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Investigation of Bacterial Contamination in Chicken Carcasses at an Abattoir in Khartoum State

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ABSTRACT

The study was conducted at a poultry slaughterhouse in Khartoum State, Sudan, to evaluate microbial contamination in poultry meat. A total of 600 swab samples were collected randomly from chicken carcasses slaughtered at modern poultry abattoir. The samples were taken from five Critical Control Points (CCPs), namely; after defeathering, after evisceration, after spray wash, after chilling and hands of workers. Total Viable Count (TVC) was carried for each sample besides isolation and identification of contaminating bacteria. With exception of the CCP after spray wash, the study revealed a statistically significant difference at P-value ($p \leq 0.05$) in the other four CCPs between the legs, backs and breast and reholding 1, reholding 2 and packing, correspondingly. The isolated bacteria were Escherichia coli, Salmonella species, Pseudomonas species, Shigella species and Staphylococcus aureus. The current results indicate that there was an increase in the level of total aerobic and coliform counts in swab samples taken from chicken carcasses and this is worrying due to their ability to cause diseases 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 slaughtering procedure.

INTRODUCTION:

Most countries specially developing one, have been worried about Food-borne diseases mainly in developing countries due to food-related problems reported cases, economic and social costs effect around the world (Zhao *et al.*, 2001).

Bacterial microorganisms such as *Salmonella* and *E. coli* normally exist in gastrointestinal tracts of several wild and domestic animals, particularly animals infrequent for human consumption (Meng and Doyle, 1998; Zhao*et al.*, 2001).Poultry meat be may contaminated with these and other pathogenic bacteria during many food chain processing from primary production to final consumer (Todd, 1997; Petersen and James, 1998; Mead, 2004).Infected people with bacterial food-borne microorganisms mainly transferred from Contaminated raw or undercooked poultry meat to them (Zhao*et al.*, 2001; Mead, 2004). *Salmonella* species are well-known of being highly adaptive and potentially pathogenic for humans and/or animals. These organisms are one of major food-borne cases of gastroenteritis. However, lower prevalence of this infection appears as bacteraemia and acute significant intestinal disease. Animals like poultry, livestock, pets and reptiles are consider as primary carriers of *Salmonella* species (Mead, 2004; Fluit, 2005).*Escheria coli* is a Gram-negative bacterium belonging to the family *Enterobacteriaceae*. Pathogenic strains of *E. coli* have the ability of causing intestinal and extra-intestinal infections in animals and poultry (Cullor, 1996). Currently, several classes of entero-virulent *E. coli* have been recognized, these include: entero-toxigenic *E. coli* (ETEC), entero-pathogenic *E. coli* (EPEC), entero-hemorrhagic *E. coli* (EHEC), entero-invasive *E. coli* (EIEC), entero-aggregative *E. coli* (EAggEC), diarrhoea-associated hemolytic *E. coli* and the cytolethal distending toxin (CLDT)- producing *E. coli* (Nataro and Kaper, 1998). Low-grade fever and digestive system clinical signs including watery diarrhea, nausea, abdominal cramps occur due to drinking water or eating food stuff contain ETEC microorganisms (Raj, 1993; Nweze, 2009; Bonyadian *et al.*, 2011). Nonetheless, refrigerated and low temperature poultry meat spoilage in aerobic atmosphere caused by *Pseudomonas* species and few types of other Gram-negative bacteria (Mead, 2004).

However, there is a paucity of data concerning the prevalence of contamination with multiple food-borne pathogens in retail meats, including poultry, in the Sudan. The objectives of this study were to determine the microbial contamination in poultry slaughterhouse and quantify the degree of contamination.

MATERIALS and METHODS:

Sampling of Broilers:

The study was conducted for a period of three months, at a modern poultry slaughterhouse in Khartoum State, Sudan. A total number of 600 swab samples were collected randomly from the legs, breast and backs of slaughtered birds in five Critical Control Points (CCPs), namely; after defeathering, after evisceration, after spray wash and after chilling and hands of workers. A sterile metal template was used to outline 10 cm^2 area on the broiler carcasses and then the area was swabbed vigorously with sterile cotton gauze wrapped around the end of a flat swab stick. These samples were taken from each CCP in the processing line of broilers and put in 10 ml of sterile 0.5% peptone water, then transported to microbiology laboratory for isolation and identification of the organisms.

Isolation and Identification Procedures:

Laboratory procedures for isolation and identification of bacteria were done as described by Barrow and Feltham (2003). The swab samples were cultured using prepared nutrient agar, nutrient broth, MacConkey agar (MCA) and Blood Agar. The plates were incubated at 37^oC for 24 hrs. Biochemical tests were conducted for identification of the isolates.

Total Viable Count:

Total Viable Count (TVC) was carried out as described by Harrigan and MacCance (1976).

Statistical 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 $\log_{10}$ cfu/cm^2 for analysis and ANOVA was performed to compare the recorded means. Statistical significance was set at P-value of ≤0.5.

RESULTS:

As shown in table I the TVC revealed that the highest contamination level after defeathering were in the back 10.20±0.26 $\log_{10}$ cfu/cm^2, then the breast 9.93±0.31 $\log_{10}$ cfu/cm^2 and the legs 8.96±0.15 $\log_{10}$ cfu/cm^2 . The TVC revealed the lowest contamination levels at packing (1.98±0.01 $\log_{10}$cfu/cm^2), reholding 2 (2.76±0.01 $\log_{10}$cfu/cm^2) reholding 1 (2.91±0.01 $\log_{10}$cfu/cm^2) and from hands of workers.

Table 1: Comparison of Mean Total Viable Counts of Bacteria ($\log_{10}$ cfu/cm^{-2}) ±Sd at Different Operational Points

Operational points	Sites			Significance
	Breast/ Reholding 1	Back/ Reholding 2	Leg/ Packing	
After defeathering	9.93±0.31	10.20±0.26	8.96±0.15	*
After evisceration	8.23±0.15	7.33±0.21	7.03±0.15	*
After spray wash	6.93±0.55	6.86±0.31	5.03±0.25	NS
After chilling	5.23±0.31	4.93±0.38	4.46±0.25	*
Hands of workers	2.91±0.01	2.76±0.01	1.98±0.01	*

* = significant difference at (P < 0.05) and NS = not significant (P > 0.05)

Isolation and identification of bacteria from the different CCPs under investigation revealed five prevailing species of bacteria as shown in Table 2. *Escherichia coli* was the most prevalent bacteria with a prevalence of 57.8% (95% CL, 47.60 - 68.00) followed by *Salmonella* species with a prevalence of 44.4% (95% CL, 34.14 - 54.66), then *Pseudomonas* species with a prevalence of 20.0% (95% CL, 11.74 - 28.26) and finally, *Shigella* species and *Staphylococcus aureus* each with a prevalence of 2.22% (95% CL, -0.820 - 5.260).

Table 2: Prevalence of Isolated Bacteria from Some Critical Control Points in a Poultry Slaughterhouse in Khartoum State

Bacteria	Number of positive	Percentage of Positive	95% CI	
			Lower	Upper
E. coli	52	57.8	47.60 - 68.00	
Salmonella species	40	44.4	34.14 - 54.66	
Pseudomonas species	18	20.0	11.74 - 28.26	
Shigella species	2	2.22	-0.820 - 5.260	
Staphylococcus aureus	2	2.22	-0.820 - 5.260	

A total of 114 bacteria were isolated and identified. In the CCP after defeathering, *Salmonella* species were the most prevalent with 6 (5.26%) positive samples from backs while *E. coli* was found in 6 (5.26%) of the legs and backs in the CCP after evisceration and also in 6 (5.26%) of the legs and breasts in the CCP after spray wash. After chilling,

Salmonella species were the most prevalent with 6 (5.26%) positive samples from breasts whereas *E. coli* was the most prevalent with 6 (5.26%) positive samples from hands of workers at reholding 1. At the same time *Shigella* species and *Staphylococcusaureus* were the least prevalent with percentages ranging from 0.00 (0.00%) to 2 (1.75%) in the five investigated critical control points (Table 3).

Table 3: Prevalence of the Isolated Bacteria in the Legs, Breasts and Backs of the Broilers Carcasses in Khartoum State

Critical Control Points	Number of Positive Samples and their Percentages (%)					
	Salmonella species	*E. coli*	*Pseudomonas* species	*Shigella* species	*Staph.Aureus*	Total
After Defeathering						
Leg	4 (3.51)	2 (1.75)	4 (3.51)	2 (1.75)	0 (00.0)	12 (10.53)
Breast	4 (3.51)	2 (1.75)	2 (1.75)	0 (00.0)	0 (00.0)	8 (7.02)
Back	6 (5.26)	0 (00.0)	4 (3.51)	0 (00.0)	0 (00.0)	10 (8.77)
After Evisceration						
Leg	0 (00.0)	6 (5.26)	2 (1.75)	0 (00.0)	0 (00.0)	8 (7.02)
Breast	4 (3.51)	2 (1.75)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Back	0 (00.0)	6 (5.26)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
After Spray Wash						
Leg	0 (00.0)	6 (5.26)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Breast	0 (00.0)	6 (5.26)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Back	2 (1.75)	2 (1.75)	4 (3.51)	0 (00.0)	2 (1.75)	10 (8.77)
After Chilling						
Leg	4 (3.51)	2 (1.75)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Breast	6 (5.26)	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Back	2 (1.75)	4 (3.51)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Hand of Workers						
Reholding 1	2 (1.75)	6 (5.26)	0 (00.0)	0 (00.0)	0 (00.0)	8 (7.02)
Reholding 2	4 (3.51)	4 (3.51)	2 (1.75)	0 (00.0)	0 (00.0)	10 (8.77)
Packing	2 (1.75)	4 (3.51)	0 (00.0)	0 (00.0)	0 (00.0)	6 (5.26)
Total	40 (35.09)	52(45.61)	18 (15.79)	2 (1.75)	2 (1.75)	114(100)

With exception of the point after spray wash, the study revealed a statistically significant difference ($p \leq 0.05$) in the other four CCPs between the legs, backs and breast and reholding 1, reholding 2 and packing, correspondingly.

DISCUSSION:

The present study demonstrated that *E. coli*, prevalent in the investigated establishment. This finding confirms the findings of Kabour, (2012) and Mohamed-Noor (2012) who isolated *E. coli*from the all investigated CCP. In this study Salmonella species only isolated from the CCPs after evisceration and after spray wash. Moreover, the results of this study revealed the isolates*Pseudomonas* species were isolated in considerable ratio. However, Shigellaspecies and *S. aureus* were detected in equal ratio.The above organisms are common in soil and water, and their finding

in poultry meat, are thought to originate from the live-bird environment (Mead, 2004).

Zhao *et al*. (2001) reported a lower prevalence of *E. coli* in the greater Washington, D.C., area in retail chicken and turkey. As in his study the rates of microbial contamination of retail meats with *E. coli* ranged from 12% in turkey to 39% in chicken. Moreover, *E coli* was studied in poultry meat products in retail markets in Finland, it was isolated from 207 out of 219 (94.50%) samples, this very high prevalence could be elaborated by that Lyhs *et al.,* (2012) who preformed PCR methods for phylogenetic groups and the susceptibility of the isolates for nalidixic acid and ciprofloxacin were also conducted. In this we think study prevalent was high (57.8%) 95% CL from 47.60 to 68.00 and this could be attributed to poor hygiene. In this study *Salmonella* species was found in 44.4% of the samples with 95% CL ranging from 34.14 to 54.66. This high prevalence might be due to sampling methods and techniques used in each study. However, *Salmonella* species are observed frequently in retail meats across the world. In Spain, Canada and Thailand prevalences of 49%, 50% and 57% of *Salmonella* species positive chicken carcasses and flocks were reported (Capita *et al.,* 2003; Padungtod *et al.,* 2006; Maharjan *et al.,* 2006; Arsenault *et al.*, 2007). These findings are similar to the prevalence reported in this study. However, various lower estimates of *Salmonella* species contamination and colonization of carcasses and flocks have been reported in many countries, with 13% (Skov *et al.*, 1999) in Denmark, 27% in the Netherlands (Jacobs-Reitsma *et al.*, 1994) and, 4.2% (Zhao*et al.*, 2001) in the greater Washington, D.C, USA, 36% in Belgium (Uyttendaele *et al.*, 1999), 14.5% in Nepal (Padungtod *et al.,* 2006; Maharjan *et al.,* 2006), 19% in the fresh and frozen poultry products in South Africa (Nierop *et al.,* 2005), 3.1% and 2.8% in chicken and turkey meat in Ireland (Jordan *et al.,* 2006), Contrary, higher prevalence of 60%, 69% and 70% were reported in Portugal (Bajaj *et al.,* 2003), India (Rose *et al.*, 1999) and France (Antunes *et al.,* 2003).Comparison of these prevalence estimates might not be aboveboard due to variations in sampling methods, characteristics of the slaughtered flocks and the variation of investigated establishments and differences in the sample size.Moreover, *Pseudomonas* species was observed in 20.0% of the samples with 95% CL ranging from 11.74 to 28.26. This prevalence is higher than what reported by Keskin and Ekmekçi (2007) in Izmir. *Shigella* species and *Staphylococcus aureus* were reported in our results at a low prevalence of 2.22% and 95% CL of -0.820 to 5.260, this result is contrary to the findings ofKozačinski *et al.* (2006) who found *enterobacteria*at a rate of 34.84% and *Staphylococcus aureus* at a rate of 30.30% in poultry meat sold on the Croatian market.In this study, the mean TVCs obtained from chicken carcasses in the following CCPs: after defeathering, after evisceration, after spray wash and hands of workers, are similar to those obtained by Kabour (2012) and Mohamed-Noor *et al.* (2012). In this study, the high prevalence in CCP might be due to distribution of the organisms especially in *E.coli* by workers un hygienic practice who used to enter the production area without wearing protecting gloves and washing their hand with soap and disinfectant after leaving the toilet. Beside, random movement of workers in and outside abattoir and from dirty zone to clean zone. In addition to the ignorance of the regular cleaning of defeather-picking machine and chilling tank,the low contamination level in some CCPscould be due to using automatic machine which decrease workers interference.

In conclusion, the current results indicate that there was an increase in the level of total aerobic and coliform counts in swab samples taken from chicken carcasses and this is worrying due to their ability to cause diseases. Sudanese abattoirs may reflect the hygienic status of chicken meat production in developing countries and the implementation of Hazard Analysis and Critical Control Points (HACCP) in poultry industry is extremely important, because it involve the constant monitoring of all slaughtering procedure.

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