Archives of Razi Institute, Vol. 73, No. 2 (2018) 95-106

Original Article

Effect of four chicken carcass transportation methods at selected room temperatures on the bacterial load of *Staphylococcus aureus, Salmonella* species, and *Escherichia coli*

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Received 12 February 2017; accepted 02 May 2017
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ABSTRACT

Pathogenic bacteria are responsible for a significant number of food poisonings in humans through infected poultries. The main objective of this study was to assess the effect of transportation of chicken carcasses at 18-24, 4-5, and 10-14 ºC on the bacterial loads of Staphylococcus aureus, Salmonella species, and Escherichia coli. This study was conducted on 180 fresh chicken carcasses (1197.0±19.88 g) randomly collected from a commercial poultry processing plant in southern Tehran, Iran, in a hot season in 2015. The sampling was performed at two stages, namely post-chilled washing and after 4 h of transportation. In the latter stage, the samples were selected from three vehicles with three types of temperatures. These vehicles included a pickup (18-24 ºC), a refrigerated car (4-5 ºC), and a refrigerated vehicle with switched off refrigerator (10-14 ºC). According to the results, the whole body carcass samples transported at the pickup temperature had the highest mean total count ($18.63\times10^6\pm2.82\times10^6$ cfu.ml-1) and was greater ($P<0.05$) than the standard limit (5×10^6). On the other hand, the samples carried by the vehicle with switched off refrigerator had the lowest total count ($0.65\pm0.04\times10^6$ cfu.ml-1). Similar results were obtained for *S. aureus*; accordingly, it reached the maximum (333.0±30.73 cfu.ml-1) at 18-24 ºC, which was lower than the national standard limit even after 4 h of transportation. In addition, the cfu values for the total count and *S. aureus* sampled from the chicken carcasses were lower than the national standard level even after 4 h of carcass transportation, with the exception of Salmonella spp. at the three vehicle temperatures and *E. coli* at the pickup temperature. It was suggested that the transportation temperature of less than 10-14 ºC could not affect the fresh chicken carcass to be contaminated with *S. aureus* and *E. coli*.

Keywords: Salmonella, *E. coli*, *Staphylococcus aureus* poisoning, chicken transportation

L'effet du transport de 4 heures à différentes températures des carcasses de poulet sur la charge bactérienne de *Staphylococcus aureus*, Salmonella spp. et *Escherichia coli*

Résumé: Les bactéries pathogènes sont responsables d'un nombre important d'empoisonnements alimentaire d'origine alimentaire chez l'homme après la consommation des volailles infectées. L'objectif principal de cette étude était d'évaluer l'effet de 4 h de transport des carcasses de poulet à 18-24 oc, 4-5 ºC et 10-14 ºC sur la charge bactérienne de S. aureus , Salmonella spp. et *Escherichia coli*. Cent quatre-vingts carcasses de poulet frais (1197,0 ± 19,88 g) ont été recueillies au hasard dans une usine de transformation de volailles commerciales dans le sud de Téhéran, durant la saison chaude en 2015. Deux prélèvements ont été effectués après un lavage réfrigéré et post-abattage, après 4 heures de transport des carcasses à trois temperatures

ambiantes par trois types de véhicules normaux et frigorifiques. La valeur moyenne du nombre total de colonies bactériennes dans lescarcasses entières transportées à température ambient atteint le maximum de 18,63 × 10^6 ± 2,82 × 10^6 cfu.ml-1et montre une valeur supérieure (p <0,05) à la limite standard (5 × 10^6) alors que dans le véhicule refroidi isolé montrait la valeur minimale de 0,65 ± 0,04 × 10^6 cfu.ml-1. Un résultat similaire a été obtenu pour *S. aureus* qui atteignait le maximumde 333,0 ± 30,73 cfu.ml-1 à 18-24 ºC, ce qui cependant restait inférieur à la limite standard nationale, même après 4 heures de transport. Il a été conclu que les valeurs du cfu pour le nombre total des colonies bactériennes et *S. aureus* obtenu à partir des carcasses de poulet restent inférieures à la limite standard nationale après 4 heures de transport des carcasses à l'exception de Salmonella spp. dans les différents types de transport et pour E. coli au niveau du ramassage. Ces résultats suggérent qu'une température de transport des poulets de moins de 10-14 oc n'affecterait la contamination des carcasses de poulet frais par *S. aureus* et *E. coli*.

Mots-clés: Salmonella, *E. coli*, empoisonnement par *Staphylococcus aureus*, transport du poulet.

INTRODUCTION

Food contamination is one of the major concerns of public health around the world. Pathogenic bacteria are responsible for a significant number of food poisoning in humans through infected poultries (Gebauer and Laska, 2011; Jetter and Cassady, 2006; Newell et al., 2010). The purpose of food packaging is to avoid wasting food products, increase shelf life, have better and easier transportation, as well as protect against the chemical and biological contaminants. The slaughtering and processing of chickens may be followed by physical, chemical, and histological changes in the carcass due to the impact of individual constituents of the meat (Petrak et al., 1999). Critical control points (CCP) have been recognized as potentially important for the transmission of Salmonella to chicken carcass during transportation and handling. Cross-contamination can occur among carcasses, chilled water, and equipment both at the post- and pre-slaughtering stages (McCrea et al., 2006). The cross-contamination in slaughterhouse is the result of the contact between chilled water tank and chicken carcass, water overflow rate, and water substitutes per carcass rearing in the tank (Ristic, 1997). According to a Brazilian law adopted for the slaughterhouses, the chilled water should be replaced every 8 h (Cavani et al., 2010). The main function of packaging is to keep the product itself. However, among the "Five P's" of marketing (i.e., product, price, packaging, place, and promotion) packaging is more important from the economic perspective (Tyagi and Kumar, 2004). The marketing of food products is also an issue of fundamental importance. Packaging can help preserve the quality, shelf life, and appearance properties to a large extent. In spite of having highly digestible proteins and low-calorie, chicken is known as a source of pathogenic contamination in human consumers (Chong, 2012). Pathogenic microorganisms, such as Salmonella, Staphylococcus aureus, and Escherichia coli, can be responsible for the poultry meat poisoning (Newell et al., 2010). In a microbial investigation on poultry meat carried out in Croatian retailer markets Kozačinski et al. (2006) investigated the fresh samples of chicken breasts with and without skin and frozen ground chicken meat. They revealed that a significant risk of meat spoilage and an increase in the number of bacteria in various pathogenic species depend on the examined parts of chicken carcass, type of packaging, and storage after transportation to the market. Rahimi et al. (2006) isolated some important foodborne bacterial pathogens from the raw materials of sausage and burger products. *S. aureus* is a bacterium, naturally found on the skin and nose of healthy people or animals, which can contaminate chicken carcass during slaughtering, packaging, storing, and transporting processes. Therefore, inappropriate handling at different levels can lead to the outbreak of food poisoning. The disease does not usually occur unless the microorganisms is transmitted to food (Ahari et al., 2008; Olins and Corry,

1999; Zargar et al., 2014). In a descriptive-analytical study conducted on 96 samples collected from four types of foods with high meat content in a military center in Tehran, Iran, 85.4% of the raw food and 12.5% of the cooked food were contaminated with excessive beyond the standard. respectively and Furthemore, 9.57% of the samples were contamination to ed with *S. aureus* enterotoxins was confirmed in 9.57% of the samples (Tavakoli et al., 2013). Salmonella is a bacterium often incriminated in the poisoning of poultry products. The outbreak of salmonellosis has been reported for decades, especially in relation to non-typhoid strains. Salmonella may transmit through poultry raising, slaughtering, and retail marketing to humans (Chong, 2012). At the retail level, it was found that 2-100% of the poultry products were contaminated by Salmonella. In a cross-sectional research, Donado-Godoy et al. assessed the prevalence of Salmonella in chilled, frozen, and freshly slaughtered chicken carcass samples in Colombia. They observed that the chilled chickens had a significantly higher risk of Salmonella contamination, compared to the frozen chickens (2012). *E. coli* is known as a major cause of bloody diarrhea. E. coli is regularly isolated from the feces of healthy cattle, sheep, goats, and poultry, and therefore can be found in manure, water, and other places. This bacterium remains on farms and is transmitted to humans through livestock and poultry retail stores by eating contaminated animal products, especially through contaminated poultry (Chong, 2012; Nayak, 2000). Chicken carcass contamination is inevitable if the incoming chickens to the slaughterhouse are the carriers of the bacteria or unless the processing and transportation steps are improved (Corry et al., 2002). The plastic crates in which live chicken are usually transported from the farm to the slaughterhouse are known as a source of cross-contamination of zoonotic pathogens, such as Salmonella (Allen et al., 2008). With this background in mind, the main objective of this study was to assess the effect of transportation of chicken carcasses carried by vehicles, namely pickup (18-24 °C), refrigerated car (4-5 °C), and refrigerated vehicle with switched off refrigerator (10-14 °C), on the bacterial load of *S. aureus*, Salmonella spp., and Escherichia coli. We also compared the results of post-slaughterhouse with those of post-chilled washing process in the selected slaughterhouse.

MATERIALS AND METHODS

Sampling and study design. This study was conducted on 180 chilled chickens (1197.0±19.88 g), randomly selected from a commercial poultry processing plant in the south of Tehran (capital of Iran) in a hot season in 2015. The chicken carcasses were sampled at two stages, namely the post-chilled washing stage performed early in the morning (pre-transportation) and after 4 h of transportation (post-transportation), for 10 days. In the post-transportation stage, the samples were selected from three vehicles with three types of temperatures. These vehicles included a pickup (18-24 °C), a refrigerated car (4-5 °C), and a refrigerated vehicle with switched off refrigerator (10-14oC). The collected samples were divided into five groups of neck, leg, thigh, groin, as well as distal and proximal muscles of the breast. The muscle biopsies from the main parts of the chicken were separately performed before and after handling the carcasses. The total count and the coagulase-positive *S. aureus*, *E. coli*, and Salmonella species were evaluated according to national standard no. 5272, 1-6806, 2946, and 181 (FDO, 2014; ISIRI, 2012a, b, c, d).

Bacteriological Analysis. After 10-fold serial dilutions in 0.1% (wt/vol) peptone solution using plate count agar at 30oC for 72 h, the enumeration of total count was performed by the pour plate technique (ISIRI, 2012d). The prepared *S. aureus* plates were incubated at 37 °C for 24-48 h. The specific colony was marked at the end of the incubation period. The colonies appeared black or gray, shiny, and convex with the diameters of 1-1.5 mm (after 24 h of

incubation) or 1.5-2.5 mm (after 48 h of incubation) that appeared transparently. A number of known and unknown colonies (usually five samples of each) were cultured in brain heart infusion broth. After 24 h of incubation at 37 °C, coagulase confirmatory test (clot formation) was performed using rabbit plasma (ISIRI, 2012c). Detection test for *E. coli* was implemented based on the two methods, including the identification of the bacteria in 1 g of food sample and enumeration of the presumptive bacteria in 1 g of food sample. Both of these methods were applied to identify the gas in Lauryl Sulfate Broth and *E. coli* medium and determine endole production following the Iranian Standard Method no. 2946 (ISIRI, 2012a). In order to detect Salmonella spp., 25 g of each sample was carefully weighted and homogenized. For the bacterial isolation, swab containing buffered peptone water was incubated at 37 °C for 16-20 h. Subsequently, 100 mL of this medium was added to 10 mL Rappaport-Vassiliadis enrichment broth (Oxoid) and incubated for 18-28 h at the temperature of 42 °C. In the next step, 1 mL of this medium was added to 9 mL Selenite Cystine Broth (Selenite Cystine Broth Base+0.4% Sodium Biselenite, Oxoid) and incubated at 35 °C for 24 h. Each of them was subcultured onto Xylose Lysine Desoxycholate agar (Oxoid); however, Brilliant Green Agar (Oxoid), along with sulphamandelate supplement (Oxoid), was also added. All plates were incubated at 35 °C for 24 h. Marked colonies were sampled, subcultured onto nutrient agar (Oxoid) and incubated at 37 °C for 18-24 h. Finally, the presumptive Salmonella was tested for oxidase (ISIRI, 2012a, b, c, d). Ultimately, in order to detect and count the colony-forming units (CFUs) of the selected bacteria and total bacterial count, three plates in triplicate were dedicated, and the bacteriological sampling was carried out.

Data analysis. The mean value was calculated for each bacterium count and total count using the triplicate plates with three samples. Total counts were expressed as 106 cfu.g-1 with the exception for the other bacterial counts that were stated in cfu.g-1 for S. aureus and *E. coli* and cfu.g-25 for Salmonella spp. The mean values of the three groups (i.e., three temperatures) and each part of the chicken body were reported for all parameters. P-value less than 0.05 was considered statistically significant. The paired sample t-test was used to determine the effect of the three types of transportation temperatures on bacterial load after the post-chilled washing stage and after 4 h of transportation in each part of the carcasses. The paired sample t-test was also utilized to compare the mean values of the total and bacterial count in whole carcass samples transported by the three vehicles of different room temperatures based on the Iranian National Standard levels as mentioned previously. In addition, the one way ANOVA was performed to show the significant variations in total count or selected bacterial counts at different temperatures of vehicles during the transportation. Subsequently, the mean values of the total and selected bacterial counts were compared using the Bonferroni test. All statistical analyses were performed in SPSS software (version 18).

RESULTS

Tables 1 to 3 present the comparison of the total bacterial count of *S. aureus*, *E. coli*, and Salmonella spp. in different parts of the chicken carcass at post-chill washing stage in a slaughterhouse and after 4 h of chicken transportation at three vehicles with different temperatures. In the carcass samples transported by the pickup (Table 1), there was a sharp increase in the mean value of the bacterial and total counts (106 cfu.g-1) after 4 h of chicken transport, compared to the post-chill washing stage (P=0.000). At this temperature (18-24 °C), the total count values for the groin and neck were 0.08±0.00 and 0.87±0.00 at the pre-transportation stage, respectively, representing the minimum and maximum values in this regard. However, at the post-transportation stage, the proximal of breast and leg had the minimum and maximum total count values (5.16±0.23 and 51.70±12.35, respectively). Based on the results obtained from the whole body samples (Table 4 and Figure 1), the total count showed a significance difference (P=0.000) at the three vehicle

temperatures. However, no significance difference was observed in the total count between the carcasses transported in the refrigerated vehicle (0.65±0.04) and those transported in the refrigerated vehicle with a switched off refrigerator (2.01±0.01) (P>0.05). Accordingly, the mean values of the total count sampled from the whole carcasses in the pickup reached the maximum (18.63×106 ±2.82×106) and was significantly higher (P<0.05) than the standard value (5×106). Nonetheless, the whole carcass samples transported by the vehicle with switched-off refrigerator had the minimum total count (0.65×106±0.048×106) that was lower than the standard level. Based on the results shown in Table 2, the pre-transportation group at 4-5 °C had the mean total counts of 0.06±0.00 and 1.37±0.06 at the minimum and maximum levels in the proximal of breast and thigh, respectively. Nevertheless, these values for the samples transported by the vehicle with switched off refrigerator (10-14 °C) were 0.69±0.02 and 3.80±0.41, respectively. The cfu of *S. aureus* (cfu.g-1) showed a higher level (P=0.000) in the post-transportation group than that of the pre-transportation group for all body parts at the three temperatures. In the pre-transportation group, groin had a minimum value of *S. aureus*, which was calculated as 3.70±0.73. Nevertheless, the S. aureus cfu of the proximal of breast had the maximum values both in the pre-transportation (89.60±1.98) and post-transportation (822.10±7.15) groups at the pickup temperature (18-24oC). Accordingly, the proximal of breast (97.90±0.34) and thigh (92.00±0.71) had the maximum cfu of *S. aureus* at the temperatures of the refrigerated vehicle (4-5 °C) and car with switched off refrigerator (14-15 °C), respectively. None of the body parts reached the maximum level of standard (5×10^2) regarding the cfu of S. aureus at these two vehicle temperatures (Tables 1-3). Likewise, the cfu of *S. aureus* estimated from the whole body samples did not meet the maximum standard limit at the temperatures of the pickup (333.03±30.73), refrigerated vehicle (49.56±3.10), and car with switched off refrigerator (44.76±4.14). No significant difference was observed between the samples transported by the refrigerated vehicle (4-5 °C) and car with switched off refrigerator (14-15 °C) in terms of the mean S. aureus (P>0.05). The cfu values were significantly lower than the Iran National Standard (5×10^2) with 99% confidence interval and according to the different temperatures. Furthermore, both pre- and post-transportation groups had lower *S. aureus* cfu than the national standard (5×102) in each body part, with the exception of proximal of breast, which showed greater cfu than the standard level (822.10±7.15). There was a significant difference (P<0.05) among the carcass samples transported at different vehicle temperatures in terms of the mean *E. coli* count. The chicken carcasses transported by the pickup had a higher mean E. coli count (81.68±9.67) than the national standard level (50.0). Based on the results of the pre-transportation group (tables 1-3) and regardless of the room temperature degrees, the mean values of E. coli (cfu.g-1) and Salmonella spp. (cfu.g-1) sampled from the chicken carcasses were significantly (P<0.05) lower than those of the different carcass parts in the post-transportation group, with the exception that the cfu value of the Salmonella spp. for the distal of breast was 0.0 in both groups. At the pickup temperature (18-24oC), only in the proximal and distal parts of the chicken carcasses, the *E. coli* cfu values were lower than the national standard limit (5×101). However, these values were lower than the standard at the car with switched off refrigerator temperature (10-14 °C) for all chicken parts. Nonetheless, at the refrigerated vehicle temperature (4-5 °C), the E. coli cfu values of the leg (65.30±0.42) and thigh (88.80±0.98) parts were slightly higher than the standard levels. The whole carcass samples transported by the refrigerated vehicle (38.43±3.76) and car with switched off refrigerator (8.76±0.45) had significantly lower *E. coli* cfu values than the standard level (P<0.05). Nevertheless, this value (81.68±9.67) was slightly more than the standard

at the pickup temperature (Table 4). The mean value of Salmonella spp. sampled from the neck part of the chicken carcass had the maximum value of 301.00±8.03. With the exception of the distal of breast (0.0), which was observed at the pickup temperature (18-24 °C), the cfu values of Salmonella spp. were obviously higher than the national standard (0.0). In addition, the cfu mean value of Salmonella spp. sampled from the whole body of carcasses were significantly (P<0.01) higher than the national standard limit (Figure 4). The samples transported by the refrigerated car (38.43±3.76) and vehicle with switched off refrigerator (8.76±0.45) had a significantly lower mean *E. coli* count, compared to the national standard (P<0.05). However, the result for Salmonella spp. was much higher than that of the national standard level. The maximum and minimum *E. coli* counts were observed among the samples transported by the pickup (278.03±5.35) and refrigerated vehicle (33.90±2.48), respectively.

DISCUSSION

The chickens arriving to slaughterhouse are generally contaminated with bacteria, especially with potential human pathogenic bacteria, such as Salmonella spp. (Geornaras et al., 1997). Based on a study conducted in Iran, about 15% of the chicken samples were positive for microbial contamination. In the mentioned study, out of the infected poultry samples, 11.3% and 2.9% of the cases were reported to be contaminated with Salmonella spp. and *S. aureus*, respectively. In addition, they observed contaminations in 59.3% and 45.7% of the packed and non-packed chickens, respectively (Soltandalal et al., 2007). In the present study, there was a significant difference among each part of the chicken carcasses transported by three vehicles at different temperatures in terms of the mean bacterial and total count values. Regarding the leg of the chicken, the total cfu of the post-transportation group was positively correlated (r=0.247) with that of the pre-transportation group (Table 1). Regardless of the transportation temperature, the proximal of breast had the lowest bacterial total count. However, the maximum level for the total count did not show a constant status in different body parts or at different temperatures. Accordingly, the mean total count at the temperature levels of <10-14 °C was lower in comparison to the national standard limits. On the other hand, this value at the temperature of > 18-24 °C had a more than 3-fold increase, compared to the Iran National Standard Limit (5×10^6). Likewise, the mean *S. aureus* count were significantly under the national standard at the temperatures of the refrigerated vehicle and car with switched off refrigerator (P=0.000). However, dissimilar to the results regarding the total count, the cfu value was rather lower than the Iran National Standard Limit (5×10^2) at the temperature of 18 °C. With respect to the worst temperature condition (18-24 °C) of this study, the cfu.g-1 of *S. aureus* was enumerated to 822.10±7.15, which is lower than the value (3019.0±247.03) obtained from a retail chicken market in another study (Álvarez-Astorga et al., 2002), but sharply greater than the Iran National Standard Limit (5×10^2). In a study conducted by Rahimi et al. (2006), it was shown that the raw materials of sausage and burger products were contaminated with *S. aureus* (68%), *E. coli* (59%), and Salmonella spp. (53%). *S. aureus* as a significant risk in chicken products can be used as an indicator of cross-contamination (Zargar et al., 2014). According to tables 1-3, the cfu values of S. aureus for the pre- and post-transportation groups at the different vehicle temperatures showed no significant correlation with the exception that the cfu values of *S. aureus* were positively correlated (r=0.338) for the leg part at the transport temperature of 4-5 °C. It was suggested that the enhancement of *S. aureus* cfu in the pre-transportation group was accompanied by an increase in *S. aureus* cfu in the post-transportation group or vice versa. Luber et al. (2006) found *S. aureus* on the contact surfaces of the chicken breasts. Similarly, in the present study, *S. aureus* was isolated from all chicken parts, transported at the three temperature levels of 18-24, 4-5 °C, and 10-14 °C. *S. aureus* reached the maximum level in the proximal

breast samples transported with the pickup and refrigerated vehicle. The mean cfu value of *S. aureus* and total count were 2.74±0.56 cfu.g-1 and 4.72±0.38 log10 cfu.g-1 at 4 °C in the proximal chicken breast fillets (Kozačinski et al., 2006), respectively. These values were lower than those obtained in the current study, which were 97.90±0.34 cfu.g-1 and $5.15\pm0.02\times10^6$, respectively, at the same temperature. Dislike these two findings, Kreyen schmidt et al. (2002) indicated that the cfu value of *S. aureus* reached the 1000 g-1 in the poultry meat samples of retailers at 10 °C. Zargar et al. (2014) demonstrated the prevalence of *S. aureus* in the samples collected from raw meat products in retail stores. They reported that about 85% of the raw and 12.5% of the cooked food were contaminated with *S. aureus*, with large variation from the standard value. It should be notified that approximately 9.6% of samples collected in another study made by tavakoli et al. (2013) were contaminated with *S. enterotoxin*. Our results showed that the cfu values of Salmonella spp. sampled from the pre- and post-transportation groups were not correlated at the pickup temperature (18-24 °C). In other words, the initial cfu in the pre-transportation group had no effects on the cfu value after 4 h of transportation. Based on Table 4 and Figure 1, *S. aureus* and Salmonella spp. increasingly grew in comparison to *E. coli*. Dharod et al. (2007) confirmed *S. aureus* as the pathogens, most frequently found on the surfaces and hands and can rapidly transmit to chicken carcass and grow promptly. Likewise, regarding the bacterial loads, significance variations ($P<0.05$) were observed at the temperature of refrigerated vehicle in comparison to the temperature of pickup and refrigerated vehicle with switched off refrigerator. Therefore, it can be concluded that the appropriate temperatures at the refrigerated vehicle and car with switched off refrigerator prohibited the growth of *S. aureus* in the chicken carcass in contrary to the pickup temperature. The samples transported in the refrigerated vehicle and car with switched off refrigerator had lower mean *E. coli* count, compared to those carried in the pickup; consequently, the formers resulted in safety condition. At the temperature condition similar to the refrigerated vehicle temperature in our study, Cohen et al. (2007) showed that S. aureus and *E. coli* counts in the chicken meat were 251.18±5.01 and 316.22±3.98 cfu.g-1, respectively, while those of our study were remarkably lower (49.56±3.10 and 38.43±3.76 cfu.g-1, respectively). Álvarez-Astorga et al. (2002) showed that *E. coli* count sampled from the chicken thigh at the temperature of 8-14 °C was 398.10 cfu.g-1, which is slightly higher than our result. Accordingly, Northcutt et al. (2003) showed the cfu value of *E. coli* sampled from the whole carcass in slaughterhouse after chilling phase was 63.09 cfu.g-1, which is greater than the values obtained in our study for each part of the carcass even after 4 h of transportation to the retail markets at the temperature of vehicle with switched off refrigerator (10-14 °C). According to the results of Table 4 and Figure 1, only the mean value of E. coli at pickup temperature (18-24 °C) showed greater level than the national standard. This could be due to different reasons, such as the elevation of temperature after 4 h of transportation in the pickup vehicle, inadequate washing the vehicle or crates, and washing the crates or vehicle by water contaminated with bacteria. There is little evidence showing that a large number of carcasses are contaminated with Salmonella spp. during packaging. This is probably due to the relatively low number of Salmonella in the body surface of the chicken during processing (Corry et al., 2002). However, similar to our results (tables 1-3), there are other studies revealing the enhancement of Salmonella spp. contamination in poultry slaughterhouse (Chambers et al., 1998; Lillard, 1989). In a study conducted in the Europe, Salmonella

Table 1: Effect of chicken transportation by pickup (18-24oC) on total and selected bacterial loads (n=9)

		Leg	Thighs	Groin	Distal of breast	Proximal of breast	Neck
Total count (10^6)	Pre-transportation	0.72±0.00 [a]	0.44±0.00 [a]	0.08±0.00 [a]	0.37±0.00 [a]	0.14±0.00 [a]	0.87±0.00 [a]
	Post-transportation	51.70±12.35 [b]	18.61±0.85 [b]	13.23±0.61 [b]	7.74±0.22 [b]	5.16±0.23 [b]	15.33±.40 [b]
	Sig.(2 tailed)	0.004	0.000	0.000	0.000	0.000	0.000
	Correlation	0.247	-0.079*	-0.020*	-0.590	-0.268*	-0.141*
Staphylococcus aureus	Pre-transportation	19.70±1.51 [a]	21.70±0.61 [a]	3.70±0.73 [a]	11.80±0.55 [a]	89.60±1.98 [a]	40.10±3.37 [a]
	Post-transportation	212.60±7.18 [b]	215.00±4.00 [b]	258.80±28.47 [b]	110.10±3.85 [b]	822.10±7.15 [b]	379.60±10.17 [b]
	Sig.(2 tailed)	.000	.000	.000	.000	.000	.000
	Correlation	-.305*	.514*	.110*	.449*	-.102*	.258*
Escherichia coli	Pre-transportation	14.20±1.28 [a]	26.40±1.71 [a]	3.20±0.46 [a]	12.40±0.84 [a]	8.20±0.64 [a]	4.50±0.93 [a]
	Post-transportation	66.30±3.56 [b]	240.30±7.95 [b]	63.80±2.13 [b]	23.40±1.11 [b]	26.30±0.95 [b]	70.00±2.36 [b]
	Sig.(2 tailed)	.000	.000	.000	.000	.000	.000
	Correlation	.001*	.243*	.249*	.369*	.331*	.161*
***Salmonella* species**	Pre-transportation	14.00±0.86 [a]	26.60±0.74 [a]	9.60±0.56 [a]	00.00	13.10±0.73 [a]	5.90±0.56 [a]
	Post-transportation	280.10±13.81 [b]	298.20±5.28 [b]	299.50±3.07 [b]	00.00	256.70±12.10 [b]	301.00±8.03 [b]
	Sig.(2 tailed)	.000	.000	.000	-	.000	.000
	Correlation	.-142*	.250*	.257*	-	-.376*	.178*

Similar superscript shows no statistical difference in each column and variable. * shows no correlation between the pre- and post-transportation groups.

Table 2. Effect of chicken transportation by refrigerated vehicle (4-5 ºC) on selected bacterial loads (n=9)

		Leg	Thighs	Groin	Distal of breast	Proximal of breast	Neck
Total count (10^6)	Pre-transportation	0.24±0.00 [a]	0.24±0.00 [a]	0.14±0.00 [a]	0.22±0.00 [a]	0.05±0.00 [a]	0.13±0.00 [a]
	Post-transportation	0.54±0.00 [a]	1.37±0.06 [b]	0.61±0.00 [b]	0.58±0.00 [a]	0.47±0.00 [b]	0.31±0.00 [b]
	Sig.(2 tailed)	0.091	0.000	0.000	0.102	0.000	0.000
	Correlation	-0.943	-0.481*	-0.565*	0.194*	-0.902	-0.867
Staphylococcus aureus	Pre-transportation	15.20 ±0.75 [a]	11.50±0.68 [a]	23.20±0.44 [a]	22.50±1.26 [a]	99.10±1.17 [a]	25.00±0.42 [a]
	Post-transportation	29.00±1.00 [b]	28.10±0.31 [b]	38.00±0.44 [b]	56.40±2.31[b]	97.90±0.34 [a]	48.00±0.21 [b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.071	0.000
	Correlation	0.338	-0.180*	0.446*	-0.377*	0.328*	-0.250*
Escherichia coli	Pre-transportation	18.90±0.94 [a]	26.60±0.66 [a]	3.30±0.39 [a]	2.80±1.13[a]	7.80±0.38 [a]	9.10±0.27 [a]
	Post-transportation	65.30±0.42 [b]	88.80±0.98 [b]	14.00±0.44 [b]	32.90±1.59 [b]	12.90±0.37 [b]	16.70±0.39 [b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.000	0.000
	Correlation	-0.380*	0.356*	0.942	0.724	0.664	0.639
Salmonella species	Pre-transportation	15.50±1.68 [a]	21.30±0.55 [a]	5.60±0.37 [a]	10.30±1.14[a]	14.10±0.34 [a]	12.60±0.37 [a]
	Post-transportation	62.50±5.88 [b]	26.90±6.42 [a]	15.30±0.36 [b]	29.30±3.49[b]	44.50±0.47 [b]	39.40±0.60 [b]
	Sig.(2 tailed)	0.000	0.400	0.000	0.000	0.000	0.000
	Correlation	-0.042	0.199*	-0.555	0.222*	-0.569*	0.778

Similar superscript shows no statistical difference in each column and variable. * shows no correlation between the pre- and post-transportation groups

spp. was found to be dominated in the Lithuanian broiler flocks and in Italy (Pieskus et al., 2008). Furthermore, Kozačinski et al. (2006) reported that the chicken breast fillets without and with skin contained a higher number of Salmonella and *S. aureus*. This is in agreement with our findings regarding the Salmonella level, which was much higher than the Iranian National Standard (0.0) even at the temperature of refrigerated

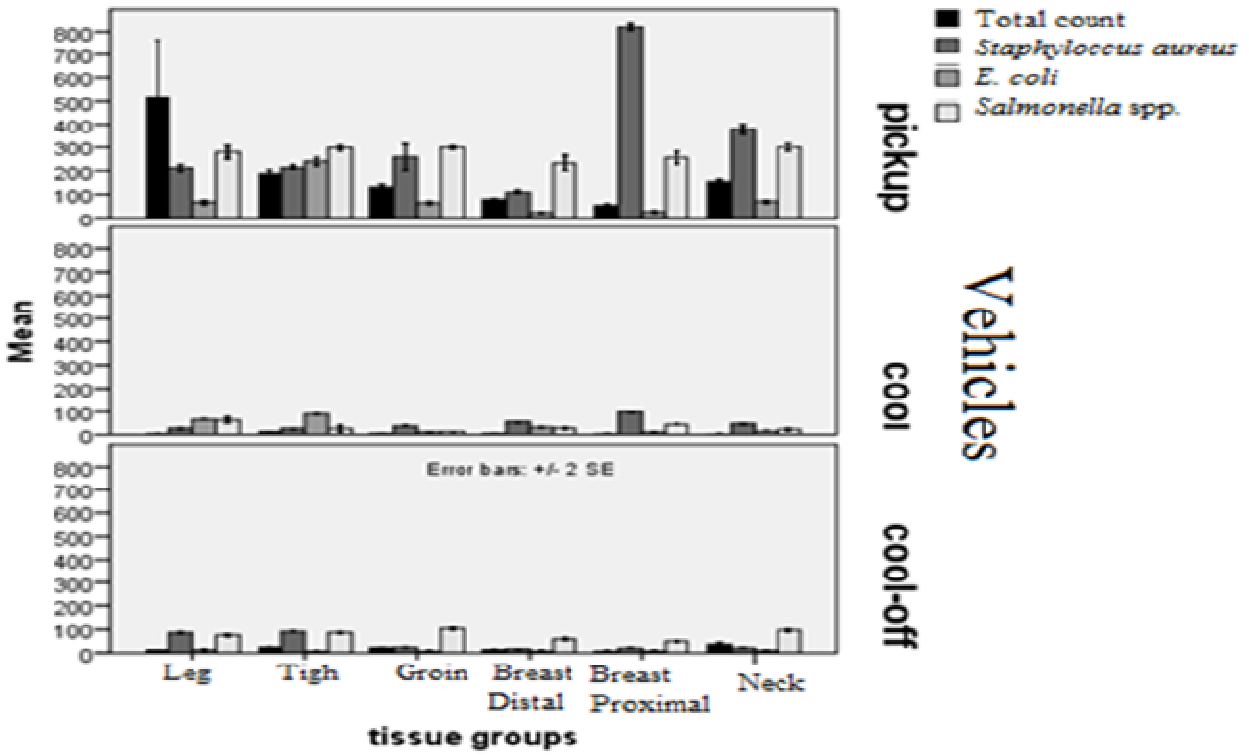

Figure 1. Mean values of total count (105 cfu) and the other selected bacteria (cfu) in each parts of body carcasses paneled by vehicle types (n=9)

vehicle, which amounted to 33.90±2.48 cfu.g-1. On the other hand, with the exception of the distal breast at the pickup temperature, our results demonstrated that Salmonella spp. had the minimum cfu of 5.90±0.56 cfu.g-1 that is higher than the Iranian National Standard (0.0). This is in line with a study performed by Cason et al. (1997) investigating broiler carcass after chilled water process (16±0.0 cfu.g-1). In a study, the prevalence rates of Salmonella spp. among the chilled carcasses were reported as 42%, 14%, and 30% (Donado-Godoy et al., 2012). Poor worker hygiene could result in Salmonella spp., *E. coli*, and *S. aureus* contamination after direct contact with raw poultry or indirect contact with contaminated food (Todd et al., 2009). In another study, the levels of Salmonella spp. in the liver, spleen, and ovaries were presented as up to 60, 280, and 960 cfu.g-1, respectively, among 4-week-old laying chickens naturally contaminated with S. enteritidis. They found that the juice or skin of the chicken carcasses may contain Salmonella at the level of 107 cfu.g-1 (Todd et al., 2009). Nonetheless, our results revealed lower Salmonella cfu values in the carcass samples transported at the temperature of the refrigerated vehicle (33.90±2.48 cfu.g-1), car with switched of refrigerator (80.13±2.57 cfu.g-1), and even pickup (80.13±2.57 cfu.g-1). The inner and outer areas of the feather plucker and chilled water tank were recognized as CCPs for controlling the contamination of the final product in the chicken slaughterhouse. In a study carried out by McCrea et al. (2006), a 37-100% reduction in Salmonella was obsereved following the implemenation of hygienic precaution. In the present study, chilled water tank was severely affected with bacterial contamination due to the lack of hygienic condition in the mentioned CCPs. Blank and Powell (1995) demonstrated that the total coliform contamination level of the inner part of the chilled water tank was greater than that of the outer part.

Table 3: Effect of chicken transportation by refrigerated vehicle with switched off refrigerator (10-14OC) on bacterial loads (n=9)

		Leg	Thighs	Groin	Distal of breast	Proximal of breast	Neck
	Pre-transportation	0.14 ±0.00 [a]	0.33± 0.00 [a]	0.26± 0.00 [a]	0.15± 0.00 [a]	0.13±0.00 [a]	0.45± 0.01 [a]
Total count cfu (10^6)	Post-transportation	1.20±0.02 [b]	2.62.00±0.05 [b]	2.42±0.05 [b]	1.35±0.04 [b]	0.69±0.02 [b]	3.80±0.41[b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.000	0.000
	Correlation	0.978	1.00	-1.00	0.703	-0.565	0.983
staphylococcus aureus	Pre-transportation	14.40±1.83 [a]	6.80±0.53 [a]	18.10±0.48 [a]	4.10±0.37 [a]	3.20±0.55 [a]	14.10±0.56 [a]
	Post-transportation	86.70±6.05 [b]	92.00±0.71 [b]	25.90±1.01 [b]	17.90±.48 [b]	22.60±0.71 [a]	23.50±0.65 [b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.000	0.000
	Correlation	0.531*	0.146*	0.116*	-0.189*	0.050*	0.285*
Escherichia coli	Pre-transportation	5.60±1.64 [a]	2.70±0.39 [a]	4.50±0.45 [a]	3.00±.61[a]	3.50±0.50[a]	8.10±0.70 [a]
	Post-transportation	13.60±2.71 [b]	6.50±0.56 [b]	6.90±0.79 [b]	7.50±0.63 [b]	7.60±0.47 [b]	10.50±1.20 [b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.000	0.030
	Correlation	0.805	0.524*	0.416*	0.625	0.513*	0.634
***Salmonella* species**	Pre-transportation	10.60±0.60 [a]	12.90±0.54 [a]	13.60±0.56 [a]	8.40±0.37[a]	7.50±0.47 [a]	13.50±0.89 [a]
	Post-transportation	77.20±1.72 [b]	87.90±1.14 [b]	105.20±1.95 [b]	60.50±1.74 [b]	52.10±1.22 [b]	97.90±2.22 [b]
	Sig.(2 tailed)	0.000	0.000	0.000	0.000	0.000	0.030
	Correlation	0.868	0.865	-0.103*	0.703	0.808	0.721

Similar superscript shows no statistical difference in each column and variable. * shows no correlation between pre- and post-transportation groups

Table 4. Mean values of total and selected bacterial counts in whole carcass samples in different vehicles after 4 hours of transportation analyzed by one-way ANOVA test, individually compared with the standard values using

	Pickup	Refrigerated vehicle	Vehicle with switched off refrigerator	Standard	Reference
Total count (10^{6}), (1 g)	18.63±2.82*	0.65±0.04**	2.01±0.15*	5×10^6	(FDO, 2014)
***Staphylococcus aureus* (1 g)**	333.03±30.73**	49.56±3.10** [a]	44.76±4.14** [a]	5×10^2	(FDO, 2014)
***Escherichia coli* (1 g)**	81.68±9.67*	38.43±3.76*	8.76±0.45**	50	(IVO, 2014)
***Salmonella species* (25 g)**	278.03±5.35**	33.90±2.48**	80.13±2.57**	0	(IVO, 2014)

In each row, *shows significant difference between each type of vehicle and standard value at the level of 0.05
And ** expresses at the level of 0.01. In each row, similar superscripts show no significant difference (α=0.05) between the vehicle types.

Mofidi et al. (2002) reported lower *E. coli* contamination level at the chilled water stage, compared to our findings. In total, it should be mentioned that in the present study, only Salmonella spp. level was greater than the standard limit in approximately 90% of the carcass body parts. This is probably due to the weak hygienic condition in slaughterhouse or lack of appropriate temperature condition during transportation, resulting in the enhancement of Salmonella spp. growth in the chicken carcasses.

As the findings of the present study revealed, the cfu values for the total count and *S. aureus* sampled from chicken carcasses were lower than the Iranian National Standard limit even after 4 h of transportation, with the exception of Salmonella spp. at different vehicle temperatures and E. coli at the pickup temperature (18-24 °C). Therefore, it can be concluded that the vehicle temperature of less than 10-14 °C during the chicken transportation could not affect the chilled chicken carcass to be contaminated with *S. aureus* and *E. coli*. However, severe Salmonella spp. contamination occurred even at the temperature of 4-5 °C.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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