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Bacteriological Quality Profiles and Prevalence of *Staphylococcus aureus*, *Salmonella* Species, and *E. coli* in Meat Samples of Sheep and Goats

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Abstract

THE HIGH nutritional value as well as traditional delicacies of beef, chevon, and mutton, are leading to an increase in the consumer demand. Even though, sheep and goat meat can become contaminated with harmful microorganisms as a result of unauthorized outdoor slaughtering, poor hygiene in slaughterhouses, and improper processing in food sites. This study aimed to identify pathogenic bacteria (including *Staphylococcus aureus, Salmonella*, and *E. coli*) in sheep and goat meat in slaughterhouses located in Qaluibya governorate, Egypt. The microbiological quality of samples was assessed by evaluating the total colony and coliform counts**.** A grand total of 100 samples were collected from the meat of sheep and goats. For instance, the average total colony count (TCC) levels for chevon meat and mutton meat samples were $3.2x10^5 \pm 0.4x10^5$ and $7.4x10^5 \pm 0.9x10^5$, respectively. The average coliform count in chevon meat samples was $4.5x10^2 \pm 0.6x102$, while in mutton meat samples it was $15x102 \pm 2.0x102$. *Staphylococcus aureus* prevalence rates were 36% and 50% while *Escherichia coli* prevalence's were 44% and 56% in chevon meat or mutton meat samples, respectively. Nonetheless, *Salmonella* species were not found in either of both meat types. It is recommended that in addition to strict hygienic measures, adequate and safe water are to be supplied by authorities for use in all slaughterhouses to improve quality and reduce contamination of slaughtered carcassess.

Keywords: Bacteria, Chevon and mutton meat, Slaughterhouses, Total colony count, public health.

Introduction

Cases of foodborne illnesses demonstrate the presence of bacteria that can cause illness in individuals who have eaten contaminated food[1] credits the increase in food contamination to the quick expansion of food production across the globe. Sufficient amenities and proper abattoirs are necessary for the sanitary preparation of meat. However, there is still a chance of bacterial contamination at the slaughterhouse. Playing a vital role in the worldwide livestock sector, it helps provide meat for millions of people [2] It is crucial to tackle a range of health and safety measures in slaughterhouses. These conditions, including the slaughter and processing of animals on dirty floors with blood and feces, pose a public health hazard [3]. The meat industry faces a major challenge from foodborne pathogens like *S. aureus, Salmonella*, and *E. coli* that contaminate fresh meat during slaughtering, dressing, and processing. The state of the animals' health pre-slaughter, the cleanliness of the slaughtering area, and the tools utilized during slaughter all play a role in the spread of S. aureus via meat and butchers [4].

The microbiological characteristics of meat are a reliable indicator of its hygiene level. Insufficient slaughterhouses, improper sanitation, and inadequate carcass processing can lead to higher levels of total bacterial colony and total coliform counts in meat [5] The levels of *Staphylococcus aureus* contamination in different kinds of meat such as pork, turkey, beef, chicken, goat, and fish were measured. The investigation found that pork had the highest level of contamination, whereas chevon had the lowest one. *Escherichia coli* and salmonellae are two of the most common harmful bacteria that cause food

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contamination. The usual route of transmission of these bacteria to humans is through food that has been tainted with water [6]

Escherichia coli presents a significant danger because it has the ability to cause a range of illnesses in humans, such as urinary tract infections, sepsis, and neonatal meningitis [7] Moreover, salmonella infections are a significant concern for public health worldwide, mainly due to being transmitted through contaminated food. In 2019, the European Union (EU) recorded 90,105 cases of human salmonellosis, with 9,718 cases specifically identified in the United Kingdom (UK). Each year, *Salmonella* species cause around 93.8 million illnesses and lead to 155,000 deaths globally [8]

Material and Methods

Sampling

A hundred meat samples (50 chevon and 50 mutton) were collected under aseptic conditions and promptly transported for bacteriological examination. The specimens were obtained from different slaughterhouses located in El-Qalubiya governorate of Egypt.

Preparation of the samples

A volume of 225 ml of sterile peptone water (0.1%) was used to immerse 25 grams of the meat samples being analyzed. Afterwards, the blend was homogenized with a stomacher for 2 minutes, producing a 1/10 dilution homogenate. After thorough mixing, 1 ml of the first dilution was transferred using a sterile pipette into another sterile tube with 9 ml of sterile buffered peptone water (1%) ISO 6887-1:2017.

Bacterial isolation and identification:

Total colony count

A weight of 25 grams of the sample were mixed with 225 ml of sterile peptone water (0.1%) and homogenized with a stomacher for 2 minutes. Afterwards, dilutions were made in increments of ten. Then, the required dilutions were poured onto plate count agar and all plates with inoculants were placed in an incubator at 30 °C for 72 hours Iso4833-1:2013 UPDATED YEAR 2022:

Coliform count

Total coliform count in each sample was carried out by utilizing violet red bile agar (VRB) at a temperature of 37ºC for a full day according to ISO4832:2006.

Isolation and identification of S. aureus

S. aureus was isolated from different meat samples following the methods recommended by ISO 6881-1:2021 Amendment:2023

Isolation of Salmonella ISO6579:2017 AMD:2020:

In order to recover and identify *Salmonella* spp., a 5 ml sample pre-enriched in peptone buffer was mixed with 45 ml of Rappaport Vassiliadis broth and incubated at 41°C for 24 hours. Afterwards, a loopful of the enriched specimen was streaked onto xylose-lysine deoxycholate (XLD) agar and left to incubate at 37 °C for 24 hours. Individual colonies were transferred onto trypticase soya agar and left to grow at 37 °C for another 24 hours.

A range of biochemical tests, such as methyl red (MR), Voges-Proskauer (VP), oxidase, catalase, urea hydrolysis, triple sugar iron agar (TSI), citrate utilization, indole, and the sulfide motility test, were carried out to verify the identification.

Results and Discussion

Ensuring the safety of meat is vital for public health, as it plays a key role in promoting health and supporting national economic growth. Consequently, significant measures need to be taken to improve safety precautions at every step of the meat supply process. Abattoirs play a crucial role in serving a significant portion of the population, so they need to prioritize meat hygiene and safety measures in order to minimize risks. As a result, this study aimed to evaluate the bacteriological safety of sheep and goat meats.

Microorganisms often found in slaughterhouses with deteriorated floors and walls, as well as the lack of an automated processing system, pose a high risk of microbial contamination. *E. coli* and *Salmonella* species are specifically included in these microorganisms. The increase in overall colony count and coliform count suggests poor hygiene practices when handling chevon and mutton meat. Moreover, the inadequate management of meat significantly contributes to the transmission of bacteria such as *E. coli* and *Salmonella* species [9].

In the current study, a combined 100 samples of chevon and mutton meats (50 samples each) were gathered from the governmental abattoirs at El-Qalubiya governorate for bacteriological analysis.

The average total colony count (TCC) in chevon meat was determined to be 3.2 x 10^5 ± 0.4 x 10^5*, with a rate of acceptability at 84%. On the other hand, mutton meat showed a TCC of 7.4 x 10^5 ± 0.9 x 10^5* with an acceptability rate of 76% (see Table 2, Figure 1). These results align with those of the study carried out by Al-Asmari et al. (2023) [10].

Sheep samples usually shows lower levels of bacterial contamination, with TCC means varying from log10 3.4 to log10 6.6 CFU/g in slaughterhouses and from log10 3 to log10 6.9 CFU/g in butcher shops. Two sheep samples from slaughterhouses had log10 5 CFU/g counts at critical limits, with one sample exceeding the standard limits at log10 6.6 CFU/g, and 17 samples were within the acceptable range. Five samples from butcher shops

had TCC values of ≥log10 6 CFU/g, exceeding standard limits, while nine samples had log10 5 CFU/g, reaching critical limits, and the rest of the samples met the standard limits. Additionally, other studies [11,12]support these results.

Coliforms and *E. coli* were found in the samples during the evaluation. The purpose of this evaluation was to examine the general cleanliness and safe procedures related to carcass management. This investigation detected coliforms in chevon meat at a mean concentration of 4.5 x 10^2 ± 0.6 x 10^2* with a 70% acceptance rate, and in mutton meat at 15 x 10^2 ± 2.0 x 10^2* with a 56% acceptance rate (see Table 3, Fig. 2). The results align wit with Al-Asmari et al.'s study[10], demonstrating that all 60 samples taken from slaughterhouses for coliform counts in camel, cattle, and sheep meat had average values of 7.5 × 10^2 MPN/g for camel, 8.1 × 10^2 MPN/g for cattle, and 4.5 × 10^2 MPN/g for sheep.

Conducting *E. coli* detection is usually done to evaluate hygiene levels of the tested target. In the present study, *E. coli* was found at a frequency of 18 (36%), with a sample acceptance rate of 64%. Chevon and mutton meat had an incidence of 25 (50%) and a sample acceptability of 50% (refer to Table 4, Fig. 3). These findings are consistent with Al-Asmari et al.[10], in which it was revealed that *E. coli* was the most common species within the isolated *Enterobacteriaceae* genera. In their study, *E. coli* was detected in every camel sample, the majority (85%) of cattle, and a minority (30%) of sheep obtained from slaughterhouses. In contrast, Ahmed et al. [13] found a reduced occurrence of *E. coli* in different meat varieties like beef, pork, chicken, and mutton, with a prevalence of 23.6%.

Inadequate hygiene practices during food processing and storage are the main causes of *Staphylococcus aureus* contamination in food.

Abdelrahman and wells[14] suggested that strains linked to humans and animals could be involved.

S. aureus was found in 22 (44%) of chevon samples and 28 (56%) of mutton samples in this study, as shown in Table 5 and Fig. 4. This is consistent with the findings of Tefera et al. [4], showing a 33.08% prevalence of *S. aureus* in the samples tested. The results also show similarities to prior research conducted by Pu et al. and waters et al. [16,17]

In this study, *Salmonella* was not found in any of the goat and sheep meat samples, in line with Mandour et al ’s discovery [18]who found no *Salmonella* spp. in beef, camel, and sheep meat. Additionally, according to GSO 1016:2014 and WHO[19,20], *Salmonella* spp. should not be present in meat and meat products intended for human consumption. However, this finding goes against the results of Al-Asmari et al. [10]who identified *Salmonella* spp. as the second most frequently detected genus, with 16 (13.3%) cases in camels, 19 (15.8%) in cattle, and 13 (10.8%) in sheep.

Conclusion

The food borne diseases associated with mutton and goat meat is less frequent than other species due to less intensive production. But further studies are needed to detect the pathogens affecting mutton and goat meat and improving hygiene in slaughtering, processing and handling of meat.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt(ethic Approval number: BUFVTM21/11/2023).

TABLE 1. Statistical analysis of Aerobic plate count (APC) (CFU/g log 10) conducted on meat samples.

Samples	Min	Max	Mean ± SE	EOS MPL (CFU/g)	Acceptability	
					No.	%
Chevon meat	$4.5x10^4$	$2.5x10^6$	$3.2x10^5 \pm 0.4x10^{5*}$	$\leq 10^6$	42	84**
Mutton meat	$8.5x10^4$	$6.3x10^6$	$7.4x10^5 \pm 0.9x10^{5*}$	$\leq 10^6$	38	76**
Total					80	80***

The results are expressed as mean ± standard error.
*A superscript asterisk indicates a significant difference among various samples (P≤0.05).
** The incidence is based on the number of each sample (n=50)
*** the total number of examined samples (n=100).
EOS MPL refers to the maximum permissible limit set by the Egyptian Organization for Standardization and Quality (EOS 3602/2013).

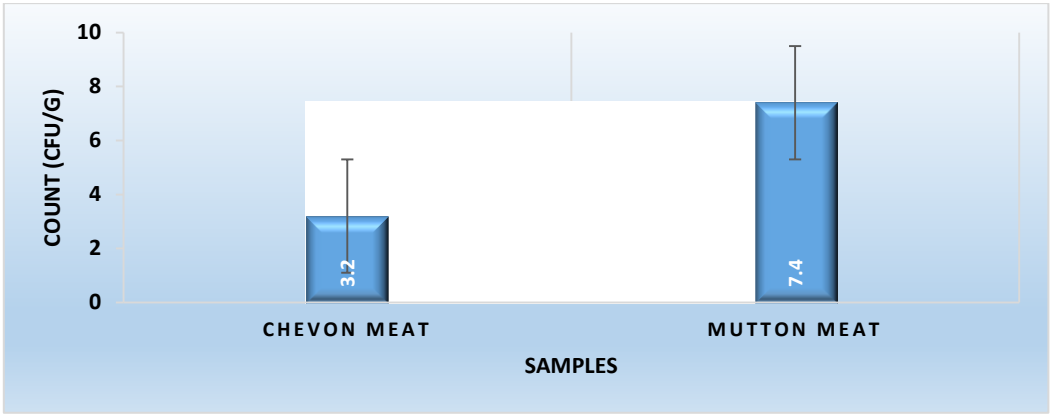

Fig.1. Mean count of APCx10^5 (CFU/g) in the examined meat samples

TABLE 2. Statistical analysis of coliform count (CFU/g log $_{10}$) of meat samples examined.

Samples	Positive samples		Min	Max	Mean ± SE	EOS MPL (CFU/g)	Acceptability	
	No.	%					No.	%
Chevon meat	35	70**	2.0x10	$1.0x10^3$	$4.5x10^2 \pm 0.6x10^{2*}$	$\leq 10^2$	35	70**
Mutton meat	42	84**	7.0x10	$2.2x10^3$	$15x10^2 \pm 2.0x10^{2*}$	$\leq 10^2$	28	56**
Total	77	77***					63	63***

The results are expressed as the mean ± standard error.

*A superscript asterisk indicates a significant difference among various samples (P≤0.05).

**The incidence is based on the number of each sample (n=50)

*** the total number of examined samples (n=100).

EOS MPL refers to the maximum permissible limit set by the Egyptian Organization for Standardization and Quality (EOS 3602/2013).

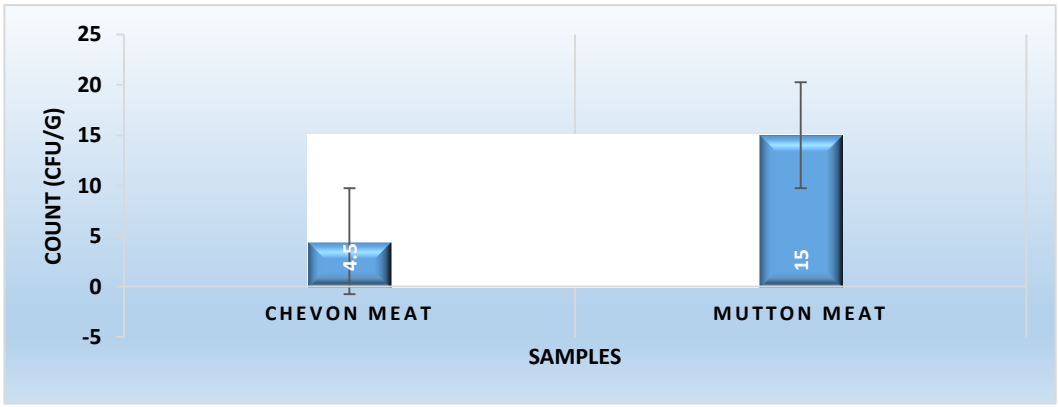

Fig. 2. Mean count of coliform x 10^2 (CFU/g) in the examined meat samples

TABLE 3. Incidence of *E. coli* (fecal type) in the meat samples that

Samples	Positive samples		Min	Max	Mean ± SE	EOS MPL (CFU/g)	Acceptability	
	No.	%					No.	%
Chevon meat	18	36**	<10	$1.0x10^2$	$3x10 \pm 0.2x10^*$	Free	32	64**
Mutton meat	25	50**	<10	$2.5x10^2$	$7x10 \pm 0.8x10^*$	Free	25	50**
Total	43	43***					57	57***

Results were represented as mean ± standard error
* superscript star means significant difference between different samples (P≤0.05)
** Incidence in relation to the number of each sample (n=50)
*** Incidence in relation to the total number of the examined samples (n=100)
EOS MPL: Egyptian Organization for Standardization and Quality maximum permissible limit (EOS 3602/2013).

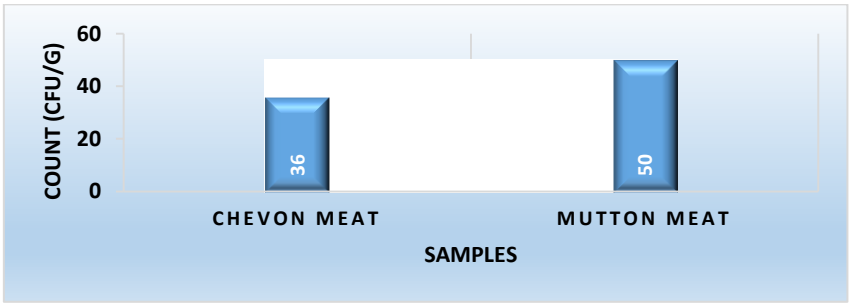

Fig. 3. Incidence of *E. coli* (fecal type) in the examined meat samples.

TABLE 4. Incidence of *Staphylococcus aureus* in the meat samples

Samples	Positive samples		Min	Max	Mean ± SE	EOS MPL (CFU/g)	Acceptability	
	No.	%					No.	%
Chevon meat	22	44**	$2.4x10^2$	$6.0x10^3$	$0.82x10^3 \pm 0.1x10^{3*}$	Free	28	56**
Mutton meat	28	56**	$1.2x10^2$	$8.2x10^3$	$2.1x10^3 \pm 0.4x10^{3*}$	Free	22	44**
Total	50	50***					50	50***

Results were represented as mean ± standard error
* Superscript star means significant difference between different samples (P≤0.05)
** Incidence in relation to the number of each sample (n=100)
*** Incidence in relation to the total number of the examined samples (n=200)
EOS MPL: Egyptian Organization for Standardization and Quality maximum permissible limit (EOS 3602/2013).

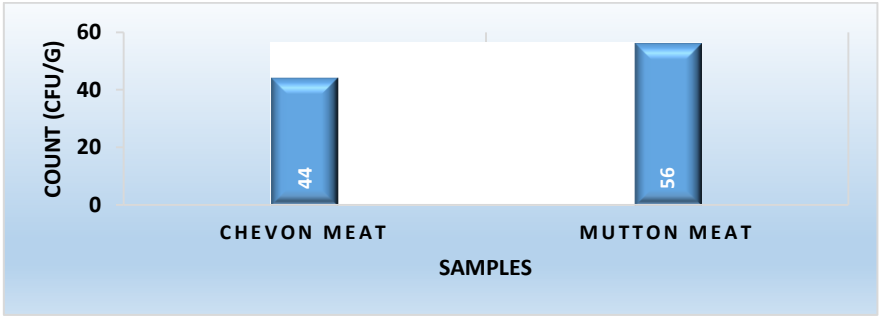

Fig. 4. Incidence of *S. aureus* in the 50 samples that were examined.

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ملامح الجودة البكتريولوجية ونسبة حدوث العزل للمكورات العنقودية الذهبية والسالمونيلا والإشريكية القولونية من لحوم الأغنام والماعز في المسالخ

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الملخص

تعتبر لحوم البقرو الماعز و الضأن من الأطباق التقليدية ويتزايد طلب المستهلكين عليها بسبب قيمتها الغذائية العالية. على الرغم من أن اللحوم الصحية من الأغنام والماعز توفر مكونات مفيدة من الناحية التغذوية، إلا أن الذبح غير القانوني في الحقول المفتوحة، وممارسات الذبح غير الصحية في المسالخ والمعالجة غير العلمية في المؤسسات الغذائية تؤدي لتلوثها بالكائنات الحية الدقيقة المسببة للأمراض..

هدفت هذه الدراسة إلى التعرف على البكتيريا المسببة للأمراض (المكورات العنقودية الذهبية والسالمونيلا والإشريكية القولونية) في لحوم الأغنام والماعز ولتقييم الجودة الميكروبيولوجية لهذه المنتجات، تم تحديد عدد المستعمرات الكلي وعدد القولونيات وعزل المكورات السبحية والإشريكية القولونية لذلك تم جمع 100 عينة من لحوم الأغنام والماعز ، وكانت القيم المتوسطة التي تم الحصول عليها للعد الكلي للمستعمرات في لحم الماعز والضان على التوالي هو:

7.4x105 ± 0.9x105 and 3.2x105 ± 0.4x105

والعد الكلي للقولونيات في لحم الماعز والضأن على التوالي كالتالي :

x102 ± 0.6x102*and 15x102 ± 2.0x102*4.5

وتم عزل المكورات السبحية في لحوم الماعز والضأن بنسبة 36% و50% على التوالي كما تم عزل الإشريكية القولونية بنسبة حدوث 44% و56% للحوم الماعز والضأن على التوالي. لكن لم يتم اكتشاف السالمونيلا في لحوم الماعزولحوم الضأن.

ولتحسين الجودة والحد من التلوث، يجب على الحكومة معالجة مسألة توفير المياه الكافية والآمنة للأنشطة وتطبيق ممارسات صحية في جميع المسالخ

الكلمات الدالة: البكتيريا، لحم الضأن، المسالخ، العدد الكلي للمستعمرات، الصحة العامة.