



REDUCTION OF MICROBIAL CONTAMINATION OF WHOLE BROILER CHICKEN CARCASSES DURING PROCESSING

F.A. Khalafalla¹, N.S. Abdel-Atty¹, Soad, A. Nasef² and Adel, S. Hanafy^{2*}

¹Faculty of Veterinary Medicine, Beni Suef University, Egypt.

²Animal Health Research Institute, Dokki, Giza, Egypt.

*Corresponding author: Adel, S. Hanafy; E-mail: vet_adel2010@yahoo.com

ABSTRACT

Contamination of broiler carcasses during processing with several microorganisms as salmonella, campylobacter, E. coli and staphylococcus aureus is frequently occurring. Scalding, defeathering, chilling are critical points at which cross contamination may occur during processing. Recently several interventions for carcass decontamination have been employed in order to reduce the levels of microbial hazards on poultry carcasses during processing; among which chlorine and organic acids are the most common. This study was carried out in a traditional poultry abattoir in Fayoum Governorate in order to improve the microbial quality of broiler carcasses. During 10 replicate a total of 160 carcasses were collected at 4 sampling points in the processing line (scalding, defeathering, final wash and chilling). Bacterial counts recovered from broiler carcasses rinse were lowered by 1.3, 1.3, 0.3 and 0.5 Log₁₀ cfu/ml after treatment of scalding water with 100 ppm Calcium hypochlorite in scalding. Adding peracetic acid (PAA) 50 ppm + 0.5 % acetic acid to the defeathering machine spraying system during defeathering lowered the count of coliforms and faecal coliforms significantly (P ≤ 0.05) by 1.5 and 1.6 Log₁₀ cfu/ml of carcass rinse, while it wasn't significant for E. coli and staphylococcus counts (1.2 and 0.95 Log₁₀ cfu/ml), respectively. Furthermore, significant differences in the reduction of all bacterial counts were observed in the washing stage (P ≤ 0.05) after treatment of broiler carcasses with a mixture of lactic acid 1 % + acetic acid 1 % in the final washing step. At chilling stage Na hypochlorite 50 ppm reduced the bacterial counts by 2, 2.2, 1.3 and 0.8 log₁₀ cfu/ml of carcass rinse for coliforms, faecal coliforms, Escherichia coli and staphylococcus aureus, respectively. E. coli, salmonellae, staphylococcus aureus and campylobacter spp. were reduced by different percentages. The used interventions effectively or significantly reduced microbial populations on broiler chicken carcasses during processing.

Keywords: Acetic acid, chlorine, decontamination, poultry processing, Peracetic acid.

Original Article:

DOI: <https://dx.doi.org/10.21608/javs.2019.62670>

Received 18 June, 2018.

Accepted 22 February, 2019.

Published in April, 2019.

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INTRODUCTION

Poultry meat is more popular in consumer market, inspite; they are frequently contaminated with wide variety of biological hazards of public health significance during processing such as Salmonella, Campylobacter, Staphylococcus aureus, Escherichia coli and Listeria., rendering it responsible for a significant number of human food poisoning cases which remains an important public health issue (Dincer and Baysal, 2004 and Scallan *et al.*, 2011). A

live healthy bird carries high numbers of different microorganisms on its feathers, skin, feet and in the intestine. The heavy bacterial loads coming the processing plant with live birds can be disseminated throughout the plant during processing (Berrang *et al.*, 2000 and Göksoy *et al.*, 2004).

Broiler slaughter's is a multi-stage operation consists of several processes as scalding, defeathering, evisceration, washing, chilling and packaging at any

point of which cross contamination can occur (**Rosenquist et al., 2006** and **Allen et al., 2007**). Although processing generally reduces overall microbial contamination of broiler carcasses (**Göksoy et al., 2004**), cross-contamination between carcasses can occur from different sources such as scalding, chilling, processing water, feather plucking, evisceration and washing, equipment and operators' hands. These operations have potential to cause a significant increase in the prevalence of bacterial pathogens in broiler carcasses (**Berrang et al., 2001** and **Nidaullah et al., 2017**).

Nowadays microbial safety and quality of commercially produced broiler carcasses are important goal and a major areas of concern. Reduction of broiler carcasses contamination and improving its shelf-life are important goals and critical objectives in relation to poultry meat production (**Okolocha and Ellerbroek, 2005**). Multiple intervention strategies are used in the processing plant to reduce and eliminate bacterial contamination on broiler carcasses during processing as single intervention not enough. Addition of decontaminant agent is an important approach to control biological hazards during poultry processing. A variety of antimicrobial treatments for control of microbial contamination and improving the safety of broiler carcasses during processing have been investigated (**Dickens and Whittemore, 1997**). The commonly used antimicrobial agents are chlorine, and organic acids.

Chlorine is generally recognized as safe (GRAS) status and the most widely antimicrobial agent used as sanitizer in commercial poultry-processing facilities. It is inexpensive and relatively effective against microorganisms found in poultry-processing environments and has the ability to kill a wide range of microorganisms on carcasses, in processing water, and on processing equipment (**Tsai et al., 1995**). Furthermore it can be used in washing, immersion chillers, or equipment sprays to reduce microbial contamination and cross-contamination (**Bailey et al., 1986; Northcutt et al., 2003** and **Bashor et al., 2004**). Organic acids such as acetic and lactic acid, recognized as safe (GRAS) interventions and are used extensively by the meat and poultry industries to reduce bacterial contamination on carcass surfaces and increase the shelf-life (**Dickson and Anderson, 1992**).

Several studies demonstrated the effectiveness of lactic acid as an antimicrobial intervention in poultry processing intervention (**Yang et al., 1998**). Acetic acid has been used by various researchers in the scald water (**Izat et al., 1989**), picking sprays (**Dickens and whittimore, 1997**), prechill (**Dickens and**

Whittimore, 1994) and chill tanks (**Dickens and Whittemore, 1995**) to reduce microbial counts of broiler carcasses. Peracetic acid (PAA) is one of the approved antimicrobials which can be used in the processing plants in both chiller applications and post-chill immersion tanks during poultry processing. Previous studies revealed that PAA is the more effective antimicrobial as compared with chlorine (**Bauermeister et al., 2008** and **Nagel et al., 2013**).

Reducing the microbial hazards on finished carcasses requires implementation of multiple-sequential interventions; previous studies have shown that even if an individual treatment does not have a significant effect, a series of treatments or overall processing may (**Stopforth et al., 2007** and **Berrang and Bailey, 2009**). Therefore the current research was conducted to study the efficacy of some decontaminant chemical agents applied to broiler carcasses in multiple interventions during some processing points to minimize the risk of cross contamination in an attempt to improve the bacteriological quality of broiler carcasses.

MATERIALS AND METHODS

The current investigation was carried out in a traditional poultry abattoir located in Fayoum Governorate; the abattoir was operating 1 kill line at a speed of 50 bird \ hour. Live birds are delivered to the slaughterhouse in transporting crates. Crates are unloaded and birds are manually hanged on the shackle line.

After slaughtering which was manually done without stunning; birds allowed to bleed completely 1-2 min, carcasses proceeded to scalding through immersion in large tank contain hot water at 53-55 °C for 2 min, then the hanged birds in the shackle line pass through series of 4 automated feather-picking machines with rubber fingers (plucker). This step is followed by manually removal of viscera after opening of the body cavity. After giblet harvesting; head, crop, lungs and feet were manually removed then birds pass through washing tank and finally immersion chilling in cold water.

2.1- Experiment design

A total of 10 replicate were conducted; on each replicate 16 broiler carcasses were collected from 4 sampling sites resulting in a total of 160 carcasses were examined. The sampling points were scalding, defeathering, washing and chilling. Four carcasses were collected in each sampling site represented by two control and two treated

carcasses. Control carcasses were collected first before application of any antimicrobial treatment on each sampling site. After all control carcasses were collected, treatment interventions were started as in table (1).

Table 1: Treatment interventions to collected

Site	Treatment
Scalding	<ul style="list-style-type: none"> • Calcium hypochlorite 100 ppm was added to scalding water. • Broiler carcasses immersed in the scalding water for 2.5 min with scalding temperature 53-55°C.
Defeathering	<ul style="list-style-type: none"> • Adding of peracetic acid 50 ppm + 0.5% acetic acid to the defeathering machine spray system. • The spray was started 15 second before carcasses entered the picker to ensure that treatment spray had completely purged the system. • The picking time was 30 second.
Final washing	<ul style="list-style-type: none"> • Adding of organic acid mixture (acetic acid 1% + lactic acid 1 %) to the washing water.
Chilling	<ul style="list-style-type: none"> • Adding sodium hypochlorite 50 ppm to the chiller tank water for 30 min immersion at temperature 4-10 °C.

carcasses

2.2- Preparation of decontaminant

A calculation was performed to determine the preferable final concentration of decontaminant in water. Calcium hypochlorite was used to prepare 100 ppm chlorine by adding 77 gm powder of calcium hypochlorite 65% to 500 litre of water while Na hypochlorite 50 ppm was prepared by adding 417 ml of Na hypochlorite 12 % to 500 litre of the chilling water. Acetic acid was used to prepare 1 % by add 510 ml of acetic acid 99.6% to 50 litre of water while lactic acid was prepared by add 575 ml of lactic acid 88% to 50 liter water.

2.3- Sampling procedure

Broiler carcasses were removed from the processing line at random at each of 4 sites (after exiting scalding tank, after picking, after final wash and after exiting chilling tank), handled with new clean latex gloves and individually placed into a separate

sterile plastic bags. Individual carcasses were subjected to whole-carcass rinses (WCR) technique with 400 ml of 0.1 % sterile buffered peptone water (BPW) added to each bag and shaken by hand for 60 sec; after shaking, carcasses were removed aseptically and the rinse liquid was poured in sterile cups. Following the collection, all the containers of the recovered rinse liquid were placed in ice box and delivered to the reference laboratory for veterinary quality control on poultry production – Fayoum branch within 1 hour for examination.

2.4- Microbiological analysis

Carcasses rinsate for each individual carcass were serially diluted in sterile 0.1% buffered peptone water (BPW) solution. Serial dilutions of the collected rinsate were prepared up to 10^{-6} for examination of the following:

- 1-MPN of Coliforms, faecal coliforms and Escherichia coli: according to (FDA, 2002).
- 2-Staphylococcus aureus count: according to ISO 6888-1 (ISO, 1999).
- 3-Salmonella spp isolation: ISO 6579 (ISO, 2002).
- 4- Campylobacter spp isolation: ISO 10272-1 (ISO, 2006).

E. coli and salmonella isolates were subjected to serological identification in the central lab in Dokki. Group O polyvalent antisera were used for serotyping test by slide agglutination technique.

Data analysis

Mean bacterial counts were converted to \log_{10} colony-forming units per ml (cfu/ml) of carcass rinse solution. Group means of bacterial counts were compared using unpaired T- test for each type of microorganism to determine significant differences in the number of bacteria recovered from treated and control carcasses. Statistical significance was set at P-value of ≤ 0.05 . The data were analyzed using GraphPad InStat version 3 for Windows 95. (GraphPad Software, 1998).

Reduction Of Microbial Contamination

Table 2: Mean log colony-forming units of bacterial counts detected per milliliter (cfu/ ml) of broiler carcasses rinse collected before and after treatments during processing

	Coliform count (MPN)			Faecal coliform (MPN)			E. coli count (MPN)			Staphylococcus aureus count		
	Control	Treated	Log ₁₀ reduction	Control	Treated	Log ₁₀ reduction	Control	Treated	Log ₁₀ reduction	Control	Treated	Log ₁₀ reduction
Scalding	5.2 ^x ± 5	3.9 ^x ±3.5	1.3	5.1 ^x ± 5	3.84 ^x ± 3	1.3	3.7 ^x ± 3	3.4 ^x ± 3	0.3	4.3 ^x ± 3	3.8 ^y ±3	0.5
Defeathering	5.5 ^x ± 5	4 ^y ± 3.6	1.5	5.4 ^x ± 5	3.8 ^y ± 3	1.6	4.7 ^x ± 4	3.5 ^x ±3	1.2	4.95 ^x ± 4	4 ^x ± 3	0.95
Washing	5.7 ^x ± 5	3.5 ^y ± 3	2.2	5.5 ^x ± 5	3.4 ^y ± 3	2.1	5.3 ^x ± 5	3 ^y ± 2	2.3	4 ^x ± 3	< 10 ^y	---
Chilling	5.6 ^x ± 5	3.5 ^y ± 3	2	5.4 ^x ± 5	3.2 ^y ±2.9	2.2	4.2 ^x ± 3	2.9 ^x ± 2	1.3	3.8 ^x ±3	3 ^x ± 2	0.8

^{x,y} Mean bacterial count values within a row with no common superscripts are significantly different for each group at (P ≤ 0.05). Counts are expressed as mean ± S.E (log₁₀ ml). Mean from 10 replications with 4 carcasses per site per replication.

Table 3: Prevalence (%) of isolated microorganisms in broiler carcass rinses collected before and after treatments during broiler processing

site	Treatment	E. coli	Salmonella spp	Staphylococcus aureus	Campylobacter spp
Scalding	Control	16\20 (80 %)	8\20 (40%)	18\20 (90 %)	18\20 (90 %)
	treated	14\20 (70 %)	4\20 (20 %)	8\20 (40 %)	12\20 (60 %)
	Reduction %	12.5 %	50%	55%	33%
Defeathering	Control	16\20 (80 %)	8\20 (40 %)	20\20 (100 %)	14\20 (70 %)
	treated	16\20 (80 %)	6\20 (30 %)	14\20 (70 %)	14\20 (70 %)
	Reduction %	Zero	25%	30%	Zero
Washing	Control	18\20 (90 %)	10\20 (50 %)	12\20 (60 %)	16\20 (80 %)
	treated	10\20 (50 %)	2\20 (10 %)	zero	4\20 (20 %)
	Reduction %	44%	80%	100%	75%
Chilling	Control	16\20 (80 %)	10\20 (50 %)	12\20 (60 %)	16\20 (80 %)
	treated	12\20 (60 %)	6\20 (30 %)	6\20 (30 %)	8\20 (40 %)
	Reduction %	25 %	40%	50%	50%

Table 4: Isolates serotypes of Salmonella and E. coli

Stage	Salmonella	E. coli
Scalding	S.Virchow, S. Aba, S. Kentucky	O1, O127, O26
Defeathering	S.Virchow, S. Aba	O119, O44
Washing	S. Aba, S. Infantis	O44, O26 , O78
Chilling	S. Aba, S.Virchow, S. Kentucky	O26, O78

DISCUSSION

Immersion scalding presents one of the earliest opportunities for carcass cross-contamination to occur earlier reports that emphasized that the efficacy of chlorine as an antimicrobial agent is affected by pH, the presence of organic material, and contact time. In this context, **Byrd and McKee (2005)** found that the efficacy of chlorine for bacterial reduction decreases with increasing pH and organic load. Traditional scalding of poultry use only one single tank which serve as a mean of cross contamination, birds entering the slaughterhouse are often heavily contaminated with faecal material, when birds are immersed in the scalding tank, dirt, fecal material, and other surface contaminants are removed this results in contaminated scald water making the scald tank a source of cross-contamination for subsequent carcasses, this agreed with that reported by (**Cason et al., 1997** and **Göksoy et al., 2004**).

Previous research treated the scalding water with chemicals such as acetic acid observed no significant difference on bacterial counts or salmonella incidence in broiler carcasses when scald water treated with acetic acid 0.5% (**Lillard et al., 1987**). Furthermore **Izat et al., (1990)** used lactic acid at concentrations of 1% in scalding and found no reduction in the incidence of salmonellae on carcasses subjected to lactic acid in the scald water. On contrast, **Sakhare et al., (1999)** found that counts of staphylococcus aureus, and coliforms were lower in birds scalded in acidified water, although, this reduction was not significant. Moreover, spray washing of carcasses with acetic or lactic acid after scalding reduced the counts of staphylococcus aureus and coliforms significantly.

Results from table (2) showed that peracetic acid 50 ppm + acetic acid 0.5% spray during defeathering reduced coliforms (MPN), faecal coliforms (MPN), E. coli (MPN) and staphylococcus aureus count by 1.5, 1.6, 1.2 and 0.95 log₁₀ cfu/ml of carcass rinse, respectively. Salmonella spp. and

during the processing operation, the scald tank is considered to be the major site of cross contamination from one carcass to another through scald water.

Calcium hypochlorite 100 ppm in scalding water reduced coliforms (MPN), faecal coliforms (MPN), E. coli (MPN), staphylococcus aureus count by 1.3, 1.3, 0.3 and 0.5 log₁₀ cfu/ml of carcass rinse, respectively. This reduction was significant only for staphylococcus aureus count while, counts of coliforms (MPN), faecal coliforms (MPN), E. coli (MPN) didn't significantly altered (table 2). Moreover, E. coli, salmonella spp, staphylococcus aureus and campylobacter spp were reduced by 12.5%, 50%, 55% and 33 %, respectively (table 3). The isolated E. coli serotypes during this stage were O1, O127, O26 while salmonella serotypes were S.Virchow, S. Aba, S. Kentucky (table 4). This finding supports

staphylococcus aureus were reduced by 25% and 30%, respectively while E. coli and campylobacter spp. remain the same (table 3). The isolated E. coli serotypes during this stage were O119 and O44 while the isolated salmonella serotypes were S.Virchow, S. Aba (table 4).

Data from table (2) showed that spray of broiler carcasses during picking with mixture of peracetic acid 50 ppm and acetic acid 0.5% reduced the count of coliforms and faecal coliforms significantly while it wasn't significant for E. coli and staphylococcus aureus counts ($p \leq 0.05$).The defeathering machine is also reported as a major site of bacterial cross contamination of broiler carcasses during processing because of contact with contaminated rubber picking fingers (**Geornaras et al., 1997**). External pressure exerted on the lower abdomen by the picker's rubber fingers induce some pressure on the carcasses so that highly contaminated fecal material is forced out from the vent contaminating carcasses during picking (**Berrang et al., 2001**).

Previous investigations have shown that, in counts of campylobacter, salmonella and staphylococcus aureus increase following plucking (**Abu-Rwaida et al., 1994; Geornaras, et al., 1997** and **Berrang et al., 2001**). Previously published reports investigated many antimicrobial interventions to lessen bacterial contamination on broiler carcasses during or after defeathering stage. A study conducted by **Berrang et al., (2011)** reported that spraying chlorine dioxide during defeathering lower numbers of campylobacter, E. coli and salmonella on broiler carcasses. Furthermore **Sakhare et al., (1999)** observed significant reduction in coliform and staphylococcus aureus counts on broiler carcasses after the acid treatment (dipping or spraying lactic or acetic acid after defeathering). On the other hand, **Lillard et al., (1987)**

found that spray solution of 0.5% acetic acid in the plucking machine spray line did not improve the bacterial quality of picked carcasses.

Little or no attention has been given to the effects of PAA as antimicrobial in the spray water of defeathering equipment. PAA is commonly used as alternative to chlorine during chilling and post chilling (**Bauermeister et al., 2008**) in this respect, **Nagel et al., (2013)** reported reductions in both salmonella and campylobacter over 2.0 log₁₀ cfu/mL when use PAA in post-chill immersion tank. Based on the result of intervention used in this study during defeathering stage it would be advisable to add 0.5% acetic acid + PAA 50 ppm to the spray water of defeathering machine to improve microbiological quality of broiler carcasses.

It is common for plants to apply any form of washing after evisceration as broiler evisceration can result in gut rupture and leakage, potentially increasing bacterial numbers on carcasses. However, using water without antimicrobial agent in washing stage may not significantly reduce carcass coliform or E. coli counts (**Northcutt et al., 2003**). Data presented here showed that the bacteriological quality of broiler carcasses was improved and the microbial count was significantly decreased ($p \leq 0.05$) after addition of acid mixture (lactic acid 1% + acetic acid 1%) during washing stage as compared with control carcasses washed with water alone, results from table (2) showed that all the bacterial count were reduced significantly at $p \leq 0.05$. Coliforms (MPN), faecal coliforms (MPN), E. coli (MPN) were reduced by 2.2, 2.1, 2.3 log₁₀ cfu/ml respectively, in addition Staphylococcus aureus count was lowered to non-detectable level. E. coli, salmonella spp, Staphylococcus aureus and campylobacter spp were reduced by 44 %, 80%, 100% and 75% respectively (table 3). The isolated E. coli serotypes during this stage were O44, O26 , O78 while the isolated salmonella serotypes were S. Aba, S. Infantis (table 4).

Northcutt et al., (2005) observed that washing with 50 ppm of chlorinated water did not significantly reduce the counts of E. coli, Salmonella or Campylobacter recovered from the carcasses rinse. Moreover, **Killinger et al., (2010)** reported that chlorine produce much smaller reduction than that observed by 2% lactic acid immersion of broiler carcass after washing which produce significant microbial reductions in coliform and salmonella levels. Several studies investigating the antibacterial efficacy of lactic acid spraying on prechill carcasses and found salmonella reductions by 0.6–1.8 log CFU/carcass (**Yang et al., 1998**). Moreover, a study conducted by **Xiong et al., (1998)** illustrated that 1% and 2% lactic acid solution sprayed on chicken skin samples brought about a 2.2 log₁₀ reduction in Salmonella on chicken

carcasses. Similarly, **Rasschaert et al., (2013)** reported that after submerging broiler carcasses in a 1.5% lactic acid solution, significant reduction of campylobacter 1.62 and 1.24 log cfu/carcass were observed compared with the control carcasses submerged in water. The results obtained during this study indicated that mixture of lactic acid 1% + acetic acid 1% combination could significantly reduce the microbial load on carcasses, minimize the cross contamination and was found to be the more effective as a decontamination intervention through the final washing stage during broiler processing.

The carcass chilling process is considered as a critical step in poultry processing. Water immersion chilling is the most widely used method to lower broiler carcass temperature during processing. Traditionally, chlorine is the antimicrobial commonly applied in commercial poultry processing plants and is allowed up to 50 ppm for the chlorination of chilled water to prevent cross-contamination and reducing bacterial contamination (**Tsai et al., 1995**). Results from table (2) showed that Na hypochlorite 50 ppm during chilling stage reduce coliforms (MPN), faecal coliforms (MPN), E. coli (MPN) and Staphylococcus aureus count by 2, 2.2, 1.3 and 0.8 log₁₀ cfu/ml of carcass rinse.

The reduction in counts of coliforms and faecal coliforms was significant ($p \leq 0.05$) while it appear to be small or non-significant reduction on E. coli and Staphylococcus aureus counts. E. coli, salmonella spp, staphylococcus aureus and campylobacter spp. were reduced by 25 %, 40%, 50% and 50%, respectively. In this context, **Stopforth et al., (2007)** noticed that the bacterial count, coliforms and E. coli were significantly lowered after using chlorine 50 ppm in chilling. Furthermore, **Yang et al., (2001)** reported reduction of salmonella and campylobacter to non-detectable levels with 30 and 50 ppm of chlorine in chill water. Also, **Mead et al., (1995)** examined the effect of process water chlorination at several stages in poultry slaughter including the chiller water and found that the numbers of campylobacter were significantly reduced.

In a study conducted by **Lillard (1980)** comparing chlorine treated chill water with non chlorinated chill water, the researcher found that the incidence of Salmonella and faecal coliforms was significantly lower in chlorine-treated chill water versus non-chlorinated chill water. This is in consistent with a previous study by **Izat et al., (1989)** in which chlorine 100 ppm in chill water reduced salmonella significantly. However, **Fabrizio et al., (2002)** used a lower concentration of chlorine (20 ppm) was used in immersion chilling treatment and yield non-significant reduction on salmonella, E. coli, and coliforms.

CONCLUSION

From data in the present study it can be concluded that decontamination of broiler carcasses at each stage of processing either by addition of chlorine to scald and chill water, spraying of carcasses during defeathering or add organic acids to washing water help in minimizing the cross contamination and improve the microbiological quality of processed carcasses. Therefore multiple sequential antimicrobial interventions is of great importance in reducing the bacterial population in broiler carcasses during processing.

ACKNOWLEDGMENT

We are extremely grateful to the members of Reference lab for veterinary control on poultry production Fayoum branch for their help in the accomplishment of this work.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript

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How to cite this article:

F.A. Khalafalla; N.S. Abdel-Atty; Soad A. Nasef and Adel S. Hanafy, 2019. Reduction of Microbial Contamination of Whole Broiler Chicken Carcasses During Processing. *Journal of Applied Veterinary Sciences*, 4(1): 5- 12.

DOI : <https://dx.doi.org/10.21608/javs.2019.62670>