Decontamination of inoculated chicken carcasses by using some microbial decontaminators

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A B S T R A C T

This study was conducted to evaluate the efficacy of three decontaminators in reducing the level of contamination in chicken carcasses which had been artificially contaminated. A grand total of forty random samples of raw chicken carcasses were purchased directly from local markets. Chicken samples were then dipped in cultures of the tested microorganisms (Salmonella spp., Staphylococcus aureus, E.coli O157:H7 and L. monoctogens) (10 samples for each microorganism). Then dipped into containers containing distilled water (control) , chlorine 30 ppm, 50 ppm and 70 ppm, trisodium phosphate 3%, 5% an 8% and lactic acid 0.75%, 1.25% and 2% , where the reduction percentage of Salmonella were 26.02 %, 26.57 %, 29.16 %, 34.88% , 36.78 %, 38.01 %, 35.42 %, 40.87 % and 54.50 %, respectively, compared to control. while the reduction of Listeria monocytogenes was 16.64 %, 20.60% , 30.43% , 21.74 %, 21.74 %, 25.14 %, 22.87 %, 30.24 % and 39.89 % respectively . Moreover , the reduction percentages of S. aureus was 14.29 %, 15.70 %, 19.58% , 8.11 %, 10.58 %, 27.69 %, 17.28 %, 21.34 % and 27.87 % , respectively . Finally, the reduction percentage of *E.coli* O157:H7 was 9.56 %, 16.61 %, 20.47 %, 17.45%, 21.48 %, 27.85 %, 18.12%, 19.97% and 29.70 %, respectively.

Keywords: chlorine, TSP, lactic acid, chicken carcasses, decontamination.

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1. INTRODUCTION

Avoiding contamination is one of the greatest challenges in meat hygiene practice to prolong the shelf-life of meat and prevent food poisoning of consumer. A substantial microbial reduction of potentially contaminated fresh poultry can be achieved by decontamination with chlorines, acid and alkaline solutions. Besides general hygienic measures during the slaughter process sodium hypochlorite, lactic acid and trisodium phosphate (TSP) seemed to be the most promising substances to reduce the overall bacterial load. Chlorine is the most frequently used antimicrobial intervention in commercial poultry processing due to its availability, low cost, and efficacy (Northcutt and Jones 2004). In general, chlorine compounds are effective against Gram-positive, Gram-negative, and acid-fast bacteria, fungi, and viruses. However, chlorine reacts with organic materials relatively easily and can quickly lose effectiveness (Barbut, 2002, Bremner and Johnstone 1996). Trisodium Phosphate (TSP) is generally recognized as safe by the US (FDA) and has been approved by USDA-FSIS at levels of 8-12% as an antimicrobial agent on raw chilled poultry carcasses that have been passed for wholesomeness (USDA, 2002). Treatment of poultry carcasses with TSP is effective in reducing populations of food spoilage bacteria as well as foodborne pathogens including *Salmonella, Escherichia coli*

O157:H7, Listeria, and Staphylococcus aureus (Capita et al., 2002).

Lactic acid has a thoroughly studied mechanism of action and generally regarded as safe status; several studies demonstrated the effectiveness of lactic acid as an antimicrobial intervention in red meat processing (Hardin et. al., 1995), and lactic acid is commonly used in commercial beef slaughter operations. Other studies have evaluated lactic acid as a poultry processing intervention (Anang et. al., 2007, Bautista et al., 1997, Kanellos and Burriel, 2005 and Okolocha and Ellerbroek, 2005). However, most antimicrobials have not been studied under mobile poultry slaughter conditions. The mechanism of action of organic acids on the microbial cell is not completely understood, but it is hypothesised that it is the undissociated molecule of the acid that is responsible for the antimicrobial activity (Podolak et al., 1996). Therefore this study was planned out to investigate the antimicrobial effect of chlorine (30 , 50 ,70 ppm) , TSP(3% , 5% , 8% 0 and lactic acid (0.75% , 1.25% , 2%) on chicken carcasses artificially inoculated with *S.typhimurium, L.monocytogenes, E.coli* O_{157}*:*H_7 *and St.aureus* .

2. MATERIAL AND METHODS

2.1. Chicken carcasses:

A grand total of forty random samples of raw chicken carcasses were purchased directly from local markets. The samples were taken and transferred directly to the laboratory using an ice box under complete aseptic conditions without any delay. The samples were divided into four equal groups (10 samples each). All samples were washed in sterile distilled water.

2.2. Preparation of microbial suspension:

Reference strains of *S. typhimurium. , St. aureus, E.coli O157:H7 and L. monoctogens* were obtained from Animal Health Research Institute. Four to five isolated colonies of the tested strains were picked up by sterile inoculating loop and inoculated in sterile peptone water 0.1% (Merck, Germany) and were then incubated at 37°C for 24 hrs.

2.3. Preparation of decontaminators:

2.3.1. Chlorine:

Sodium hypochlorite solution 4% (Oxford laboratory) was used to prepare 30 pmm, 50 pmm and 70 ppm by dissolving 0.75ml, 1.25ml and 1.75 ml in 100 ml sterile distilled water.

2.3.2. Trisodium phosphate:

Tri-sodium orthophosphate 98% (Alpha chemika) was used to prepare 3%, 5% and 8 % solution by dissolving 3.06 ml, 5.1 ml and 8.16 ml in 100 ml sterile distilled water.

2.3.3. Lactic acid:

Pure lactic acid (Oxford laboratory) was used to prepare 0.75%, 1.25% and 2% solution by dissolving 0.833 ml, 1.388 ml and 2.222 ml in 100 ml sterile distilled water.

2.4. Artificial contamination of samples of chicken carcasses with the tested microorganisms:

Forty chicken samples were dipped in 500 ml sterile peptone water 0.1% (Merck, Germany) containing 24 hrs-old cultures of the tested microorganisms (*S. typhimurium, St. aureus , E.coli* O_{157}*:*H_7 *and L. monoctogens*) (10 samples for each microorganism). Subsequently, the inoculated chicken samples were left for 30 min. at room temperature (25^{o}C) to allow attachment and absorption of the inoculated bacteria. Then, the tested microorganisms *(S. typhimurium, St. aureus, E.coli* O_{157}*:*H_7 *and L. monoctogens*) were enumerated in the artificially contaminated samples to get the initial load before dipping treatments were performed.

Salmonella typhimurium was enumerated on XLD agar, while, *St.aureus* was

enumerated on Baird Parker agar media, *E.coli* O_{157}:H_7 was enumerated on sorbitol MacConkey agar media and finally *L. monoctogens* was enumerated on Oxford agar media.

2.5. Application of the tested decontaminators:

Every ten contaminated chicken sample with known load of the tested microorganisms (*S. typhimurium, L. monoctogens, St. aureus and E. coli* O_{157}:H_7) were dipped into containers containing 500 ml of chlorine 30 ppm, 50 ppm and 70 ppm, trisodium phosphate 3%, 5% an 8% and lactic acid 0.75%, 1.25% and 2% at room temperature (25 ± 1oC) for 15 min. The control samples were dipped in 500 ml sterile distilled water. All the containers were properly labeled. Solutions covered all surface of the chicken samples. After dipping in the microbial decontaminators, all samples were removed and left at room temperature for 15 min. to allow the decontamination effect. The experiment was repeated five times. *Salmonella typhimurium, St. aureus, E.coli* O_{157}:H_7 *and L. monoctogens* counts were conducted using the serial dilutions and spread plate technique after decontamination to determine the reduction percentages. The experiment was repeated five times.

2.6. Statistical analysis:

The data was statistically treated by one-way ANOVA using SPSS program for windows (Version 16) (SPSS Inc. Chicago, IL and USA) and Duncan's post hoc test with $p < 0.05$ considered to be statistically significant.

3. RESULTS

3.1. Reduction by Chlorine :

Table (1) reported that initial load of *S. typhimurium* was 7.34 log CFU/g, then it was reduced to 5.43±0.55, 5.39±0.44 and 5.20±0.45 log CFU/g when dipped in 30 ppm, 50 ppm and 70 ppm. Chlorine solutions, respectively, with a reduction percentages of 26.02 %, 26.57% and 29.16 % as shown in table (2) and figure (1). *St.aureus* initial count was 5.67 log CFU/g (1) , after dipping the counts were reduced to 4.86±0.12 , 4.78±0.13 and 4.56±0.21 log CFU/g , respectively , where the reduction percentages were 14.29% , 15.70 % and 19.58 % , respectively (table 2 and figure 1). *E.coli*O_{157}:H_7 counts inoculated on chicken parts were reduced when dipped in 30 ppm, 50 ppm and 70 ppm. Chlorine solutions from 5.96 log CFU/g (initial load) to 5.39± 0.32 , 4.97±0.20 and 4.74±0.20 log CFU/g , respectively , with reduction percentages of 9.56 %, 16.61% and 20.47 % (table 2 and figure 1). Finally, following treatment with chlorine solutions, reduction in *L. monocytoges* counts was shown from 5.29 log CFU/g (initial load) to 4.41±0.33, 4.20±0.38 and 3.68±0.63 log CFU/g in case of 30 ppm, 50 ppm and 70 ppm. , respectively (Table 1). Moreover, the results shown in table (2) and figure (1) revealed that counts were reduced in percentages of 16.64 %, 20.60 % and 30.43 % when treated by 30 ppm, 50 ppm and 70 ppm. chlorine solutions , respectively .

3.2. Reduction by TSP :

Salmonella typhimurium initial count was 7.34 log CFU/g , which was decreased to 4.78±0.20 , 4.64±0.37 and 4.55±0.18 log CFU/g by using 3 , 5 and 8 % TSP, respectively, as shown in table (1). Regarding the results in table (2), the reduction percentages were 34.88%, 36.78% and 38.01 % when treated with 3%, 5% and 8 % TSP, respectively, which was shown in figure (1). Regarding *E.coli*O_{157}:H_7, the initial microbial count was 5.96 log CFU/g, when using TSP with concentrations of 3 %, 5 % and 8 % the counts decreased to 4.92±0.78, 4.68±0.23 and 4.30±0.30 log CFU/g, respectively (table 1). Moreover, the reduction percentages were 17.45%, 21.48 % and 27.85 %, respectively. (Table 2 and figure 1)

Staphylococcus aureus initial count was 5.67 log CFU/g, which was reduced to 5.21±0.37, 5.07±0.37 and 4.10±0.36 log CFU/g, respectively (table 1). Results in table (2) and figure (1) showed that the reduction percentages in *S. aureus* count were 8.11, 10.58 and 27.69 %. While *L. monocytogens* was reduced from initial count of 5.29 to 4.14±0.24, 4.14±0.31 and 3.96±0.22 log CFU/g, respectively, table (1). Such concentrations reduced L.monocytogens in percentages of 21.74%, 21.74 % and 25.14 %, respectively, table (2), and figure (1).

3.3. Reduction by lactic acid :

Salmonella typhimurium count was 7.34 log CFU/g (initial count), it was reduced to 4.74±0.17, 4.34±0.23 and 3.34±0.23 log CFU/g, when inoculated chicken parts dipped in lactic acid solution in concentrations of 0.75 %, 1.25 % and 2 %, respectively. (table 1). On the other hand, the reduction percentages of *S.typhimurium* treated with the same concentrations were 35.42%, 40.87% and 54.50 %, respectively. (table 2, figure 1). *St. aureus* count was decreased from 5.67 log CFU/g to 4.69±0.26, 4.46±0.34 and 4.09±0.29 log CFU/g, respectively (table 1). Where the reduction percentages achieved in the experiment were 17.28 %, 21.34 % and 27.87 % for the 3 concentrations of lactic acid used, respectively (table 2 , figure 1) . Moreover, *E.coliO157:H7* initial count was decreased from 5.96 log CFU/g to 4.88±0.07, 4.77±0.12 and 4.19±0.26 log CFU/g, respectively (table 1), where the counts were reduced in percentages of 18.12%, 19.97% and 29.70 %, respectively. (table 2, figure 1). Finally, *L. monocytogenes* count, the initial load was decreased from 5.29 log CFU/g to 4.08±0.39, 3.69±0.26 and 3.18±.27 log CFU/g, respectively (table 1). The reduction percentages were 22.87%, 30.24% and 39.89 %, respectively (table 2).

Table (1): Effects of various concentrations of decontaminators on counts of food borne pathogens (log CFU/g) in chicken carcasses samples.

	Salmonella spp	Staph. Aureus	E. coli O157:H7	L. monocytogenes
Initial load	7.34	5.67	5.96	5.29
Control (D.W.)	7.34±0.38a	5.67±0.38a	5.96±0.22a	5.29±0.32a
Chlorine 30	5.43±0.55b	4.86±0.12bcd	5.39±0.32b	4.41±0.33b
Chlorine 50	5.39±0.44b	4.78±0.13cd	4.97±0.20c	4.20±0.38bc
Chlorine 70	5.20±0.45bc	4.56±0.21d	4.74±0.20c	3.68±0.63d
TSP 3%	4.78±0.20cd	5.21±0.37b	4.92±0.78c	4.14±0.24bcd
TSP 5%	4.64±0.37d	5.07±0.37bc	4.68±0.23c	4.14±0.31bcd
TSP 8%	4.55±0.18d	4.10±0.36bc	4.30±0.30d	3.96±0.22bcd
Lactic acid 0.75%	4.74±0.17d	4.69±0.26cd	4.88±0.07c	4.08±0.39bcd
Lactic acid 1.25%	4.34±0.23d	4.46±0.34de	4.77±0.12c	3.69±0.26cd
Lactic acid 2%	3.34±0.23e	4.09±0.29e	4.19±0.26d	3.18±.27e

The values represent mean ± SD of three experiments. Mean values with different letters within the same column indicate significant difference ($P < 0.05$).

Table (2): Reduction % in food borne pathogens chicken carcasses samples dipped in different concentrations of decontaminators

	Salmonella spp.	Staph. Aureus	E. coliO157	L. monocytogenes
Chlorine 30	26.02	14.29	9.56	16.64
Chlorine 50	26.57	15.70	16.61	20.60
Chlorine 70	29.16	19.58	20.47	30.43
TSP 3%	34.88	8.11	17.45	21.74
TSP 5%	36.78	10.58	21.48	21.74
TSP 8%	38.01	27.69	27.85	25.14
Lactic acid 0.75%	35.42	17.28	18.12	22.87
Lactic acid 1.25%	40.87	21.34	19.97	30.24
Lactic acid 2%	54.50	27.87	29.70	39.89

4. DISCUSSION

Chlorines are powerful germicides where its molecules penetrate the bacterial cell wall and react with key enzymes to prevent normal respiration and carbohydrate metabolism (Chmielewski & Frank, 2003). Similar results were reported by Mart et al. (2013), where they reduced *Salmonella* contaminating chicken meat by 2.32 log cfu/g by treating inoculated parts with 50 ppm chlorine solution. On the other hand lower decontaminating effect was reported by Fabrizio et al. (2002), where *salmonella* counts were reduced 0.86 log CFU/g by 20 to 50 ppm. chlorine solution . On contrary, higher reduction of *Salmonella* contaminating chicken meat was achieved by Whyte et al. (2001), where the mean count was 1.04 log cfu /g, 25 ppm. chlorine solution caused complete decontamination, where *Salmonella* was not detected after dipping. The same reduction in *St. aureus* count achieved by 70 ppm chlorine solution in this study (from 5.67±0.38 to 4.56±0.21 log cfu/g) was reported by Aksoy (2003), although they used 200 ppm chlorine solutions . On the other hand, Hecer et al. (2007) reduced 28.57% of *St. aureus* on chicken carcasses using 30 ppm chlorine solution. Higher reduction percent of *E.coli*O_{157}:H_7 was reported by Hecer et al. (2007), where 30 ppm chlorine solution could reduce 31.25% of *E.coli* on chicken carcasses. Moreover, Sheen et al. (2012) indicated that permanent damage may have occurred to some *E. coli* O_{157}:H_7 cells, with chlorine exposure. The *E. coli* O_{157}:H_7 count reduction was around 3.0 log cfu/ml at chlorine levels of 25 - 50 ppm. On contrary, Northcutt et al. (2005) reported that adding chlorine at 50 mg/l to the water in a broiler inside–outside bird spray wash station did not have any effect on the numbers of *E. coli*. Regarding reduction of *L.monocytogenes*, higher decontaminating effect was reported by Sheen et al. (2011) who stated that chlorine levels of 25 ppmto 50 ppm reduced *L. monocytogenes* by 2.5 log cfu/mL in vitro. Moreover, in the presence of 45 ppm chlorine, *L. monocytogenes* counts in chicken breast meat were reduced by about 0.12 log most probable number/g (Goncalves et al., 2005). Another study made by Tsai et al. (1992) indicated that 40 ppm chlorine reduced *L. monocytogenes* in poultry by only 37% to 50% in 3 to 5 min. Lower reduction in *L. monocytogenes* count was achieved by Oh et al. (2014), where the bacterial populations were reduced by 0.03, 0.06, and 0.22 log cfu/g after treatments of 50 ppm, 100 ppm, and 200 ppm chlorine. At the cell membrane level, TSP at a high pH (pH 12) helps to remove fat films and exerts surfactant or detergent effect. The loss of cell viability, membrane integrity and

disruption of cytoplasmic and outer membranes of the microorganisms (Capita et. al., 2002). Lower reduction in *salmonella* count was reported by Del Rio et al. (2006) where *S. typhimurium* initial count was 5.5 log cfu/g, which was reduced to 3.5 log cfu/g during decontamination using 12 % TSP. Moreover, Fabrizio et al. (2002) reduced 1.41 log10 *Salmonella* count by decontaminating chicken meat by 10 % TSP. Lillard (1994) found that dipping chicken carcasses in a 10% TSP solution for 15 minutes reduced *Salmonella* levels by 2 log10 cycles. On the other hand complete decontamination of *Salmonella* inoculated on chicken meat was achieved by Whyte et al. (2001), where *Salmonella* count was 1.04 log cfu/g which in not detected after application of 10 % TSP dipping. On the other hand, nearly similar results were achieved by Capita et al. (2002), where they reduced *L. monocytogens* count from 7.02 to 5.78 log cfu/g using 8% TSP solution. Moreover, higher reduction in count was reported by Aksoy (2003) where the initial *St. aureus* count was 5.74 log cfu/g, which was decreased to 2.58 log cfu/g by dipping inoculated chicken meat in 12 % TSP solution. While in case of *E.coliO157*, Somers et al. (1994) reported that *E. coli O157:H7* (105 cfu/cm^2 of biofilm cells) was eliminated by a 30-second treatment with 1% TSP. On contrary, lower reduction in *L.monocytogens* count was reported by Del Rio et al. (2006), where the initial count was 6.25 log cfu/g, which was decreased to 5.25 log cfu/g by dipping inoculated chicken meat in 12% TSP solution. The antimicrobial activity occurs through the diffusion of lactic molecules in to microbial cells until equilibrium is reached, in accordance with the pH gradient, causing membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis and accumulation of toxic anions and ultimate death of microbial cells (Ibrahim et al. 2008) . Similar results of *S. typhimiurium* reduction were reported by Dan et al. (2012), who reduced 2.89 log$_{10}$ of *salmonella typhimurium* count by dipping chicken meat in 1 % and 2 % lactic acid. While 100 % reduction percent was achieved in the same study when the sample was treated by 3 % lactic acid. Moreover, Sudershan et al. (2011) examined the decontaminating effect of 0.5%, 1%, 2% and 3% lactic acid on *Salmonella*, where the count was reduced from 2.17 log cfu/g to not detectable level. Moreover, Mulder et al., (1978) reported 2 log cfu/g reductions in *Salmonella* count on chicken meat treated by 1% lactic acid. Moreover, higher reduction percentages were achieved by Hecer and Guldas (2011), where *E. coliO157* counts were reduced as 16% and 56% with lactic acid solution (0.5 and 1.0%). On the other hand, relatively higher reduction percentage was achieved by Aksoy (2003) who decreased *St. aureus* count on chicken meat from 5.74 to 2.93 log cfu/g by dipping inoculated samples in 2 % lactic acid solution. While, nearly similar results were reported by Sudershan et al (2011) where they examined the effect of different concentration of lactic acid (0.5%, 1%, 2% and 3%) on *St. aureus* contaminating chicken meat , where counts reduced from 3.08 log cfu/g to 1.79 log cfu/g . Finally, higher reduction in *L. monocytogens* count was reported by Dan et al. (2012), where the counts decreased 1.56 to 4.45 log$_{10}$ after dipping chicken meat in 1% and 2% lactic acid solutions. From the obtained results, it could be realized that *S.typhimurium* was the most reduced microorganism, followed by L. monocytogenes, then *E.coliO157* and finally *S. aureus*, which was the least reduced microorganism. On the other hand this study proved that lactic acid 2% was the best decontaminator (among the examined decontaminators), where it achieved the highest reduction percentage.

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