 cm^2 or 10 ml of an appropriate solution on a surface of 50×80 cm^2.

Selective agar media were used for the enumeration of pathogens: Mannitol Egg Yolk Polymixine Agar (Merck, Darmstadt, Germany) for *B. cereus*, incubated for 18–40 h at 30 °C; Mannitol Lysine Crystal Violet Brilliant Green Agar (Oxoid) for *S. enteritidis*, incubated for 18–24 h at 37 °C; Baird Parker Egg Yolk-Tellurite Agar (Oxoid) for *Staph. aureus*, incubated for 24–48 h at 37 °C; and Columbia-Blood-Preston Agar (Columbia agar base (Oxoid) containing 5% lysed, defibrinated sheep blood (bioTrading Benelux, Mijdrecht, The Netherlands) and modified Preston Campylobacter supplement (Oxoid)) for *C. jejuni*, incubated micro-aerobically for 40–48 h at 42 °C.

2.3. Recovery of pathogens from surfaces

2.3.1. Direct contact method using contact plates

The recovery of the viable counts from surfaces was carried out using contact plates (Ø 55 mm, Greiner Bio-one, Glos, UK) with appropriate agar media. Applicator Count-Tact (bioMérieux, Marcy l'Etoille, France) was used during the sampling to obtain a consistent sampling condition of 10 s contact time with 500 g pressure per plate.

To generate countable numbers at high contamination levels, the agar of the contact plates was suspended in 50 or 100 ml sterile PSS and subsequently homogenised in a Stomacher® (type 400 Circulator, Seward, Laboratory Blender, England) at 260 rpm for 60 s. Appropriate dilutions were spread on the selective media using a spiral inoculation apparatus (Eddy Jet, IUC, Barcelona, Spain).

2.3.2. Single versus five contact plates on the same area

This experiment was carried out to examine whether any bacteria were still present on the surface after sampling using a single contact plate. The surfaces were contaminated with low numbers to obtain countable numbers on the contact plates. Therefore, 1 ml of the test suspension of an appropriate dilution was put on 20×20 cm^2 stainless steel surface to obtain a concentration of approximately 10^3 CFU/100 cm^2. For *B. cereus* spores, the level of contamination was approximately 10^2 CFU/100 cm^2. Polyester fiber-tipped applicator swabs (Falcon™, Becton Dickinson, Sparks, USA) were used to spread the test suspensions over the surface.

The surface was sampled immediately after artificial contamination using a contact plate as described above and subsequently with four other contact plates. The recoveries of pathogens using a single plate were compared to the recoveries using five plates.

2.4. Survival of pathogens on stainless steel surfaces

The effects of different contamination levels on the survival of pathogens on dry surfaces for an extended period of time were investigated. For *S. enteritidis* and *Staph. aureus*, three different contamination levels on 20×20 cm^2 surface area were examined: high, moderate and low levels. For the survival experiment of *C. jejuni*, two levels were examined: high and moderate. The test suspensions were prepared as described before.

The contaminated surfaces were placed in a laminar hood without airflow at room temperature (22–25 °C, 40–45% RH). The viable counts were determined using a single contact plate as described above immediately after contamination and at different time intervals for an extended period of time.

For the determination of the effect of food residues on the survival of pathogens, the surfaces were contaminated with 1 ml of pathogen suspension in com-

mercial sterilised milk or a suspension of raw chicken breast fillet (obtained from local retail supermarket). The suspension of raw chicken breast fillet was negative for selected pathogens, enumerated using appropriate selective media. The pathogen suspensions were prepared by diluting the cultures in PSS as described previously, but for the final dilutions, 9 ml of commercial sterilised milk or a suspension of raw chicken breast fillet in PSS (1:9, blended at 260 rpm for 60 s) were used. These experiments were carried out using low contamination levels.

2.5. Cross-contamination via sponges and surfaces to foods

This experiment was carried out to determine the transfer rates of the pathogens from artificially contaminated sponges to stainless steel surfaces and subsequently from these surfaces to the foods. Kitchen sponges (Lola, $9 \times 7 \times 3$ cm; Nedac, Duiven, The Netherlands) were artificially contaminated with 10 ml of an appropriate dilution of bacterial suspension and used to wipe 50×80 cm^2 stainless steel surfaces. The contaminated surfaces were placed in a laminar hood without airflow at room temperature (22–25 °C, 40–45% RH).

To simulate subsequent contamination of food, slices of cucumber or roasted chicken fillet (± 0.5 cm thick, approximately 25 cm^2) were put on the surface for 10 s, with and without pressure of 500 g per slice. The numbers transferred to the food were determined by suspending the food in 50 ml sterile PSS and subsequently homogenising in a Stomacher® for 60 s at 260 rpm. Appropriate dilutions were spread on the selective media using a spiral inoculation apparatus. The levels of bacteria on the surfaces were investigated using a single contact plate as described above.

The transfer rates to cucumber or roasted chicken fillet were determined immediately after artificially contamination and 15 min after contamination. The transfer rates were calculated based on the numbers of microorganisms present on surfaces that were recovered by a single contact plate, using the formula:

$$\% \text{ transfer rate} = N_f/N_s \times 100\%$$

where N_f = CFU recovered from food; N_s = CFU on surfaces recovered by contact plate.

2.6. Scanning electron microscopy

To visualise the arrangement of the cells on the dry surfaces, stainless steel (1.5×1.5 cm^2) specimens were prepared. These surfaces were contaminated with pathogens as described for the survival experiments and with pathogens suspended in water. After the exposure to room temperature for particular periods of time, they were sputter-coated with 10 nm platinum and viewed in a JSM-6300F Scanning Electron Microscope (JEOL, Massachusetts, USA).

2.7. Statistical analyses

Each experiment, except scanning electron microscopy, was repeated at least three times at different days and no less than two replicates were used in each experiment. Data analyses were performed on the SPSS for Windows 95/98/NT/2000, release 10.1. A p-value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. Recovery using contact plates

The recoveries of *S. enteritidis*, *Staph. aureus, C. jejuni* and *B. cereus* spores from surfaces using contact plates are shown in Table 1. The recoveries were calculated based on the contamination numbers that were applied on surfaces. Approximately 10–20% of spores or cells were removed during the spreading of the test organisms on the surfaces by the swabs (data not shown).

The recovery was dependent on the test organism ($p = 0.01$). A single contact plate recovered 18% of the *B. cereus* spores applied on the surface. Since spores may survive on dry conditions for a long period of time (Peng et al., 2001), slowly drying of surfaces will hardly affect the numbers of viable spores. The difficulty of detachment was probably due to the ability of the spores to attach to the surfaces. It has been found that the spores of *B. cereus* adhered to stainless steel surfaces better than vegetative cells (Peng et al., 2001). The recovery of *S. enteritidis* by a single contact plate with this technique was 23%. Although *S. enteritidis*, being Gram-neg-

Table 1
Recovery of pathogens from stainless steel surfaces using a single contact plate and five consecutive plates immediately after contamination

Organism	*n*	Contamination (CFU/cm^2)	Recovery count numbers		Recovery percentages[a]		Comparison of recovery using single and five plates[b] (%)
			Single plate (CFU/cm^2)	Five plates (CFU/cm^2)	Single plate (%)	Five plates (%)	
B. cereus spores	50	4 ± 0	1 ± 0	1 ± 1	18 ± 6	33 ± 11	55 ± 8
Staph. aureus	10	12 ± 4	5 ± 2	10 ± 3	46 ± 14	88 ± 26	52 ± 3
S. enteritidis	14	50 ± 14	11 ± 2	20 ± 5	23 ± 6	42 ± 12	57 ± 5
C. jejuni	10	119 ± 22	8 ± 4	17 ± 8	7 ± 3	14 ± 7	49 ± 4

n indicates the number of experiments, with two parallel sampling for each experiment.
± indicates the standard deviation.
[a] Calculated as ((counts of single plate/contamination) × 100%) or ((counts of five plates/contamination) × 100%).
[b] Calculated as ((counts of single plate/counts of five plates) × 100%).

ative, might be sensitive to drying on surfaces, similar recovery to *B. cereus* spores was obtained. The lowest recovery was found for *C. jejuni* (7%), indicating that this strain was likely to be susceptible to the experimental conditions: dry surfaces and direct exposure to the air (de Boer and Hahne, 1990; Humphrey et al., 2001). *Staph. aureus* was recovered in higher numbers (46%) than the other test organisms, probably due to its clump-like structure that may provide a higher chance of detaching more cells during sampling.

The recovery using a single contact plate represented 50–60% of the recovery using five consecutive plates. This result indicated that, after the first sampling, bacteria were still found on surfaces. By further sampling on the same spot, the bacteria were recovered in decreasing numbers (Fig. 1). Tebbutt (1991) suggested that this was probably influenced by whether the bacteria have formed clumps on the surfaces. Since there was no abrasion during the sampling, clumps were not broken and organisms were not released easily from the test surface (Scheusner, 1982). However, the scanning electron micrographs showed no clumps of bacteria on the surfaces contaminated with low levels (10^3 $CFU/100$ cm^2), except for *Staph. aureus* that formed clump-like structures in vivo as well (results not shown). This indicated that the cells were spread over the surfaces. The contact method is easy to use, but it might have limiting factors as it is based on the detachment of microorganisms from surfaces. The cells or spores that adhered to the surfaces would not be detached easily. Some bacteria have been found to be able to adhere to the stainless steel surfaces after short contact times (Mafu et al., 1990).

Niskanen and Pohja (1977) indicated that the contact plate method was suitable for a flat, firm surface, considering both recovery and repeatability, whereas swabbing was better for flexible and uneven surfaces and for heavily contaminated surfaces. However, for heavily contaminated surfaces, contact plates may be used for sampling, if the agar is homogenised and subsequently the bacterial content of the homogenate is examined (Baumgart and Kussmann, 1975 in Niskanen and Pohja, 1977). During quantitative assessment of bacteriological contamination in domestic settings, the contact plate method was satisfactory for differentiation of hygiene

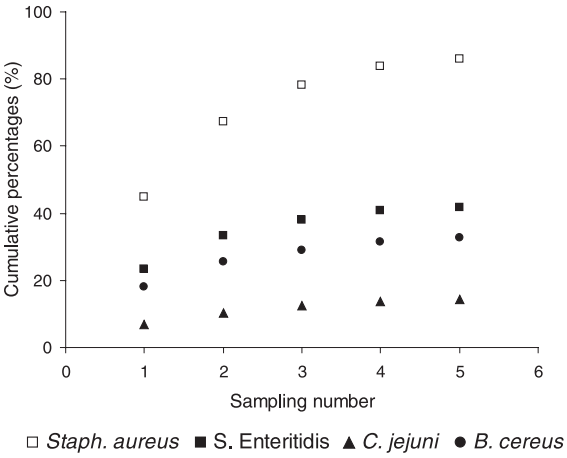

Fig. 1. Cumulative percentages of microorganisms recovered from stainless steel surfaces on the same spot using five consecutive contact plates. The data obtained from 10 experiments for *Staph. aureus* (□), 14 experiments for *S. enteritidis* (■), 50 experiments for *B. cereus* (●) and 10 experiments for *C. jejuni* (▲).

level at environmental sites while facilitating the handling of large numbers of samples in a field survey (Scott et al., 1984). Both the contact method and the swab method are based on the detachment of surface-bound microorganisms. Validation and comparison studies on the recovery of microorganisms from the test surface by swabbing, contact agar plate and Hygicult TPC dipslide, with a known microbial load spread onto the test surface, indicated that the method did not differ in practical terms either in yield or in precision (Salo et al., 2000). The sampling methods recovered 25–30% at the lowest, 18–20% at the middle and 16–21% at the highest levels of microorganisms from the test surfaces, with contamination numbers of 1.4, 10.7 and 43.6 CFU/cm^2, respectively.

Our study showed that the first contact plate sampled the fraction of cells that were easily removed. Since it has been known that a single contact plate represents 50–60% of the total recovery, this technique was used in further experiments. This procedure was considered to be similar with the transfer process of microorganisms from surfaces to food products.

3.2. Survival on stainless steel surfaces

The survival of *S. enteritidis*, *Staph. aureus* and *C. jejuni* on stainless steel surfaces is indicated in Fig. 2. Three different contamination levels were used for the experiments with *S. enteritidis* and *Staph. aureus*, whereas two initial levels were examined for *C. jejuni*. The results indicated that the survival of bacteria decreased rapidly, especially when the initial numbers on the surfaces were low. *Staph. aureus* could be detected on dry surfaces for at least 96 h at high and moderate contamination levels, while at low levels, the cells decreased below the detection limit (log cells numbers (N) = 0.62 CFU/100 cm^2) within 48 h after contamination. The surviving bacteria decreased rapidly within 4 h, in which the cell numbers declined for about 1 or 2 log units, dependent on the initial levels of contamination. For *S. enteritidis*, the viable cells could still be detected after 96 h when a high initial level was present, but at moderate initial numbers, the amount of the surviving cells decreased within 24 h below the detection limit. At low contamination levels, the count of *S. enteritidis* decreased within 1 h below the detection limit.

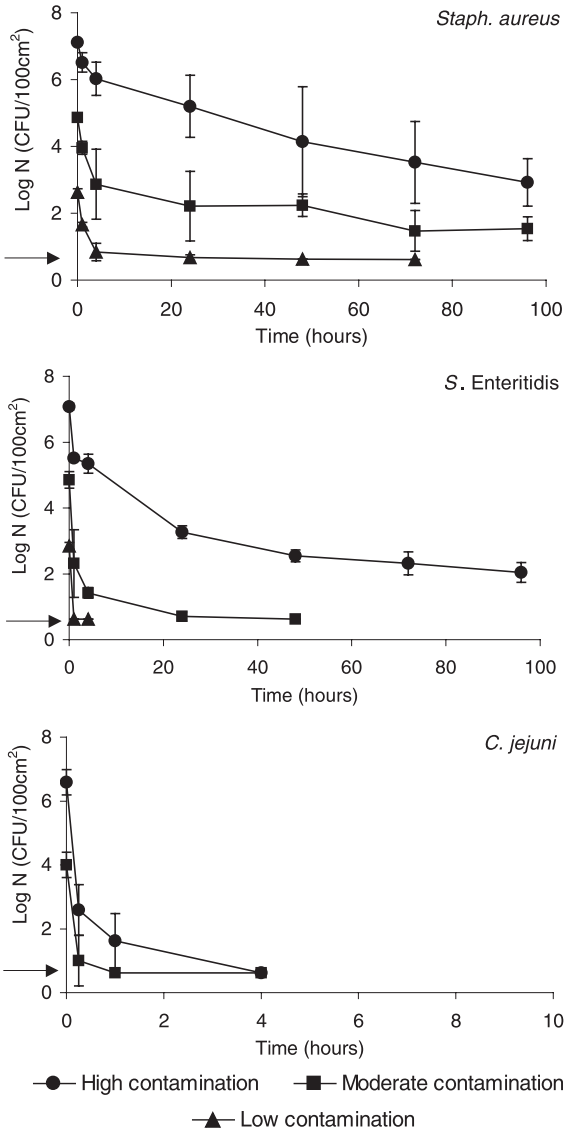

Fig. 2. Survival of pathogens on stainless steel surfaces at room temperature (22–25 °C, 40–45% RH) at different contamination levels; (●) high, approximately 10^7 CFU/100 cm^2; (■) moderate, approximately 10^5 CFU/100 cm^2; and (▲) low, approximately 10^3 CFU/100 cm^2. Experiments with *C. jejuni* were carried out only at high and moderate contamination levels. The arrow bar (→) indicates the detection limit of sampling using a single contact plate (1 CFU/plate, correlated with 0.62 CFU/100 cm^2 log unit). The bars indicate the standard deviation from three experiments.

The survival of these bacteria also decreased rapidly within 4 h, with 1.7 and 3.4 log reductions when high and moderate initial levels were present, respectively.

After 4 h, the cell numbers declined less rapidly. *C. jejuni* was the most susceptible to air-drying on surfaces at room temperature, since the results indicated that at high contamination levels, a 5 log unit reduction was obtained and the numbers decreased below the detection limit within 4 h. For this reason, the experiments at low levels were not carried out for *C. jejuni*.

The characteristics of an organism and its surrounding environment are among the important factors that may affect the survival of bacterial cells on surfaces. The presence of particular surface structures such as flagella, pili and extracellular polysaccharides have been suggested to affect the adhesion and survival of bacteria (Peng et al., 2001). Scanning electron micrographs of surfaces that were contaminated with high levels of bacteria in water suspension show clumps of bacteria (results not shown). Clumps structure might form some protection to the innermost cells against drying (Tebbutt, 1991). The scanning electron micrographs demonstrated also that some cells were found in crevices on the stainless steel surfaces.

The food residues on the surfaces improved the survival of pathogens (data not shown). When a low level of *S. enteritidis* in saline solution was applied to surfaces, the numbers decreased 2.2 log units after 1 h. In the presence of milk residues, the number of *S. enteritidis* decreased 1.0 log unit, whereas in the presence of chicken fillet suspension, the numbers decreased 1.9 log units. Since chicken fillet was suspended in peptone saline solution (1:9), this suspension might contain less nutritional compounds than the milk, resulting in less protection on the survival on surfaces. Residues of chicken fillet suspension improved the survival of *C. jejuni* on the surfaces as well. The rate of decreasing survival on surfaces was reduced from 5.0 to 4.4 log unit reduction after 1 h. The food residues, while slowly drying, probably formed a layer that might protect the cells on surfaces, resulting in a prolonged survival.

Our study has highlighted the fact that pathogens may remain viable on dry stainless steel surfaces and present a (re)-contamination hazard for considerable periods of time. The presence of residual foods on these surfaces may have an important role as it may improve the survival.

3.3. Cross-contamination via sponges and stainless steel surfaces

The risk of cross-contamination during regular domestic cleaning is important since kitchen sponges were found to be potential vehicles of pathogens in domestic kitchens (Hilton and Austin, 2000) and pathogens were able to survive in kitchen sponges for at least weeks (Kusumaningrum et al., 2002). In this study the transfer rates of pathogens from artificially contaminated sponges to stainless steel surfaces were investigated (Table 2). The sponges that were contaminated with 10 ml of pathogen suspension, resulting in moisture contents of 50–65% (w/w), were used to wipe the surfaces and the transmission was determined directly after this artificial contamination. The transfer rates (21–43%) were not dependent on the test microorganisms ($p = 0.07$) or on the contamination levels ($p = 0.30$). After the transmission process, some bacteria remained on the sponges (10^5–10^6 CFU/sponge, data not shown), which are also indicated by the transfer rates that were less than 50%. These residual bacteria could still be harboured and transferred during subsequent use of the sponge.

Table 2
Transfer of pathogens from sponges[a] to stainless steel surfaces immediately after contamination

Organism	*n*	Contamination (log CFU/10 ml)	Count numbers		Transfer rate
			Sponges (log CFU/sponge)	Surfaces[b] (log CFU/4000 cm^2)	Sponges to surfaces (%)
Staph. aureus	3	High (8.8 ± 0.2)	9.0 ± 0.2	8.6 ± 0.2	38 ± 12
	6	Moderate (6.7 ± 0.1)	6.8 ± 0.1	6.4 ± 0.2	41 ± 17
S. enteritidis	3	High (9.3 ± 0.1)	9.4 ± 0.2	8.8 ± 0.2	29 ± 23
	6	Moderate (7.3 ± 0.1)	7.3 ± 0.0	6.6 ± 0.2	21 ± 8
C. jejuni	3	High (9.4 ± 0.1)	9.4 ± 0.1	9.0 ± 0.0	43 ± 10
	6	Moderate (8.5 ± 0.1)	8.4 ± 0.1	7.8 ± 0.1	28 ± 13

n indicates the number of experiments, with two parallel sampling for each experiment.
± indicates the standard deviation.
[a] The sponges were artificially contaminated with 10 ml of appropriate pathogen suspension.
[b] Sampled using a single contact plate.

Table 3
Transfer of pathogens from stainless steel surfaces[a] to cucumber ($n=6$) with or without pressure of 500 g/slice

Organism	Moment of sampling	Count numbers			Transfer rate	
		Surface[b] (log CFU/cm^2)	Cucumber		Surface to cucumber	
			Pressure (log CFU/cm^2)	No pressure (log CFU/cm^2)	Pressure (%)	No pressure (%)
Staph. aureus	Direct after contamination	2.8 ± 0.2	2.9 ± 0.1	2.8 ± 0.1	117 ± 48	95 ± 30
	15 min after contamination	2.9 ± 0.1	2.8 ± 0.3	2.7 ± 0.2	100 ± 59	74 ± 41
S. enteritidis	Direct after contamination	3.0 ± 0.2	3.0 ± 0.2	2.8 ± 0.2	105 ± 26	65 ± 21
	15 min after contamination	3.1 ± 0.3	3.0 ± 0.3	2.8 ± 0.3	90 ± 27	50 ± 18
C. jejuni	Direct after contamination	4.2 ± 0.2	4.4 ± 0.1	4.4 ± 0.1	185 ± 75	177 ± 72
	15 min after contamination	3.8 ± 0.5	3.7 ± 0.8	3.9 ± 0.5	134 ± 89	153 ± 99

n indicates the number of experiments, with two parallel sampling for each experiment.
± indicates the standard deviation.
[a] The surfaces were contaminated with artificially contaminated sponges with moderate contamination level (log CFU/sponge); *Staph. aureus* (6.8 ± 0.1), *S. enteritidis* (7.3 ± 0.0) and *C. jejuni* (8.4 ± 0.1).
[b] Sampled using a single contact plate.

The transfer rates of pathogens from stainless steel surfaces to cucumber slices and to roasted chicken fillet slices are shown in Tables 3 and 4, respectively. The transfer rates were based on the numbers of microorganisms that were recovered from surfaces using a single contact plate, which represents 50–60% of total recovery using five consecutive plates. This resulted, for some cases, in transfer rates that were more than 100% when bacteria were found more on food slices than on contact plates. When the transfer rates were determined based on the contamination numbers that were applied on the surfaces, the values were less than 100% (data not shown).

According to the statistical analysis, the moment of sampling (immediately or 15 min after contamination) did not affect the transfer rate of pathogens to cucumber ($p=0.26$ with pressure, $p=0.46$ without pressure), and to roasted chicken fillet when no pressure was applied ($p=0.84$). The transfer rate to roasted chicken fillet was dependent on the moment of sampling when pressure was applied ($p=0.02$). Furthermore, the type of microorganisms did not influence the transfer rates to roasted chicken fillet slices (with pressure $p=0.77$, without pressure $p=0.52$) and to cucumber slices when pressure was applied ($p=0.06$). When a cucumber slice was placed on a

Table 4
Transfer of pathogens from stainless steel surfaces[a] to roasted chicken fillet ($n=3$) with or without pressure of 500 g/slice

Organism	Moment of sampling	Count numbers			Transfer rate	
		Surface[b] (log CFU/cm^2)	Roasted chicken fillet		Surface to roasted chicken fillet	
			Pressure (log CFU/cm^2)	No pressure (log CFU/cm^2)	Pressure (%)	No pressure (%)
Staph. aureus	Direct after contamination	2.9 ± 0.2	2.8 ± 0.1	2.7 ± 0.1	76 ± 36	62 ± 28
	15 min after contamination	2.9 ± 0.2	2.8 ± 0.0	2.7 ± 0.0	74 ± 17	56 ± 20
S. enteritidis	Direct after contamination	3.1 ± 0.3	3.1 ± 0.2	2.8 ± 0.1	94 ± 42	49 ± 21
	15 min after contamination	3.0 ± 0.0	2.8 ± 0.4	2.9 ± 0.0	55 ± 21	32 ± 9
C. jejuni	Direct after contamination	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.1	101 ± 42	66 ± 26
	15 min after contamination	3.7 ± 0.4	3.4 ± 0.2	3.5 ± 0.4	24 ± 16	70 ± 83

n indicates the number of experiments, with two parallel sampling for each experiment.
± indicates the standard deviation.
[a] The surfaces were contaminated with artificially contaminated sponges with moderate contamination level (log CFU/sponge); *Staph. aureus* (6.8 ± 0.1), *S. enteritidis* (7.3 ± 0.0) and *C. jejuni* (8.4 ± 0.1).
[b] Sampled using a single contact plate.

contaminated surface without pressure, the transfer rate was dependent on the type of microorganism ($p = 0.00$). However, from practical point of view, cross-contamination clearly took place from surfaces to food slices, with or without pressure. Overall, the transfer rates were varied from 50% to more than 100% for cross-contamination to cucumber slices, and from 25% to 100% for transmission to roasted chicken fillet slices. Roasted chicken fillet slices contained less moisture than cucumber and consisted of some fat. The moisture contents of cucumber surfaces might positively affect the ease of retrieval of bacteria from surfaces.

The level of pressure used in our study, i.e. 500 g per slice (approximately 20 g/cm^2) was comparable to the pressure applied during sampling with the contact plates. Furthermore, the application of pressure in this experiment was to simulate the regular practice during food preparation in the kitchen. This pressure, however, was lower than that applied by Sattar et al. (2001) who used a pressure of 0.2 kg/cm^2 with a contact time of 10 s to study the bacterial transfer from fabrics to hands and other fabrics. It was found that bacterial transfer from moist donor fabrics using recipients with moisture was always higher than that to and from dry ones (Sattar et al., 2001).

Chen et al. (2001) found that bacterial transfer rates varied by more than five orders of magnitude (0.0005% from hand to spigot, to 100% from chicken to hand) depending on the nature of the surfaces involved in the cross-contamination. A transfer rate of 100% was identified as the maximum value, although in some cases it was found that the recipients demonstrated higher numbers of microorganisms than the contributor of contamination. The least variability was observed for cutting board-to-lettuce cross-contamination, which was likely due to the relatively homogeneous surface of the cutting board. Our study resulted in less variability of the transfer rates due to the semi-controlled experimental conditions.

Cogan et al. (2002) indicated that after meal preparation with *Salmonella*-contaminated chickens, *Salmonella* counts of more than 10^3 CFU/5 cm^2 were found on 5% of the chopping boards. This level is comparable with the moderate contamination applied in our survival experiments. If it is assumed that the same numbers are present on stainless steel work surfaces, based on our results, the surviving numbers would be reduced to 10 CFU/5 cm^2 after 1 h. If foods are prepared on these surfaces, with a transfer rate of 30% and 50% to roasted chicken fillet and cucumber slices (without pressure), respectively, the numbers of *Salmonella* would be 15 and 25 CFU, respectively, on 25-cm^2 food slices. These numbers could represent a potentially infective dose, particularly when growth is taking place, since current estimates suggest that the infectious dose for *Salmonella* may be up to 10^6 but could be as low as 10–100 cells (Cogan et al., 2002).

The risk of food-borne infection associated with cross-contamination depends on two factors: the level of contamination on the surfaces and the probability of its transfer to the foods being consumed (Bloomfield and Scott, 1997). Our study has shown that *S. enteritidis*, *Staph. aureus* and *C. jejuni* were still viable on dry stainless steel surfaces for hours (*C. jejuni*) or days after contamination (*S. enteritidis* and *Staph. aureus*), dependent on their initial numbers. These pathogens were readily transferred from kitchen sponges to stainless steel surfaces and from these surfaces to the foods. Systematic studies on the risks of pathogen transfer associated with surface cleaning using contaminated sponges provide quantitative data from which risks assessment in domestic setting could lead to reduce, prevent or eliminate cross-contamination in the kitchen.

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