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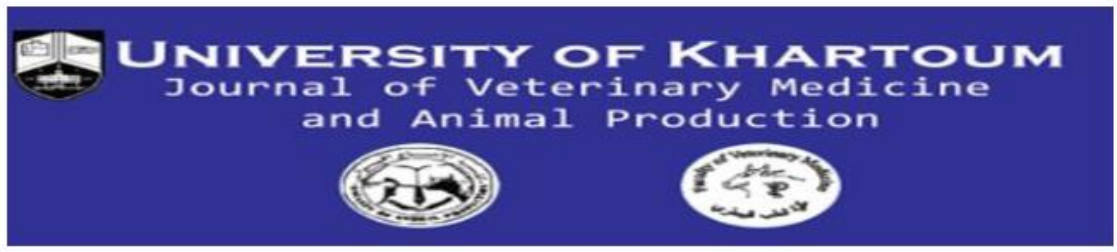

Bacterial Contamination of Sheep Carcasses in El-Kadroo Slaughterhouse in Khartoum State, Sudan

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Abstract

This study aimed to determine bacterial contamination on sheep carcasses at El-Kadroo slaughterhouse in Khartoum State, Sudan. The study was conducted during the period from January to April 2016. Ninety swab samples were collected from three sites of sheep carcass including shoulder, flank and rump (30 samples from each site) after the last washing. The total viable count (TVC) was used to assess bacterial contamination. Isolation and identification of the bacteria performed by using standard bacteriological and biochemical methods. Samples taken from the three parts had the highest TVC of $4.93x10^8$ cfu⁄cm^2 for flanks, $2.1x10^8$ cfu⁄cm^2 for shoulder and $6.3x10^7$ cfu⁄cm^2 for rump respectively ($P<0.021$). Bacteria isolated from the samples were *Staphylococcus* spp.79 (37.7%), *Bacillus* spp. 56 (26.7%), *Corynebacterium* spp. 26 (12.4%), *Klebsiella* and *Kurthia* 7(3.3%), while *Actinobacillus, Streptococcus, Listeria monocytogenes*, *Aerococcus* spp., *Nocardia* spp., *Eschericia, Nocardia* and *Pseudomonas* spp. with isolation rate of 1to 6 (0.5 -2.9%) of which some may be pathogenic and of public health concern. Variations in TCV and various type of bacteria were observed at different sites of the sheep carcasses is an indication of low standards of handling practices from pre-slaughter to post–slaughter, abattoir facilities, and equipments.

Key words: sheep carcasses, bacterial contamination, pathogenic bacteria, El-Kadroo, Khartoum.

المستخلص

هدفت هذه الدراسة لتحديد االتلوث الباكتري في ذبائح الضان بمسلخ الكدرو بولاية الخرطوم. تم جمع 90 عينة مسحة من ثلاث مواقع في ذبيحة الضان شملت الكتف والخاصرة والكفل (30 عينه لكل موقع) بعد آخر غسيل. إستخدم العد

الكلي الحي للباكتريا لقياس التلوث الباكتري. تم عزل وتحديد نوع الباكتريا بواسطة الطرق الباكترولوجية القياسية والاختبارات البيوكيميائية.من نتائج العينات التي جمعت من الثلاثة مواقع، كانت الخاصرة هي الأعلى بمعدل $4.93x10^8$ وحده موكنه للمستعمرة لكل سنتمتر مربع ثم الكتف بمعدل $2.1x\ 10^8$ وحده موكنه للمستعمره لكل سنتمتر مربع واقلها الكفل بمعدل 6.3x107 وحده موكنه للمستعمره لكل سنتمتر. لقد تم عزل الموكورات 79 (37.7%) ، الباسيلس 56(26.7%)،(P<0.02) مربع على التوالي الكوراينباكتريوم 26(12.6%)، المايكروكوكس 15(7.15)، الكليبسيلا والكورثيا 7(3.3%)، كما أن الأكتينوباسلس والسبحيات واللسترية والايروموناس والنوكارديا والإشريشيا القولونية والسودوموناس كان معدل العزل ما بين 1 الى 6 (0.5-2.9%) والتي منها باكتريا ممرضة ولها علاقة بالصحة العامة. لوحظ التباين في العد الكلي الباكتري وانواع الباكتريا على مختلف مواقع ذبائح الضان ويعتبر ذلك مؤشر على تدني مستوى الممارسة والمعاملة قبل وبعد الذبح و مكونات ومعدات المسلخ.

الكلمات المفتاحية: ذبائح الضأن، التلوث الباكتري، الباكتريا الممرضة ، مسلخ الكدرو، الخرطوم.

Introduction

Meat is one of the highly perishable foods because of its high nutritional contents, and may result in oxidative rancidity, discolorations, moldiness, off flavor and sliminess that renders the meat unacceptable and unfit for human consumption (Ajiboye*, et al.,* 2011). Bacteria which are responsible for the most food-borne diseases contaminate meat directly and indirectly from animal excreta at slaughter process (Yen, 2003). Hennlich and Verne (1990) emphasized that hygienic measures promote the quality and safety of meat and increase its shelf life. The transfer of contamination through the airborne route is one of the most significant areas of high-careful production (Burfoot *et al.,* 2000*)*.

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments (Gill and Robert, 1998; Abdallah*, et al*., 2009a). Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* pose a threat to consumer's health (Gustafson and Borch, 1993; Samelis*et al*., 2001). Norman *et al.,* (2006) indicated that the contamination of meat at the end consumers level, correspond to the combination of contaminations at different stages of meat preparation including the slaughtering, transportation and marketing.

In Sudan hygienic measures to control microbial contamination of meat is unsatisfactory. Storage at refrigerator temperatures is still one of the most effective practices for improving the safety of fresh meat. According to Ministry of Agriculture and Animal Resources and Irrigation, Khartoum State records of the year 2016, the number of slaughtered sheep in El-Kadro slaughterhouse was 55800. The microbial assessment of mutton intended for export from El-Kadro slaughterhouse was found relatively high count, but without critical contamination level according to the Standards (El Hassan*et al*., 2011). A higher contamination level on flank sites and lower contamination level on rump sites during skinning was recorded by Ali (2007). Several researchers have reported that the meat is contaminated with high level of *Klebsiella Pneumoniae, Enterobacter* spp*., Pseudomonas Aeruginosa, E. coli, Salmonella* spp*., Serratiamarcescens, Proteusvulgaris, Staphylococcus aureus* and *Bacillus*spp. (Okonko*et al*., 2010;Collins and Thato, 2011).

Many species of bacteria were isolated from mutton and the most prevailing bacteria was *Staphylococcus aureus*, *Klebsiella spp*. and *Escherichia coli* respectively (Abdalla *et al*., 2009b). In most developing countries, the absence or non-respect of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and nonpathogenic microorganisms (FAO, 2004).

Food Safety and Inspection Service and USDA (1998), emphasized that Processing operations are presently required to have Sanitation Standard Operation procedures (SSOP`s) and functional Hazard Analysis Critical Control Points (HACCP) systems, to improve food safety through purchase requirements. According to Mackey and Roberts (1990), it is necessary to conduct monitoring exercises under the (HACCP) system, using automated methods to measure microbial loads, because traditional inspection procedures had failed to improve the microbiological condition of carcass meat. Therefore, the safety of meat has been in the forefront society consumers in recent years, evidence exists that the challenges of meat safety will continue in future. The objectives of the present study are to access the microbial contamination of the slaughtered sheep carcasses at El-Kadaroo slaughterhouse in Khartoum State, Sudan.

Materials and methods

Collection of samples:

A total of 90 swab samples were collected randomly from three sites of sheep carcasses including shoulders, flanks and rumps (30 for each) after the last washing using sterile cotton swabs according to Thrusfield, (1995). Swabs were placed into ice box containing ice then transported to the Department of Preventive Medicine, Faculty of Veterinary Medicine University of Khartoum for microbiological analysis. The collected swabs were examined for total viable count (TVC), isolation and identification of bacterial contamination of sheep carcasses.

Culturing of the collected samples:

The samples were cultured onto blood agar and MaConkay agar (Oxoid, CM 270 UK) plates and incubated in an aerobic incubator (Scott Scientific, UK) at 37ºC for 18-24 hours. After incubation the plates were checked for visible bacterial colonies, and plates that show no growth were further incubated for 24 hours before they recorded for no growth. Colonies were sub cultured onto nutrient agar then incubated at 37ºC for 24 hours for purification; selected colonies were preserved at 4˚C in a refrigerator (Coldair, Sudan) for further analysis.

Identification of the isolates:

The identification of the isolates was performed using standard bacteriological methods of identification according to Barrow and Felltham (1993) for primary tests and secondary biochemical tests. Primary tests included Gram reaction, growth in air, growth an aerobically, motility, oxidase, catalase, fermentation of glucose and oxidation fermentation (OF) test. Secondary biochemical tests used according to each genus or group of microorganisms.

Total Viable count (TVC):

The total viable count was carried out according to Quinn, *et al.,* (2000). Tenfold Serial dilutions was prepared by taking 1ml from the original samples and added to the test tube containing 9ml of peptone water. From the homogenized

dilutions number 10^{-4}, 10^{-5}, and 10^{-6} respectively 0.05ml from each suspension was taken, poured onto nutrient agar then spread by bend Pasteur pipette and incubated at 37˚C for 24hours to detect bacterial growth. The average value of bacterial colonies from each duplicated Petri-dishes for the same dilution was taken. Colonies were calculated by colony counter. Using the following formula:
Colony count = (average value× dilution factor) =CFU/cm^2

C.F.U/ml refers to colony forming unit per ml
Cut-of (critical) point
Fresh meat 10^6(Graceyet *al*., 1999).

Statistical analysis

The data were analyzed using SPSS software (Statistical Package for Social Sciences, version 11.5, SPSS Inc. and Chicago, IL, USA). And all bacterial count was convert to log10 CFU/cm2, for analysis and T- test procedure was performed.

Results

Total viable counts

In this study, the level of mean TVC of bacteria in flank, shoulder and rump was 8.6926 (CFU\$cm^2$), 8.3209 CFU\$cm^2$and 7.8002 CFU\$cm^2$respectively (Table 1). TVC difference between shoulder and rump was not significance (*P*= 0.74). There was significant difference in TVC between shoulder and flank (*P*= 0.021), the difference was highly significant between flank and rump (*P* = 0,001).

Table 1: Average contaminant bacteria at shoulder, flank and rump of slaughter sheep (CFU/cm^2)

Parts	Mean	Anti-log
Shoulder	8.3209	2.1×10^8
Flank	8.6926	4.93×10^8
Rump	7.8002	6.3×10^7

Isolation and identification

From the examined parts, frequent bacterial growth was recorded; 30 (93.3%) from shoulder, 29(96.6%) from flank and 28(93.3%) from rump (Table 2) and the total number of isolates was 209. Fourteen different bacterial genera were detected from the three sites (Table 3) shoulder, flanks and rump.

Table2: Percentage of sheep carcasses contamination

Sources of samples	Total number of samples	Bacterial growth number in (B.A* agar)	percentage
Shoulder	30	28	93.3%
Flank	30	29	96.6%
Rump	30	28	96.6%
Total	90	86	95.6%

A higher number of isolates, grown in blood agar, was recorded from flanks 80 (38.3%), followed by rumps 61 (29.1%) and shoulders 68 (32.6%). Fourteen different genera of bacteria were detected form the all samples of the three parts based on biochemical tests. The most detected genus was *Staphylococcus*. (37.7%), followed by *Bacillus spp*.56 (26.7%) and the least genera were *Eschericia* 2(0.96%) and *Citrobacter* 3 (1.4%) as shown in Table 3.

Table 3: Total number of bacterial genera detected from shoulder, flank and rump

Genus	Shoulder	Flank	Rump	Total
Staphylococcus	24	40	15	79(37.8%)
(Bacillus	22	14	20	56(26.8%)
Crynobacterium	10	10	6	2612.4%)
Micrococcus	2	8	5	15(7.1%)
Klebsiella	2	3	2	7(3.3%)
Kurthia	2	1	4	7(3.3%)
Actinobacillus	2	2	2	6(2.9%)
Streptococcus	Non	Non	4	4(1.9%)
Citrobacter	Non	1	2	3(1.4%)
Eschericia	2	Non	Non	2(0.9%)
Aerococcus	1	Non	Non	1(0.96%)
Nocardia	1	Non	Non	1(0.5 %)
listeria	Non	1	Non	1(0.5 %)
Pseudomonas	Non	Non	1	1(0.5 %)
Total	**68**	**80**	**61**	**209(100%**

Discussion

The presence of microorganisms on meat and other foods has been reported worldwide. The high levels of microbial presence on meat increase the chances of meat to get spoiled within the shortest possible time. This was in line with the work of Zweifel and Stephan, (2003) who obtained data concerning the microbiological contamination of sheep carcasses at the abattoirs. In this study, the highest TVC was 4.93×10^8 CFU/cm^2 and the lowest was $6.3x10^7$CFU/cm^2. There was no TVC significant differences between samples from

shoulder and rump (P=0.74) whilst samples taken from flanks and shoulders, and flanks and rumps were found significant (P=0.02 and P=0.001) respectively. The level of total viable bacterial count was higher in flanks than shoulders and rumps and could be due to eviscerating area. These finding were in agreement with the results of others (El Hassan et al., (2011) and Hamdan, (2015). The high total bacterial counts recorded in this study also showed the microbial diversity in these parts, condition of the carcasses and without adhering to good manufacturing practices could result in the increased level of total bacterial count in the meat products (Bhandare *et al*., 2007;Ahmed *et al*., 2012).The results recorded in the present study revealed a high percentage of bacterial contamination of the three parts of shoulders 73%, flanks 88% and rumps 73% after treatment at skinning, evisceration and washing. These findings in agreement with the published results of Gill and Barker (1998) and Abdalla *et al*., (2009) who reported that meat can be contaminated by bacteria during skinning operation.

The various sheep carcasses have different hygienic levels as far as handling, and processing is concerned. This might have accounted for the variations in both bacteria isolates and total aerobic counts at the various carcasses processing as shown in tables 1 and 3 respectively. This was in line with the work of Zweifel and Stephan, (2003). This study revealed that the predominant bacteria of public health importance isolated from the three parts of sheep carcasses were *Staphylococcus* spp.*Bacillus* spp.*Corynebaterium*spp.,*Micrococcus*spp.,*Klebsiella*spp., *Kurthia*spp.,*Streptococcus*spp., *Actinobacillus* spp., *Citrobacter* spp.,*E. coli*, *Listeria*, *Aerococcus*spp.,*Nocardia* spp., and *Pseudomonas spp*. This finding was in agreement with others (Kahraman *et al*., 2005; Amel, 2009; Nouichi; Hamdi, 2009). In this a study, *Salmonella* spp.was not detected in all examined samples and this was in accordance with the reports of others (El-Ghareeb *et al*., (2014); Hamdan, (2015). However, *Salmonella* spp. was not detected in this study and could be explained by sampling problem (number and size of selected sites.

The presence of the microbial isolates on the carcasses is worrying due to their ability to cause diseases. Improper/unhygienic handling by workers, sanitary conditions at the various slaughtering processes, and environmental conditions could be the most probable sources of contamination.

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