

## INSPECTION OF MICRO FLORA CHANGES IN BROILER CARCASS DURING SLAUGHTER PROCESSING OPERATION

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**Abstract.** *The poultry slaughter processing in abattoirs may result in carcass contamination with different pathogens. This survey was carried out to evaluate the slaughtering process effects on hygienic status of poultry carcass slaughtered in Kabul industrial abattoir. For this purpose, samples had taken from breast skin surface with swab and template randomly. Therefore, counting of total aerobic plate count, coliforms, E.coli and Staphylococcus aureus and detecting of Salmonella spp. were done in different slaughtering processes. The results showed that the cooling in chillers significantly ( $p < 0.05$ ) decreased the total aerobic plate count ( $1 \log 10 \text{ cfu/cm}^2$ ), coliforms ( $0.27 \log 10 \text{ cfu/cm}^2$ ), E.coli ( $0.47 \log 10 \text{ cfu/cm}^2$ ) and Staphylococcus aureus ( $1.46 \log 10 \text{ cfu/cm}^2$ ) in poultry carcass. In the evisceration stage the highest contamination was observed in comparison with other stages. Salmonella spp. was detectable in all stages of processing. Although the carcass bacterial load was decreased after cooling in chillers, cross contamination with some bacteria such as Salmonella and Staphylococcus aureus necessitates the need for modifying slaughter line and effective staff training regarding hygiene. Furthermore, special attention should be paid to salmonella spp. infection control in poultry farms during rearing period.*

**Keywords:** *Carcasses, Contamination, Bacteria, poultry, Pathogens, micro-organisms, slaughter.*

workers) and live bird (contents of the digestive tract, skin contamination, etc.) (Cox and Pavic., 2010).

Therefore, in case of non-compliance with health standards, the poultry slaughterhouse can have a significant effect on the creation and spread of contamination of carcasses (Izat et al., 1989).

Today, the food industry invests a significant part of its financial resources to ensure the health quality of its products, which is due to the economic losses caused by the microbial spoilage of food and also the emergence of foodborne diseases in consumers (Escudero et al., 2007).

One of the ways to reduce pathogenic bacterial agents for humans is to monitor the microbial quality of poultry meat during production, storage and distribution (Gill and Badooni., 2005).

Recently, in order to improve health control during poultry slaughtering, the HACCP system is implemented, with the implementation of which the health risks of food items are minimized or completely prevented. And

### 1. Introduction

Poultry meat is one of the favorite protein sources for humans, the consumption of which has increased in recent decades in many countries of the world. Some reasons for the popularity of poultry meat include its cheapness, low fat content and high nutritional value (Van., 2002).

However, consumption of chicken meat contaminated with pathogens can cause some diseases in humans. Chicken breeding usually takes place on the floor of the hall, which can lead to their contamination with various bacteria, but in many cases, these birds can carry pathogenic bacteria for humans without showing any symptoms (Keener et al., 2004).

In many countries, one of the biggest challenges for the poultry industry is the contamination of this product with microorganisms such as Salmonella and Campylobacter (Sun and Ockerman., 2005).

Contamination of poultry carcass with pathogenic microorganisms originates from two main sources of the slaughterhouse environment (process, equipment and hands of

parts was done separately. All the samples were transferred to the laboratory near the ice for microbial tests (Gill et al., 2005; Jamshidi et al., 2008).

### **3.2. Microbial Tests**

Total count of aerobic bacteria (TVC): First, serial dilution using 0.1% sterile peptone water solution was performed on the samples obtained. Then, 100 microliters of each dilution was placed on the Kant agar plate medium by the plate counting method. The surface was cultured and the plates were kept in an incubator for 2 days at a temperature of 35 degrees Celsius. For the overall count, plates with 30-300 colony were selected.

Counting of coliform bacteria and *Escherichia coli*: One milliliter of serial dilutions was cultured in two layers of VRBA media and placed in an incubator at 35°C for 24 hours. Colored cells with a diameter of 0.5 mm or more along with the surface of bile acid deposition were counted in plates containing 15-150 colonies.

From the above plates, 10 colonies were selected and inoculated on brilliant green agar tubes and Durham tubes, and then the tubes were placed in an incubator at 35°C for 24 hours.

The tubes producing turbidity and gas were confirmed as coliform bacteria and the final coliform count is obtained by multiplying the number of positive tubes divided by 10 and multiplying to the counted colony

In order to count *Escherichia coli*, the brilliant green agar tubes with gas were inoculated into EC agar tubes containing Durham tubes and kept in incubator at a temperature of 45.5 degrees Celsius for 48 hours. EC tubes with gas were inoculated into EMB environment and for 24 hours were placed in incubator at 35 degrees Celsius.

The positive colony were examined by microscopy and IMVIC test

If the bacteria was gram negative and in terms of indole bacteria was positive or negative, MR was positive, VP was negative and citrate was negative, it was considered as *Escherichia coli*. And it was calculated as a percentage of the numbered colony in the initial environment of VRBA. Counting of *Staphylococcus aureus*: one milliliter of the

on the other hand, the trust of the consumer is attracted to this category of food products (Tsola et al Morar et al., 2008).

In most studies, the presence of pathogenic and indicator organisms such as *Salmonella* and *Escherichia coli* on the surface of poultry carcasses has been used as a tool to evaluate the health quality of poultry slaughter lines (Akhondzadeh and Misaghi., 2007).

Therefore, considering the importance of the health quality of poultry meat as one of the important sources of providing animal protein and the effect of slaughtering processes on the contamination of chicken carcasses, this study aims to evaluate the contamination status of poultry carcasses with pathogenic bacteria, as well as the role of different slaughtering processes on the microbial load count and the presence of indicator microorganisms during the killing stages to determine the stages the danger was carried out in Kabul industrial abattoir.

**2. Aims:** Aim of the study to evaluate Microbiological quality of the chicken carcasses in farms and slaughterhouses

### **3. Materials and methods**

#### **3.1. Sampling**

This descriptive-cross-sectional study was conducted to evaluate the microbial changes of chicken carcasses during processing in the Kabul industrial slaughterhouse. Based on the history obtained from the studied flocks, the average rearing period of slaughtered chickens was 40 days and their average weight was 2.3 kg, which they were transported to the slaughterhouse with the same ration and vaccination from the existing meat farms in Kabul province, which has a moderate climate. Also, the average number of herds slaughtered was around 3000 pieces, and the drug-free period before slaughtering was well observed in them.

Random sampling was done from 6 flocks in total 60 samples in the spring and summer seasons using swabs and sampling plates from the surface of breast skin area of the chicken after skinning, emptying the internal organs and cooling in chillers.

Then the swabs were placed in tubes containing 0.3% sterile peptone water. In order to aspire to the sanitary quality of water in the scalding and chiller parts, sampling of these

different stages of the poultry slaughter process, including the swap samples from the skin surface of the carcass after feathers removing, after removing byproducts, After cooling, as well as sampling of scald water and first and second chiller water are shown in tables number one and two, respectively.

The results showed that in all stages of chicken carcass processing in the slaughterhouse, the total count of aerobic bacteria was high, and among them, the internals discharge stage had the highest level of pollution compared to other stages of carcass processing ( $P < 0.05$ ).

Although the scalding stage led to the initial reduction of microorganisms and none of the studied bacteria were identified. Based on the obtained results, the total count of aerobic bacteria in the water of the first chiller was significantly higher than the water of the scald and the second chiller ( $P < 0.05$ ). However, the steps of cooling poultry carcasses in chillers led to a decrease in the total count of *Escherichia coli* and *Staphylococcus aureus*.

successive dilutions mentioned as 0.3, 0.3 and 0.4 was inoculated into Brad-Parker agar medium and surface cultured.

Plates containing 15-150 colonies with black colony characteristics and a light ring around them were selected to count *Staphylococcus aureus*.

Isolation of *Salmonella*: First, pre-enrichment was done in lactose agar environment for 24 hours at 37 degrees Celsius.

Then, one milliliter of lactose agar was transferred into selenite cysteine and tetrathionate environments and placed in incubator at temperatures of 35 and 45 degrees Celsius for 24 hours. Then, the media in which the growth had taken place were cultured on the *Salmonella-Shigella* agar environment. Colony with black center were examined and tested on TSI, LIA and Karbamait environments for confirmation.

#### 4. Result

After conducting microbial tests, the average results of the total count of aerobic bacteria, the count of coliforms, the count of *Escherichia coli*, the count of pathogenic aureus, and the isolation of salmonella related to the

Table 1. The result of microorganism counts on the skin surface of the poultry carcass during the slaughter process (cfu/cm<sup>2</sup>)

Slaughter process	Number of samples	Total count of aerobic bacteria	Coliform bacteria	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i>
Feathers removal	20	$5,03 \times 10^4 \pm 0,04^a$	$1,87 \times 10^4 \pm 0,03^a$	$1,70 \times 10^4 \pm 0/05^a$	$2,61 \times 10^4 \pm 0/05^a$	+
Evacuation of by-products	20	$6,50 \times 10^5 \pm 0,07^b$	$2,53 \times 10^5 \pm 0,03^b$	$2,75 \times 10^5 \pm 0,04^b$	$1,88 \times 10^5 \pm 0/07^b$	+
Cleaning and chilling	20	$4,05 \times 10^4 \pm 0,06^c$	$1,60 \times 10^4 \pm 0,08^c$	$1,18 \times 10^4 \pm 0,10^c$	$1,05 \times 10^4 \pm 0,11^c$	+

Table 2. The results of microorganism counts in scalding water and chillers during the slaughter process (cfu/cm<sup>3</sup>)

Slaughter process	Number of samples	Total count of aerobic bacteria	Coliform bacteria	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella</i>
Scald water	20	$3,71 \times 10^4 \pm 0,03^a$	-	-	-	-
First chiller water	20	$5,55 \times 10^4 \pm 0,03^b$	$2,85 \times 10^4 \pm 0,03^a$	$2,55 \times 10^4 \pm 0,11^a$	$2,25 \times 10^4 \pm 0,13^a$	+
Second chiller water	20	$3,45 \times 10^3 \pm 0,07^c$	$2,37 \times 10^3 \pm 0,08^b$	$1,50 \times 10^3 \pm 0,25^b$	$1,25 \times 10^3 \pm 0,17^b$	+

as well as the aerosols in the air (Geornaras et al., 1997; Goksoy et al., 2004; Jamshidi et al., 2008, Mead and Scott., 1994). Based on the results of this study, the highest level of contamination in terms of the total count of aerobic bacteria, coliforms, *Escherichia coli* and *Staphylococcus aureus* was observed in the byproduct removing stage.

In many slaughterhouses, including the slaughterhouse under study, the process of emptying byproducts is done manually, it seems that the total microbial load increases due to the surface contamination of the carcass with the microbial flora of the digestive system, as well as inappropriate handling during the inspection stage, therefore avoiding cross contamination is great importance with fecal material in the phase of internal emptying, especially when the bowels exit (Hue et al., 2011).

In a study that was conducted to determine the critical control points along the slaughter line, the highest level of fecal streptococci contamination belonged to the stage of emptying the intestines. Also, due to the high volume of contamination, the poultry intestine was introduced as one of the most serious critical control points in the poultry slaughterhouse ( Javadi and Farshbaf., 2011).

The cooling stage of the carcass is important in terms of controlling microbial growth, in which the microbial load can be reduced by using cold water.

Several studies have shown that the cooling stage is the most effective stage to reduce the microbial load on the surface of the poultry carcass during the slaughter process ( Akhondzadeh et al., 2004; Akhondzadeh and Misaghi., 2007; Gonzalez et al., 2006; Javadi and Razavilar., 2007; Northcutt et al., 2006, Tsola et al., 2008).

However, if you do not pay attention to the sanitary condition of water coolers, there is a possibility of *Listeria monocytogenes* contamination of chicken carcasses after leaving the cooler (Akhondzadeh and Misaghi., 2007).

The present study showed that the microbial load of carcasses decreases significantly after cooling but this decrease in microbial load is not enough to create a favorable mi-

## 5. Discussion

Contamination of poultry meat with food-borne pathogens can endanger the health of the consumer as a serious risk, especially in cases such as unnecessary handling, insufficient cooking or storage in unfavorable conditions. Poultry sent to the slaughterhouse usually have a high microbial load in the skin and digestive tract, especially in terms of pathogenic microorganisms such as salmonella and campylobacter (Geornaras et al., 2007; Tsola et al 2008).

Extensive research has been done on the amount and factors affecting the contamination of poultry carcasses in slaughterhouses, which shows the importance of chicken processing conditions in slaughterhouses (Akhondzadeh et al., 2004; Akhondzadeh and Misaghi., 2007; Jamshidi et al., 2008; Javadi and Razavilar., 2007 Mofidi et al., 2011; Mokhtarian., 2009).

The presence of birds with dirty skin and contaminated with fecal compounds during processing in the slaughterhouse can lead to an increase in the total microbial load of the carcasses during the scalding stage. In the present study, despite the high temperature of the scalding water at about 60 degrees Celsius, the total count of aerobic bacteria was high at this stage, which indicates the insufficient flow of water temperature and the accumulation of pollution in it. On the other hand, the reason for not isolating pathogenic bacteria in scalding water can be due to the high temperature of scalding water. Therefore, a solution to reduce this problem is the use of a multi-stage scalding, in which poultry are entered into several scalding tanks, so that the level of microbial load on the surface of the chicken is reduced (Mead., 1997; Mead et al., 1993).

Also, by continuously replacing scalding water with fresh water and using chlorine compounds, the surface microbial load of carcasses can be reduced by more than one logarithmic cycle (James et al., 2006).

Many researchers have shown that the feathers removing stage of carcass microbial load is at high levels, which is consistent with the results of the present study. One of the reasons for the high microbial load of the carcass at this stage can be the transfer of contamination from the equipment to the carcass

should be taken to kill salmonella-free flocks before salmonella-positive flocks. Considering the presence of Salmonella in the carcasses studied in this study, the above strategy is recommended ( Hue et al., 2011; Rasschaert et al., 2007).

Although the microbial load of the carcasses decreases after cooling in successive coolers, but due to cross-contamination with some microorganisms such as Salmonella and Staphylococcus aureus, the improvement of the slaughter line and the effective training of workers in relation to health and safety It is also necessary to pay special attention to reducing the contamination of herds with salmonella during breeding.

### 6. Conclusion

Based on the results of this study, the highest level of contamination in terms of the total count of aerobic bacteria, coliforms, Escherichia coli and Staphylococcus aureus was observed in the byproduct removing stage. The cooling stage of the carcass is important in terms of controlling microbial growth, in which the microbial load can be reduced by using cold water. In present study, after the processing of carcasses in a slaughterhouse, Salmonella bacteria were isolated and identified from all the carcasses. Slaughter of salmonella-positive herds can lead to the contamination of carcasses and the slaughter chain. It is also necessary to pay special attention to reducing the contamination of herds with salmonella during breeding. By observing health regulations in the stages of breeding, slaughtering, packaging and transportation, carcasses with hygienic quality and free of pathogenic microorganisms are offered to the market.

microbial level (Javadiand Farshbaf., 2011; Keener et al., 2004 and Tsola et al., 2008).

So that the average logarithm of Escherichia coli count after the cooling phase of the carcasses was equal to 1.08 cfu/cm<sup>2</sup> of the bird's skin surface. Also, the microbial load in the water of the second chiller showed a greater decrease compared to the water of the first chiller, and among the reasons for this decrease, one can imagine the lower temperature and higher flow of water in the second chiller, as well as the slow flow of clean water and the longer duration of the first chiller (Akhondzadeh et al., 2004).

In a study conducted by the Ministry of Agriculture in 2012, the level of contamination of poultry carcasses in Kabul slaughterhouses after the carcasses were removed from the second chiller was investigated. Their results showed that despite the high number of infected with salmonella, but the average number of salmonella in them is low. 69% of the total carcasses evaluated by them, were infected with salmonella (Ministry of Agricultural and Livestock., 2008).

The 2010 study of the Ministry of Agriculture shows that from 60 neck skin swap samples of poultry carcass after the cooling stage, 6 samples (6.11%) were found to be contaminated with Salmonella (Javadi and salawati., 2010).

In the present study, after the processing of the carcasses in the slaughterhouse, Salmonella bacteria were isolated and identified from all the carcasses. Slaughter of salmonella-positive herds can lead to the contamination of carcasses and the slaughter chain. In order to reduce the cross-contamination of poultry carcasses with salmonella, measures

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## ПРОВЕРКА ИЗМЕНЕНИЙ МИКРОФЛОРЫ В ТУШКАХ БРОЙЛЕРОВ ВО ВРЕМЯ УБОЙНОЙ ОБРАБОТКИ

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**Аннотация.** Процесс убой птицы на скотобойнях может привести к заражению тушек различными патогенами. Это исследование было проведено с целью оценки влияния процесса убой на гигиенический статус тушек домашней птицы, забитых на промышленной скотобойне в Кабуле. С этой целью образцы были взяты с поверхности кожи молочной железы с помощью тампона и шаблона случайным образом. Таким образом, подсчитывается общее количество аэробных бактерий, кишечной палочки, *E.coli* и золотистого стафилококка и выявляется *Salmonella spp.* были изготовлены в различных процессах забоя скота. Результаты показали, что охлаждение в чиллерах значительно ( $p < 0,05$ ) снизило общее количество аэробных бактерий ( $1 \log 10$  кое/см<sup>2</sup>), кишечной палочки ( $0,27 \log 10$  кое/см<sup>2</sup>), кишечной палочки ( $0,47 \log 10$  кое/см<sup>2</sup>) и золотистого стафилококка ( $1,46 \log 10$  кое/см<sup>2</sup>). в тушке домашней птицы. На стадии потрошения наблюдалось самое высокое загрязнение по сравнению с другими стадиями. *Salmonella spp.* был обнаружен на всех этапах обработки. Несмотря на то, что бактериальная нагрузка на туши снизилась после охлаждения в холодильных камерах, перекрестное заражение некоторыми бактериями, такими как сальмонелла и золотистый стафилококк, требует модификации линии убой и эффективного обучения персонала правилам гигиены. Кроме того, особое внимание следует уделять борьбе с инфекцией *Salmonella spp.* на птицефабриках в период выращивания.

**Ключевые слова:** туши, контаминация, бактерии, домашняя птица, патогены, микроорганизмы, убой.