

**ORIGINAL RESEARCH PAPER**

**STUDY OF MICROBIAL CONTAMINATION OF BROILERS IN  
MODERN ABATTOIRS IN KHARTOUM STATE**

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This study was carried out to investigate the contaminating microorganisms that can be found on the breast, legs and backs of broilers during processing. A total of 81 swab samples from 27 carcasses were collected randomly from chicken carcasses slaughtered at modern poultry abattoir in Khartoum State, the Sudan. The samples were taken from 9 Critical Control Points (CCPs), namely; after bleeding with feathers, after scalding, after defeathering, after evisceration, after spray wash, after chilling and packing, workers' hand, knives and Scalding water. Total Viable Count (TVC) was carried for each sample besides isolation and identification of contaminating bacteria. The study revealed a statistically significant difference at *P*-value ( $p < 0.05$ ) in the 9 CCPs between the legs, backs and breast respectively. The isolated bacteria were *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus* and *Salmonella* species. Sudanese abattoirs may reflect the hygienic status of chicken meat production in the developing countries and the implementation of Hazard Analysis and Critical Control Points (HACCP) in poultry industry is extremely important, because it involves the constant monitoring of all steps of the process.

**Keywords:** broilers carcasses, microbial contamination, HACCP

### **Introduction**

Poultry is an important part of the animal food and the volume of their production, marketing and consumption is increasing to satisfy the public demand worldwide within the last decades (Bryan, 1980; Anand *et al.*, 1989; Mead 1997). Modern poultry processing requires a high rate of throughput to meet consumer demand, as poultry meat can easily be contaminated with microorganisms, due to many factors, as nutrients, high water activity and neutral pH (Kabour, 2011). However, healthy broilers entering slaughter processing might be highly contaminated by microorganisms, including food borne pathogens such as *Salmonella* species, *Campylobacter* species and other bacteria and these pathogens tend to disseminate

in the processing plant (Mead *et al.*, 1994). They can be found on the surfaces of feet, feathers, skin and also in the intestines. During processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the processing operation (Kabour, 2011).

The procedure for converting a live, healthy bird into a safe and wholesome poultry product provides many opportunities for micro-organisms to colonize on the surface of the carcasses. During the various processing operations, opportunities exist for the contamination of the carcass from the environment, the process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcass to carcass. Some processing operations increase contaminating micro-organisms or encourage their multiplication (Kabour, 2011). As a result, the microbial population changes from mainly Gram-positive rods and micrococci on the outside of the live chicken to Gram-negative micro-organisms on the finished product (Bryan, 1980; Thomas and McMeekin., 1980; Roberts, 1982; Grau, 1986; Bailey *et al.*, 1987; Connor *et al.*, 1987; Banwart, 1989; Mead, 1989). Efforts should be made to prevent the build-up of contamination peaks during processing. Rinsing of the carcasses, especially during defeathering and evisceration is therefore of great importance (Mead, 1982; Anand *et al.*, 1989; Mead, 1989). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin.

Studies conducted over the last few years show that the sites most heavily contaminated are the neck skin and less frequently on the back and the area around the vent. Fewer organisms are found around the breast, legs and under the wings (Bryan, 1980; Thomas and McMeekin., 1981; Mead, 1982; Grau, 1986; Anand *et al.*, 1989). The presumable reason for the neck skin being the most heavily contaminated is that the washings from the rest of the carcass run down the neck while the carcass hangs on the conveyor (Connor *et al.*, 1987).

The study was carried out to investigate the contaminating micro-organisms which can be found on the breast, legs and backs of broilers during processing.

## **Materials and Methods**

### ***Sampling of Broilers***

A total number of 81 swabs from 27 carcasses were collected randomly from the legs, breast and backs in 9 Critical Control Points (CCPs), namely; after bleeding with feathering, after scalding, after defeathering, after evisceration, after spray wash, after chilling and packing, hand of worker, knives and scalding water (Bryan, 1980). Samples were collected over a period of three months every one week. A sterile metal template was used to outline 10 cm<sup>2</sup> area of the thigh region on the broiler carcasses and then the area was swabbed vigorously with sterile cotton gauze wrapped around the end of a flat swab stick. Three samples were taken from each CCP in the processing line of broilers. The organisms were removed from each swab by shaking for of few minutes in 10 ml of sterile 0.5% peptone water. The collected swabs of each carcass were marked, numbered and transported promptly on ice to the laboratory.

### **Bacterial colony count**

The total viable count (TVCs) of the isolated microorganism was carried out according to the method of Harrigan and MacCance (1976). A serial dilution of each sample was made to form ( $10^{-1}$  up to  $10^{-5}$ ). Generally the sample is diluted in ten-fold increments to make the resulting math simple, this was done by preparing five sterile, labeled beakers from (1) to (5). From this solution 10 ml was pipette into beaker (2) which contains 90 ml of distilled water to yield a total volume of 100 ml to form 10. The process continued until serial dilution of original bacterial suspension in beaker number (5) was made. Each dilution was spread out on a disposable Petri-dish contained a solidified agar medium, MacConkey's, Nutrient agar, blood agar.

Then 0.1-0.2 ml of the dilution was taken out, this was done by sterile bent spreader. Then all plates incubated upside down at 37°C. After 24 hours the number of all colonies on the plate (between 30-300) was counted for each dilution and the mean count was determined. Each colony forming unit represented a bacterium that was present in the diluted sample, therefore the concentration of viable bacteria per milliliter in initial sample can be calculated and expressed in CFU /ml.

### **Bacterial identification**

The morphology of colonies on agar media were examined microscopically, smears were made from clean slides fixed with heat and subjected to Gram stain and examined under oil immersion. In addition to that, the identification has also been based mainly on biochemical tests (Barrow and Feltham, 2003).

### **Biochemical tests**

The Enteropluri-test was done as described by the producer company (LIOFILCHEM Bacteriology Products Via Scozia Zona Ind. Le - 64026 Roseto D.A –Italy). An isolated colony from agar medium was picked up by needle of the Enteropluri-test system without penetrating into the agar, afterwards that the needle was inoculated throughout the sectors of the system. After that the system was incubated at 37C for 24 hours. Finally the change in colour in the different sectors was observed for positive reactions and the bacterium was identified by following instructions on codebook. Indole test by using Kovac's reagent (3-4 drops) was added, then positive pink – red colour has developed within 15 minutes for *E. coli*.

The catalase test was done by mixing a dense culture with 2 drops of H<sub>2</sub>O<sub>2</sub> and looking for bubbles. The presence of bubbles indicates a positive test and the organisms considered to be Staphylococcus (Barrow and Feltham, 2003).

### **Data analysis**

The data were analyzed with SPSS software (Statistical package for the social sciences version 20, IBM/SPSS. Descriptive statistics were used to analyze the data. In addition, all TVCs bacteria were converted to log<sub>10</sub> CFU cm for analysis. ANOVA was performed. Statistical significance was set at P- value of <0.5.

## Results

The study revealed a statistically significant difference at *P-value* ( $p \leq 0.05$ ) in the 9 CCPs between the legs, backs and breast respectively. As shown in Table 1, the TVC revealed the highest contamination level of the backs recorded after defeathering  $9.99 \pm 0.01 \log_{10}$  CFU/cm<sup>2</sup> while the highest contamination level of the breasts was after chilling and packing  $1.86 \pm 0.01 \log_{10}$  CFU/cm<sup>2</sup> but the highest contamination level of the legs was after scalding  $9.96 \pm 0.01 \log_{10}$  CFU/cm<sup>2</sup>.

**Table 1.** Comparison of mean total viable count of bacteria ( $\log_{10}$  CFU/cm)  $\pm$  SD at different operational points from different sites on carcasses (N =81)

Operational points/Sites	Leg	Back	Breast	Significance
After bleeding with feather	9.53 $\pm$ 1.000	9.94 $\pm$ 5.508	9.80 $\pm$ 1.000	*
After scalding	9.96 $\pm$ 1.528	9.93 $\pm$ 2.000	9.60 $\pm$ 0.543	*
After defeathering	9.35 $\pm$ 2.082	9.99 $\pm$ 1.000	9.76 $\pm$ 1.000	*
After evisceration	8.94 $\pm$ 2.572	9.93 $\pm$ 1.528	9.84 $\pm$ 1.482	*
After spray wash	2.97 $\pm$ 2.517	2.61 $\pm$ 2.000	2.30 $\pm$ 0.537	*
After chilling and packing	1.44 $\pm$ 0.528	1.43 $\pm$ 0.528	1.86 $\pm$ 0.528	*
Hand of workers	2.91 $\pm$ 1.767	2.76 $\pm$ 1.438	1.98 $\pm$ 1.722	*
Knives	9.94 $\pm$ 1.528	9.36 $\pm$ 1.528	9.26 $\pm$ 1.498	*
Scalding water	9.78 $\pm$ 1.528	9.33 $\pm$ 1.324	9.85 $\pm$ 1.528	*

\*Significant difference at P value of <0.05.

**Table 2.** Bacteria species isolated from different operational points (N = 81)

Operational points/Sites	<i>E. coli</i>	<i>S. aureus</i>	<i>S. albus</i>	<i>Salmonella</i>
After Bleeding with Feathering	0 (0.00%)	2 (11.11%)	0 (0.00%)	0 (0.00%)
After Scalding	0 (0.00%)	2 (11.11%)	0 (0.00%)	0 (0.00%)
After Defeathering	2 (11.11%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
After Evisceration	2 (11.11%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
After Spray Wash	1 (5.56%)	0 (0.00%)	0 (0.00%)	1 (5.56%)
After Chilling and Packing	1 (5.56%)	1 (5.56%)	0 (0.00%)	0 (0.00%)
Hand of Worker	1 (5.56%)	1 (5.56%)	0 (0.00%)	0 (0.00%)
Knives	0 (0.00%)	0 (0.00%)	2 (11.11%)	0 (0.00%)
Scalding Water	2 (11.11%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	9 (50.00%)	6 (33.33%)	2 (11.11%)	1 (5.56%)

Isolation and identification of gram positive bacteria 8 (9.1%) and gram-negative 10 (12.3%) bacteria at different operational points under investigation revealed 4 species of bacteria as shown in Table 2. After bleeding with feathering and after scalding there was *Staphylococcus aureus* (11.11%), after defeathering and after evisceration there was *Escherichia coli* (11.11%), after spray wash *Escherichia coli* and *Salmonella* (5.56%), after chilling and packing *Escherichia coli* and *Staphylococcus aureus* (5.56%), workers' hand *Escherichia coli* and *Staphylococcus aureus* (5.56%), knives *Staphylococcus albus* (11.11%) and scalding water *Escherichia coli* (11.11%).

## Discussion

Broilers arriving to the poultry slaughter house for processing are generally highly contaminated with bacteria, especially with potential human pathogenic bacteria, such as *Coliform* and *Salmonella* (Göksoy *et al.*, 2004). In this study, the mean TVCs obtained from chicken carcasses after slaughter with feathers, after scalding, after defeathering, after evisceration, and scalding water were higher than those reported by Göksoy *et al.* (2004), while the mean TVCs obtained from chicken carcasses after spray wash and after chilling and packing were lower. This difference could be due to environmental and transportation conditions. Moreover, our findings are also higher than the findings of Kabour (2011) who reported mean TVCs  $7.69 \pm 2.6$  in legs,  $7.49 \pm 1.6$  in backs and  $8.38 \pm 2.1$  in breasts after defeathering. But the mean TVCs obtained from chicken carcasses after spray wash and after chilling and packing were lower than those reported. It is obvious that the variability of microbial counts indicates the need for use of prerequisite programs. The reduction of the microbial contamination in this study is in agreement with Rahkio and Korkeala (1996), who said that the enforcement of hygienic practice such as regular disinfection of working tools and workers' hands is important in reducing the microbiological contamination of carcasses. John *et al.* (2000) reported that the reduction of bacterial contamination during slaughtering after using a degree of sanitation.

Another study by Jeffery *et al.* (2003) revealed that the workers' hands and the equipments were the sources of meat contamination; these results are in accordance with the present results. The elimination of contamination sources by practicing good sanitary measures will reduce the occurrence of microorganisms. Appropriate methods should be applied during slaughtering operations, using adequate water and disinfection.

Sudan is a tropical country, with ambient temperatures conducive for the growth of microorganisms, which can rapidly render meat unsafe for human consumption. The levels of microbial contamination in Sudanese abattoirs may reflect the hygienic status of poultry meat production in the developing world.

Qualitative bacterial examination of carcass samples in this study revealed results that are in agreement with the findings of Ahmed, (2004) and Kaboor, (2011). The presence of *Escherichia coli* as a contaminant of fresh meat which is reported here has also been reported by several workers. Ahmed (2004) and Kaboor, (2011) suggested faecal contamination as a cause of the large numbers of isolates of *E. coli* recovered. The isolation of bacteria from workers in this study, and the presence of *E. coli* can be attributed to carcass contaminated with the gastrointestinal contents during processing.

An important observation in the present study is that *Salmonella* spp. was isolated from the samples examined. This is in contrast with the result of Ahmed (2004). The highest contamination levels recorded in the point of washing in all sites (flank, hind leg and shoulder) may be due to unclean management during washing, this is in agreement with (Ali, 2007) who reported the highest level of contamination with regard to the critical control point found in the washing point.

Also Göksoy *et al.* (2004) demonstrated the presence of *Staphylococci* species at different stages of processing. This finding is similar to our result but contrary to the findings of Kabour (2011) who did not detect any *Staphylococci* species in his study. Industries must implement this food safety program to serve both internal and external market (Jimenez *et al.*, 2002; Mead, 2004). Sudanese abattoirs may reflect the hygienic status of chicken meat production in the developing countries.

## Conclusion

The present study demonstrates the degree of the microbial contamination during processing of broilers carcasses. The results also indicate that the viable count for microorganisms causing public hazards is appropriate for analysis. Therefore, application of hygienic measurements appears to be important to reduce the contamination of bacteria in abattoirs. So the implementation of Hazard Analysis and Critical Control Points (HACCP) in poultry industry is extremely important, because it involves the constant monitoring of all steps of the process.

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