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Research Article

Evaluation of Safety Attributes of Meat Purchased at Small Scale Butcheries, Wad-Medani City, Gezira State, Sudan

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Abstract

Objective: This article aimed to investigate safety attributes of meat purchased at small scale selling points at Wad-Medani City, Sudan.

Methods: Standard microbiological procedures were performed to determine the microbiological qualities of beef meat and offals during three seasons; summer, winter and autumn.

Results: The microbiological analysis revealed that the highest contamination was detected in the summer since the total viable counts (TVC) were 6.5 x 10^7 cfu/g and TVC was 12.9 x 10^7 cfu/g in offals. A high count of yeast and molds was (3.5 x 10^2 cfu/g) in offals, while a lower count (0.93 x 10^2 cfu/g) was found in meat. The data showed that the prevalence of most of the pathogenic bacteria of both meat and offals were influenced by the season, in that; a higher percentage of *Salmonella* spp (83.3%), *Staphylococcus aureus* (100%) and *E. coli* (83.3%) during autumn, whereas; *Bacillus cereus* showed a high percent (66.7%) in winter. All the isolated bacteria were found with high proportions in offal samples, in that: *E. coli, Salmonella spp, Staphylococcus aureus* and *Bacillus cereus* were found in 100%, 94.4%, 94.4% and 66.7% of the samples, respectively and for meat samples, these bacteria were found in 83.3%, 66.7%, 61.1% and 55.6% of the samples, respectively.

Conclusion: The results cleared that, the highest contamination level was detected at offal. There is high contamination in the meat selling centers due to a lack of health requirements in meat shops, meat handling, and lack of attention to personal hygiene. The meat and offals must be subjected to proper cooking prior to consumption, in addition, there is a need for the application of suitable control measures to prevent meat cross and post-contamination.

Keywords: *Microbiological Analysis; Contamination; Salmonella; Yeasts; Cooking*

Introduction

Sudan has a huge animal resource, estimated to be more than 106 million head 30.37 million cattle, 4.80 million camels, 40.21 million sheep and 31.32 million goats [1].

Meat, including beef, is a significant source of high quality nutrients, like proteins of high biological value amino acids, and minor components for people. The protein and essential amino acids content of meat usually compensates for deficiencies in diets made mainly of cereals and other vegetable proteins [2]. Its fats, amino acids, and minerals as well as micronutrients like selenium, zinc can easily be absorbed. It also supplies vitamins, which are important components of the diet, which make it a good substrate for possible microbial growth. As a result, in its raw state meat is easily susceptible to colonization by microorganisms [3]. Therefore, it is important that meat processing should be guided by a food safety system, including the schematic layout of the production process so that possible sources of contamination can be identified [4]. Monitoring and verification procedures must be established to maintain the production of a hygienically acceptable product by controlling the key steps in the production process where the hazards were identified [5].

The microorganisms that are usually prevalent in raw meat include *Escherichia coli* and *Escherichia coli 0157: H*, *Coliforms, Staphylococcus aureus, Salmonella, Clostridium* and *Bacillus*, this microbiota has been associated with food-borne illness outbreaks and even death to many people each year [5]. These pathogen types were having been associated with several diseases in both humans and animals; some *E. coli* is able to produce toxins that can cause very serious illnesses in humans, such as HC and HUS [3]. *Staphylococcus aureus* is one of the most frequent pathogens that cause foodborne outbreaks. It is responsible for staphylococcal food poison by producing heat-stable toxins [6].

In Sudanese communities, the opportunity for contamination of the meat exists, amongst others, from the slaughter floor. Whether in raw or processed meat, both usually contain bacteria or other microorganisms [5]. In Sudan, hygienic measures to control microbial contamination of meat it unsatisfactorily applied. Storage at refrigerated temperatures is still one of the most effective practices for improving the safety of fresh meat. However, some butcheries still use poor refrigeration; in addition, the retail raw meat in most butcheries is presented exposed to environmental pollution, which might lead to increased bacterial contamination, and also poor personal hygiene.

Objective of the Study

The objectives of this study were to evaluate of Safety of beef meat and offal samples collected from Wad-Medani local market, central Sudan.

Materials and Methods

Study area

This study was carried out in Wad Medani capital of the Gezira State the city is located between 14° 24'N-14.4° N longitude and 33° 31'E- 33.517° E latitude, 136 Km southeast to Khartoum on the west bank of the Blue Nile.

Samples

A total of 108 samples were examined in this study, divided into three seasons every season there were 18 samples of beef meat collected from 6 sites (butcher shops) in sterilized containers from 6 locations during three seasons (summer, winter and autumn) at small scale butchers market, Wad Madani, central Sudan, these locations included AlsugAlkabeer (AK), AlsugAlsageer (AS), AlsugAlmarkazy (AM), Alkariabah (AK), Aldibagah (AD), Alsinniat (AN).

Submission of samples

All samples were kept in a sterile ice container and transported within 3 hrs to the Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, the University of Khartoum for further analysis.

Microbiological analysis

For the preparation of serial dilutions, 10g sample (meat or offal) were homogenized with 90 ml of distilled water by shaking for several minutes, from this suspension, 1 ml was taken from the dilution and transferred to another tube containing 9 ml sterile distilled water to make serial dilutions up to 10^6. Plate count agar (PCA), Potato dextrose agar (PDA), Paired-parker agar and *Bacillus cereus* agar were used for enumeration the total counts of bacteria [7], yeast and mould, *Staphylococcus aureus* and *Bacillus cereus* in all collected meat and offal samples, respectively. All plates with the h exception to the PDA plates were incubated at 37°C for 48 hours, while PDA plates were incubated at 30°C for 72 hours. The plates containing 30 - 300 colonies were counted and multiplied by the dilution factor to express microbial ac colony-forming unit (cfu/g). The *Staphylococci* and *Salmonella* count were estimated accordingly.

The test for coliform bacteria was carried out by using the most probable number (MPN) technique, which is composed of 3 stages:

1. **Presumptive coliform test:** 1 ml of suitable dilution of the beef meat (10^{-1}, 10^{-2}, 10^{-3}) was inoculated in tubes each containing nine milliliters of MacConkey broth (enrichment medium), fitted with Durham tubes, the tubes were incubated at 37°C 48 hrs. Growth and gas production after 24 and 48 hrs were recorded. Gas production constituted a positive test.

2. **Confirmed coliform test:** All fermentation tubes from the presumptive test showing gas in 24 hrs at 37°C were utilized in the confirmation test. The medium used in this test was Brilliant Green Bile BGB lactose broth. Each tube contained 10 ml of the medium fitted with Durham tubes. A loopful, from presumptive test tubes, were transferred to each BGB tube, and then incubated at 37°C for 48 hrs. coliform was calculated from the Most probable number (MPN) via (MPN) tables [8].

3. **Confirmed *E. coli* test:** For further confirmation of *E. coli,* any tubes showing the positive result on E C broth streaked on Eosin Methylene Blue (EMB). The plates were incubated at 37°C for 48 hrs colonies with green metallic sheen gave a positive test [7].

Detection of *Salmonella*

Twenty-five grams of samples were aseptically weighed and mixed well with 225 ml sterile peptone water and incubated at 37°C for 24 hours. Then 10 ml were aseptically drawn and added to 100 ml selenite cystine broth. The broth was incubated at 37°C for 24 hours. Using a loopful, streaking was carried out into solidified bismuth sulfite agar plates. The plates were incubated at 37°C for 72 hours. Black metallic shine discrete colonies indicated the presence of *Salmonella*.

Identification and characterization of bacteria isolated

The bacterial isolates were examined microscopically under the oil immersion lens. The shape, size, and Gram stain of the isolated cell were observed. The isolates were then identified and characterized based on both morphological characteristics and biochemical tests. Growth morphology tests included: colony shape, size, and surface features. The structures assigned to bacteria *cocci* (round), *bacilli* (rods), or *spirochetes* (corkscrew) can be readily seen via light microscopy, bacterial isolates were further differentiated at the time of microscopic examination by staining the gram stain is one of the most useful and commonly used tools to differentiate bacteria beyond the genus level.

Statistical analysis

The data were analyzed using statistical analysis system software (Statistics 8) comparison between samples were tested using analysis of variance (ANOVA) test to determine whether there were significant differences between means, then LSD was used. Percent of bacteria isolated during three seasons were analyzed using statistical analysis system software statistical package for the social sciences (SPSS).

Results

The influence of different seasons and types of beef meat on microbial load

Microbiological characteristics of the different meat samples (fresh meat and offal) and three different seasons (summer, autumn and winter) are shown in figure 1, 2 and table 1.

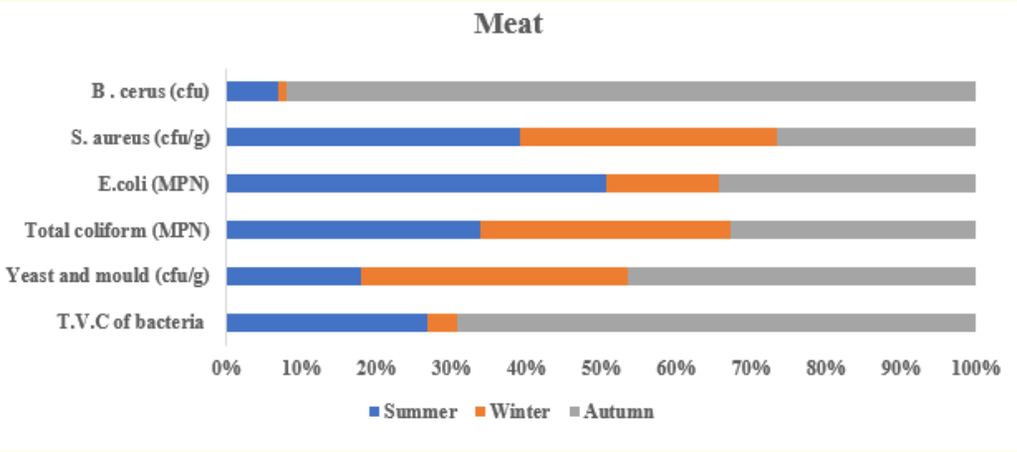

Figure 1: *Microbiological characteristics of meat samples at various seasons (summer, autumn, and winter).*

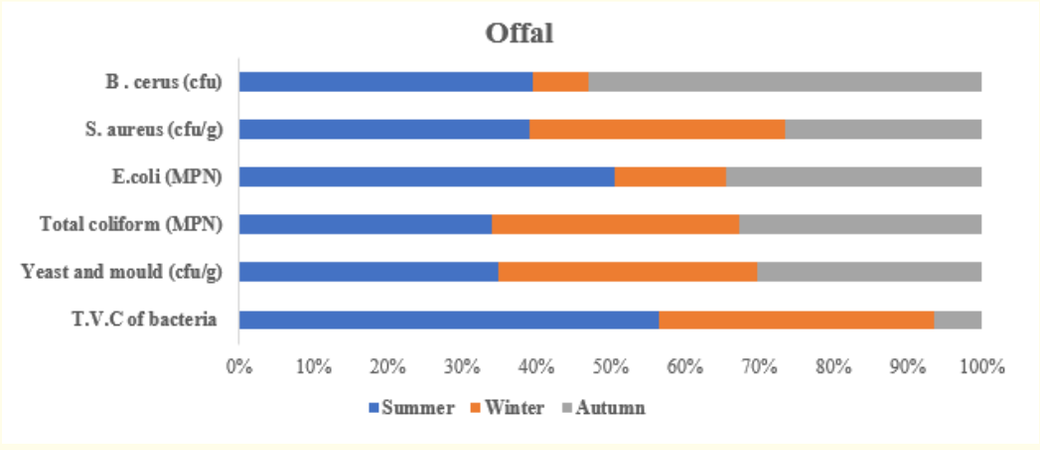

Figure 2: *Microbiological characteristics of offals samples at various seasons (summer, autumn, and winter).*

Type	TVC of bacteria (cfu/g)	Yeast and mould (cfu/g)	Total coliform (MPN)
Meat	$1.5\times10^{6\,a}$	0.9×10^{2a}	16.3^{a}
Offal	7.7×10^{7b}	3.5×10^{2b}	29.6^{b}
S.E	0.8	0.7	0.1

Table 1: *Comparison between meat and offal of microbiological characteristics (Average of three seasons).*

a,b : Mean values in the same column having different superscripts, differ significantly ($p < 0.05$).

S.E: Standard Error; T.V.C = Total Viable Count; S. aureus = Staphylococcus aureus; B. cereus = Bacillus cereus.

Effect of season on the prevalence of bacteria

The count of bacteria isolated from meat and offal during the three seasons was shown in figure 3-5.

Comparison of prevalence of bacteria (*Salmonella* spp, *E. coli, Staphylococcus aureus* and *Bacillus cereus* isolated from meat samples during different seasons were presented in figure 3, the data showed higher isolates number *Salmonella* spp, *Staphylococcus aureus* and *E. coli* whereas, isolates of *Bacillus cereus* showed high numbers during winter.

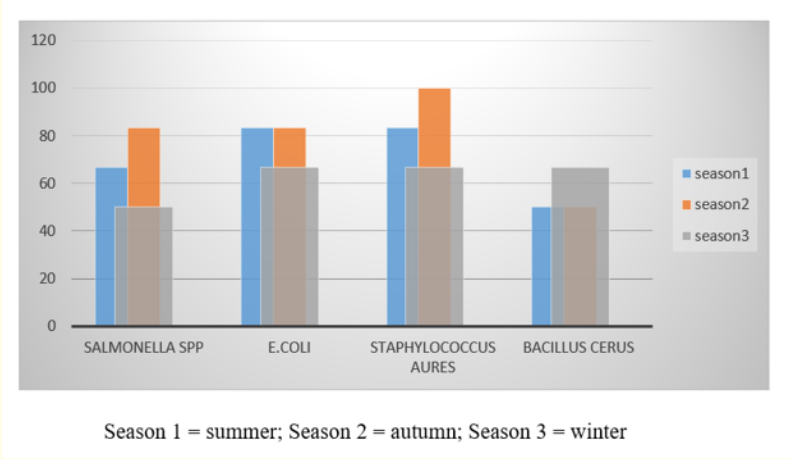

Figure 3: *The prevalence of bacteria isolated from meat samples during different seasons.*

For offal samples, as indicated in figure 4, *E. coli* was isolated from samples during all seasons. However, the number of *Salmonella* spp. isolates were similar to those of *Staphylococcus aureus* during autumn and summer (100%), moreover, both organisms were found in higher numbers (83.3%) during winter. Similarly, *Bacillus cereus,* was found with a high number (83.3%) during summer.

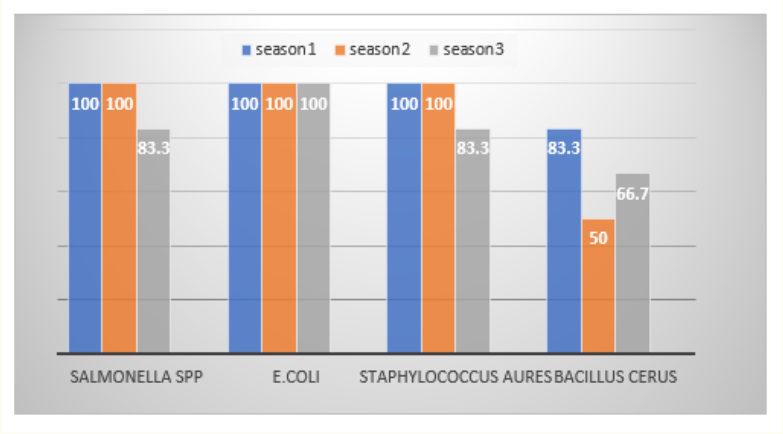

Figure 4: *The prevalence of bacteria isolated from offal samples during different seasons.*

All bacteria isolated showed high prevalence in offal samples as shown in figure 5. *Salmonella* spp*, E. coli, Staphylococcus aureus* and *Bacillus cereus* were found with a percentage of 100%, 94.4%, 94.4% and 66.7%, respectively. However, 83.3%, 66.7%, 61.1% and 55.6% of meat samples contained *Salmonella* spp*, E. coli, Staphylococcus aureus and Bacillus cereus*, respectively.

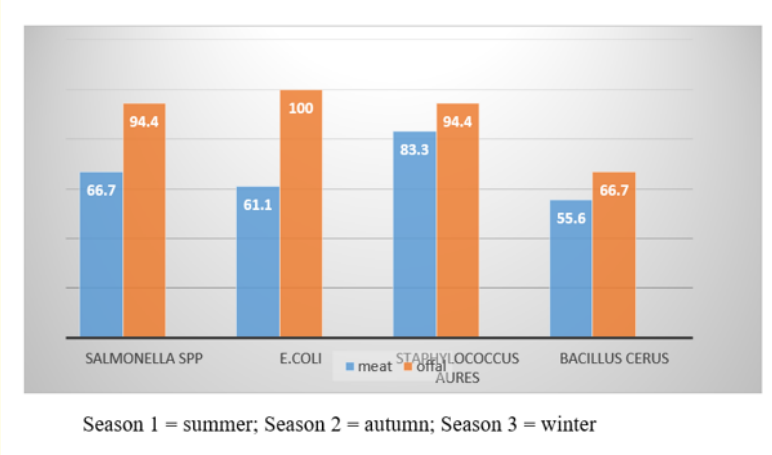

Figure 5: *Comparison of percentage of bacteria isolated from offal and meat samples.*

Discussion

This study intends to lay out the shelf-life and microbiological quality of meat. The microbiological profiles of beef meat obtained from the carcasses and offals were examined. Practically 60% of the raw beef samples from little butcheries in Gezira State were inadmissible, which shows the low microbial quality and shelf lives of these products, the meat, and unseemly timeframes of realistic usability of these items. Poor hygiene during handling might be one justification for the generally high bacterial loads.

The results indicated significantly higher contamination with microbes (TVC) during the summer season (6.5×10^7) however. The bacterial load in this study exceeded that determined by [9], which reported one million colonies per gram of fresh, conditioned, and frozen meat. The result was also higher than that reported by Mohamed [10] who reported (4.7×10^5 CFU/gm), (1.3×10^5 CFU/gm) in beef and camel meat, respectively.

Offals often have higher initial contamination and are more likely to be contaminated with pathogens than carcass meat. This study revealed that the TVC of offal was higher than that of meat. However, the highest TVC of bacteria (12.9×10^7 cfu/g) was determined in the offal at summer season. Sheridan and Lynch [11] attributed the increases in bacterial load in meat products to slow cooling.

Microbial analysis showed that there were no significant effects of seasons on the count of yeast and molds ($P < 0.05$) between meat and offal which contained 0.93×10^2 and 3.5×10^2, respectively. Moreover, a relatively high count of yeast and molds in the offal was 3.7×10^2 in summer and winter. These results were lower than that reported by Sulieman., *et al* [12].

Foodborne diseases connected with meat are a worldwide general wellbeing worry because of the great gamble of bacterial contamination of meat by a few sorts of microorganisms [13].

The presence of a higher number of organisms makes the meat more prone to spoilage and may serve as a tool for the transmission of pathogenic strains. The diseases of the gastrointestinal tract are very common in this part of the world and they are mainly transmitted through contaminated food and water. It is largely due to improper handling, unhygienic conditions. Several studies in Sudan have been conducted to see the microbiological quality of meat and meat products. In one such study Abd Elkareem [14] isolated *Salmonella* spp and *E. coli* from raw meat. Similar results were obtained in another study Ahmed [15] who isolated a variety of microorganisms.

The study indicated that the effect of season on total coliforms load was non-significant ($p < 0.05$). In addition, there were significant differences ($p < 0.05$) between meat and offal, in that, the coliform count was 29.57 and was 16.28 in offal and meat, respectively at summer. These results were agreed with Ayesha Zafar., *et al*. [16] who mentioned all the beef samples were contaminated with coliforms and 9 out of 10 samples had > 1100 cfu/g coliform count. These indicator organisms clearly showed that these meat samples were contaminated with fecal pollution and may transmit a variety of bacterial and viral diseases [16]. Adentunji and Awosanya [17] reported that portable water was an essential requirement in the quality assurances of meat produced at the abattoir.

In this study, there was a significant difference at ($p < 0.05$) in *E. coli* load of meat and offal during the three seasons. It has been recognized that *E. coli* is a useful indicator of the quality of meat (Modern reference on *E. coli* and quality of meat). These indicator organisms clearly showed that these meat samples were contaminated with fecal pollution and might transmit a variety of bacterial and viral diseases. In this study, it high *E. coli* load in offal was determined in summer. This study agreed with many investigators revealed who detected high contamination by *E. coli* in meat products [12,15,17].

As for *Staphylococcus aureus* in meat and offal, the data in this study revealed that there were non-significant differences ($p < 0.05$) of season and meat type, although high contamination was found in offal during autumn.

Analysis of *Staphylococcus* spp revealed that the counts of this microbe exceeded the maximum acceptable limits reported by the SSMO which indicates that the acceptable *Staphylococcus* spp limits are 5×10^2 cfu/g and the level of maximum count is 1×10^3 cfu/g. On the other hand, the determined values matched the findings of Ahmed (2018) [15] for the count of *Staphylococcus aureus.*

Meat and offal were contaminated with high levels of *Bacillus cereus*, however, there was a significant difference ($p < 0.05$) during Autumn. The counts of the microbe were 10.73×10^2, 11.48×10^2 and 146.17×10^2 during winter, summer, and autumn, respectively. The high contamination agreed with that reported by Ahmed [15] who isolate *Bacillus cereus* from Khartoum samples.

Conclusion

This study revealed that fresh meat products available in Wad-Medani markets were seriously contaminated with a variety of microorganisms (some of them are pathogens.

High microbial contamination of beef meat as well as offals sold at small butchers was found, this could be attributed to an improper meat safety management system in which meat is prone to microbial contamination by various sources, in addition, meat handling and lack of attention to personal hygiene.

The effect of the season (summer, winter, and autumn) on the count of bacteria varies from significant differences to non-significant differences. A high total viable count of bacteria, total coliform, and *E. coli* was observed during summer, however *S. aureus* and *B. cereus* were higher during autumn, however, some of these bacteria are potential pathogenic microorganisms The high isolation rate (100%) in *E. coli, Salmonella* and *S. aureus* were during summer and autumn.

However, the application of the HACCP system could largely contribute in meat safety.

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