Effect of some chemical decontaminants on the survival of *Listeria monocytogenes* and *Salmonella* Typhimurium with different attachment times on chicken drumstick and breast meat

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Abstract The aim of this study was to investigate the efficacy of some decontaminant agents on the survival of *Salmonella* Typhimurium and *Listeria monocytogenes* with different attachment periods to chicken drumstick with skin and skinless breast meat. For this purpose, *S. Typhimurium* and *L. monocytogenes* were given periods of 0.5 (30 s), 20 and 210 min to attach to the chicken drumstick and breast meat. At the end of the each attachment period, the meat samples were treated with lactic acid, (2 and 4%), cetylpyridinium chloride (0.5%) and acidified sodium chlorite (1200 ppm). In the drumstick sample treated with cetylpyridinium chloride, the reduction level of *L. monocytogenes* with 30 s attachment period was 3.2 log₁₀ CFU/ml while the reduction level was found to be 2.2 log₁₀ - CFU/ml with 20 min attachment period. Decontamination with acidified sodium chlorite resulted in reduction of 1.8 log₁₀ CFU/ml in *S. Typhimurium* attached to the chicken drumstick for 30 s while the reduction levels of *S. Typhimurium* with 20 and 210 min attachment periods were 1.2 and 1.3 log₁₀ CFU/ml, respectively. The results indicated that some antimicrobial agents have more strong effect on *L. monocytogenes* and *S. Typhimurium* on the chicken meat parts in the first 30 s of attachment. However, there were no changes in the efficacy of the decontaminants on the survival of *L. monocytogenes* and *S. Typhimurium* on chicken meat when the attachment time of these bacteria were extended from 20 min to 210 min.

Keywords *Listeria monocytogenes* · *Salmonella* Typhimurium · Attachment period · Chicken meat · Decontamination

Introduction

Poultry meat is an important food source for humans. On the other hand, poultry meat has been noted as important reservoir of some foodborne pathogen bacteria such as *Salmonella* spp., *Listeria monocytogenes* and some of other pathogen bacteria (EFSA 2015; Dias et al. 2016). Many chemical decontaminants have been tested to eliminate those pathogens on poultry meat (Sohaib et al. 2016). Despite all these efforts, *Salmonella* spp. and *L. monocytogenes* continue to remain as a significant public health problem in poultry meat industry (Dias et al. 2016).

As it is known, the first step in meat contamination is bacterial contact to meat surface and then attachment. From past to present, factors affecting bacterial attachment to meat surfaces and bacterial attachment mechanism have been investigated (Kinsella et al. 2007; Zulfakar et al. 2013; Giaouris and Nesse 2015). As understood from those studies, the procedure of bacterial attachment to surfaces is a complex phenomenon.

Bacterial contamination of chicken carcasses during slaughter process is unavoidable (Dias et al. 2016). Slaughter process of a chicken may take 3–4 h (the period from scalding tank to packaging), and bacterial contamination may occur at any time of the production process. On the other hand, many decontamination studies have been conducted to eliminate or reduce the foodborne pathogens on chicken meat, and in those studies the contact times that were given to bacteria for the attachment to meat surface are highly variable (changing from 2 to 60 min) (Dikici

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et al. 2013; Lee et al. 2014; Karuppasamy et al. 2015). It has been reported that *Salmonella* cells that firmly attached to poultry skin are significantly more resistant to chemical interventions (Mikolajczyk 2015). Although many studies have been conducted to understand the mechanism of bacterial attachment and the resistance of firmly attached bacteria to chemical interventions (Giaouris 2015), there is no information whether there is an interaction between different bacterial attachment periods to chicken meat surface and the effectiveness of chemical agents used for decontamination interventions.

As mentioned above, bacterial contamination of chicken meat may occur at any time during slaughter process. The objective of this study was to investigate whether there is a change in the efficacy of some decontaminant agents on the survival of *S. Typhimurium* and *L. monocytogenes* with different attachment periods to chicken drumstick with skin and skinless breast meat.

Materials and methods

Chemicals

L (+) Lactic acid solution (88–92%) and Cetylpyridinium chloride (CPC) were purchased from Sigma-Aldrich (Seelze, Germany). Peptone water and Sodium chlorite were obtained from Merck (E. Merck, Darmstadt, Germany). Acidified sodium chlorite (ASC, 1200 ppm wt/v, pH 2.46) was prepared by adding citric acid (Tekkim, Turkey). Tryptic Soy Broth (TSB) and Xylose Lysine Deoxycholate Agar (XLD) were obtained from Acumedia (Baltimore, Maryland). Palcam Agar Base and its supplement (SR0150E) were from OXOID (Basingstoke, England).

Bacterial inocula

Three *S. Typhimurium* (NCTC 12416, NCTC 74 and ATCC 14028) and three *L. monocytogenes* (N 7144, RSKK 472 and 476 (Refik Saydam National Public Health Agency-Turkey)) strains were used in this study. Each strain was individually grown in tubes containing 10 ml of tryptic soy broth (TSB) at 37 °C for 18 h. Following incubation, the cultures were centrifuged at $4192 \times g$ for 10 min at 5 °C, and then the resulting pellets were washed in 0.1% sterile peptone water and re-centrifuged to remove organic residues. The pellets of each strain were suspended in 0.1% sterile peptone water. To prepare the bacterial cocktail, these suspensions were combined in a separate tube. This bacterial cocktail that composed of *S. Typhimurium* (approximately $9.0 \log_{10}$ CFU/ml) and *L.*

monocytogenes (approximately $9.3 \log_{10}$ CFU/ml) was used immediately.

Inoculation of chicken meat samples, and treatments

The chicken drumstick with skin and skinless breast meat were obtained from a local supermarket on the day of experiment. Before inoculation procedure, breast meat samples were cut to 3 pieces of about 3–4 cm in size with sterile scalpel. For the inoculation, 0.5 ml bacterial cocktail that composed of *S. Typhimurium* and *L. monocytogenes* was spread on the chicken drumstick and breast meat samples using a sterile disposable spreader. After inoculation, the attachment period of 0.5 and 20 min at room temperature (22–25 °C) and 210 min at refrigeration temperature (4 °C) were given to the cultures for the bacterial attachment to the chicken meat samples. The reason of choosing 0.5 min (30 s) attachment period is that this period was practically the shortest duration to spread the bacteria to all over the chicken meat surface and then starting decontamination procedures in a laboratory condition. The attachment period of 20 min was one of the average attachment periods chosen in many decontamination studies. The reason of choosing 210 min (3.5 h) attachment periods at refrigeration temperature was for simulating the average maximum time spent for chilling chicken carcasses in air-chilling room in slaughterhouse. At the end of the each attachment period, a sample was taken and dipped into a decontaminant solution for 1 min. The chemicals used were lactic acid (2 and 4%), CPC (0.5%) and ASC (1200 ppm). Physiological saline solution (sterile 0.85% NaCl) was used for the control treatment. For each treatment, duplicate sample were used.

Microbiological sampling

At the end of the each attachment period, two chicken drumsticks and two breast meat samples were used for microbiological analyses. The sample placed into a sterile stomacher bag and added 100 ml of 0.1% sterile peptone water. The sample was rinsed by manually massaging for 2 min, and then 1 ml solution was taken and serially diluted in 0.1% sterile peptone water. One hundred microliters of diluted suspensions were surface plated on Xylose Lysine Deoxycholate Agar and Palcam Agar Plate to enumerate *S. Typhimurium* and *L. monocytogenes*, respectively. After incubation at 35 °C for 24–36 h, characteristic colonies were counted.

Statistical analyses

Three independent replicates were performed on different days. Before calculation of means and standard deviations,

microbiological data were converted to $\log_{10}$ CFU/ml values. The data were analyzed using Analysis of Variance (ANOVA) appropriate to meat type $\times$ treatment groups $\times$ attachment time to determine fixed effects and interactions between variables. All analyses were conducted by using Statistical Analyses System (SAS Institute, Carry, NC). Significance was determined at the $P \leq 0.05$ level.

Results and discussion

The attachment procedure is highly complex event and may be affected from many factors (such as cell surface charge, cellular constituents, hydrophobicity, bacterial population density, culturing temperature, characteristic of surface etc.) (Goulter et al. 2009; Zulfakar et al. 2013; Giaouris and Nesse 2015). To our knowledge, there is no study investigating the efficacy of decontaminant agents on survival of pathogenic bacteria with different attachment periods to poultry meat parts.

The results obtained from control groups showed that the reduction levels of both *L. monocytogenes* and *S. Typhimurium* on the chicken drumstick and breast meat were approximately the same after the samples were dipped into the physiological saline solution for 1 min (Tables 1, 2). These results may be interpreted as loosely attached or unattached bacteria counts are very few (between 0.03 and 0.5 $\log_{10}$ CFU/ml) for both bacteria, and although the attachment periods of the bacteria were extended, there was no significant change in the number of the attached bacteria. Based on this result, it can be said

that *L. monocytogenes* and *S. Typhimurium* can moderately or firmly attach to chicken skin or meat surface in time less than 0.5 min (30 s).

The reduction levels of *L. monocytogenes* with different attachment periods to the chicken meat parts and treated with different chemical agents were shown in Table 1. Depending on the meat type, initial inoculation levels of *L. monocytogenes* changed between 6.0 and 6.8 $\log_{10}$ CFU/ml. After all attachment periods, the reduction levels of *L. monocytogenes* in the samples treated with CPC (0.5%) were higher than those of the samples treated with other chemicals ($P < 0.05$). After the attachment period of 30 s and decontamination with CPC, the reduction level of *L. monocytogenes* on the chicken drumstick was 3.2 $\log_{10}$ - CFU/ml, and this reduction level decreased to 2.2 and 2.0 $\log_{10}$ CFU/ml as the attachment periods extended to 20 and 210 min. The result of the study showed that the resistance of *L. monocytogenes* against CPC increased as the attachment period extended ($P < 0.05$). However, the result in the skinless breast meat was different. The resistance of *L. monocytogenes* against CPC did not increase when the attachment period was extended from 30 s to 20 min or 210 min on the skinless breast meat ($P > 0.05$). Besides, the longer attachment periods did not cause an increase in the resistance of *L. monocytogenes* against lactic acid (2 and 4%) and ASC (1200 ppm) on the chicken drumstick with skin and the skinless breast meat (Table 1).

The reduction levels of *S. Typhimurium* with different attachment periods to the chicken meat parts and treated with different chemical agents were shown in Table 2. Depending on the meat type, initial inoculation levels of *S. Typhimurium* changed between 6.0 and 6.8 $\log_{10}$ CFU/ml.

Table 1 Efficacy of some decontaminant agents on reduction of *Listeria monocytogenes* with different attachment periods to the chicken drumstick with skin and skinless breast meat ($\log_{10}$ CFU/ml $\pm$ SD)

Treatments		Inoculation level	Attachment period (min) and reduction level of bacteria		
			0.5	20	210
Chicken drumstick with skin	Control ^a	6.4 ^A $\pm$ 0.25	0.5 ^{Av} $\pm$ 0.23	0.3 ^{Avx} $\pm$ 0.21	0.03 ^{Av} $\pm$ 0.23
	2% lactic acid	6.0 ^A $\pm$ 0.35	0.6 ^{Av} $\pm$ 0.13	0.4 ^{Avx} $\pm$ 0.18	0.5 ^{Avx} $\pm$ 0.18
	4% lactic acid	6.7 ^A $\pm$ 0.23	0.9 ^{Bvx} $\pm$ 0.26	0.8 ^{Bvx} $\pm$ 0.22	0.9 ^{Bvx} $\pm$ 0.20
	0.5% CPC	6.6 ^A $\pm$ 0.18	3.2 ^{Bz} $\pm$ 0.17	2.2 ^{Cyz} $\pm$ 0.17	2.0 ^{Cy} $\pm$ 0.15
	1200 ppm ASC	6.6 ^A $\pm$ 0.15	1.4 ^{Bxy} $\pm$ 0.13	1.1 ^{Bxy} $\pm$ 0.20	1.1 ^{Bx} $\pm$ 0.19
Skinless chicken breast meat	Control ^a	6.5 ^A $\pm$ 0.20	0.4 ^{Av} $\pm$ 0.12	0.2 ^{Av} $\pm$ 0.12	0.3 ^{Avx} $\pm$ 0.20
	2% lactic acid	6.2 ^A $\pm$ 0.23	0.7 ^{Bv} $\pm$ 0.21	0.7 ^{Bvxy} $\pm$ 0.16	0.5 ^{ABvx} $\pm$ 0.23
	4% lactic acid	6.8 ^A $\pm$ 0.13	0.8 ^{Bvx} $\pm$ 0.11	0.8 ^{Bvx} $\pm$ 0.14	0.9 ^{Bvx} $\pm$ 0.20
	0.5% CPC	6.7 ^A $\pm$ 0.23	2.1 ^{By} $\pm$ 0.15	2.5 ^{Bz} $\pm$ 0.13	2.0 ^{By} $\pm$ 0.35
	1200 ppm ASC	6.8 ^A $\pm$ 0.16	0.7 ^{Bv} $\pm$ 0.26	0.7 ^{Bvx} $\pm$ 0.20	0.8 ^{Bvx} $\pm$ 0.19

^{ABC}The means with different superscript in the same line are significantly different ($P < 0.05$)

^{vxyz}The means with different superscript in the same column are significantly different ($P < 0.05$)

^aPhysiological saline water (0.85% NaCl)

Table 2 Efficacy of some decontaminant agents on reduction of *Salmonella* Typhimurium with different attachment periods to the chicken drumstick with skin and skinless breast meat ($\log_{10}$ CFU/ml $\pm$ SD)

	Treatments	Inoculation level	Attachment time (min) and reduction level of bacteria		
			0.5	20	210
Chicken drumstick with skin	Control ^a	6.1 ^A $\pm$ 0.24	0.4 ^{Avxy} $\pm$ 0.23	0.5 ^{Avxy} $\pm$ 0.20	0.3 ^{Avx} $\pm$ 0.40
	2% lactic acid	6.0 ^A $\pm$ 0.38	1.1 ^{Bz} $\pm$ 0.23	1.0 ^{By} $\pm$ 0.19	1.1 ^{By} $\pm$ 0.23
	4% lactic acid	6.4 ^A $\pm$ 0.04	1.3 ^{Byz} $\pm$ 0.15	1.3 ^{Bxy} $\pm$ 0.12	1.4 ^{By} $\pm$ 0.19
	0.5% CPC	6.6 ^A $\pm$ 0.09	1.1 ^{Bvxyz} $\pm$ 0.28	1.3 ^{Bxy} $\pm$ 0.18	0.8 ^{Bvx} $\pm$ 0.21
	1200 ppm ASC	6.6 ^A $\pm$ 0.14	1.8 ^{Cz} $\pm$ 0.19	1.2 ^{Bvxy} $\pm$ 0.23	1.3 ^{Bxy} $\pm$ 0.16
Skinless chicken breast meat	Control ^a	6.5 ^A $\pm$ 0.30	0.3 ^{Av} $\pm$ 0.32	0.4 ^{Av} $\pm$ 0.10	0.2 ^{Av} $\pm$ 0.30
	2% lactic acid	6.2 ^A $\pm$ 0.12	0.3 ^{ABvx} $\pm$ 0.20	0.7 ^{Bvxy} $\pm$ 0.11	0.6 ^{ABvxy} $\pm$ 0.17
	4% lactic acid	6.4 ^A $\pm$ 0.06	0.9 ^{Bvxyz} $\pm$ 0.18	1.4 ^{By} $\pm$ 0.19	1.2 ^{Bxy} $\pm$ 0.13
	0.5% CPC	6.5 ^A $\pm$ 0.15	1.1 ^{Bxyz} $\pm$ 0.15	1.0 ^{Bvxy} $\pm$ 0.17	0.7 ^{Bvx} $\pm$ 0.16
	1200 ppm ASC	6.8 ^A $\pm$ 0.15	0.9 ^{Bvxy} $\pm$ 0.19	1.0 ^{Bvx} $\pm$ 0.13	1.2 ^{Bvxy} $\pm$ 0.15

^{AB}The means with different superscript in the same line are significantly different ($P < 0.05$)

^{vxyz}The means with different superscript in the same column are significantly different ($P < 0.05$)

^aPhysiological saline water (0.85% NaCl)

After the attachment period of 30 s on the chicken drumstick with skin and the decontamination processes, the most effective chemical in the reduction of *S. Typhimurium* was ASC (1200 ppm). After the attachment period of 30 s and decontamination with ASC, the reduction level of *S. Typhimurium* on the chicken drumstick was 1.8 $\log_{10}$ - CFU/ml, and this reduction level decreased to 1.2 $\log_{10}$ - CFU/ml when the attachment period was extended to 20 min. The result of the study showed that the resistance of *S. Typhimurium* against ASC increased when the attachment period was extended from 30 s to 20 min ($P < 0.05$), however there was no change in resistance when the attachment period was extended from 20 to 210 min ($P > 0.05$). The results also showed that there were no changes in the efficacy of the chemicals on the survival of *S. Typhimurium* on the skinless breast meat when the attachment periods were extended (Table 2).

The chicken skin contains many feather follicles and crevices (Zhang et al. 2013). In the present study, it was expecting that the chemicals used would cause markedly more reduction in the number of *S. Typhimurium* and *L. monocytogenes* that attached to the skinless breast meat when compared to those that attached to the chicken skin. However, at the end of the decontamination processes, it was not observed marked bacterial reduction on the skinless breast meat when compared with the reduction on the chicken drumstick with skin. It has been reported that chicken breast fascia allows the greatest bacterial attachment compared to cut chicken muscle, cut beef muscle and beef fascia (Frank 2001; Zulfakar et al. 2012). It seems that the chicken breast fascia can protect the bacteria as much as the chicken skin does.

These results indicate that some antimicrobial agents have more strong effect on *L. monocytogenes* and *S. Typhimurium* on the chicken meat parts in the first 30 s of the attachment. It is impossible to know when the contamination will occur in real-life situation in poultry slaughterhouse or during chicken meat processing. On the other hand, application of decontaminant agents constantly is not practical or possible due to cost, sensorial effect and legal dose limit etc.

It has been noted that bacterial cells compose adhesive structures such as curli and exopolysaccharide and production of bacterial adhesins are highly influenced by the medium surrounding the cell (Zulfakar et al. 2012; Giaouris et al. 2014). Because of that, attachment ability of the bacteria that are grown in nutritious media in a laboratory may be different from the bacteria growing in wild environment. It should be noted that, the results of this research were obtained using *L. monocytogenes* and *S. Typhimurium* that were grown in TSB under a laboratory condition, and the results may be different for the bacteria growing in a wild environment or under different conditions.

In this study, the samples purchased from a supermarket were not analyzed for the presence of natural *Salmonella* and *Listeria monocytogenes*. It is expected that the samples purchased from supermarket may contain no *Salmonella* and *Listeria monocytogenes* or may contain at very low concentrations (may be less than 1 $\log_{10}$ CFU/g, at most 2 $\log_{10}$ CFU/g). In this study, initial inoculation levels for both bacteria were between 6.0 and 6.8 $\log_{10}$ CFU/ml which was about 10,000 times higher than the count that is likely to be found on the samples. Because of that, no

significant interference was expected by natural *Salmonella* and *Listeria monocytogenes*.

Consequently, effect of some chemicals used for carcass decontamination may be influenced by the extension of bacterial attachment period to meat surfaces. Detailed researches concerning this issue are needed to understand if there is an interaction between the bacterial attachment time on chicken meat surface and the efficacy of decontaminants, and in this way it can be performed more effective carcass interventions.

Compliance with ethical standards

Conflict of interest None.

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