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Research Note

Effect of Peracetic Acid Solutions and Lactic Acid on Microorganisms in On-Line Reprocessing Systems for Chicken Slaughter Plants

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ABSTRACT

During poultry slaughter and processing, microbial cross-contamination between individual chickens is possible, as well as from one slaughter animal to the next without direct contact. One option for reducing the risk of cross-contamination is to decrease the number of microorganisms on contact surfaces by using disinfectants. The aim is to decontaminate the surfaces coming into direct contact with the carcasses. In the present study, the effectiveness of different disinfectants was investigated in laboratory settings, simulating the conditions in the slaughterhouses and in a chicken slaughterhouse. For this, an artificial residue substance (consisting of yeast extract, albumin, and agar) was developed, tested, and included in the assays. Two disinfectants were tested under laboratory conditions: lactic acid (5 and 6.67%) and peracetic acid (0.33 and 0.5%). At the slaughterhouse, peracetic acid (0.021%) was used. In the laboratory tests, it was found that the peracetic acid solution had the highest disinfection potential with respect to an *Escherichia coli* strain (reduction >4 log CFU mL^{-1}) at 0.5% without an artificial residue substance. The tested lactic acid solutions also showed the highest disinfection potential against a *Pseudomonas aeruginosa* strain, without an artificial residue substance. When applying the artificial residue substance, the reduction potential of lactic acid and peracetic acid was decreased to less than 1.4 log CFU mL^{-1}. Application of peracetic acid in the slaughterhouse reduced the number of total aerobic bacteria by more than 4 log CFU mL^{-1} and the number of *Enterobacteriaceae* by more than 3 log CFU mL^{-1}, depending on the place of sampling.

HIGHLIGHTS

- Peracetic acid and lactic acid decreases *E. coli* and *P. aeruginosa* numbers in vitro.
- Sanitation in place reduces the number of bacteria in a chicken slaughterhouse.
- The number of total aerobic bacteria and *Enterobacteriaceae* was significantly reduced.

Key words: Chicken slaughter plant; Disinfection; Extended spectrum beta-lactamase

During the slaughtering process, chicken carcasses come into contact with various surfaces, such as hooks, metal surfaces, and conveyor belts. Almost all slaughter and processing steps pose a threat of cross-contamination between individual carcasses or meat parts. These steps pose a risk of cross-contamination with various microorganisms (e.g., shown by tests for *Salmonella* contamination at the slaughter line). Rasschaert et al. *(21)* demonstrated that slaughter lines contaminated with *Salmonella* are able to contaminate *Salmonella* free broiler slaughter lots with just this microorganism during slaughtering.

Slaughterhouse visits have shown that it takes less than 5 s for two carcasses or pieces of meat to contact the same surface. To reduce the risk of cross-contamination from surface contact, decontamination (especially in-process decontamination) is necessary. To achieve this, different procedures are possible, such as treatment of the carcass surfaces with disinfectants. However, this procedure is not currently legal in the European Union, except for the application of lactic acid in the treatment of bovine carcasses *(7–9, 16, 25)*. Alternatively, the use of hot water to treat surfaces with direct contact to carcasses or the carcasses itself is possible, but this may lead to high humidity and changes in the protein structure of the meat or skin *(19)*.

Cold decontamination methods of contact surfaces with disinfectants is another possibility. Multiple studies have shown the effectiveness of product treatments with different disinfectants that could also be used to reduce the number of microorganisms on surfaces *(2, 8)*. The German Veterinary Medical Society has listed various disinfectants for the food

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sector having different active substances *(12)*. However, among the challenges and limitations of the application of disinfectants on slaughter lines are the short reaction times, low temperature, and high protein levels and moisture on the surfaces.

The aim of this study was to investigate the effectiveness of different disinfectants in reducing the number of microorganisms on contact surfaces in slaughterhouses during the slaughtering process. In this context, the influence of the disinfectants on the microorganisms was investigated in a laboratory study and directly in a slaughterhouse.

MATERIALS AND METHODS

Strains used. Laboratory tests were carried out on two strains, *Escherichia coli* 10714 (extended spectrum β-lactamase–TEM-52, phylogroup B1, isolated from a chicken meat sample) and *Pseudomonas aeruginosa* (ATCC 15442). In accordance with EN 13697:2015-06, single strains were used *(11)*. Strains were stored at −20°C in Luria-Bertani-Miller broth (Merck Millipore, Darmstadt, Germany) containing 25% (v/v) glycerol and cultured at 37°C on tryptic soy agar (TSA; Oxoid, Wesel, Germany) for 24 h. Colony material was passaged in Luria-Bertani-Miller broth to a concentration of 8 to 9 log CFU mL^{-1}. The concentration was verified by inoculating 50 μL of appropriate dilutions by the drop-plating method on TSA. Incubation conditions were 37°C for 24 h. The suspension was stored at 4°C for 24 h until use.

Preparation of artificial residue substance. To simulate residue content on relevant slaughterhouse surfaces, an artificial residue substance was prepared. For that, 100 μL of the microorganism suspensions (8 to 9 log CFU mL^{-1}) were mixed with 100 μL of albumin from chicken egg white (Sigma-Aldrich, St. Louis, MO) and yeast extract (Mast Group, Bootle, UK; final concentration of each: 0.5 g/100 mL). Finally, 800 μL of agar solution (Oxoid; final concentration 0.2 g/100 mL at 50°C) was added (agar concentration was intended to simulate the texture of the residues). These mixtures were stored at 4°C until the agar gelatinized.

For preparing the samples without the artificial residue substance, 900 μL of maximum recovery diluent (Merck Millipore) was added to the microorganism suspension instead of the protein, yeast extract, and agar solution.

Disinfectants. Different disinfectants were chosen for a prescreening of their effectiveness. For these preliminary trials, high concentrations of disinfectants and a treatment time for 60 s without the artificial residue substances were tested at 7°C.

The prerequisite for inclusion of a disinfectant in the experiments was a reduction of the number of microorganisms by more than 2 log CFU mL^{-1} under these conditions. The prescreening was applied to disinfectants containing triamine (0.38%), citric acid (6.67%), sodium chlorite (6.67%), quaternary ammonium cation (0.8%), formic acid (5%), L–lactic acid (LA; 6.67%), and peracetic acid (PAA; 0.5%). Only LA and PAA were able to reduce *E. coli* 10714 and *P. aeruginosa* ATCC 15442 by more than 2 log CFU mL^{-1} and thus were included in the in vitro trials.

For in vitro trials, LA solution (Merck Millipore) was diluted to concentrations of 5 and 6.67%. A PAA solution (1+1 Wofasteril SC Super, Kesla Hygiene, Bitterfeld-Wolfen, Germany), with two components, was mixed immediately before use and adjusted to concentrations of 0.33 and 0.5%. Both disinfectants were diluted in water with standardized hardness (described in EN 13697:2015-06) *(11)*. All reported concentrations describe the final concentration of disinfectant after mixing with the microorganism suspension.

For the in vivo trials at the slaughterhouse, Inspexx 210 (Ecolab, Monheim, Germany) was used. This disinfectant is a solution of acetic acid, octanoic acid, hydrogen peroxide, PAA, and peroctanoic acid. PAA, the active substance, had an application concentration of 0.021%.

In vitro treatment. Disinfectant effectiveness was tested at 7°C. All materials were adjusted to that temperature before the experiments started. Tests were carried out in accordance with the provisions of EN 13697:2015-06 *(11)*. Briefly, 100 μL of each bacterial suspension (with or without artificial residue substances) was applied to steel disks. Agar pieces (radius of 0.26 mm), the maximum diffusion distance, were thereby generated. Thereafter, 50 μL of individual disinfectant solutions were added to the surface of the microorganism suspension. For the controls, disinfectant solution was substituted with water. After a defined reaction time of 5, 30, or 60 s, the disks with the solution were moved into 9.85 mL of neutralization medium with 5 g of glass beads (diameter, 4 mm). Samples were shaken for 5 min to release microorganisms from the gelatinized agar. The bacterial count was verified by adding 50 μL of the microorganism suspension with the drop-plating method to nonselective TSA.

In vivo treatment. In a chicken slaughterhouse, swab samples were taken from seven different contact surfaces directly after the stopping of the conveyor system. The surfaces examined were the lung vacuum device, neck skin cutter, cropping machine, intestine trays, eviscerator, abdominal skin trimmers, and the vent cutter. During the slaughter and processing, surfaces were continuously sprayed with the disinfectant Inspexx 210 as part of a sanitation-in-place system. The concentration of PAA was verified semiquantitatively with the Merckoquant Perex-Test (Merck) and quantitatively by titration with potassium permanganate and sodium thiosulfate, as described by the manufacturer of the disinfectant *(6)*. On days with sanitation, the disinfectant was sprayed throughout the whole production day on the surfaces with product contact, which were subsequently examined. On control days, the surfaces were left untreated.

From each sampling surface, six samples of both treated and untreated surfaces were taken on different days. Sampling took place after 12 h of operation time. This corresponds to approximately 120,000 slaughtered chickens. Swab samples (Copan, Brescia, Italy) were taken from each surface. For each sampling point, sampling area and size were defined, and samples were taken from the same area and size per each sampling point throughout the experiments. The collected swabs were put into 2.5 mL of neutralization medium (Henkel, Düsseldorf, Germany) to inhibit the effect of the disinfectant. The samples were stored at refrigeration temperatures until examination.

For bacterial counting, Luria-Bertani-Miller medium was added to the swabs at a dilution factor of 1:10. After homogenization for 1 min, cell counts were established by drop plating on plate count agar (Oxoid) to determine the number of total aerobic bacteria (TAB) and on MacConkey agar (Oxoid) to determine the number of *Enterobacteriaceae*. Plate count agar was incubated for 72 h at 30°C, and MacConkey agar was incubated for 24 h at 37°C.

Statistical analysis. The in vitro experiments were repeated three times separately for both concentrations with each

disinfectant. The lower detection limit of the method was 1.3 log CFU mL^{-1}. For statistical analyses, all results below the detection limit were set to 1.3 log CFU mL^{-1}. The statistical analyses of the reduction of the microorganisms by the disinfectants compared with the untreated control were performed with the nonparametric Dunnett test by using the "mctp" function from the R package "nparcomp" *(14)*. These analyses were conducted with the statistical software R, Version 3.5.1 *(20)*. The statistical analyses of the influence of the artificial residue substances on the efficiency of the disinfectants were performed with the nonparametric exact Mann-Whitney U test for independent samples by using SPSS (version 21, IBM SPSS Statistics for Windows, IBM Corporation, Armonk, NY) *(23)*. Results were considered significant at $P < 0.05$.

The in vivo experiments were repeated six times separately with and without treatment with disinfectants. Each sample was investigated in duplicate. The lower detection limit of the method was 2.3 log CFU mL^{-1}. For statistical analyses, all results below the detection limit were set to 2.3 log CFU mL^{-1}. Statistical analyses were performed with the nonparametric exact Mann-Whitney U test for independent samples by using SPSS (version 21) *(23)*. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The aim of the present study was to investigate the effectiveness of sanitation in place for continuous reduction of bacterial numbers on contact surfaces in a chicken slaughterhouse. In the first step, PAA and LA solutions were tested in vitro for their effectiveness in reducing the number of *E. coli* and *P. aeruginosa* in laboratory experiments. In the second step, the effectiveness of a PAA-containing disinfectant was tested in vivo.

Reduction of *E. coli* and *P. aeruginosa* in vitro. Application of 0.33% PAA for 30 and 60 s showed significant reductions of *E. coli* 10714 by approximately 2 and 4 log CFU mL^{-1}, respectively. After a 5-s treatment, no significant changes in cell numbers were observed. When applying 0.5% PAA, a similar trend was observed, with even higher reduction rates at 30 s (approximately 3 log CFU mL^{-1}), but with significant reductions already after 5 s (Table 1). When *E. coli* 10714 was incorporated into the artificial residue substance, significantly lower reduction rates were observed after 30 and 60 s. Here, only 0.5 to 1.3 log CFU mL^{-1} reductions were observed.

Application of LA solutions at 5 and 6.67% resulted in lower reduction rates for *E. coli* 10714 (Table 2) compared with the reduction by PAA. No reductions were seen after a 5-s treatment for both concentrations. Reduction rates of 0.4 log CFU mL^{-1} (30 s) and 1.2 log CFU mL^{-1} (60 s) were detected by the 5% LA solution application. Higher reduction rates were seen at 6.67% with 1.3 log CFU mL^{-1} (30 s) and 2.4 log CFU mL^{-1} (60 s). The presence of artificial residue substances protected *E. coli* 10714, resulting in no reduction of cell numbers.

In general, higher reduction rates were observed for *P. aeruginosa* ATCC 15442 under similar treatment conditions (Table 2). PAA treatment with both concentrations leads to reductions in *P. aeruginosa* ATCC 15442 cell numbers over 4 log CFU mL^{-1} (60 s) and 2.3 to 3.7 log CFU mL^{-1} (30 s) (Table 1). After 5 s, reduction rates were already significant (up to 0.7 log CFU mL^{-1}). The addition of artificial residue substances limited the reduction of the bacteria significantly.

A similar picture was observed for LA solutions: reduction rates of approximately 4 and more than 4 log CFU mL^{-1} at 30- and 60-s treatments and only slight reductions after 5 s (Table 2). The addition of artificial residue substances also limited the reduction of the bacteria by LA significantly.

To summarize, the tests show that during a treatment time of 5 s, it is possible to reduce the investigated strains (up to 0.7 log CFU mL^{-1}) significantly ($P < 0.05$) with PAA at this high concentration in the absence of the artificial residue substance. After 30-s reaction time, the cell count of *E. coli* 10714 was decreased significantly ($P < 0.001$) by almost 3 log CFU mL^{-1} by 0.5% PAA. *P. aeruginosa* ATCC 15442 was decreased by almost 4 log CFU mL^{-1} ($P < 0.001$) by a solution of 5% LA. In the presence of artificial residue substances, the effectiveness of both disinfectants decreased significantly.

Some components of slaughterhouse residues, such as fat, would reduce the efficacy of the disinfectants even further. Water soluble components cannot penetrate fatty residue on work surfaces, and the effectiveness of basic disinfectants, such as the PAA product, are also negatively influenced by saponification error *(4, 10)*.

The effectiveness of PAA is described in several publications and summarized in a scientific opinion statement of the European Food Safety Authority. According to this statement, PAA is commonly used in scalding baths, chiller baths, and as supplement in spray washes *(8)*. In the setup of Dankert *(5),* for example, chicken carcasses were sprayed with different concentrations of PAA for a few seconds. He determined a reduction of 0.8 log CFU g^{-1} of *E. coli* after a 45-s treatment with 0.0095% PAA *(5)*. Nagel et al. *(15)* dipped chicken carcasses for 20 s in 0.1% PAA solution. They observed a reduction of *Salmonella* Typhimurium by 2.1 log CFU mL^{-1} via carcass rinse *(15)*. Both reported treatments have in common that the PAA solution was present or applied in such a way that the concentration of PAA was less influenced by degradation reactions than in a single treatment event, as described in the present study.

Effectiveness of sanitation in place for reducing bacterial numbers on surfaces. The influence of a disinfectant containing 0.021% PAA on the number of TAB and *Enterobacteriaceae* on different contact surfaces was investigated in a chicken slaughterhouse. Samples were taken at the end of the respective production day, after approximately 120,000 chickens were slaughtered. The number of TAB on all contact surfaces showed a significant reduction ($P < 0.05$) when 0.021% PAA was applied throughout the whole production day. The TAB were reduced by 4.4 log CFU mL^{-1} at the lung vacuum device, >3.5 log CFU mL^{-1} at the neck skin cutter and the intestine trays, 2 log CFU mL^{-1} at the eviscerator, 1.9 log CFU mL^{-1} at the abdominal skin trimmers, 1.8 log CFU mL^{-1} at the vent cutter, and 1.7 log CFU mL^{-1} at the cropping machine (Table 3).

TABLE 1. *The number of* E. coli *10714 and* P. aeruginosa *15442 after PAA treatment in the absence (–) or presence (+) of the artificial residue substance*[a]

		Mean ± SD (log CFU mL^{-1})[b]			
		E. coli 10714		*P. aeruginosa* 15442	
Concn (%)	Treatment time (s)	–	+	–	+
	0	5.5 ± 0.1	5.4 ± 0.1	5.7 ± 0.4	5.6 ± 0.3
0.33	5	5.4 ± 0.1	5.3 ± 0.1	5.1 ± 0.4*	5.3 ± 0.4
	30	3.5 ± 0.4***	4.9 ± 0.2***[c]	3.4 ± 0.5***	5.0 ± 0.6[c]
	60	<1.3 ± 0.0***	4.1 ± 0.6***[c]	<1.3 ± 0.0***	4.8 ± 0.2*[c]
0.5	5	5.2 ± 0.2***	5.3 ± 0.3	5.0 ± 0.5*	5.5 ± 0.4
	30	2.5 ± 0.4***	4.8 ± 0.3**[c]	2.0 ± 1.1***	4.7 ± 0.4*[c]
	60	<1.3 ± 0.0***	4.2 ± 0.7***[c]	<1.3 ± 0.0***	4.4 ± 0.5**[c]

[a] Statistical analyses of the reduction were performed with the Dunnett test. The statistical analyses of the influence of the artificial residue substance were performed with the exact Mann-Whitney U test.
[b] The level of significance is shown next to the respective number. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. 1.3, detection limit.
[c] Significant difference induced by the presence of the artificial residue substance ($P < 0.01$).

For *Enterobacteriaceae,* large reductions were seen at all sampling points by PAA application (at or below detection limit of our procedure). Except for the results at the eviscerator, all results show a significant ($P < 0.05$) reduction of the *Enterobacteriaceae*. The *Enterobacteriaceae* were reduced by >3.5 log CFU mL^{-1} at the lung vacuum device, >2.2 log CFU mL^{-1} inside the intestines trays, >2.1 log CFU mL^{-1} at the neck skin cutter, >1.4 log CFU mL^{-1} at the vent cutter, >1.5 log CFU mL^{-1} at cropping machine, >1.2 log CFU mL^{-1} at the eviscerator, and >0.7 log CFU mL^{-1} at the abdominal skin trimmers (Table 3).

The literature is inconstant in describing changes in bacterial load on carcasses along the slaughter line: studies show both increasing and decreasing numbers of different microorganisms, such as *Campylobacter, E. coli,* and *Salmonella* on the carcasses during the slaughtering and cutting process without sanitation in place. Interherd cross-contaminations by *Campylobacter* and *Salmonella,* for example, are also described *(1, 3, 17, 18, 21, 22, 25, 26)*. Thus, continuous sanitation of contact surfaces can contribute to reducing or preventing cross-contamination between individual slaughtered chicken or slaughter lots.

The results of our study demonstrate significant ($P < 0.05$) reductions (up to 4.4 log CFU mL^{-1}) of TAB and *Enterobacteriaceae* on almost all sampled contact surfaces in the slaughterhouse, due to continuously spraying 0.021% PAA on the surfaces. Rodrigues et al. *(22)* have reported reduction rates of TAB of 3.8 log CFU per area unit on surfaces in a poultry slaughterhouse in a sanitation standard operating procedure at the end of a processing day. This procedure included a hot water treatment, followed by application of a 0.075% PAA solution, with 15-min reaction time after a single spraying treatment *(22)*. Comparing the number of TAB on days with sanitizer treatment to the bacterial number on days with no treatment, the results of the present study are similar. It can be concluded that continuous spaying of a PAA solution enables a continuous reduction of bacterial

TABLE 2. *The number of* E. coli *10714 and* P. aeruginosa *15442 after LA treatment in the absence (–) or presence (+) of the artificial residue substance*[a]

		Mean ± SD (log CFU mL^{-1})[b]			
		E. coli 10714		*P. aeruginosa* 15442	
Concn (%)	Treatment time (s)	–	+	–	+
	0	5.7 ± 0.1	5.3 ± 0.1	5.7 ± 0.4	5.6 ± 0.3
5	5	5.7 ± 0.1	5.3 ± 0.1[c]	5.4 ± 0.5	5.5 ± 0.4
	30	5.3 ± 0.1***	5.3 ± 0.1	1.8 ± 0.4***	5.4 ± 0.3[c]
	60	4.4 ± 0.4***	5.4 ± 0.1[c]	<1.3 ± 0.0***	5.3 ± 0.4[c]
6.67	5	5.7 ± 0.2	5.5 ± 0.2	5.3 ± 0.5	5.6 ± 0.4
	30	4.4 ± 0.4***	5.4 ± 0.1[c]	<1.3 ± 0.0***	5.3 ± 0.3[c]
	60	3.3 ± 0.4***	5.5 ± 0.1[c]	<1.3 ± 0.0***	5.4 ± 0.3[c]

[a] Statistical analyses of the reduction were performed with the Dunnett test. The statistical analyses of the influence of the artificial residue substance were performed with the exact Mann-Whitney U test.
[b] The level of significance is shown next to the respective number. *** $P < 0.001$. 1.3, detection limit.
[c] Significant difference induced by the presence of the artificial residue substance ($P < 0.01$).

TABLE 3. *Number of TAB and* Enterobacteriaceae *determined on the respective surface by swab sampling without (–) and with (+) surface treatment with sanitizer*[a]

	Mean ± SD (log CFU mL^{-1})[b]			
	TAB		*Enterobacteriaceae*	
Sampled surface	–	+	–	+
Lung vacuum device	7.3 ± 1.0	2.9 ± 0.6**	5.8 ± 0.9	2.3 ± 0.4**
Neck skin cutter	5.8 ± 0.4	<2.3 ± 0.0**	4.4 ± 0.6	<2.3 ± 0.0**
Cropping machine	4.9 ± 0.7	3.2 ± 0.8**	3.8 ± 0.6	2.3 ± 0.2**
Intestine trays	5.8 ± 0.9	2.3 ± 0.7**	4.5 ± 1.0	2.3 ± 0.5**
Eviscerator	4.5 ± 0.7	2.5 ± 1.1*	3.5 ± 0.8	2.3 ± 0.3
Abdominal skin trimmers	4.2 ± 0.8	2.3 ± 0.5**	3.0 ± 0.5	<2.3 ± 0.0*
Vent cutter	5.1 ± 0.4	3.3 ± 0.5**	3.7 ± 0.6	2.3 ± 0.2**

[a] The statistical analyses of the influence of the sanitation treatment were performed with the exact Mann-Whitney U test.
[b] The level of significance is shown next to the respective number. * $P < 0.05$; ** $P < 0.01$. 2.3, detection limit.

numbers on the treated surfaces that could subsequently lead to reduced cross-contamination.

Note that the slaughter lines had to be turned off to perform the sampling necessary for the present study. As a result, disinfectant treatment time was increased to approximately 3 min. Additionally, the rinse effect was improved by the octanoic acid acting as a surfactant.

The results of Soares et al. *(27)* have shown that a continuous water treatment of modular and smooth conveyor belts has no reducing effect on different microorganisms, such as aerobic mesophilic bacteria and *Enterobacteriaceae,* at the surfaces. Similar results were reported by Julião et al. *(13)*. Additionally, Julião et al. *(13)* reported no differences in the number of microorganisms on the chicken meat itself. Both the extended treatment time and the enhanced rinsing effect could result in an increased reduction of microorganisms in the slaughterhouse samples compared with the in vitro results in the present study. However, the number of microorganisms on the surfaces is reduced by the continuous spraying of disinfectant.

In conclusion, the results determined in the laboratory show that a treatment time of 5 s (corresponding to the maximum time intervals between contact of individual carcasses with contact surfaces in praxi) is, under certain circumstances, sufficient to reduce the number of *E. coli* 10714 and *P. aeruginosa* ATCC 15442 significantly ($P < 0.05$), up to 0.7 log CFU mL^{-1} with the tested PAA solutions in the absence of artificial residue substances. The reduction of the investigated microorganisms was increased significantly by increased treatment times. However, these effects were significantly negatively impacted by the presence of the artificial residue substances. The reduction of TAB and *Enterobacteriaceae* in the slaughterhouse was significant ($P < 0.05$) at almost each station (except the reduction of *Enterobacteriaceae* at the eviscerator).

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