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Research article

Campylobacter colonisation of slaughterhouse surfaces may be affected by ultra-thin silica coating

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Abstract: Campylobacteriosis is the most reported gastrointestinal zoonotic disease worldwide and is caused by the consumption of inadequately heated and contaminated food, especially poultry meat. This may result from cross-contamination events during poultry slaughtering and cutting processes. Carcass contact surfaces in slaughterhouses, such as plucking fingers of rubber or stainless-steel surfaces, are high-risk points for contamination, with intestinal contents likely containing *Campylobacter* bacteria that may result in the cross-contamination of subsequent carcasses. Modification of these food contact surfaces by coating can be beneficial in combating bacterial contamination, as already applied in the packaging materials of the food industry. The aim of this study was to compare the attachment, growth and detachment of *Campylobacter jejuni* on uncoated and nanoscale silicon dioxide coated stainless steel and plucking fingers during laboratory experiments. The coating partly resulted in significantly reduced attachment and an improved detachment of the target organism on stainless steel. In contrast, there was no significant decrease in *Campylobacter* adherence to the coated plucking fingers as compared to the uncoated ones. However, a significantly higher reduction of recultivable bacteria on the coated plucking fingers was observed during a five-hour period. In future studies, specific coating parameters should be investigated to further support development, and thus a better adaptation of the coating to the environmental conditions.

Keywords: coated materials; cross-contamination; food contact surfaces; nanomaterials; poultry

1. Introduction

Campylobacteriosis is currently the most common bacterial cause of a notifiable diarrheal disease worldwide, with over 400–500 million infection cases each year [1,2]. The species most frequently associated with human pathogenic infection is *Campylobacter (C.) jejuni*. One of the main causes of human infection is the consumption of insufficiently heated contaminated poultry meat [3]. Within poultry, broiler meat represents the most relevant source of contamination due to the comparatively higher consumption quantity [4]. An entire broiler flock can easily become colonised with *C. jejuni* during its lifespan at the farm level [5]. When *Campylobacter* colonised birds are processed, cross-contamination of chicken meat can occur within a slaughter batch or even between different batches [6,7]. During slaughter, defeathering and evisceration are the most high-risk points for cross-contamination with *Campylobacter*, as an increased exchange of organic material between carcasses may occur [8,9]. The pressure on carcasses during defeathering can cause the intestinal content to leak, and thus be transferred by the plucking fingers to the subsequent broiler carcasses [10]. During evisceration, any damage to the gastrointestinal tract may result in leakage of potentially *Campylobacter*-positive faecal material, as well as contamination of the slaughter line equipment and subsequent carcasses [11]. However, even cleaning and disinfecting the slaughter line after slaughtering *Campylobacter*-positive flocks does not guarantee the complete removal of pathogens from abiotic surfaces [12].

Various risk assessments have shown that even a moderate reduction in *C. jejuni* concentration on carcasses (>1 $\log_{10}$ per carcass) can lead to a significant decrease in the risk of infection in humans, thereby resulting in a 50% to 90% decline in campylobacteriosis cases [13,14]. Thus, a reduction of the *Campylobacter* prevalence at any stage of the chicken meat production is crucial. The most efficient strategy to achieve contamination reduction on chicken meat is the prevention of cross-contamination between batches of birds at the slaughterhouse [15], where contact surfaces, including plucking fingers and stainless-steel equipment, are considered high-risk points [16].

One way to reduce cross-contaminations could be the modification of the surface structure. From nature, there are examples of surfaces that exhibit a certain structure like pillars in a nanoscale range. Such surfaces' structures may lead to a reduced interaction with bacteria. Kelleher et al. [17] were able to demonstrate that the surface structure of cicada wings, which consists of nanoscale hexagonally-packed, uniform pillars, exhibited a bactericidal effect against gram-negative bacteria such as *Pseudomonas fluorescens*. Several other studies have shown that various types of engineered nanostructured surface topographies, which may be constructed by different methods such as electron beam lithography and nanoimprint lithography [18], can also influence bacterial cells such as *Listeria innocua* and *Staphylococcus epidermidis* and either reduce or even prevent their attachment or the formation of biofilms [18–20]. Besides the construction of a material that originally exhibits a nanostructured surface, it is also possible to use coating to modify the surface structure of an already existing material. In the medical sector, the application of such nanotechnology-based coating to provide a nanoscale layer on top of the original surface is a well-established practice [21]. First, nanoscale coating applications are already used for food packaging materials [22,23]. In order to achieve the nanostructuring of commonly used and existing surfaces, it seems most sensible to modify them with the aid of a coating; in this way, a direct adaptation to the respective conditions can take place. Various materials such as $SiO_xC_yH_z$ [24] or metal derivates such as chromium nitride or titanium nitride [25] have already been investigated with regard to their suitability as coating materials

for stainless steel. Silicon dioxide is another material that is becoming increasingly important in this context, especially in view of its low-cost production and good biocompatibility [26]. Recent studies have demonstrated some efficacy of silicon-dioxide nanoparticles against certain types of bacteria such as *Pseudomonas* [27,28].

Therefore, the aim of the current study was to investigate the influence of a nanoscale silicon dioxide coating on typical poultry slaughterhouse surfaces, namely stainless steel and plucking fingers, for the first time on *C. jejuni*. The focus of the experiments was on the attachment, bacterial growth and detachment behaviour of this foodborne pathogen.

2. Materials and methods

2.1. Campylobacter cultivation and suspensions

The *C. jejuni* isolate BFR-CA-19285 originating from chicken meat samples was provided by the German Federal Institute for Risk Assessment (BfR, Berlin, Germany) using a culture swab and was cryopreserved at the Institute of Food Hygiene Leipzig at −80 °C (Cryobank, Mast Group Ltd., Germany). A single bead was spread on a blood agar plate (Columbia Agar with Sheep Blood Plus, Oxoid GmbH, Germany) for reactivation and microaerobically incubated (85% N_2, 10% CO_2 and 5% O_2) for at least 48 h at 37 °C using the TRILAB-system (TRILAB, SN: 0139.0015, Jenny Science AG, Switzerland) with corresponding anaerobic pots. This reactivated culture was stored under microaerobic conditions and used for experimental setups for up to one week.

Colony material from the reactivated culture was spread on a new blood agar plate and microaerobically incubated for 32 h at 42 °C to obtain a fresh working culture.

For each experimental approach, about half of the bacterial material from this fresh working culture was stirred into 5 mL of Brain Heart Infusion Broth (BHI, TN1216, sifin diagnostics GmbH, Germany) and incubated without agitation microaerobically for 16 h at 42 °C with stainless-steel discs. For every experimental approach, the bacterial concentrations of these solutions were separately determined by a surface plating technique on modified Cefaperazone Charcoal Desoxycholate Agar plates (mCCDA, Oxoid GmbH, Germany) and were generally at about 8.0×10^8 cfu/mL. Then, those solutions were either used directly or diluted with BHI to obtain the bacterial density required for the respective experiment.

For attachment and detachment experiments with plucking fingers, colonies from a blood plate were stirred into 15 mL of BHI and incubated without agitation microaerobically for 16 h at 42 °C to achieve about 5.0×10^7 cfu/mL. It was either used at this concentration or diluted with BHI when appropriate for the respective experiment. The bacterial culture for growth experiments on plucking fingers was analogously prepared to the stainless-steel experiments.

To mimic slaughterhouse conditions of protein contamination, filter-sterilised bovine serum albumin solution (BSA; A6588; VWR International GmbH Germany) was added as interfering substance to the bacterial suspension of each experimental approach to a final concentration of 0.3% (based on DIN EN 13697:2019-10).

2.2. Surfaces

Discs (20mm diameter and one mm height) of stainless-steel type 304, grade 2B polished (X5CrNi1810-2B; GK Formblech GmbH, Germany), that is commonly used in slaughter line equipment, were utilised in this study.

Commercial thermoplastic rubber plucking fingers (20 mm bore diameter; hard version; total length: 97.5 mm; Westfalia Werkzeug company GmbH & Co. KG, Germany) were used for the respective experiments.

Stainless-steel discs and plucking fingers were pretreated by cleaning and sterilisation. First, they were soaked for 60 min in a 5% Decon solution (Decon™ Decon90, Fisher Scientific GmbH, Germany), rinsed with distilled water and then degreased for 15 min in a 95% 2-propanol solution (Carl Roth GmbH, Germany). Subsequently, surfaces were rinsed again with distilled water and dried in a biosafety cabinet by evaporation. To generate ready-to-use surfaces, stainless-steel discs were subjected to hot air sterilisation at 180 °C for 30 minutes and plucking fingers were subjected to autoclaving at 121 °C for 15 minutes. A nanoscale layer of silicon dioxide was applied by Nanopool GmbH (Germany) by utilising its commercial product, Liquid Glass Metall, to generate the coated surfaces used in this study. Unused stainless-steel discs and plucking fingers were used for each experiment.

2.3. Experimentation design

Attachment, growth and detachment experiments were repeated three times on three coated and three uncoated discs or plucking fingers. Experiments were carried out for *Salmonella* Enteritidis and *Escherichia coli* for stainless-steel discs as described in detail elsewhere [29].

Briefly, three different types of attachments were simulated for stainless steel at an ambient temperature (25 °C ± 5 °C). First, 50 µL of a bacterial suspension (approx. 3.5×10^7 cfu/disc) was pipetted onto the discs to simulate the dripping of liquid. Second, to simulate the pressure of carcasses against the slaughterhouse equipment, a silicone plug was dipped into 50 µL of a bacterial suspension (approx. 8.0×10^8 cfu/mL) and was pressed onto the discs. Third, to imitate the gliding of broiler carcasses along surfaces such as guide rods, 20 µL of the bacterial suspension (approx. 8.0×10^8 cfu/mL) was smeared onto the discs using a silicone plug.

Two types of attachments were simulated for plucking fingers at an ambient temperature (25 °C ± 5 °C) using bacterial suspensions with approx. 5.0×10^7 cfu/mL. In the first case, each rubber finger was immersed in 20 mL of the bacterial suspension located in a centrifuge tube (TPP Techno Plastics Products AG, Switzerland) and briefly vortexed to remove air bubbles to simulate liquid spilling. After an exposure time of one minute, each finger was tapped three times for every third of its surface on a glass dish rim to remove any excess fluid. For the second treatment, the bacterial suspension was brushed onto the plucking fingers three times lengthwise for each third of their surfaces using silicone brushes (Vivess Grillpinsel, Rewe, Germany) to simulate the contact with carcasses and feathers during defeathering.

For both materials, the *C. jejuni* numbers were determined every hour for five hours at 30 °C. Each stainless-steel disc was inoculated with a bacterial suspension by pipetting (approx. 4.0×10^5 cfu/disc). Afterwards, three uncoated and three coated discs were placed in the incubator at each hourly examination timepoint for a total of five hours. Additionally, to simulate slaughterhouse conditions, a

small Petri dish containing distilled water was placed inside to achieve a relative humidity of about 70%. Fingers were immersed in a 20 mL bacterial suspension (approx. 5.0×10^6 cfu/mL) for one minute and then tapped three times for every third of their surface on a glass dish rim to remove excess fluid and were transferred to a centrifuge tube. The rim of the fingers sealed the tube to achieve a relative humidity of about 70%. In total, a rack containing three tubes with uncoated and three tubes with coated plucking fingers for every of the five hourly examination timepoints was placed in the incubator.

The detachment of *Campylobacter* cells from both types of surfaces was examined by utilising hot water and a mildly alkaline protein and fat dissolving detergent (0.5%; Eiweiß-Fettlöser flüssig, Ernst GmbH & Co.KG, Germany). Stainless-steel discs were contaminated with approx. 3.0×10^7 cfu/disc; after an incubation time of five minutes, the discs were either rinsed with 1 mL distilled water (78–80 °C) or covered with foam produced from the detergent, as described by Schumann-Muck et al. [29]. After five minutes of exposure, the foam was rinsed with distilled water (78–80 °C) and residual bacteria were recovered from discs. Plucking fingers were treated by immersing into 20 mL of a bacterial suspension of approx. 5.0×10^7 cfu/mL for one minute and then tapped three times per third of their surface on a glass dish rim to remove any excess fluid. Afterwards, they were either dipped into distilled water (78–80 °C) and subsequently tapped on a glass dish rim one time per third of their surface to remove any excess fluid or immersed in a foam produced from 7.5 mL of detergent by manual vertical shaking for 10 seconds. After five minutes of exposure in foam, the fingers were rinsed with distilled water (78–80 °C) and any excess fluid was removed by tapping, as previously described.

2.4. Bacterial recovery and evaluation

Campylobacter bacteria were recovered from the discs according to DIN EN 13697:2019-10 by placing the discs with the contaminated side down in a 25 mL beaker filled with 2.1 g of three-millimetre sterile glass beads and 10 mL of a sodium chloride peptone solution. Then, the beaker was shaken for five minutes at 150 rpm (shaker RS-OS 5, Phoenix Instrument GmbH, Germany).

The method of Arnold [30] was applied for the bacterial recovery from the plucking fingers. The finger was cut above the third rib with sterile scissors, placed in a centrifuge tube filled with 10 mL of a sodium chloride peptone solution and vortexed for 15 seconds at 2,500 rpm.

Decimal dilutions of the sodium chloride peptone solution (0.85% NaCl, 0.1% peptone) were spread on mCCDA plates (Oxoid GmbH, Germany) and microaerobically incubated for 24 h at 42 °C.

2.5. Statistical analysis

The bacterial counts were $\log_{10}$ transformed for the statistical analyses. Regarding the bacterial attachment, the differences of originally inoculated and recovered bacteria of three discs or plucking fingers per triplicate were averaged and the mean log values of three repetitions were calculated. Standard deviations were calculated from the mean log values of the three repetitions. The results were expressed as attachment reduction, and the calculated value represented the number of *Campylobacter* that were not able to attach to the surface. The bacterial growth was determined as the mean log value of the triplicate recovered bacterial count of a given time point minus the recovered bacterial count at time point zero. These differences of three repetitions were averaged to the overall mean log values and standard deviations were also calculated from these averaged means. The detachment of *Campylobacter* was calculated as the mean log of three repetitions of the differences from the

originally inoculated and recovered bacterial count of three discs or plucking fingers per triplicate. Differences between the coated and uncoated discs and plucking fingers per treatment (attachment/detachment) and per time point (growth) were statistically analysed by an unpaired t test at an alpha level of significance of 0.05. All statistical analyses were executed by Prism 9 (Graph Pad, USA).

3. Results

3.1. Bacterial attachment

When the bacterial suspension was applied to stainless steel, an average of 0.63$\log_{10}$ cfu were unable to adhere to the uncoated discs; an average of 0.76 $\log_{10}$ cfu were unable to adhere for the coated discs. This difference was not statistically significant ($p = 0.09$) (Figure 1). However, when stainless-steel surfaces were contaminated by pressing, there was a significant difference between the treatments. More bacteria were unable to attach to the coated discs (0.57 $\log_{10}$ cfu) as compared with the uncoated discs (0.28 $\log_{10}$ cfu; $p = 0.0005$) (Figure 1). When the bacterial suspension was applied by smearing, there was no significant difference ($p = 0.26$) between the average inability to adhere on coated and uncoated discs (0.40 $\log_{10}$ cfu or 0.33 $\log_{10}$ cfu, respectively; Figure 1).

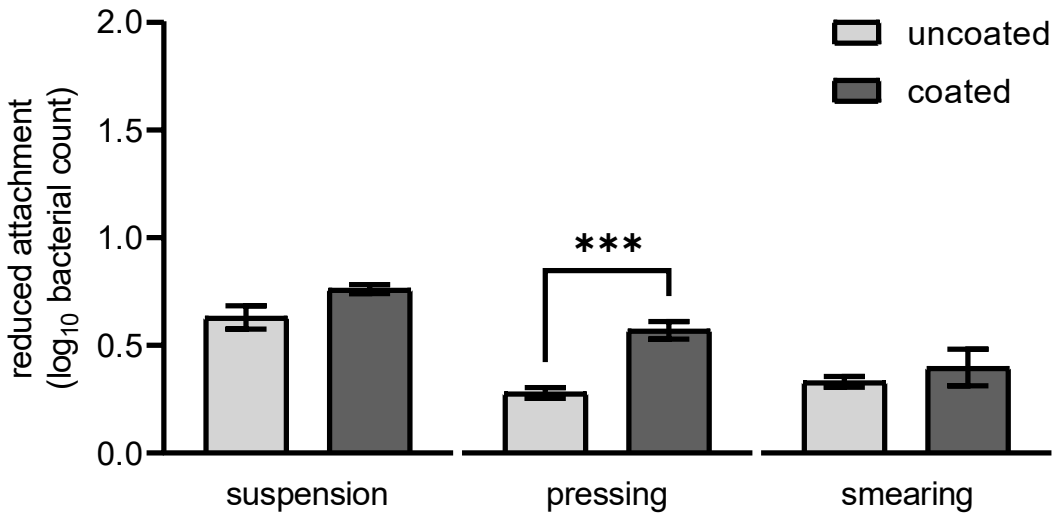

Figure 1. Reduction of *C. jejuni* attachment after application by suspension, pressing or smearing on uncoated (light grey) and coated (dark grey) stainless-steel discs. The mean values ± standard deviation of three repetitions are shown.

With regard to the plucking fingers, 1.5 $\log_{10}$ cfu did not adhere to the uncoated fingers as compared with 1.28 $\log_{10}$ cfu that did not adhere to the coated fingers when they were immersed in the bacterial material. The difference was not significant ($p = 0.20$; Figure 2). When the bacterial suspension was applied with a brush, *Campylobacter* attachment was similar for coated (1.16 $\log_{10}$ cfu) and uncoated (1.11 $\log_{10}$ cfu) surfaces ($p = 0.79$; Figure 2).

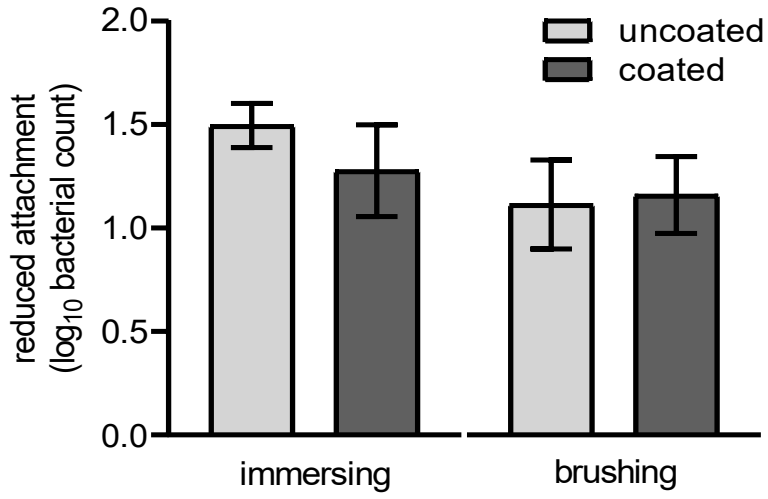

Figure 2. Reduction of *C. jejuni* attachment after application by immersing or by brushing on uncoated (light grey) and coated (dark grey) plucking fingers. The mean values ± standard deviation of three repetitions are shown.

3.2. Bacterial growth

During the five-hour monitoring on both tested surfaces, a reduction of the *Campylobacter* population was observed. The respective hourly counts are presented in Table 1. There was no statistically significant difference in the bacterial count decline between coated and uncoated stainless-steel discs at any time point (Figure 3a). Contrariwise, a statistically significant and higher reduction was detected on the coated fingers in comparison to the uncoated ones from the second hour and onwards (Figure 3b).

Table 1. *C. jejuni* growth (log_{10} cfu; difference from hour zero; approx. 4.0 x 10^5 cfu applied per disc; original inoculum on plucking fingers: approx. 5.0 x 10^6 cfu/mL) on uncoated and silicon-coated stainless-steel discs and plucking fingers over a period of five hours. The mean values (±standard deviation) of three experiments are shown.

Timepoint [h]	Stainless-steel discs		plucking fingers	
	Uncoated	Coated	Uncoated	Coated
0	0	0	0	0
1	−0.04 (±0.05)	−0.03 (±0.04)	−0.19 (±0.05)	−0.23 (±0.02)
2	−0.13 (±0.06)	−0.09 (±0.08)	−1.00 (±0.05)	−1.08 (±0.04)
3	−0.23 (±0.15)	−0.20 (±0.18)	−1.26 (±0.02)	−1.38 (±0.06)
4	−0.49 (±0.11)	−0.44 (±0.14)	−1.46 (±0.02)	−1.85 (±0.02)
5	−2.54 (±0.66)	−1.91 (±0.46)	−1.84 (±0.06)	−3.09 (±0.16)

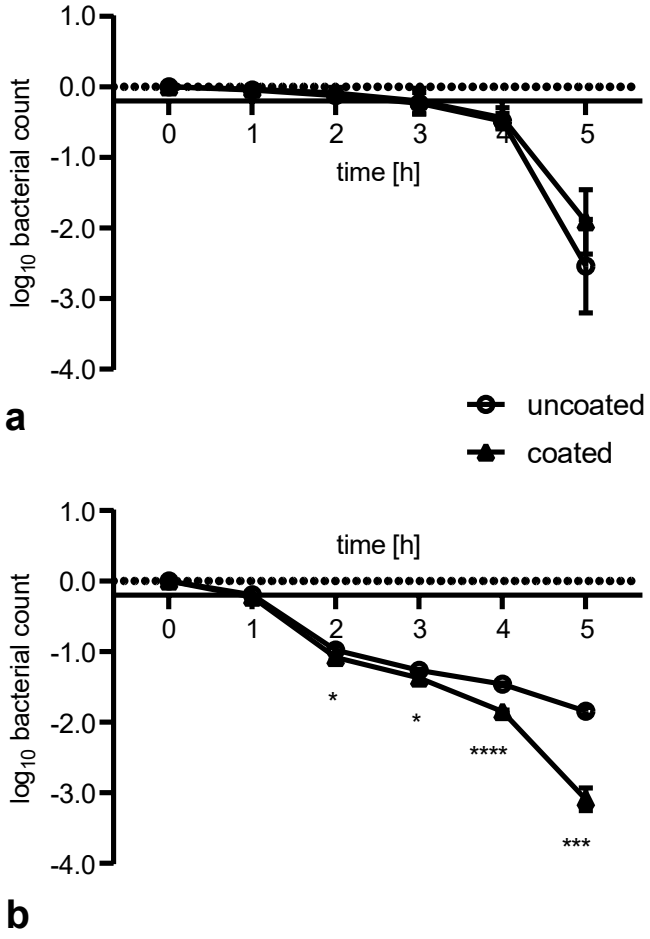

Figure 3. *C. jejuni growth* on uncoated (**ɵ**) and coated (-▲-) stainless-steel discs (a) or plucking fingers (b). The mean values ± standard deviation of three repetitions are shown.

3.3. Bacterial detachment during simulated cleaning

By rinsing stainless-steel discs with hot water, 1.96 $\log_{10}$ cfu were detached from the uncoated discs, while 1.72 $\log_{10}$ cfu were detached from the coated ones ($p = 0.16$; Figure 4). In contrast, a similarly slight, but inverse, significant difference was found in regard to coating when cleaned with foam, with 2.64 $\log_{10}$ cfu being detached from coated discs and only 2.51 $\log_{10}$ cfu from the uncoated ones ($p = 0.02$; Figure 4).

For plucking fingers, *Campylobacter* cells detached slightly better from uncoated surfaces than from coated surfaces (Figure 5). When hot water was used for cleaning, this slight difference was statistically significant (2.54 $\log_{10}$ cfu vs. 2.47 $\log_{10}$ cfu; $p = 0.04$); however, it was not significant when the surfaces were cleaned with detergent foam (2.19 $\log_{10}$ cfu vs. 2.13 $\log_{10}$ cfu; $p = 0.65$).

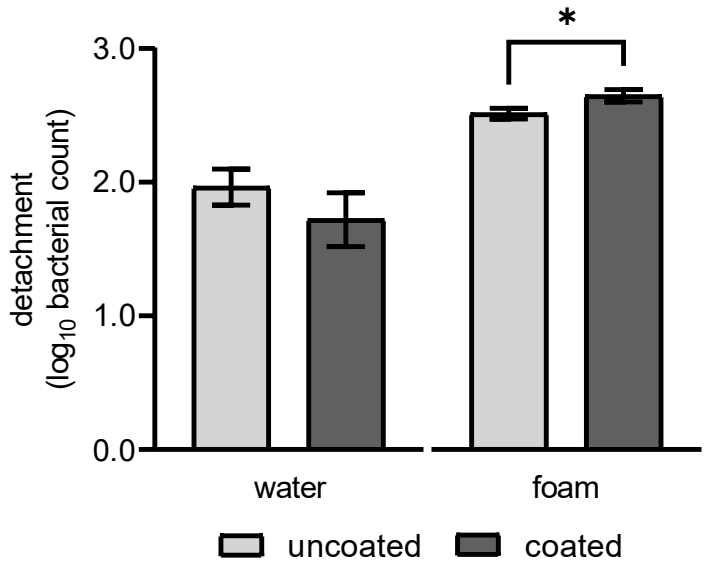

Figure 4. Detachment of *C. jejuni* after cleaning with water or foam from uncoated (light grey) and coated (dark grey) stainless-steel discs. The mean values ± standard deviation of three repetitions are shown.

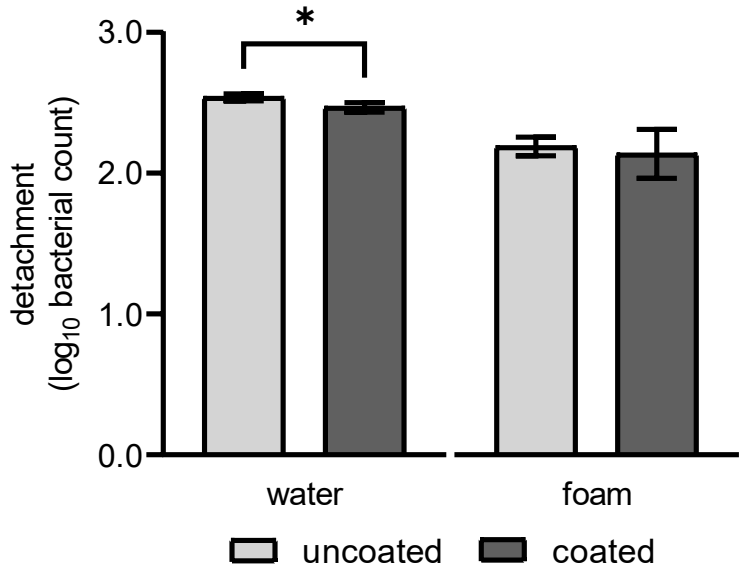

Figure 5. Detachment of *C. jejuni* after cleaning with water or foam from uncoated (light grey) and coated (dark grey) plucking fingers. The mean values ± standard deviation of three repetitions are shown.

4. Discussion

4.1. Bacterial attachment

Although stainless steel is widely used in the food processing industry due to its good surface properties, such as a long-lasting corrosion resistance, stability, and its relatively low initial cost [31], this material provides a good substrate for bacterial attachment. Flint et al. [32] were able to demonstrate that *Streptococcus thermophilus* highly adhered to stainless steel when compared to a glass surface. One way to reduce bacterial attachment to stainless steel can be to modify its surface (e.g., with a nanoscale coating). Verma et al. [33] showed that coating steel with silica–titania core–shell nanoparticles lead to an OD reduction of *E. coli* (OD_{600} 0.9 vs. 0.19 on uncoated and coated surfaces, respectively). In our study, we showed that on a nanoscale coated and, thus, modified stainless-steel surface, there is, in parts, a statistically significant attachment reduction of *C. jejuni* cell numbers when compared to unmodified stainless steel. A similarly positive result with regard to the nanoscale modification of surfaces was also obtained by Feng et al. [25], who were able to demonstrate that an alumina surface with nanoscale pores of 15 nm resulted in a 40% decrease in the attachment of *Listeria innocua* and 25 times less adhesion of non-pathogenic *E. coli* compared to a nanosmooth topography. The results indicate that some nanoscale modification of surfaces can cause a difference in bacterial adherence, which can be explained in several ways. On the one hand, there may be a change in surface free energy and free charge due to nanostructuring, which affects biofilm formation and is essential for the successful attachment of most bacteria [34]. On the other hand, the coating can lead to a change in surface wettability. As demonstrated elsewhere [29], the application of an identical silicon dioxide coating on stainless steel increased the water contact angle from 56.7° to 115.4°, which signifies a more hydrophobic character and a decreased wettability of the coated surfaces. The hydrophobic surface characteristics may inhibit bacteria from adhering to surfaces. Nguyen et al. [35] reported adhesion prevention of 13 *C. jejuni* strains on polyurethane compared to less hydrophobic glass and stainless-steel surfaces. In contrast, other studies have found that the increased hydrophobicity of a surface enhances the adhesion of *Salmonella* [36] and a specific *C. jejuni* strain [37]. In these studies, it is assumed that the increased attachment of the bacteria to the surfaces results from interactions between the hydrophobic bacterial surface and the hydrophobic contact surface.

In the study presented here, plucking fingers showed a tendency towards a decreased attachment of *C. jejuni* on the uncoated fingers when immersed. This is consistent with the results of Arnold and Silvers [16], where the rubber used for the fingers had a lower bacterial adhesion capacity and biofilm formation than stainless steel, which resulted in decreased bacterial adhesion.

It should be noted that, in the present study, the contact time of surfaces with the bacterial suspension is relatively short at five minutes for stainless steel and one minute for the plucking fingers; however, these values were chosen to be indicative of commercial slaughterhouse operations.

4.2. Bacterial growth

In the current study, a reduction of *C. jejuni* numbers was recorded on either stainless steel or plucking fingers during the observed period of five hours. As confirmed by others, the target organism is mostly incapable of reproducing outside of the preferred environment (i.e., the intestinal tract of birds) due to the lack of microaerobic conditions and the correct growth temperature [38,39].

Similar reduction results were recorded by Kusumaningrum [40], where the used *C. jejuni* strain, even in high concentrations of 10^7 cfu/100 cm^2 at room temperature on stainless steel, showed a drop below the detection limit after an experimental period of four hours. This indicates that certain *Campylobacter* strains attached to surfaces show a strong susceptibility to air drying at temperatures above 20 °C, as well as to a direct contact with an aerobic environment [41,42]. Although we prevented the applied bacterial suspension from drying by establishing a high humidity in the experimental setup, the *Campylobacter* numbers also quickly decreased.

Although it is known that different *Campylobacter* strains do display a diverse behavior in regard of certain characteristics and, therefore, some of the undermentioned aspects may not be generally valid, and an attempt of comparing with the existing literature is made. The observed reduction was similar for uncoated and silicon dioxide-coated stainless-steel discs. This is contradictory to what was reported by Zakarienė et al. [43] for another kind of coating. In their study, the development of bacterial counts of *C. jejuni* and *Listeria monocytogenes* on stainless steel coated with diamond-like carbon Ag nanocomposites was compared with uncoated one. They observed a statistically significantly lower concentration of *C. jejuni* on the coated stainless steel as compared with the uncoated one after 30 minutes of inoculation in the culture-based enumeration ($p \leq 0.05$). A similar result was obtained in another study for molybdenum oxide nanocomposite coating, in which the attachment of *C. jejuni* was also compared between uncoated and coated stainless steel after four and 24 hours. There, the logarithmic difference of 2.11 between the mean values of uncoated and coated stainless steel after 24 h showed that the coating has a great activity against *C. jejuni* and leads to a reduction of the latter [44].

In contrast to the findings for stainless steel, in the present study, the decrease in bacterial numbers of *C. jejuni* was significantly enhanced on coated plucking fingers from hour two onwards. A similar finding was reported by Nguyen et al. [45] for a different experimental set-up, as they reported that a nanoscale gold coating of polystyrene resulted in a 57% decrease in the detectability of *Pseudomonas aeruginosa* and a 20% decrease in the detectability of *Staphylococcus aureus* within 18 hours as compared to smooth surfaces.

4.3. Detachment

In the current study, the coating had no significant effect on the detachment of *C. jejuni* from stainless-steel surfaces through water. This contrasts with other data in which naturally nanostructured surfaces exhibited a water-repellent effect that resulted in the self-cleaning of surfaces [46]. In contrast, there was a statistically significant, albeit small, improvement in the detachment of the target organism from the coated surface when using detergent foam. This foam was produced from an alkaline cleaner with surfactants, which are particularly important for the removal and emulsification of fats. An important effect of alkaline cleaner is the ability to swell, and thus improve the detachment of proteins from contaminated surfaces [47]. This is an important aspect in the present study, as all experimental procedures involved the addition of BSA to simulate a high degree of protein contamination. In general, proteins are present on slaughter lines and can have an influence on the interaction between bacteria and surfaces. As noted by Singh et al. [48], the attachment of *E. coli* to nanostructured titanium surfaces with different topographies can be influenced by the addition of proteins. On the one hand, this can occur through the inhibition of bacterial adhesion by the protein layer, which results from the formation of protein clusters on the surfaces to be examined. On the other hand, the protein layer may affect the nanoscale surface due to the flattening that occurs, and thus leads to increased bacterial adhesion. This

can explain the low effect of the stainless-steel surface coating in this study during the hot water detachment experiments. The addition of the alkaline cleaner causes swelling of the adherent proteins, which breaks down the protein layer. This can lead to an increased removal of the bacteria attached to the protein layer. Additionally, it is possible that when rinsing with hot water after treatment with foam, a water-repellent effect can take place, which is caused by the hydrophobicity of the coated surface [46]. This can lead to the improved self-cleaning of the nanoscale surface, and thus to the improved removal of bacteria still attached to the surface. Although hot water may denature bacterial cells and affect protein integrity, which could result in an overall lower detection rate of *Campylobacter* cells from surfaced cleaned with hot water, it is unlikely that nanoscale coating would have an impact on the extent of such general effects. Therefore, it is also unlikely that the presented differences between coated and uncoated surfaces are biased by using hot water for simulated cleaning.

In this study, a slightly higher detachment of the target organisms from the uncoated plucking fingers was recorded when the fingers were treated with hot water, whereby the difference to the coated fingers even proved to be statistically significant. This minimal difference may be due to a decreased attachment as recorded in the tendency of this study. As discussed above, since Arnold and Silvers [16] found that the rubber surface of the plucking fingers itself inhibited the attachment of the bacterial suspension obtained by rinsing the chicken carcass, it is reasonable to conclude that this leads to the increased rinsing off the bacteria that were not able to attach themselves on the uncoated surface.

It is questionable whether the results of this study can be used in a generalized way for *Campylobacter*. First, the study results must be preliminary comprehended in view of the used strains, as only the chicken-derived strain *C. jejuni* BFR-CA-19285 was used in the current study, since its origin made it relevant to the study and it was found to be most cultivable under the current laboratory conditions. It is known that variations exist between different strains with regard to their survivability, cell surface properties and colonization ability [37,49,50]. Because of that, the present results are difficult to transfer to other strains and experiments should be conducted using a *Campylobacter* cocktail before final conclusions can be drawn. Second, only the development of culturable *C. jejuni* was investigated in the experiments. Studies have shown that *Campylobacter* can enter a viable but non-culturable (VBNC) state under stressful situations [51]. Although the *in vitro* invasion of eukaryotic cells has been shown to some extent, the ability of this stage to revert to the infectious state has not yet been definitively demonstrated [52–55], which is also a reason why only culturable *Campylobacter* were assessed in this study.

5. Conclusions

Modification of slaughterhouse surfaces using coatings may represent a new approach for reducing cross-contamination during the slaughter process, and thus for reducing the bacterial load of chicken meat. In the current study and for the first time, the effects of a nanoscale silicon dioxide coating on the behavior of *C. jejuni* were investigated in various tests by comparing coated and uncoated stainless-steel surfaces and plucking fingers.

On stainless steel, the coating appeared to have only a minimal effect in terms of the attachment and detachment of the *Campylobacter* strain studied. In contrast, it appeared to influence the reaction of the strain on plucking fingers in that the bacteria tended to adhere more strongly to the coated ones and were better detached from the uncoated ones.

This suggests that the type of coating needs to be adapted to the environment, particularly in regard to possible use on plucking fingers, in order to achieve a greater effect and a significant reduction in the bacterial load on the surfaces. Further studies on the interactions between the nanoscale surfaces and the bacteria with regard to specific parameters of the coating such as structure, surface charge and roughness would provide additional insights.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Victoria Blaeske. The first draft of the manuscript was written by Victoria Blaeske and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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