Bacterial Contamination of Beef Related to Hygiene Practices in
Slaughterhouses in Khartoum State, Sudan

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Abstract
The present study was conducted at Elsabloga slaughterhouse, Khartoum State, Sudan to evaluate the level of bacterial contamination in beef using Total Viable Counts (TVCs) and identification of the isolates. Checklist following international standards was also used in different slaughterhouses (n = 13) in Khartoum State in order to assess good hygiene practices. Statistical Package of Social Science (SPSS) for Windows was used for data analysis and both descriptive and analytical statistics were used for this purpose. The main results of the checklist revealed that absolute absent of documentation of training programme 100% (n = 13). Washing hands policy including use of sanitizer and or gloves was limited 30.8% (n = 4). Relatively the same observation was obtained for wearing of protective clothing, footwear, hair covering, gloves, etc. 15.4% (n = 2). In addition, fitness to work related to consideration of annual medical certificate was not applied in all slaughterhouses in Khartoum State 100% (n = 13). Similar finding was reported for absent of using microbiological swabbing to determine the effectiveness of sanitizers used 100% (n = 13). Moreover, only 30.8% (n = 4) was recorded for storage and handling of final carcasses under refrigerator. On the other hand, a total of 300 swab samples were collected from beef (neck, shoulder, flank and rump) (75 for each site) after the last washing. According to the Sudanese standards and cut off level for meat and meat product, high Total Viable Counts (TVCs) were recorded for different sites of the carcasses in Elsabloga slaughterhouse. For instance, the mean log TVCs for shoulder, neck, flank and rump were 7.64 CFU/cm², 7.48 CFU/cm², 6.94 CFU/cm² and 7.00 CFU/cm² with 95% confidence interval (7.24 - 8.03), (7.03 - 7.93), (6.45 - 7.43) and (6.64 - 7.36) respectively. Analytical statistic using One Way ANOVA revealed that no significant difference with respect to the mean log TVCs and different sites of the carcasses (F- value = 2.615, P value = 0.051). Furthermore, a total of 309 bacterial isolates from beef were identified in the mentioned slaughterhouse based on microscopic, cultural and biochemical properties. The most dominant bacteria isolated were Micrococcus spp. 26.9% (n = 83), Bacillus spp. 19.1% (n = 59), Staphylococcus spp. 17.8% (n = 55), Enterobacteriaceae 12.9% (n = 40), Pseudomonas spp. 5.5% (n = 17), Corynebacterium spp. 4.8% (n = 15), Aerococcus spp. 2.7% (n = 8), Moraxella spp. 2.3% (n = 7). In conclusion, the level of bacterial contamination in beef at Elsabloga slaughter house was very high as well as relatively poor hygiene practices were recorded in different slaughterhouses in Khartoum State which constitutes a real public health hazard. Hence, enforcement of Good Hygiene practices (GHP) is required at abattoirs in Sudan to decrease the microbial contamination and improve the quality of meat.

Keywords: Bacterial Contamination; Hygiene Practices; Slaughter Houses; Sudan

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Introduction

Dirty animals must be cleaned before entering slaughterhouse to prevent contamination of meat. About 90% of the food poisoning caused by meat and meat products may be attributed to post-mortem operations and handling and about 10% are the cases due to diseases already present in the living animal. Cattle slaughter operations, such as bleeding, dressing and evisceration, expose sterile muscle to microbial contamination that were present on the skin, the digestive tract and in the environment [1,2]. In addition, Roberts [3] found that the sources of bacterial contamination of meat are hides, hooves, soil adhering to hide, intestinal contents, air, water supply, knives, saws, hook, floors and workers.

Nazik [4] investigated the level of bacterial contamination in beef at slaughterhouse in Khartoum State and found highest mean Total Viable Counts (TVCs) 7.79 ± 0.17 (CFU/cm²), 7.92 ± 0.15 (CFU/cm²) and 7.69 ± 0.2 (CFU/cm²) in shoulder, flank and rump. The same author stated that Micrococcus spp., Staphylococcus aureus, Bacillus spp. and E. coli were the most predominant bacteria isolated from beef. On the other hand, study on Hazard Analysis Critical Control Points (HACCP) in slaughterhouse in Khartoum state by Ghihan [5] revealed that the most dominant organism isolated from different critical points was Staphylococcus aureus and there was no E. coli or Salmonella spp. observed. In contrast, Sari Eldin [6] confirmed presence of Salmonella spp. in meat in slaughterhouse in Khartoum state. Moreover, estimation of bacterial contamination in indigenous bovine carcasses in Khartoum State was done by Abdalla., et al [7]. The authors found high bacterial Total Viable Counts (TVCs) in different sites of the carcasses as well as Staphylococcus aureus and E. coli were isolated. In the same study the authors, explained that the level of contamination on bovine carcasses was much higher and the levels of microbial contamination in Sudanese abattoirs may reflect the hygiene status of meat production in the developing countries.

Objective of the Study

Therefore, the objectives of this study are:

1. To assess good hygiene practices using checklist following international standards in different slaughterhouses in Khartoum State, Sudan.
2. To estimate the level of an aerobic bacterial contamination using Total Viable Counts (TVCs) in Elsabloga slaughterhouse, Khartoum State, Sudan.
3. To identify the aerobic bacteria cause microbial contamination in Elsabloga slaughterhouse, Khartoum State, Sudan.

Materials and Methods

Study area

This study was conducted at Elsabloga slaughterhouse (swab samples) in Omdurman as well as checklist on hygiene practices was also used in different types of slaughterhouses in Khartoum State.

Data collection

Information associated with general hygiene practices was recorded using checklist following international standards in different slaughterhouses in Khartoum State as follow:

- **Khartoum**: Elshaha (slaughterhouse).
- **Khartoum North**: Elkadro (slaughterhouse), Eelshahid Nasr Eldin (slab), Eleilafon (slab), Om Dwan Ban (slab), Abu Dleiga (slab).

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- **Omdurman:** Elsabloga (slaughterhouse), Karary Elwatani (slaughterhouse), Ganawa (slaughterhouse), Elsalam (slab), Soog Elmashia (slab), Elbaraka (slab), Karary Elmahalia (slab).

Hygiene practices in checklist including: staff training, personal hygiene practices and facilities, fitness to work, sanitation programme, water supply, refrigeration and storage, storage rooms, monitoring and control and final storage of carcass.

**Sampling**

A total of 300 swab samples were taken from 75 bovine carcasses from different sites of the carcasses (shoulder, neck, flank, and rump) after the last washing. The sampling area of 100 cm² was wiped with sterile wet swab. Swabs were put in thermos flasks containing ice and immediately transferred to the laboratory at Faculty of Veterinary Medicine, University of Khartoum for bacteriological examinations in the same day.

**Samples preparation**

Each sample was immersed in 10 ml of sterile peptone water and incubated at 37°C for one hour.

**Total viable counts (TVCs)**

A Total Viable Counts (TVCs) was done as described by Miles and Misera [8]. Sterile peptone water was used as diluents to make ten-fold dilution from each sample. A drop of 0.02 ml was mounted onto sterile nutrient agar plates for aerobic bacterial count and spread over the plate. Then 10⁻⁴, 10⁻⁵ and 10⁻⁶ were cultured in duplicates. The plates were incubated at 37°C for 24 - 48 hours. Then the average number of colonies was multiplied by the dilution factor to give the number of colonies forming units (CFU) per ml and divided by ten to give the number of colonies forming unit per cm².

**Isolation and identification of bacteria**

Isolation and identification of aerobic bacteria were done as described by Barrow and Feltham [9]. About 0.02 ml of cultured peptone water was inoculated onto blood agar and Mac Conkey agar plates and incubated at 37°C for 24 hours. All bacterial isolates were purified by several sub-culturing from single well separated colonies on nutrient agar plates and then incubated at 37°C for 24 hours. Pure isolates were visually examined for bacterial growth, and then examined microscopically using Gram’s stain as well as biochemical tests were done.

**Statistical analysis**

Statistical analysis was done using Statistical Package of Social Science (SPSS) for Windows version 20.0. Descriptive statistic such as counts and percentage were used for some variables. While, mean, standard deviation, standard error and 95% confidence interval were used for Total Viable Counts (TVCs) which converted to log 10 for analysis. Analytical statistic using One Way ANOVA was employed for comparison of mean log TVCs at different sites of the bovine carcasses. Interpretation was done according to Sudanese Standards (SDS) 341. (2015) cut off level from meat and meat products, Minimum limit 10⁵ CFU/cm² (log 10⁵ = 5 CFU/cm²) and Maximum limit 10⁶ (log 10⁶ = 6 CFU/cm²) [10]. All results were presented as tables, graphs and plates.

**Results**

**Checklist related to hygiene practices in different slaughterhouses in Khartoum State, Sudan**

Good hygiene practices were evaluated in different slaughterhouses (n = 13) in Khartoum State using checklist method following international standards. The main results revealed that absolute absent of documentation of training programme 100% (n = 13). Washing hands policy including use of sanitizer and or gloves was limited 30.8% (n = 4). Relatively same observation was obtained for wearing...
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of protective clothing, footwear, hair covering, gloves, etc. 15.4% (n = 2). Furthermore, fitness to work related to consideration of annual medical certificate was not applied in all slaughterhouses in Khartoum State 100% (n = 13). Similar finding was reported for absent of using microbiological swabbing to determine the effectiveness of sanitizers used 100% (n = 13). Thermometers in coolers and freezers in the most of the slaughterhouses was absent 92.3% (n = 12) as well as using +4°C or colder for chilled bone - in storage rooms was limited 30.8% (n = 4). Limitation of monitoring of temperature of all refrigerated storage and production areas was also observed 15.4% (n = 2). Moreover, only 30.8% (n = 4) was recorded for storage and handling of final carcasses under refrigerator. The rest of the details results are summarized in table 1-4 and plate 1-3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Documentation of training program</td>
<td>0 (0.0%)</td>
<td>13 (100.0%)</td>
</tr>
<tr>
<td>2. Washing hand policy including use of sanitizer and or gloves.</td>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
</tr>
<tr>
<td>3. Presence of cleaning policy</td>
<td>7 (53.8%)</td>
<td>6 (46.2%)</td>
</tr>
<tr>
<td>4. Wearing of protective clothing, footwear, hair covering, gloves, etc.</td>
<td>2 (15.4%)</td>
<td>11 (84.6%)</td>
</tr>
<tr>
<td>5. Keep knives, aprons and steels clean and tidy.</td>
<td>6 (46.2%)</td>
<td>7 (53.8%)</td>
</tr>
</tbody>
</table>

**Table 1:** Results of checklist on hygiene practices related to staff training in different slaughterhouses in Khartoum State, Sudan.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of designated area for employees to leave outer garments.</td>
<td>5 (38.5%)</td>
<td>8 (61.5%)</td>
</tr>
<tr>
<td>All employees wearing outer garments suitable for the operation.</td>
<td>9 (69.2%)</td>
<td>4 (30.8%)</td>
</tr>
<tr>
<td>Washing hands before interring production area and after using toilet.</td>
<td>8 (61.5%)</td>
<td>5 (38.5%)</td>
</tr>
<tr>
<td>Eating and drinking in slaughterhouse hall.</td>
<td>13 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Smoking during work.</td>
<td>13 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

**Table 2:** Results of checklist on hygiene practices related to personal hygiene practices and facilities in different slaughterhouses in Khartoum State, Sudan.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of written sanitation programme for slaughterhouses.</td>
<td>0 (0.0%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Visual examination of the plant and equipments before production to insure cleaning.</td>
<td>12 (92.3%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Using of microbiological swabbing.</td>
<td>0 (0.0%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Regular microbiological analysis of water in slaughterhouse.</td>
<td>2 (15.4%)</td>
<td>11 (84.6%)</td>
</tr>
<tr>
<td>Is all water used in processing plant is potable?</td>
<td>11 (84.6%)</td>
<td>2 (15.4%)</td>
</tr>
</tbody>
</table>

**Table 3:** Results of checklist on hygiene practices related to sanitation programme and water supply in different slaughterhouses in Khartoum State, Sudan.

### Table 4: Results of checklist on hygiene practices related to refrigeration and storage rooms in different slaughterhouses in Khartoum State, Sudan.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using +4°C or colder within 24 hours of slaughtering.</td>
<td>2 (15.4%)</td>
<td>11 (84.6%)</td>
</tr>
<tr>
<td>Finishing carcass stored and handled under refrigerator.</td>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage room</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of thermometers in all coolers and freezers.</td>
<td>1 (7.7%)</td>
<td>12 (92.3%)</td>
</tr>
<tr>
<td>Using refrigeration for meat in storage room.</td>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
</tr>
<tr>
<td>Using +4°C or colder for chilled bone-in storage</td>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
</tr>
</tbody>
</table>

**Plate 1:** Carcass washing in slaughterhouse, Khartoum State, Sudan.

**Plate 2:** Meat inspection by veterinarian in slaughterhouse, Khartoum State, Sudan.
Total viable counts (TVCs) of beef at Elsabloga slaughterhouse in Khartoum State, Sudan

According to the Sudanese standards and cut off level for meat and meat product, highest Total Viable Counts (TVCs) were recorded for different sites of the bovine carcasses in Elsabloga slaughterhouse. For instance, the mean log TVCs for shoulder, neck, flank and rump were 7.64 CFU/cm², 7.48 CFU/cm², 6.94 CFU/cm², and 7.00 CFU/cm², respectively with 95% confidence interval (7.24 - 8.03), (7.03 - 7.93), (6.45 - 7.43) and (6.64 - 7.36) respectively. Analytical statistic using One Way ANOVA revealed that there was no significant difference with respect to the mean log TVCs and different sites of bovine carcasses (F-value = 2.615, P value = 0.051). The results are presented in table 5 and figure 1.

<table>
<thead>
<tr>
<th>Site</th>
<th>No examined</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>95% confidence Interval for mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoulder</td>
<td>72</td>
<td>7.64</td>
<td>1.70</td>
<td>0.20</td>
<td>(7.24 - 8.03)</td>
</tr>
<tr>
<td>Neck</td>
<td>71</td>
<td>7.48</td>
<td>1.90</td>
<td>0.22</td>
<td>(7.03 - 7.93)</td>
</tr>
<tr>
<td>Flank</td>
<td>71</td>
<td>6.94</td>
<td>2.07</td>
<td>0.25</td>
<td>(6.45 - 7.43)</td>
</tr>
<tr>
<td>Rump</td>
<td>71</td>
<td>7.00</td>
<td>1.51</td>
<td>0.18</td>
<td>(6.64 - 7.36)</td>
</tr>
<tr>
<td>Total</td>
<td>285</td>
<td>7.26</td>
<td>1.82</td>
<td>0.11</td>
<td>(7.05 - 7.48)</td>
</tr>
</tbody>
</table>

*Table 5: Descriptive statistic of log TVCs (CFU/cm²) for different sites of bovine carcasses at Elsabloga slaughterhouse, Khartoum State, Sudan.*

*Cut off level: Minimum 10⁵ CFU/cm² (log 10⁵ = 5 CFU/cm²) Maximum 10⁶ (log 10⁶ = 6 CFU/cm²) [10].*

Types of bacterial isolates of beef at Elsabloga slaughterhouse in Khartoum State, Sudan

High percentage of Grams positive bacteria 77.7% (n = 240) was observed in beef at Elsabloga slaughterhouse in Khartoum State, Sudan. While, only 22.3% (n = 69) was reported for Grams negative bacteria. The most bacterial isolates predominately were Micrococcus spp. 26.9% (n = 83), Bacillus spp. 19.1% (n = 59), Staphylococcus spp. 17.8% (n = 55), Enterobacteriaceae 12.9% (n = 40), Pseudomonas spp. 5.5% (n = 17), Corynebacterium spp. 4.8% (n = 15), Aerococcus spp. 2.7% (n = 8), Moraxella spp. 2.3% (n = 7). The results are shown in figure 2 and table 6.
### Discussion

Food borne diseases are diseases resulting from ingestion of bacteria toxins and cell produced by microorganism presents in food. The presence of these microorganisms in meat food should receive particular attention because their presence indicates public health hazard. On the other hand, the guidelines to ensure safe meat handling are recorded by World Health Organization (WHO), Food and Agricultural Organization (FAO) and Codex Alimentarius Commission such as Hazard Analysis Critical Control Point (HACCP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP). Pre-requisites programs (PRPs) are the foundation for building a HACCP system and may include: training, premises and equipment, storage, maintenance, cleaning/sanitation, residue control program, services (water and pest control), waste management and product recall/withdrawal and traceability.

Evaluation of good hygiene practices using checklist following international standards was done in different slaughterhouses in Khartoum State in present study. For instance, limitation regarding washing hands policy, documentation of training program, presence of written sanitation programs, presence of annual health medical certificate and wearing of protective clothing, footwear, hair covering, gloves, etc. were observed. Similar findings were obtained by Chepkemoi., *et al.* [11], who explained that in Kenya, meat handling practices among small medium enterprise (SME) butcheries did not meet the minimum sanitation and hygiene practices. In addition, the same authors stated that seventy percent and 82% of operation in Nairobi and Isiolo counties respectively did not wear protective clothing as well as a percent of 94% and 88% of operators respectively did not have medical certificates. Washing hands were also observed in most of them 60% and 82% respectively. As seen from the study, using refrigeration for meat in storage room was reported only in 30.8% slaughterhouses in Khartoum State and the same percentage was also reported for the storage of final carcasses and handling under refrigerator. Similarly, Chepkemoi., *et al.* [11] stated that 58% and 27% of operators in Nairobi and Isiolo counties respectively used closed vehicles.
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to transport meat from the slaughterhouse to the butcheries. They also explained that the metallic containers or transport vehicles used were not refrigerated as the distance covered. Furthermore, hygiene status of the abattoirs was assessed by Akinnibosun and Imade [12] in Edo State in Nigeria. Results obtained from mentioned study indicated that the overall hygienic status of the abattoirs was bad, indicating that no systems and no written records exits, and lay workers without any training were expected to apply hygiene standards.

On the other hand, according to the Sudanese standards and cut off level for meat, highest Total Viable Counts (TVCs) were recorded for the different sites of beef (over all mean log TVCs = 7.26 CFU/cm²) in Elsabloga slaughterhouse, Khartoum State. Similar findings were obtained by Nazik [4] in slaughterhouse in Khartoum State, who stated that the mean TVCs was (7.79 ± 0.17 CFU/cm²) in the shoulder, (7.92 ± 0.15 CFU/cm²) in the flank and (7.69 ± 0.2 CFU/cm²) in the rump of the beef. Moreover, a study on evaluation of the bacteriological contamination in indigenous cattle in slaughterhouses, Khartoum State by Abdalla, et al. [7] revealed that the mean Total Viable Counts (TVCs) of bacteria after skinning, evisceration and washing operations at shoulder, neck and rump was statistically significant. The authors were also recorded statistically significant difference for mean (TVCs) between knives and workers hands during the three operations.

In the current research work, the most dominant bacteria isolated were Micrococcus spp., Staphylococcus spp., Bacillus spp., Enterobacteriaceae, Pseudomonas spp. and Corynebacterium spp. These findings agree with Nazik [4], who isolated similar organisms with high percentage at slaughterhouse in Khartoum State. Furthermore, Abdalla, et al. [7] explained that twelve species of bacteria were isolated as indicator of bacteriological contamination in indigenous cattle in slaughterhouses in Khartoum State and the highest prevalence rate was reported for Staphylococcus aureus 10.54%, Klebsiella spp. 10.12% and E. coli 8.86%. In addition, Ghihan [5] stated that the most dominant bacteria isolated from different critical points at slaughterhouse in Khartoum State was Staphylococcus species and there was no E. coli or Salmonella spp. observed [13,14].

Conclusion

1. Relatively poor hygiene practices using checklist following international standards were recorded in different slaughterhouses in Khartoum State, Sudan.
2. High level of bacterial contamination using Total Viable Counts (TVCs) beef (Over all mean log TVCs = 7.26 CFU/cm²) was found in beef at Elsabloga slaughterhouse in Khartoum State, Sudan.
3. The most dominant bacteria cause microbial contamination of beef at Elsabloga slaughterhouse in Khartoum State, Sudan were: Micrococcus spp., Bacillus spp., Staphylococcus spp., Enterobacteriaceae, Pseudomonas spp., Corynebacterium spp., Aerococcus spp. and Moraxella spp.

Recommendations

1. There is an urgent need for using strict meat hygiene systems which control contamination in the slaughterhouses including: sanitation of equipments and machines, personal hygiene.
2. Attention should be paid regarding training programme for the workers and all staff about the good hygiene practices.
3. Enhancement of good manufacturing practices and good hygiene practices is required at abattoirs in Sudan to decrease the microbial contamination and improve the quality of meat.

Bibliography

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