Investigation of *Salmonella spp*. and *Escherichia coli* and *staphylococcus aureous* at six points in Traditional poultry slaughtering process in Khartoum State Sudan.

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ARTICLE INFO	ABSTRACT
	The study was aimed to isolate and to identify three types of bacteria at six different operational processes in Traditional poultry slaughtering process in Khartoum state. 90 swab samples were collected from carcasses of broiler chicken at six processing stages (Scalding, defeathering, evisceration, after washing, after chilling, hands of Employees.) during slaughtering process. Traditional methods were used to determine the isolation and identification of three bacteria (*Salmonella spp., Escherichia coli and Staphylococcus aureus)*. The results revealed that, the highest contamination level was at Evisceration which showed high total bacterial count (mean ($\log_{10}$CFU/ml) 7.43 ±0.16)) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp.,* 10(5.7%) samples were positive for *Escherichia coli, and* 8(4.6%) samples were positive for *Staphylococcus aureus, the low level contamination after Chilling which showed* (mean($\log_{10}$CFU/ml) 7.30 ±0.14) and the numbers of bacteria isolated were 14(7.3%) samples were positive for *Salmonella spp.,* 7(4%) samples were positive for *Escherichia coli and* 10(5.7%) samples were positive for *Staphylococcus aureus*. The results at scalding stage showed total bacterial count (mean ($\log_{10}$CFU/ml) 7.28±0.11) 14(8%) and the numbers of bacteria isolated as follows samples were positive for *Salmonella spp.,*6(3.4%) for *Escherichia coli, and 9*(5.1%) for *Staphylococcus aureus* The results at defeathering stage showed total bacterial count (mean ($\log_{10}$CFU/ml**)** 7.43±0.08) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for

Salmonella spp., 8(4.6%) for *Escherichia coli, and* 6(3.4%) for *Staphylococcus aureus.* The results after washing stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.36±0.11) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp., 3*(1.07%) for *Escherichia coli, and* 10 (5.7%) for *Staphylococcus aureus.* The results at employees stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.37±0.16) and the numbers of bacteria isolated as follows 12 (6.9%) samples were positive for *Salmonella spp., 10* (5.7%) for *Escherichia coli, and12* (6.9%) for *Staphylococcus aureus.* The statistical analysis of results revealed that, there was significant difference at P-Value (P≤ 0.01) in all Different Operational Points in traditional slaughtering process in Khartoum state.

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) Poultry meat can simply contaminated with microorganisms, thus modern processing needs an elevated rate of through put to meat consumers demand (Kabour 2011). Skin of poultry carcasses always exposed to high average rate of microorganisms, they can be pathogenic causing 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 (Sandrou and Arvanitoyannis,1999) .These systems present some advantages over traditional methods, results obtained in slaughter houses suggested that HACCP systems can maintain or even improved food safety(Cates et al ,2001). 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 & Hathaway 1995).Also dirty work hands, clothes,

equipments of slaughterhouse. Acts as intermediated sources of contamination of meat (Gill, 1998; Gilmour et al; 2004).

Obtaining poultry meat is a similar process in all the slaughterhouses, with some differences in specific stages. Basically, this process consists on a highly coordinated system of different operations aimed at slaughtering the birds, removing the inedible portions of the carcasses and preserving the edible portions for distribution to consumers (Sams and McKee 2010). The poultry slaughtering process involves the following phases: stunning and bleeding, scalding, defeathering, evisceration, washing and chilling. The whole process can be divided in two basic areas: the "dirty zone", including stunning, bleeding, scalding, defeathering and evisceration stages and the "clean zone" including washing and chilling (Escudero-Gilete et al., 2005).

Chicken carcasses have higher pathogenic and spoilage bacterial counts than most other foods, where carcass can be contaminated at several points throughout the processing operation during scalding, de-feathering and evisceration as well as cross

contamination from other birds and processing and employees . All slaughter-process should implement HACCP or similar food safety management system to control all food hazards in the slaughtering processes to ensure the production of safe and wholesome food for human consumption. The objective of the study A total number of 90 swab samples were collected from the surfaces of broiler meat carcass at the Traditional slaughter processes 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.

Total 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 based mainly on the procedure of Barrow and Feltham (1993) The TBC was conducted by making of a 10-fold serial dilution of each sample. Five sterile test tubes were labeled 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.

were to isolate and indentify the (*Salmonella spp., Escherichia coli and Staphylococcus aureus)* at six processing stages during slaughtering process in Traditional poultry slaughtering processes in Khartoum state.

Materials and Methods:
 Sampling:
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 (1993) .
 The collected swab samples were cultured   . All plates were incubated at 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.*

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*.(Table 1). Isolation and identification of bacteria at different operational points under investigation revealed that highest contamination level at the Traditional Slaughtering process *at Evisceration*

which showed high total bacterial count (mean (log$_{10}$CFU/ml) 7.43 ±0.16)) and the numbers of bacteria isolated were 12(6.9%) samples were positive for *Salmonella spp.,* 10(5.7%) samples were positive for *Escherichia coli, and* 8(4.6%) samples were positive for *Staphylococcus aureus, the low level contamination **after Chilling which** showed* (mean(log$_{10}$CFU/ml) 7.30 ±0.14) and the numbers of bacteria isolated were 14(7.3%) samples were positive for *Salmonella spp.,* 7(4%) The results at defeathering stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.43±0.08) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp.,* 8(4.6%) for *Escherichia coli, and* 6(3.4%) for *Staphylococcus aureus.*

The results after washing stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.36±0.11) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp., 3*(1.07%) for *Escherichia coli, and* 10 (5.7%) for *Staphylococcus aureus.*

samples were positive for *Escherichia coli and* 10(5.7%) samples were positive for *Staphylococcus aureus.* .in table1(Fig 1).The types of bacteria from other stages were showed the results at scalding stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.28±0.11) 14(8%) and the numbers of bacteria isolated as follows samples were positive for *Salmonella spp.,*6(3.4%) for *Escherichia coli, and 9*(5.1%) for *Staphylococcus aureus*

The results at employees stage showed total bacterial count (mean (log$_{10}$CFU/ml) 7.37±0.16) and the numbers of bacteria isolated as follows 12 (6.9%) samples were positive for *Salmonella spp., 10* (5.7%) for *Escherichia coli, and12* (6.9%) for *Staphylococcus aureus.*

The statistical analysis of results revealed that, 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 traditional slaughtering process in Khartoum state

Table 1: Mean Total Viable Counts of Bacteria (log10 CFU/ml) at Different Operational Point in traditional slaughtering processes. Table1

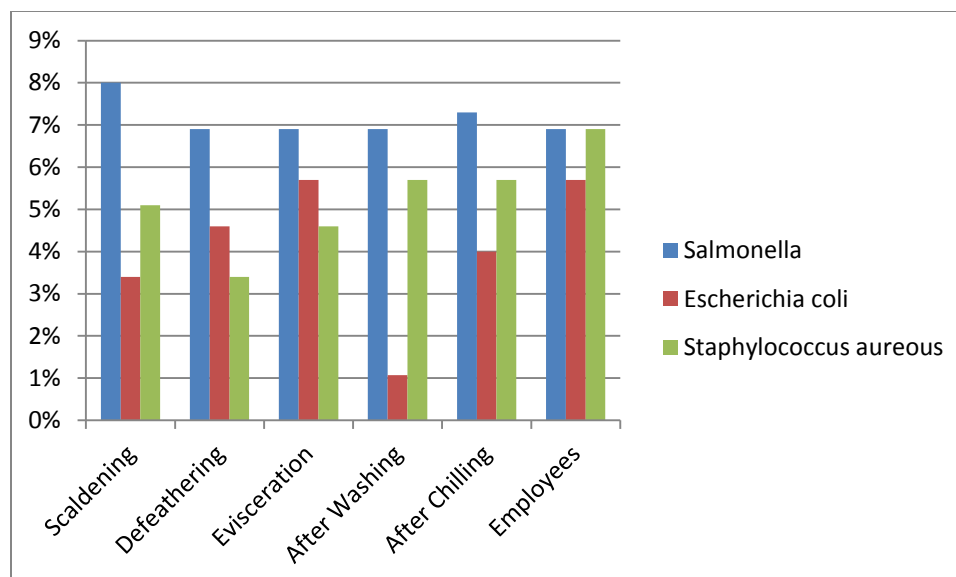| CCP	Traditional
Scalding	7.28±0.11
Defeathering	7.43±0.08
Evisceration	7.43±0.16
After Washing	7.36±0.11
After Chilling	7.30±0.14
Employees	7.37±0.16

±SD = Standard Deviation
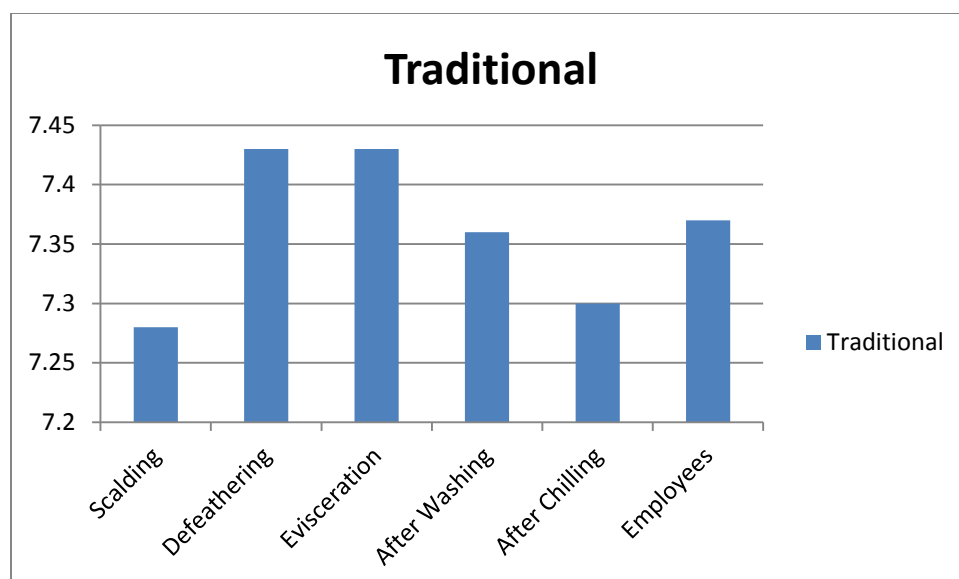
Fig1: Mean Total Viable Counts of Bacteria (log10 CFU/ml) ±Standard Deviation at Different Operational Point in traditional poultry slaughtering processes

Table 1: Numbers of bacteria and percentages were isolated from the Traditional poultry Slaughtering Processes (*Salmonella spp. and Escherichia coli and Staphylococcus aureous*).

Critical control points	No. of Samples	Salmonella spp.		Escherichia coli.		Staphylococcus aureous		Phases %
		Frequency	%	Frequency	%	Frequency	%	
Scalding	15	14(8%)		6(3.4%)		9(5.1%)		29(16.57%)
Defeathering	15	12(6.9%)		8(4.6%)		6(3.4%)		26(14.86%)
Evisceration	15	12(6.9%)		10(5.7%)		8(4.6%)		30(17.14%)
After Washing	15	12(6.9%)		3(1.07%)		10(5.7%)		25(14.29%)
After Chilling	15	14(7.3%)		7(4%)		10(5.7%)		31(17.71%)
Employees	15	12(6.9%)		10(5.7%)		12(6.9%)		34(19.43%)
TOTAL	90	76(42.4%)		44(25.01%)		55(32%)		175(100%)

Fig2: Isolated Bacteria (*Salmonella spp. and Escherichia coli and Staphylococcus aureous)* from the Traditional poultry Slaughtering processes

Discussion: In this study the total bacterial viable count (TVCs) obtained from the result showed that the high level of contamination was detected in defeathering stage (mean($\log_{10}$CFU/ml) 7.43 ± 0.08) and the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp.,* bacterial population associated with water from the scald tank, rubber fingers at the exit of defeathering machine (Feathers generally may contaminate external surface of the carcass skin during early processing stages.

Presence of *salmonellae* in chicken meat may be attributed 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). *Escherichia coli* has been isolated world-wide from at carcasses muscles

8(4.6%) samples were positive for *Escherichia coli, and* 6(3.4%) samples were positive for *Staphylococcus aureus),* This data in accordance to the finding of Mead (2004) and Georanras et al, (1997). who reported that substantial decrease in TBCs Contamination may occur due to (Contamination of poultry properly due to increased used antimicrobials (Miranda et al., 2008; Adetunji 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).

Low contamination was detected after chilling stage (mean ($\log_{10}$CFU/ml) 7.30 ± 0.14). *14*(7.3%) samples were positive for *Salmonella spp.,* 7(4%) samples were positive for *Escherichia coli and 10*(5.7%) samples were positive for *Staphylococcus aureus.*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) who

reported 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.

CONCLUSIONS The contamination in the traditional poultry processes in Khartoum State which causing by *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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