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Evaluation of the Safety Status of Beef for Domestic Consumption during Processing at Three Slaughterhouses in Omdurman, the Sudan

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Abstract: *A cross-sectional study was conducted, from June/2011 to March/2012, to evaluate the safety status of beef during slaughtering and carcass processing at three slaughterhouses in Omdurman, the Sudan. A total of 350 swab samples were collected from 30 randomly selected cattle carcasses from different anatomical sites, hands of workers and knives for estimating the Total Viable Counts (TVCs) and isolating the contaminating bacteria. The TVCs levels were not significantly different at p-value of p≤0.05 between the three slaughterhouses and ranged from 3.19±0.11 to 6.90±0.99 log_{10} cfu/cm^2 at Al-Huda slaughterhouse, from 3.15±0.49 to 6.43±0.25 log_{10} cfu/cm^2 at Al-Sabaloogah slaughterhouse and from 3.23±0.11 to 8.33±0.82 log_{10} cfu/cm^2 at Al-Salam slaughterhouse. The highest recorded levels of TVCs after skinning were from the neck at Al-Huda slaughterhouse (6.90±0.99 log_{10} cfu/cm^2) and Al-Salam slaughterhouse (8.33±0.82 log_{10} cfu/cm^2), respectively, but from hands of workers at Al-Sabaloogah slaughterhouse (6.43±0.25 log_{10} cfu/cm^2). Moreover, the highest levels of TVCs after evisceration were from hands of workers (5.47±0.47 log_{10} cfu/cm^2), shoulder (6.25±0.23 log_{10} cfu/cm^2) and brisket (7.77±0.96 log_{10} cfu/cm^{-2}) at the three slaughterhouses, respectively. In after wash the highest levels of TVCs were from the neck at the three slaughterhouses; 4.48±0.37 log_{10} cfu/cm^2, 6.38±0.73 log_{10} cfu/cm^2 and 6.39±0.80 log_{10} cfu/cm^2. Certain bacteria have been detected on the surfaces of the investigated carcasses, including: E. coli, Salmonella species, Pseudomonas species, Shigella species, Staphylococcus species and Streptococcus species. This study showed that the level of contamination on bovine carcasses was higher than the acceptable value set by the EU Commission. However, involving good sanitary measures during slaughtering processes will lead to the reduction of the amount microorganisms. Extensive education and training programs for workers are needed.*

***Keywords*:** *Beef safety, bacterial contamination, slaughterhouse, HACCP, Sudan*

I. Introduction

Because meat contains high amount of vital substances like proteins, essential amino acids, B complex vitamins and minerals, that are essential for maintaining good health, it is considered one of the most important components of human foods [1, 6, 22]. Due to this richness in composition, meat is also considered a good medium for the growth and propagation of many pathogenic bacteria such as *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* species, *Escherichia coli* O157:H7 and numerous other bacteria [1, 6, 7, 10, 26, 27]. These pathogenic bacteria can cause food-borne diseases or illnesses after consuming them in contaminated meat [4, 26, 27].

Microbial contamination of cattle carcasses during handling and processing, such as bleeding, skinning, evisceration and storage in slaughtering plants and retail establishments is an unavoidable, and at the same time, undesirable problem while converting live animals to meat for human consumption [21, 26, 28]. The main source of carcass contamination is the fecal material that could come in contact with carcasses through direct deposition as well as by indirect means. Others sources of carcass contamination include: unhygienic equipments, workers, installations and air [1, 5, 22, 25]. However, in a general sense, cattle and their environment are an important source of pathogenic bacteria and contamination of meat and meat products which can afterwards be transmitted to human beings [8, 9, 25].

Developed countries have adopted mechanized or automated slaughtering techniques that ensure the reduction of the amount of exposure of carcasses to atmospheric contaminants and to manipulation. In addition, the implementation of the Hazard Analysis Critical Control Point (HACCP) system allows them to regulate the control of the general hygiene of the chains of production [13, 14]. However, in the Sudan, slaughtering procedure is mainly artisanal and manual. Few studies highlighted the inadequacy or even lack of hygiene in slaughterhouses in Khartoum including Abdalla *et al*. [1, 21, 25]. Therefore, this work was carried out to provide a deeper understanding of the hygiene and safety states of beef for local consumption during the processes of converting live cattle to consumable meat and to evaluate them.

II. Materials and Methods

2.1. Study Area and Slaughterhouses

This study was carried out at Al-Huda, Al-Sabaloogah, and Al-Salam slaughterhouses. The three slaughterhouses are in Omdurman, Khartoum State, the Sudan. Khartoum State is located in the semi-arid savannah belt, with an average annual rainfall of 100-200 mm and a long dry season from September to June. A wide range of production systems are practiced ranging from household subsistence to large-scale commercial farming; for milk, meat, and poultry are operational in the state. The state today has an estimated population of 7 million people.

2.2. Bleeding and Processing of Animals

The main animals slaughtered at the studied slaughterhouses are cattle, sheep and some goats and camels in separate processing halls and lines.

When animals arrive at the slaughterhouse, they undergo a thorough ante-mortem examination with animals having any visually detectable health problem and disease symptoms excluded. Poor body condition, behavioural disorders, diarrhoea, skin diseases, profuse discharges and apparent pregnancy in females usually disqualify animals from being slaughtered. However, aspects of animal welfare are also observed during ante-mortem examination like giving the trekked and/or transported animals an enough resting period and providing them with clean drinking water and shadow. Moreover, the diseased ones are isolated, thoroughly examined and treated. When the animals pass the ante-mortem examination, they are slaughtered in the Islamic way of slaughtering which guarantees a perfect bleeding and as result the meat could be good stored. In the post-mortem examination the carcasses are examined visually and physically; they should not be yellowish or congested or have any detectable pathological lesions. Then after, certain muscles and lymph nodes are incised and inspected to that ensure the carcasses are wholesome and not likely to prone any risks to consumers [34].

2.3. Study Design and Sampling Strategy

A cross-sectional design, from June/2011 to March/2012, was employed in this study as described by Thrusfield [29]. A total of 350 swab samples were taken from 30 carcasses from four different anatomical sites as follows: brisket, shoulder, neck and rump. The samples were collected from three operational points or critical control points (CCP), namely; after skinning, after evisceration and after wash, respectively. The 30 carcasses were randomly selected; 10 from each slaughterhouse, in addition, 50 samples taken from worker hands and the knives.

2.4. Sample Collection

The different anatomical sites on each carcass were sampled by the swab technique; an area of 100 cm^2 was marked with a sterile frame (10 cm × 10 cm) for each site on the carcass according to Abdalla et al. [1, 21, 25] and Magdaa et al. [30].

2.5. Total Viable Count

As described by Barrow and Feltham [3] and Abdalla *et al*. [1, 25] the TVC was carried out by making of a serial dilution of each sample (form 10^{-1} up to 10^{-5}). Ten-fold increments were done by preparing five sterile and labelled test tubes from (1) to (5). From the test tube (1), a solution of 1 ml was taken into the test tube (2) which contains 9 ml of distilled water to yield a total volume of 10 ml to form 1. The process continued until serial dilution of original bacterial suspension in the test tube (5) was made. Each dilution was spread out on a disposable Petri-dish contained a solidified agar medium (MacConkey agar (MCA), Nutrient agar and blood agar). All the plates were then incubated upside down at 37°C. After 24 hours the number of all colonies on the plate was counted for each dilution and the mean count was determined. Each bacterium i.e. a colony forming unit (cfu), which was in the diluted sample, represented a colony; therefore, the concentration of viable bacteria per Millilitre (ml) in initial sample was calculated and expressed in cfu/cm^2.

2.6. Isolation and Identification of the Bacteria

The isolation and identification of the bacteria were done as described by Barrow and Feltham [3]. The swab samples were cultured using prepared Nutrient Agar, Nutrient Broth, Selenite Cystine Broth, Deoxycholate Citrate Agar (DCA), Salmonella Shigella Agar, MacConkey Agar (MCA), Blood Agar and Mannitol Salt Agar (MSA). The broth tubes and agar plates were incubated at 37^{o}C for 24 hours. Afterwards, the morphology of colonies on agar media were examined macroscopically, smears were then made from clean slides fixed with heat and subjected to Gram stain and examined under oil immersion and the biochemical tests for species identification were parallel conducted.

2.7. Data analyses

The data were analyzed using the software Statistical Package for the Social Sciences version 18.0 (SSPS Inc. and Chicago, IL, USA). All bacterial counts were converted to $\log_{10}$ cfu/cm^2 for analysis. Analysis of Variance (ANOVA) was performed to evaluate the differences in the levels of TVCs between the different operational points/critical control points. Moreover, the statistical significance was set at a *p-value* of ≤ 0.05.

III. Results

There were no statistically significant differences at p-value ($p \leq 0.05$) between the levels of the TVCs obtained from the brisket, shoulder, neck and rump of the cattle carcasses processed at the three slaughterhouses (Table 1, 2 and 3). At Al-Huda slaughterhouse the highest contamination levels recorded after skinning and after wash were from the neck, 6.90±0.99 $\log_{10}$ cfu/cm^2 and 4.48±0.37 $\log_{10}$ cfu/cm^2, respectively. While the highest contamination level among the TVCs estimated after evisceration was from knives (5.47±0.47 $\log_{10}$ cfu/cm^2). Moreover, at Al-Sabaloogah slaughterhouse the highest contamination levels recorded from hands of workers (6.43±0.25 $\log_{10}$ cfu/cm^2), shoulder (6.25±0.23 $\log_{10}$ cfu/cm^2) and neck (6.38±0.73 $\log_{10}$ cfu/cm^2) at after skinning, after evisceration and after wash, correspondingly. Furthermore, at Al-Salam slaughterhouse the highest contamination level after skinning (8.33±0.82 $\log_{10}$ cfu/cm^2) and after washing (6.39±0.80 $\log_{10}$ cfu/cm^2) were from the samples taken from the surface of the neck but the highest contamination level after evisceration was from the samples taken from the brisket (7.77±0.96 $\log_{10}$ cfu/cm^2).

The morphology of the colonies on agar media, Gram stained smears and the biochemical tests for species identification led to the detection of seven species of bacteria on the surfaces of the carcasses (Table 4). *E. coli* was detected on 53.3% (95% CI, from 35.45 to 71.15) and *Shigella* species were detected on 13.3% (95% CI, from 1.15 to 25.45).

IV. Discussion

The level of the TVC was set and agreed to be a criterion for assessing and evaluating the microbial contamination of carcasses and a useful mean to know the hygienic and safety states of meat [13]. According to the Decision 2001/471/EC of the EU Commission, the acceptable value of TVC was set at 2.0 $\log_{10}$ cfu/cm^2 [13]. However, at the three investigated slaughterhouses, the TVCs ranged from 3.19±0.11 to 6.90±0.99 $\log_{10}$ cfu/cm^2, 3.15±0.49 to 6.43±0.25 $\log_{10}$ cfu/cm^2 and from 3.23±0.11 to 8.33±0.82 $\log_{10}$ cfu/cm^2; all of them had TVCs above the acceptable level set by Decision 2001/471/EC of the EU Commission. However, some of the levels of the TVCs recorded in the present study were similar to what have been concluded by Frank and Mallion [31] who found out that the bacterial count of a freshly processed carcass could range from 10^2 to 10^6 cfu/cm^2, while on the other hand, some were higher. The findings of this study were not significantly diverse form those reported by Abdalla *et al*. [1], who reported TVCs that ranged from 2.73±0.04 to 3.74±0.02 $\log_{10}$ cfu/cm^2 from indigenous bovine carcasses, in Khartoum. Our results were also similar to the TVCs (3.0±0.59 to 6.0±0.33 log10 cfu/cm^2) reported from sheep carcasses at El-Kadero slaughterhouse by Abdalla *et al*. [25] and to the TVCs (1.0×10^7 cfu/cm^2) estimated in a slaughterhouse in Omdurman by Elamin [24]. The TVCs were also agreeing with those ones reported by El-Hassan et al. [32] who assessed the microbiological validity of mutton purported for export to international markets from El-Kadero export slaughterhouse and recorded TVCs of 1×10^3 - 6×10^6 cfu/cm^2. The processing methods adopted in Sudanese slaughterhouses are analogous and the hygienic standards too. Generally in the Sudanese slaughterhouses, there are no strict hygienic measures in place and many practices and conditions might lead to cross contamination and facilitate bacterial growth and, hence, result in high levels of TVCs. These practices and conditions include: there are no demarcations between the clean and dirty areas in the processing halls, the working personnel do not wear the recommended protective clothes and they move freely to and from the clean and dirty areas. Knives are cleaned in same water more than once, in addition to that, this water is not heated, and no disinfectants are used for the same purpose. These knives are used for processing more than one animal before being washed and they are sharpened by the same device. As well, carcasses are cut into pieces by the same device without cleaning it and the ambient temperature in the processing halls is not adjusted at the wanted and recommended degrees. This would confirm the conclusions of Abdalla *et al.* [21] and Magdaa *et al*. [30] who indicated that the main sources of

contamination during the processes of converting live animals to consumable meat are the slaughtered animals themselves, the environment and the working personnel. Furthermore, Magdaa *et al*. [30] found a significant reduction in the TVCs of bacteria on the surfaces of sheep carcasses when the working personnel did wear the recommended protective clothes (gloves, apron, mask and caps).

The microbiological quality of beef has been investigated in many countries like Ethiopia, by Gebeyehu *et al*. [12] who found a mean aerobic plate count of 1.62×10^5 cfu/cm^2, in Nigeria by Clarence *et al*. [19] who detected TVCs of 3.02×10^3 - 5.01×10^3 cfu/g, in Algeria by Nouichi and Hamdi [26] who found the superficial bacterial contamination levels of 4.48±0.63 log cfu/cm^2, in Switzerland by Zweifel and Stephan [13] who reported a mean log TVCs that ranged from 2.5 to 3.8 log cfu/cm^2 and Zweifel *et al*. [14] who recorded mean log TVCs that ranged from 2.1 to 3.1 log cfu/cm^2. In Australia the $\log_{10}$ mean of the aerobic plate count was 3.13 cfu/cm^2 and 2.42/cm^2 and 2.52/g, respectively [15, 16, 17]. But in India, Bhandare *et al*. [33] noted an average of 6.06 log cfu/cm^2 and Mukhopadhyay *et al*. [20] a level of as high as 8.5×10^5 - 8.7×10^{10} cfu/g. In Trinidad and Tobago, Badrie *et al*. [18] found TVCs of 10^2 - 10^5 cfu/g. In general, investigating different anatomical sites and critical control points or operational areas and variations of the sampling methods could lead to a wide dissimilarity in the levels of carcass contamination as could be noticed in the aforementioned studies [11].

Abdalla *et al*. [1] and Gill [6] found significant statistical differences ($p\leq0.05$) between the degrees of bacterial contamination on the surfaces of carcasses during processing, nevertheless, this study did not come to comparable results. Interestingly, the highest contamination levels were from neck, knives, hands of workers and brisket respectively. This might probably be due to that muscles of the neck are the first part of the animal to be exposed to the ambient environment during bleeding. Also spilling out of the bacteria rich rumenal contents through the cut esophagus and that the carcass is washed from rump downwards to the neck, could all together result in concentration of the bacteria on the surface of the neck. Likewise, neither the washing towel nor the water, which is kept in an open container, are changed until the whole carcass is washed. Viscera brisket contact after evisceration could explain the high levels of TVCs at the brisket. Another conceivable justification to the differences of the levels of the TVCs at the critical control points could be due to multiple contacts of carcasses with contaminated slaughtering utensils, including; knives and hands of workers during manipulation of the carcass [26]. The results of Amine *et al*. [27] revealed that at after evisceration the bacterial count is high due to fecal contamination and the neck is most contaminated site.

In the present study *E. coli*, *Salmonella* species, *Pseudomonas* species, *Shigella* species, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus* species were isolated from the surfaces of the investigated carcasses. These findings did confirm the findings of many former studies in which numerous bacterial species have been detected on the surfaces of cattle carcasses being processed for human consumption. Nouichi and Hamdi [26] identified different serotypes of *Salmonella*; like *Salmonella anatum* and *Salmonella abortus* subspp. *ovis*. Phillips et al. [15] detected *E. coli* on 10.30% and in 5.10% of the investigated cattle carcasses and boneless beef samples as well as coagulase-positive *staphylococci* on 24.30% of the carcasses and in 17.50% of the boneless beef. *Salmonella* species on 0.20% of carcasses and in 0.10% of boneless beef were detected too. Many other bacterial species were recovered from the surfaces of cattle carcasses including: *Corynebacterium* species, *Lisleria* species, *Staphylococcus* species, *Micrococcus* species, *Bacillus* species, *Actinomycetes* species, *Actinobacillus* species, *Chromobacterium* species and *Enterobacteria* species [1, 2, 6, 7, 10, 16, 21, 23, 25, 27]. Moreover, Elamin [24] isolated *Staphylococcus*, *Micrococcus*, *Corynebacteria*, *Kurthia*, *Enterobacteria* and *Pseudomonas*. While Vanderlinde *et al*. [17] indicated that 0.59% of the investigated carcasses were found positive for Listeria monocytogenes, 0.16% were positive for *Campylobacter jejuni*/*coli*, 0.22% were positive for Salmonella species, and 29% were positive for coagulase-positive *Staphylococcus* species.

V. **Conclusion and Recommendations**

In conclusion, this study showed that the level of contamination on bovine carcasses was much higher than the acceptable value set by the EU. However, to attain the international requirements and acceptable value set by the EU Commission, involving good sanitary measures during slaughtering processes that will lead to the reduction of the amount and/or removal of the microorganisms and other hazards should be stressed on. Hazard Analysis Critical Control Point (HACCP) should be applied properly during slaughtering operations by using sufficient clean heated water and safe disinfectants. To make all these, extensive education and training programs on hygiene for workers should immediately be started.

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Table 1: The Total Viable Counts (log_{10} cfu/cm^2) of the Bacteria Found on the Four Anatomical Sites of Cattle Carcasses at Three Operational Points/Critical Control Points in Al-Huda Slaughterhouse (from June/2011 to March/2012)

Anatomical Sites	Operational Points/Critical Control Points			p value
	After Skinning	After Evisceration	After Washing	
Brisket	4.54±0.26	5.06±0.44	3.28±0.15	NS
Shoulder	4.54±0.27	3.40±0.32	4.08±0.53	NS
Neck	6.90±0.99	4.39±0.21	4.48±0.37	NS
Rump	3.51±0.27	3.24±0.18	3.19±0.11	NS
Knives	3.40±0.27	5.47±0.47	3.35±0.17	NS
Hands of Workers	3.70±0.14	3.28±0.01	4.30±0.35	NS

* = statistically significant, NS = not statistically significant

Table 2: The Total Viable Counts (log_{10} cfu/cm^2) of the Bacteria Found on the Four Anatomical Sites of Cattle Carcasses at Three Operational Points/Critical Control Points in Al-Sabaloogah Slaughterhouse (from June/2011 to March/2012)

Anatomical Sites	Operational Points/Critical Control Points			p value
	After Skinning	After Evisceration	After Washing	
Brisket	6.16±0.45	6.20±0.55	4.31±0.10	NS
Shoulder	6.30±0.41	6.25±0.23	5.24±0.50	NS
Neck	6.36±0.39	6.22±0.28	6.38±0.73	NS
Rump	3.27±0.33	3.15±0.49	3.26±0.53	NS
Knives	6.20±0.48	4.86±0.57	3.83±0.44	NS
Hands of Workers	6.43±0.25	5.19±0.31	5.66±0.22	NS

* = statistically significant, NS = not statistically significant

Table 3: The Total Viable Counts (log_{10} cfu/cm^2) of the Bacteria Found on the Four Anatomical Sites of Cattle Carcasses at Three Operational Points/Critical Control Points in Al-Salam Slaughterhouse (from June/2011 to March/2012)

Anatomical Sites	Operational Points/Critical Control Points			p value
	After Skinning	After Evisceration	After Washing	
Brisket	4.40±0.38	7.77±0.96	3.65±0.28	NS
Shoulder	4.30±0.84	4.99±0.78	4.39±0.46	NS
Neck	8.33±0.82	3.81±0.26	6.39±0.80	NS
Rump	3.23±0.11	5.21±0.40	3.45±0.50	NS
Knives	6.75±1.30	3.29±0.06	4.06±0.34	NS
Hands of Workers	6.12±0.13	3.65±0.28	4.49±0.44	NS

* = statistically significant, NS = not statistically significant

Table 4: Number of Carcasses Contaminated with Bacteria at Al-Huda, Al-Sabaloogah and Al-Salam Slaughterhouses in Omdurman, Khartoum State (from June/2011 to March/2012)

Bacteria	No. of tested carcasses	No. of positive carcasses	% of Positive carcasses	95% CI	
				Lower	Upper
Escherichia coli	30	16	53.3	35.45 - 71.15	
Salmonella species	30	11	36.7	19.45 - 53.95	
Pseudomonas species	30	13	43.3	25.57 - 61.03	
Shigella species	30	4	13.3	1.15 - 25.45	
Staphylococcus aureus	30	10	33.3	16.44 - 50.16	
Streptococcus species	30	9	30.0	13.60 - 46.40	
Staphylococcus epidermidis	30	7	23.3	8.17 - 38.43	