

Decontamination of knives used in a slaughterhouse by a commercial non-thermal UV-C treatment

Susanne Raschle, Claudio Zweifel, Katrin Zurfluh, Roger Stephan

Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland

Abstract

To assess the antimicrobial effect of a commercial UV-C system, knives inoculated with *Escherichia coli* and *Staphylococcus aureus* as well as naturally contaminated and collected from the wet and clean area of a slaughterhouse knives were examined. For inoculated knives, UV-C treatment for 30 s reduced mean *E. coli* counts by 5.1 log CFU cm⁻² and mean *S. aureus* counts by 4.5 log CFU cm⁻². The presence of blood lowered mean reductions to 3.4 log CFU cm⁻² for *E. coli* and to 2.5 log CFU cm⁻² for *S. aureus*. The presence of fat had a greater negative impact on the efficacy of the UV-C treatment resulting in mean reductions <1.8 log CFU cm⁻². For naturally contaminated knives from a slaughterhouse, total viable counts (TVC) before UV-C treatment varied considerably (wet area: 2.0-6.0 log CFU cm⁻², clean area: 1.0-3.0 log CFU cm⁻²). UV-C treatment for 30s reduced mean TVC by 0.8 log CFU cm⁻² (wet area) and 0.6 log CFU cm⁻² (clean area), but the effect varied greatly between individual knives. Thus, under commercial conditions, the antibacterial effect of UV-C for the decontamination of knives is affected by the presence of additional contaminations like blood or fat. The adequate cleaning of the knives prior to UV-C decontamination is therefore of central importance.

Introduction

Strict adherence to good practices of slaughter hygiene along with risk-based preventive measures (HACCP approach) is crucial to ensure food safety at slaughter (Nørrung & Buncic, 2008; Sofos, 2008). Because healthy food-producing animals can be carriers of bacterial pathogens causing human disease (EFSA/ECDC, 2017). Despite all advancements in slaughter technologies, complete prevention of microbial contamination of carcasses during slaughter

can hardly be warranted. To limit contamination by equipment and utensils like knives, an effective cleaning and disinfection regime is of great importance.

For knives used during slaughter, in-process cleaning and disinfection should be performed after each use. The most widespread procedure also mentioned in the European legislation (EU Regulation 853/2004) is hot-water decontamination at 82°C in a water bath. Its efficacy is affected by various framing conditions and limitations (Eustace *et al.*, 2008; Goulter *et al.*, 2008; Leps *et al.*, 2013; Taormina & Dorsa, 2007), which include but are not limited to: The presence of organic matter (which has to be removed prior cleaning); the hot-water exposure time (on which there is no consensus); the maintenance of the required hot-water temperature (leading to high energy consumption and steam formation); the affected sharpness and increased wear of the knives. Hence, there is interest in (non-thermal) alternatives for knife decontamination and the legal framework allows the use of alternatives with an equivalent effect to hot-water treatment (EU Regulation 853/2004). Various alternatives to hot-water treatment and combinations of hot water with chemicals have been evaluated for knife decontamination in the meat industry (Barbosa *et al.*, 2016; Brasil *et al.*, 2017; Eustace *et al.*, 2008; Leps *et al.*, 2013; Taormina & Dorsa, 2007). Ultraviolet (UV) light is commonly used in the food industry for the decontamination of surfaces (*e.g.* packaging material), water, or air (Bintsis *et al.*, 2000) and it could constitute a promising, non-thermal alternative for knife decontamination. The UV spectrum can be subdivided into a number of ranges: The long-wave UV-A (315-400 nm, "black-light"), the medium-wave UV-B (280-315 nm, responsible for human sunburn), and the short-wave UV-C (200-280 nm). The UV-C rays have an antimicrobial (germicidal) effect at a wavelength of 254 nm. However, studies investigating the effect of UV-C for the decontamination of knives used in the meat industry are lacking in the literature, in particular studies performed under routine operations during slaughter. Therefore, the aim of the present study was therefore to determine the bacterial reductions obtained by a commercial non-thermal UV-C system (i) on knives inoculated with *Escherichia (E.) coli* or *Staphylococcus (S.) aureus* (with and without additional contamination) and (ii) on naturally contaminated knives under commercial conditions in a slaughterhouse.

Correspondence: Roger Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland.
Tel.: +41.44.6358651 - Fax: +41.44.6358908
E-mail: roger.stephan@uzh.ch

Key words: UV-C decontamination; Knives; Inoculation; Natural contamination; Slaughterhouse.

Acknowledgments: The authors thank the staff of the slaughterhouse involved in this study for facilitating access to their operations and for the assistance with the collection of data.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Received for publication: 12 February 2019.
Revision received: 22 April 2019.
Accepted for publication: 29 April 2019.

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Licensee PAGEPress, Italy
Italian Journal of Food Safety 2019; 8:8107
doi:10.4081/ijfs.2019.8107

Materials and Methods

UV-C decontamination device

For UV-C decontamination of knives, the ME3-S device (Sterilsystems, Mautendorf, Austria) with dimensions of 215 mm (width) × 600 mm (height) × 160 mm (depth) and a weight of 8.9 kg was used (Figure 1). The device was equipped with an integrated water cleansing (using cold water) and two UV-C emitters (wavelength of 254 nm; power requirement of 2 × 13 W). The UV-C emitters (equipped with splinter shields) and the electronics were integrated in the housing and waterproof. The housing consisted of stainless steel and of plastic at the top forming the three blade insertion positions 1 to 3. After insertion, blades were automatically cleaned by the integrated cold-water jet and decontaminated by UV-C. The exposure time recommended by the manufacturer was 30 s.

Laboratory study with inoculated knives

For knife inoculation, *E. coli* ATCC

25922 and *S. aureus* ATCC 29213 strains were used. *E. coli* and *S. aureus* were selected as representatives of gram-negative and gram-positive bacteria, respectively. After growing of strains on plate count agar (Oxoid AG, Pratteln, Switzerland; 24 h at 37°C), several *E. coli* or *S. aureus* colonies were collected with a sterile cotton swab and used for knife inoculation. Examined knives (stainless-steel blade) comprised two types of knives widely used in the meat industry (Swibo No. 5.8411.25 and 5.8411.26, Victorinox, Ibach-Schwyz, Switzerland). To evaluate the effect of UV-C on inoculated *E. coli* or *S. aureus*, three experimental layouts were investigated: clean knives, knives contaminated with blood, and knives contaminated with fat. Clean knives were inoculated by rubbing the *E. coli* or *S. aureus* containing cotton swab in designated blade areas (2 x 5 cm² on each knife). After drying (about 5 min), knives were inserted in the ME3-S device (Sterilsystems) and treated for 30 s (using positions 1 to 3 of the device) or for 45 s (using position 1 of the device). For the contamination experiments, sheep blood (Oxoid AG) and pork fat bought at retail were used. After inserting in sheep blood or rubbing over pork fat, cotton swabs were rubbed over designated blade areas (2 x 5 cm² on each knife). Blade areas were then inoculated with *E. coli* or *S. aureus* and further treated (UV-C for 30 s) as described above.

Field study with naturally contaminated knives from a slaughterhouse

The UV-C ME3-S device (Sterilsystems) was used for knife decontamination in a Swiss slaughterhouse (slaughter capacity: >20 million kg per year). The UV-C device was thereby tested during the slaughter of cattle and sheep, both in the wet area (after removal of hooves) and in the clean area (after trimming) of the slaughterhouse. The examined, naturally contaminated knives (stainless-steel blades) were of various sizes and types. Knives were inserted in the UV-C device and treated for 30 s (using positions 1 to 3 of the device).

Collection of samples

Sampling was performed by swabbing: Sterile cotton swabs moistened with 0.85% saline solution were rubbed over designated blade areas before UV-C treatment (about 5 cm²) and after UV-C treatment (about 5 cm²). The number of replicates (before and after UV-C treatment) tested in the laboratory study for each *E. coli* and *S. aureus* was 100 for clean knives (50 for 30 s of exposure; 50 for 45 s of exposure), 16 for knives

contaminated with blood, and 15 for knives contaminated with fat. In the field study examining naturally contaminated knives, 50 replicates from the wet area and 31 replicates from the clean area of the slaughterhouse were analyzed. Microbiological examinations were carried out immediately (laboratory study) or within 16 h (field study) after sampling.

Microbiological examinations

Samples were homogenized for 30 s in 5 mL of 0.85% saline solution in a stomacher, of which 1 mL was used to prepare decimal dilution series (0.85% saline solution). Aliquots of 0.1 mL were then transferred by the spreading method to plate count agars (Oxoid). Agars were used to determine the counts of *E. coli* and *S. aureus* (24 h at 37°C; laboratory study) or total viable counts (TVC; 72 h at 30°C; field study). Counts were calculated as CFU cm⁻² and the detection limit was 10 CFU cm⁻².

Data analysis

Colony counts were expressed as log CFU cm⁻². A log value of zero was used for counts below the detection limit. For naturally contaminated knives, results were only considered when TVC before UV-C treatment were equal to or above the detection limit. Mean values and standard deviations before and after UV-C treatment and mean reductions obtained by UV-C treatment were then calculated. Statistical analysis was performed using JMP 13.0 (SAS Institute, Cary, NC, USA). The level of significance was set at $\alpha=0.05$ ($P<0.05$). With regard to UV-C decontamination of knives, t-tests were used to analyze differences (i) between reductions of *E. coli* and *S. aureus* (30 s or 45 s of exposure; blood or fat con-

tamination), (ii) between reductions after 30 s and 45 s of exposure (*E. coli* or *S. aureus*), (iii) between reductions on knives with and without additional contamination (blood or fat contamination, *E. coli* or *S. aureus*) or between reductions in the presence of blood and fat contamination (*E. coli* or *S. aureus*), and (iv) on naturally contaminated knives between TVC before and after UV-C treatment (wet or clean area) or between TVC from the wet and clean area (before and after UV-C and reductions). Moreover, analysis of variance (ANOVA) and the Tukey HSD test were used to analyze differences between reductions at the three positions of the UV-C device (knives with or without blood or fat contamination, *E. coli* or *S. aureus*; naturally contaminated knives from the clean or wet area).

Results

Reductions of inoculated *E. coli* and *S. aureus* on knives by UV-C treatment under laboratory conditions

In the laboratory experiments with inoculated knives (but lacking additional contaminations; Table 1), mean log counts before UV-C treatment were 5.98 log CFU cm⁻² for *E. coli* (n=50) and 6.65 log CFU cm⁻² for *S. aureus* (n=50). Mean reductions obtained by UV-C treatment for 30 s accounted for 5.12 log CFU cm⁻² for *E. coli* and 4.53 log CFU cm⁻² for *S. aureus*, but differences were not significant. With regard to the three insertion positions of the UV-C device (Figure 1), the lower reductions obtained at position 3 were striking ($P<0.05$), in particular as respective counts

Table 1. Decontamination of knives used in the meat industry with UV-C (ME3-S, Sterilsystems GmbH): Reductions of inoculated *E. coli* and *S. aureus* after 30s of UV-C exposure (n=50 for each bacterial species).

		<i>E. coli</i> ^a		<i>S. aureus</i> ^a	
		\bar{x}	SD	\bar{x}	SD
Before UV-C treatment	Total	5.98	0.68	6.65	0.52
	Position 1 ^b	5.88	0.82	6.55	0.46
	Position 2	6.16	0.57	6.71	0.58
	Position 3	6.01	0.44	6.70	0.55
After UV-C treatment	Total ^b	0.86	1.53	2.12	2.19
	Position 1 ^c	0.24	0.85	0.47	0.85
	Position 2	0.36	1.28	1.40	1.98
	Position 3	2.58	1.56	4.48	1.01
Mean log reductions by UV-C	Total	5.12	-	4.53	-
	Position 1	5.64	-	6.08	-
	Position 2	5.80	-	5.31	-
	Position 3	3.43	-	2.22	-

^a \bar{x} and SD, mean log CFU cm⁻² and standard deviation; *E. coli*: position 1, n=25; position 2, n=13; position 3, n=12. *S. aureus*: position 1, n=18; position 2, n=15; position 3, n=17. ^bKnives intended for insertion at the respective position of the ME3-S device. ^cKnives UV-C treated at the respective position of the ME3-S device.

before UV-C treatment were comparable (Table 1). This could be due to the fact that the light intensity or the angle is different in this position. By increasing the UV-C exposure time to 45 s, mean reductions accounted for 6.3 log CFU cm⁻² for *E. coli* (n=50) and 6.1 log CFU cm⁻² for *S. aureus* (n=50). Compared to the reductions after 30 s of UV-C exposure (and only considering results from position 1 of the UV-C device), *E. coli* reductions were increased on average by 0.7 log CFU cm⁻² (P<0.05), whereas *S. aureus* reductions were comparable.

The antibacterial effect of UV-C on inoculated knives with additional blood contamination (n=16 for each bacterial species) or fat contamination (n=15 for each bacterial species) was analyzed (Table 2). Compared to the results from knives without additional contamination, obtained reductions were clearly lower (P<0.05) and differed on average by 1.72 to 3.63 log CFU cm⁻² from those of clean knives (depending on the type of contamination and the tested bacterial species). Fat contamination thereby showed a greater negative impact on the efficacy of UV-C than blood contamination (P<0.05 for *E. coli* or *S. aureus*). Reductions of *S. aureus* were lower (P<0.05) than those for *E. coli* in the presence of blood (on average by 0.9 log CFU cm⁻²), whereas reductions of *E. coli* and *S. aureus* were comparable in the presence of fat. With regard to the three insertion positions of the UV-C device (Figure 1), reduc-

tions tended to be comparable for the respective bacterial species and type of contamination, but comparisons were hampered by the limited number of samples.

Reductions of total viable counts on naturally contaminated knives by UV-C treatment under commercial conditions in a slaughterhouse

In the field experiments with naturally contaminated knives, the UV-C device (ME3-S, Sterilsystems) was tested during the slaughter of cattle and sheep, both in the wet area and in the clean area of a slaughterhouse (Figure 2).

Before UV-C treatment, TVC results on knives from the wet area ranged from 2.0 to 6.0 log CFU cm⁻² (on average 3.8 log CFU cm⁻²) and those on knives from the clean area from 1.0 to 3.1 log CFU cm⁻² (on average 1.5 log CFU cm⁻²). Mean reductions obtained by UV-C treatment for 30 s accounted for 0.8 log CFU cm⁻² (wet area; P<0.05) and 0.6 log CFU cm⁻² (clean area; P<0.05), but differences in reductions on knives from the wet and clean area were not significant (Table 3). When considering individual knives, UV-C treatment only yielded reductions on 35 (70%) knives from the wet area (by 0.3 to 3.1 log CFU cm⁻²) and on 15 (63%) knives (by 0.5 to 3.1 log CFU cm⁻²). On the remaining knives, UV-C treatment had no effect or even increases were observed (up to 1.7 log CFU cm⁻²).

Table 2. Decontamination of knives used in the meat industry with UV-C (ME3-S Sterilsystems GmbH): Reductions of inoculated *E. coli* and *S. aureus* obtained on knives contaminated with blood (reflecting protein contamination) or fat after 30s of UV-C exposure (using positions 1 to 3 of the device).

	<i>E. coli</i> ^a		<i>S. aureus</i> ^a	
	\bar{x}	SD	\bar{x}	SD
Blood Contamination (n=16)				
Before UV-C treatment	6.78	0.30	7.48	0.36
After UV-C treatment	3.38	1.52	4.99	1.15
Mean log reductions by UV-C	3.40	-	2.49	-
Fat Contamination (n=15)				
Before UV-C treatment	6.86	0.50	7.34	0.26
After UV-C treatment	5.37	1.67	5.62	0.95
Mean log reductions by UV-C	1.49	-	1.72	-

^a \bar{x} and SD, mean log CFU cm⁻² and standard deviation.

Table 3. Decontamination of knives used in the meat industry with UV-C (ME3-S, Sterilsystems GmbH): Reductions of total viable counts (TVC) on naturally contaminated knives originating from the wet area (n=50) and clean area (n=24) of a slaughterhouse after 30s of UV-C exposure.a

	<i>E. coli</i> ^a		<i>S. aureus</i> ^a	
	\bar{x}	SD	\bar{x}	SD
Before UV-C treatment	3.75	0.89	1.52	0.59
After UV-C treatment	2.97	0.71	0.89	1.05
Mean log reductions by UV-C	0.78	-	0.63	-

^aTVC results from untreated and corresponding treated knife (from the clean area) only considered when TVC before UV-C treatment \geq detection limit (1.0 log CFU cm⁻²). ^b \bar{x} and SD, mean log CFU cm⁻² and standard deviation.

After UV-C treatment, all 50 knives from the wet area and 12 (50%) knives from the clean area still showed TVC above the detection limit (wet area: 1.6 to 4.6 log CFU cm⁻²; clean area: 1.0 to 3.0 log CFU cm⁻²).

When comparing TVC reductions obtained by UV-C treatment on knives from the wet and clean area of a slaughterhouse, reductions were generally comparable and differed on average by less than 0.2 log CFU cm⁻² (despite the significant differences in the initial contamination, P<0.05). With



Figure 1. ME3-S device (Sterilsystems GmbH, Mauterndorf, Austria) for UV-C decontamination of knives with the different blade insertion sites (positions 1 to 3).

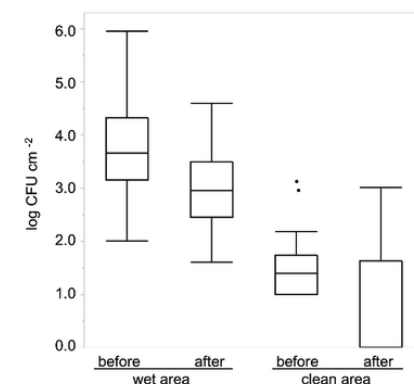


Figure 2. Total viable counts (TVC) on naturally contaminated knives from the wet area (after removal of hooves; n=50) and the clean area (after trimming; n=24 because results were only considered when TVC before UV-C treatment \geq detection limit) of a slaughterhouse before and after UV-C treatment for 30s with the ME3-S device (Sterilsystems GmbH).

regard to the three insertion positions of the UV-C device (Figure 1), TVC reductions tended to be comparable for knives from the wet or the clean area of the slaughterhouse, but comparisons were hampered by the limited and varying numbers of samples.

Discussion and Conclusions

Referred to the company, the ME3-S device was tested in accordance with the standard EN13679 and a disinfection effect of >4 log reduction on knives was achieved. In our study, under laboratory condition, without fat or protein, the obtained mean TVC reduction at a 30s exposure time resulted in 5.1 log CFU cm² for *E. coli* and 4.5 log CFU cm² for *S. aureus*. However, a reduction of the contamination of >4 log could not be achieved under running conditions in a slaughterhouse. The organic material can limit the effect of UV light in at least two ways: the bacteria get a place to hide, and aerosols and water around the knife after cleansing may form shadow zones for the light, and thereby lower the intensity of light exposure. Moreover, the time until enumeration was different for laboratory and industry knives. There may be growth of bacteria between the sampling and enumeration for the abattoir knives which could lead to a possible bias in these results. For the knife decontamination in slaughterhouses in Switzerland as well as in Europe a water disinfection method at a minimum of 82°C or an equivalent procedure is required (EU Regulation 853/2004). Eustace *et al.* (2008) recorded, that the TVC contamination on knives in slaughterhouses is reduced by an average of 1 to 2.5 log steps using the current rinsing and sanitizing procedures. Bell and Hathaway (1996) found a mean TVC reduction of 2.6 log CFU cm² by rinsing knives in warm water (44°C) and immersing them in an 82°C water bath. Compared to other researches, our study showed a comparably

mean TVC after the decontamination process with UV-C.

Leps *et al.* (2013) investigated, among others, a combination of water bath with ultrasound (in a laboratory setting). The use of ultrasound at 40°C and 50°C tempered water for 30s affected the bacterial load only minimally (mean TVC reduction by ca. 0.5 log/10 cm²), whereas an ultrasound treatment with 60°C for 30s resulted in a mean TVC reduction of at least 4 log CFU 10cm². Additionally, a combination of 40°C tempered water with lactic acid (2%) showed an efficient way to eliminate microorganisms (mean TVC reduction of 2.3 log CFU 10cm² in 5s). Brasil *et al.* (2017) evaluated the effect of ultrasound on knives in chlorinated water (20°/25°C) with/without combination using neutral detergents. These authors concluded that a UV-C treatment seems not to be practicable if compared with the common method (hot water treatment at 82°C).

Nevertheless, UV-C treatment has many advantages compared to the current procedure (low electricity cost and water consumption, no water steam). A future use of UV-C treatment might still be an option. Future studies should be performed to evaluate whether an effective prewash and a higher spray temperature in combination with UV-C treatment leads to more desirable outcomes in a commercial slaughterhouse setting.

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