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Detection of *Salmonella* spp. and *Escherichia Coli* in Poultry Carcasses at Abattoir in Khartoum State Sudan

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ABSTRACT

The aim of the study was to investigate the contamination of poultry carcasses at abattoir by *Salmonella* spp. and *Escherichia coli*. Sixty swab samples were collected from carcasses of broiler chickens. The study covered six stages of poultry meat processing and these were hands of employees, defeathering, evisceration, after washing, after chilling, and packing. Isolation and identification of *Salmonella* spp. and *Escherichia coli* were also done. The highest contamination level by Total Viable Counts caused by *Salmonella* spp. 6(11.11 %) at defeathering than *Escherichia coli* 2(3.71%). While the lowest contamination level at after chilling. Statistically, there was significant difference at P-Value ($P \leq 0.05$) in six stages from results the two species of bacteria predominant in abattoir processing that affected on safety and quality of poultry meat. Application of HACCP can be reduced bacterial contamination.

INTRODUCTION

Most countries have been worried about food-borne diseases nearly in developing countries due to food problems reported cases economic and social costs effect around the world (Zhao *et al*., 2001). The microorganisms in different part of carcass ,carried out on food from origin animal, particularly poultry product, contribute significantly to food- borne disease in humans , during processing, a high proportion of this organisms will be removed and will result in reducing the incidence of illnesses but further contamination may occur at any stage of processing operation (Kabour, 2011).

Skin of poultry carcasses always exposed to high average rate of microorganisms, they can pathogenic that cause food-borne illness as well as food spoilage, they series of microorganisms on the surface of carcasses which can be canalized in order to indicate the microbial quality , the level of hygiene in production and handling and the correct maintenance of cold chain (Sandrou and Arvanitoyannis,1999) .These systems present some advantages over traditional methods and results obtained in study from eight slaughter houses suggested that HACCP systems can maintain or even improved food safety(Cates *et al;* 2001). Due to defeathering the microorganisms are widely distributed under normal circumstances and are spread over the skin during scalding and defeathering on inner and outer surfaces during evisceration of the further processing (Bailey *et al*; 1987). Quality of poultry meat during slaughtering and packing and hygienic status of slaughterhouse (Lillard, 1990). Monitoring of all steps of process aiming the food safety of final product HACCP in poultry industry is extremely important involving the constant, this safety program to serve both internal and external market (Jimenez *et al*; 2002, Mead 2004 , Galhardo *et al*; 2006). The contamination and or cross - contamination of carcasses, during slaughter process were demonstrated and results indicated presence of bacteria potential public health significances. (Doyle, 1991; Biss and Hathaway 1995). Also dirty worker hands, clothes, equipments of slaughterhouse. Acts as intermediated sources of contamination of meat (Gill, 1998; Gilmour *et al*; 2004). Dirty worker hands, clothes equipments of slaughterhouse acted as intermediated sources of contamination of meat (Gill, 1998; Gilmour *et al*; 2004; Abdelsadig 2006, Abdalla *et al.*, 2009). HACCP in poultry is extremely important because it involves the constant monitoring of all steps of the process, aiming the food safety of final product, industries must implement this food safety program to serve both external and internal market (Jimenez*et al*., 2002, Mead 2004; Galhardo *et al*., 2006). The aim of this study was to determine the contamination of poultry carcasses by *Salmonella* species and *Escherichia coli* at abattoir in Khartoum state.

MATERIALS AND METHODS

Study area: The Samples collected from broiler abattoir located in Khartoum state.

Collection of samples: Total of 60 swab samples were obtained from carcasses of broiler chickens. The samples were taken from six critical control points CCPs after evisceration, hands of employees, defeathering, after washing, before chilling, after chilling, packing. Samples were collected in sterile tubes and preserved in ice and transferred to laboratory of microbiology for culturing.

Bacterial counts: For bacterial counts, the total plate count (TPC) was carried out as described by Barrow and Feltham (2003). The TPC was conducted by making of a 10-fold serial dilution of each sample. Five sterile test tubes were labelled from 1 to 5. From the test tube 1, a volume of 1 ml was added into the test tube 2 to make a total volume 10 ml. The process continued until a serial dilution form 10-1 to 10-5 was achieved. Each dilution was then cultured by the pouring plate method using the standard plate count agar medium and cultured plates were then incubated at 37°C for 24 hours. After that, the number of all colonies was counted for each dilution and the mean count was determined. Each colony represented a bacterium or colony forming unit (cfu)

that was in the diluted sample, this is why, and the number of viable bacteria per Millilitre (ml) in the sample was calculated and expressed in cfu / cm^2. The *Enterobacteriace* Enumeration (EE) was carried out by using the pouring plate method and violet red bile glucose agar (VRBGA). A volume of 1 ml of the diluted sample was transferred to a Petri dish. Then 15 ml of tempered VRBGA in a 45°C water bath was added. The inoculum was carefully mixed with the medium and the mixture was allowed to solidify. After complete solidification, an overlay with 10 to 15 ml was made. Plates containing completely solidified mixtures were incubated at 37°C for 24 - 48 hours. The count was expressed as *Enterobacteriace* per cm^2 as follows: the number of computed colonies was divided by the inoculated volume which is multiplied by the dilution factor.

Isolation and identification of bacterial: The standard procedures for isolation of *Escherichia coli* and *Salmonella* species were conducted by using the surface plate method and the respective selective media as described by Barrow and Feltham (2003). The collected swab samples were cultured onto prepared, violet red bile agar (VRBA), Oxford Listeria agar, mannitol salt agar (MSA), tryptic soy agar (TSA) and Baird Parker agar. All plates were incubated at 37°C for 24 hours. For confirmation of *E. coli*, any growth on the VRBA was subcultured onto Brilliant green agar and into trypton water and incubated at 44oC for 24 hours. For isolation of *Salmonella* species, samples were first cultured into buffered peptone water and kept at 37°C for 24 hours, then subcultured into Selenite cystine broth base for 24 hours at 37°C, and finally subcultured onto xylose lysine deoxycholate agar (XLDA) for 24 hours at 37°C.

Statistical analyses: The generated data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0, IBM/SPSS. All bacterial counts were converted to $\log_{10}$ cfu/cm. Descriptive statistics were performed and Analysis of Variance (ANOVA) with a *p-value* of ≤ 0.05 likewise.

RESULTS

The study revealed isolation and identification of two types of bacteria in different operational points. *Salmonella* spp. and *Escherichia coli* (Table 1). Isolation and identification of bacteria at different operational points under investigation revealed at defeathering stage 6(11.18%) for *Salmonella* spp. and 4(7.42%) for *Escherichia coli* whereas chilling stage 8(16.66%) *Salmonella* spp. and 2(1.85%) for *Escherichia coli* 2(1.85%).

Table 1: Number and percentage of Bacteria isolated in different Operational Points at Khartoum State

Points	*Salmonella* spp.	*Escherichia coli*	Total
Evisceration	7(5.5%)	3(5.55%)	10(11.05%)
Employees	8(11.11%)	2(3.71%)	10(14.82%)
Defeathering	6(11.18%)	4(7.42%)	10(18.60%)
After Washing	9(16.66%)	1(1.85%)	10(18.51%)
After Chilling	8(16.66%)	2(1.85%)	10(18.51%)
Packing	8(14.81%)	2(3.70%)	10(18.51%)
Total	47(75.92%)	13(24.08%)	60(100.00%)

In table 2 , the TVC revealed the highest contamination at defeathering point (Mean 6.50) and Std. Deviation (±0.09) , and lowest contamination after chilling point (Mean 6.40) at Std Deviation (±0.16). The study revealed a statistically Significant Differerence at ($P \leq 0.05$) after Defeathering and after Chilling (Table 2)

Table 2 : Mean and Standard Deviation of Total Viable Counts of Bacteria($Log_{10}cfu/cm^2$) at Different Operational Points

Points	Mean ±Std	Significant Difference
Evisceration	0.17±6.41	**
Employees	6.46±0.08	**
Defeathering	6.50±0.09	**
After washing	6.43±0.12	**
After chilling	6.40±0.16	**
During Packing	6.43±0.12	**

Std: standard Deviation

DISCUSSION

In this study the total bacterial viable count (TVCs) obtained from the result showed lower contamination after chilling stage, and highest contamination at defeathering stage. This data in accordance to the finding of (Mead 2004) who reported that substantial decrease in (TVCs) Contamination which may occur due to bacterial population associated with water from the scald tank, rubber fingers at the exit of defeathering machine (Georanras *et al*., 1997). Feathers generally may contaminate external surface of the carcass skin during early processing stages. The highest level of viable aerobic bacteria recovered from the samples. Also the result in agreement with the findings of Ramires *et al* 1997 and Hinton *et al*., 2000). Who reported that broiler carcass can contaminated by bacteria when contact with ingesta or feces from eliminatory tract during grow - out . Drewnaik *et al* ., (1984) found that there was build-up of bacteria on the skin of chicken during dressing and evisceration, they also found that the procedures after dressing which include washing with pressurized sprays water decreases the bacteria present on the skin of poultry. Defeathering or picking achieved by passing the birds through rows of rotating rubber fingers that remove the feathers and help squeeze the remaining blood. Mechanical pickers and other items used for processing must be constructed to ensure clean lines (Houston 1985). It represents another chance for cross - contamination, consider microorganism like *Salmonella* have been shown to attach firmly to poultry skin and rubber fingers act as transmitters for contamination. The extent of cross - contamination during plucking is governed by the hygiene of scalding process. Alternatives have been developed including simultaneous scalding and plucking and steam scalding .These minimize cross - contamination with Salmonella spp. Salmonella are more frequently isolated from carcasses after defeathering than following any other processing operation (MeBride *et al*., 1980). Following hot or hard scalding, defeathering damages and removes the epidermal layer and exposes a new surface layer to contamination. Contamination either during primary operation (e.g. slaughtering) or further processing & handling (cross - contamination during processing human to food contamination via food handlers). *Escherichia coli* has been isolated world-wide from at (Contamination of poultry properly due to increased used antimicrobials (Miranda *et al*., 2008; Adesiji *et al*., 2011). Also due defearthening the microorganisms are

widely distributed under normal circumstances and are spread over the skin during scalding and defeathering on inner and outer surfaces during evisceration of the further processing(Bailey *et al*., 1987). On study of the effect of processing procedures and overall environmental and hygienic condition of the microbiological quality and safety, found heavily contamination at scalding and defeathering with Salmonella and *Escherichia coli* (Abu-Ruwida *et al*., 1994). This data is in agreement with the data of this study. The presence of Escherichia Coli in fresh meat can be attributed to carcass contamination with the gastrointestinal content during processing .The contamination levels recorded in the point of washing in all sites carcasses in this study may be due to unclean management during the washing , this is agree with (Ali, 2007)

In this study, the Mean TVCs obtained from chicken carcasses in following CCPs; after defeathering, after evisceration, after spray water and hands of employees are similar to that obtained by Kabour (2011). Furthermore, the Mean TVCs are confirming the reports of Mohamed Noor *et al*., (2012), who found the same TVCs of some CCPs including: after defeathering, after evisceration, after chilling and employees hands at the same time.

CONCLUSIONS

There is contamination in an automatic poultry slaughterhouse in Khartoum State. *Salmonella* species and *Escherichia coli* were isolated from poultry meat at all stages of processing. The highest contamination was shown at defeathering stage and the lowest contamination after chilling stage. Most of poultry slaughter houses are not applying HACCP System.

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