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Investigation of *Salmonella spp.* and *Escherichia coli* and *Staphylococcus aureous* at six points in an automatic poultry slaughter house in Khartoum State Sudan

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ABSTRACT

The present study was conducted to investigate the microbial contamination of poultry meat carcasses at an automatic abattoir slaughter processing caused by three types of bacteria *(Salmonella spp. and Escherichia coli and Staphylococcus aureus)* which was isolated and indentified in Khartoum State. 90 swab samples were collected from carcasses of broiler chicken at six stages (Scalding, defeathering, evisceration, after washing, after chilling, hands of Employees.) during slaughtering process. The experiments were conducted to determine isolation and identification for three bacteria *(Salmonella spp. and Escherichia coli and Staphylococcus aureus)*The results revealed that, the high contamination was detected in defeathering stage(mean (log_{10}CFU/ml**)** 7.09± 0.13 and the numbers of bacteria isolated as follows 12(10.4%) samples were positive for *Salmonella spp., 4*(3.5%) samples were positive for *Escherichia coli, and* 11(9.6%) samples were positive for *Staphylococcus aureus*, the low contamination was after washing stage (mean(log_{10}CFU/ml**)** 6.68 ± 0.15) and the numbers of bacteria isolated as follows 15(13%) samples were positive for *Salmonella spp.,*3(2.6%) samples were positive for *Escherichia coli.spp, and* 2(1.8%) samples were positive samples for *Staphylococcus aureus.* Statisticaly there was significant difference at P-Value (P≤ 0.01) in all Different Operational Points (scalding, defeathering, evisceration, after spray wash, after chilling and hands of workers.) in automatic abattoir slaughter.

INTRODUCTION:

Microbiological status of broiler carcasses depends on several factors, as infection level of living birds and /cross contamination during processing (Abu-Ruwaida et al, 2004. Meat hygiene and safety. It is generally assumed that preventing visible contamination or removing visible contamination from carcasses will enhanced the microbiological safety of meat. They have a potential impact on the food safety or bacteriological quality of poultry carcasses. Heemskerk (2005). 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).

MATERIALS AND METHODS:

Sampling: A total number of 90 swab samples from an automatic slaughtering processes and employees hands were collected carcass surfaces of broiler meat by swabbing methods technique in sterile test tubes 6 Critical Control Points (CCPs), namely; scalding, defeathering, evisceration, after spray wash, after chilling and hands of workers. The collected swabs of each carcass were marked, numbered and transported promptly on ice to the laboratory in College of Veterinary Medicine, Sudan University of Science and Technology for microbiological analysis.

Bacterial examination*:* Smears were made from colonies on agar media, by clean slides fixed with heat and subjected to Gram stain and examined under microscopic oil immersion. In addition to that, the identification has been also based mainly on the procedure of Barrow and Feltham (2003) The TBC 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 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). Isolation and identification of bacterial: The standard procedures for isolation and identification of *Salmonella species, Escherichia coli*

and Staphylococcus aureus 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 . All plates were incubated at

Data analysis: The data were analyzed with SPSS software (Statistical package for social science version 20, IBM/SPSS). Descriptive statistics were used to analyze the data. In addition, all TVCs bacteria were converted to log10 CFU/cm2 for analysis. ANOVA was performed. Statistical significance was set at P- value of≤ 0.05.

RESULTS:

The study revealed the isolation and identification of three types of bacteria in different operational points. (*Salmonella spp., Escherichia coli and Staphylococcus aureus*.) The results revealed that high level of contamination was detected at defeathering stage (mean (log_{10}CFU/ml**)** 7.09 ± 0.13) and the numbers of bacteria isolated as follows 12(10.4%) samples were positive for *Salmonella spp., 4*(3.5%) samples were positive for *Escherichia coli.spp, and* 11(9.6%) samples were positive for *Staphylococcus aureus*, low contamination was detected after washing stage(mean(log_{10}CFU/ml**)** 6.68 ± 0.15) and the numbers of bacteria isolated as follows 15(13%) samples were positive for *Salmonella spp.,*3(2.6%) samples were positive for *Escherichia coli. spp,* 2(1.8%) samples were positive for *Staphylococcus aureus*

37°c for 24 hours. The media used were Dexoycholate Citrate Agar for *Salmonella spp. colonies,* MaConkey Agar: for *Escherichia coli Colonies* and Mannitol Salt Agar*: for Staphylococcus aureus.*

The types of bacteria isolated from other stages as follows:

At Scalding (mean (log_{10}CFU/ml**)** 7.01±0.21) and the numbers of bacteria isolated as follows12 (10.4%) samples were positive for *Salmonella spp.,* 3(2.6%) for *Escherichia coli and* 2(1.8%) samples for *Staphylococcus aureus.*

At Evisceration (mean (log_{10}CFU/ml**)** 6.86±0.15) and the numbers of bacteria isolated as follows15 (13%) samples were positive for *Salmonella spp.,* 1(0.9%) for Escherichia *coli.spp, and* 1(0.9%) for *Staphylococcus aureus*

At after chilling (mean (log_{10}CFU/ml**)** 6.84±0.29) and the numbers of bacteria isolated as follows15 (13%) samples were positive for *Salmonella spp.,* 1(0.9%) for *Escherichia coli. and1* (0.9%) for *Staphylococcus aureus.*

At Employees (mean (log_{10}CFU/ml**)** 6.74±0.18) and the numbers of bacteria isolated as follows 13 (11.3%) samples were positive for *Salmonella spp.,* 2(1.7%) for *Escherichia coli.spp, and* 2(1.8%) for Staphylococcus *aureus*

Statistically there was high significant difference at P-Value (P≤ 0.01) in all Different Operational Points (scalding, defeathering, evisceration, after spray wash, after chilling and hands of workers.) in automatic abattoir slaughter.

Table (1): Mean and Standard Deviation at six operation processes in an automatic slaughter house:

CCP	Automatic
Scalding	7.01±0.21
Defeathering	7.09±0.13
Evisceration	6.86±0.15
After Washing	6.68±0.15

After Chilling	6.84±0.29
Employees	6.74±0.18

±Sd = Standard Deviation

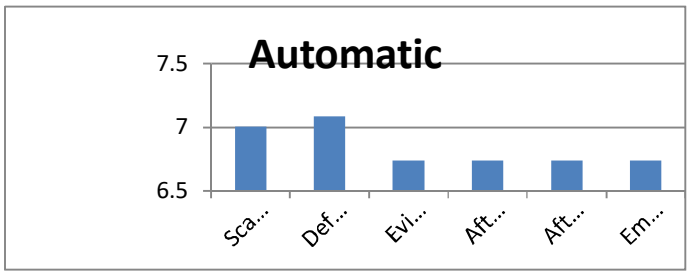

Figure (1): Mean and Standard Deviation at six Operation Processes in at an automatic slaughter house

Table (2): Numbers and percentages of bacteria (*Salmonella spp. and Escherichia coli and Staphylococcus aureus)* which were isolated at An Automatic poultry slaughter house

Critical control points	No. of Samples	*Salmonella.* Frequency %	*Escherichia coli.* Frequency %	*Staphylococcus aureus* Frequency %	Phases %
Scaldening	15	12(10.4%)	3(2.6%)	2(1.8%)	17(14.78%)
Defeathering	15	12(10.4%)	4(3.5%)	11(9.6%)	27(23.48%)
Evisceration	15	15(13%)	1(0.9%)	4(3.6%)	20(17.39%)
After Washing	15	15(13%)	1(0.9%)	1(0.9%)	17(14.78%)
After Chilling	15	15(13%)	1(0.9%)	1(0.9%)	17(14.78%)
Employees	15	13(11.3%)	2(1.7%)	2(1.8%)	17(14.78%)
TOTAL	90	82(71.1%)	12(10.5%)	21(18.6%)	115(100%)

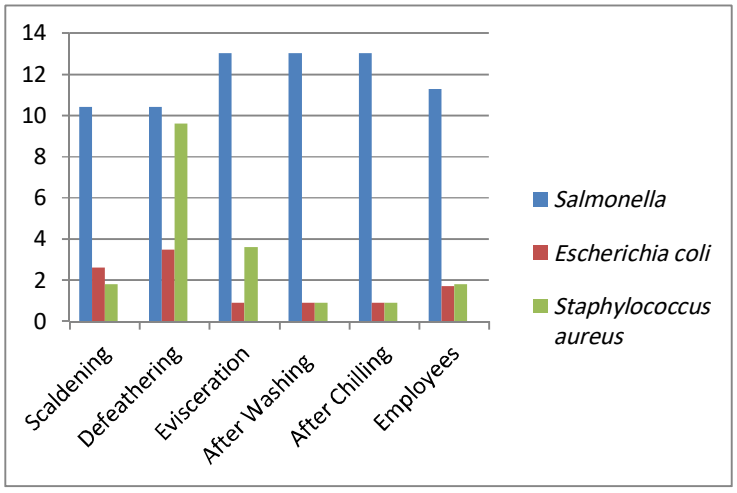

Figure 2: Load of contamination by (Salmonella spp. and Escherichia coli and Staphylococcus aureus).at An Automatic poultry slaughter house

DISCUSSION:

In this study the total bacterial viable count (TVCs) obtained from the result tamination was detected in defeathering stage (mean (log_{10}CFU/ml**)** 7.09 ± 0.13) and the numbers of bacteria isolated as follows 12(10.4%) samples were positive for *Salmonella spp., 4*(3.5%) samples were positive for *Escherichia coli., 11*(9.6%) samples were positive samples for *Staphylococcus aureus. This data in accordance to the finding* of Mead (2004) who reported that substantial decrease in TBCs Contamination 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.

Presence of *salmonellae* in chicken meat may be attributed to the healthy state of the living bird which carries *salmonellae*, bad hygienic conditions during slaughtering, cross contamination either from other birds, instruments, machines, workers, scalding tanks, defeathering machines, crop removal, manual evisceration , during slaughter, intestinal contents can spill and contaminate the muscle and organs of the chicken, which is the important source of presence of *Salmonella* in meat and chilling tanks Paiao et al., (2013).This data agree with the present results. *Escherichia coli* have 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 surface (Bailey et al., 1987).

showed high level of con

In this study the total bacterial viable count TVCs obtained from the result showed lower contamination was detected after washing stage (mean(log_{10}CFU/ml**)** 6.68 ± 0.15) and the numbers of bacteria isolated as follows 15(13%) samples were positive for *Salmonella spp.,*3(2.6%) samples were positive for *Escherichia coli.spp, and* 2(1.8%) samples were positive for *Staphylococcus aureus.*

The presence of *Escherichia Coli* in fresh meat can be attributed to carcass contamination with the gastrointestinal content during processing .The contamination levels recorded at the point of washing in all sites carcasses may be due to unclean management during the washing, Ali (2007) but this data disagree with present study.

CONCLUSIONS:

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

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