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Microbiological Evaluation of Fresh Retail Rabbit Meat Cuts from Zagazig City, Egypt

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Abstract

The present study was conducted to evaluate the microbiological status of rabbit carcasses marketed in Zagazig City, Sharkia province, Egypt. Eighty random samples of fresh rabbit meat cuts representing shoulder, loin, ribs, and thigh regions (20 of each) were obtained from different rabbit markets in Zagazig City during 2022. Aerobic plate count (APC), *Enterobacteriaceae*, total mould and yeast counts were determined in the collected samples, as well as the isolation and identification of *E. coli* and *Salmonella* spp. The results showed that the mean values of APC and *Enterobacteriaceae* were $1.1x10^6$ and $4.7x10^4$ CFU/g for shoulders, $9.6x10^5$ and $5.7x10^4$ CFU/g for ribs, $1.0x10^6$ and $5.1x10^4$ CFU/g for loins, $1.2x10^6$ and $6.0x10^4$ CFU/g for thigh samples, respectively. Moreover, the total mould and yeast count ranged from $2.6x10^4$ to $1.9x10^5$ with mean values of $9.7x10^4 \pm 1.1x10^4$, $8.2x10^4 \pm 9.9x10^3$, $9.5x10^4 \pm 1.4x10^4$ and $9.8x10^4 \pm 8.5x10^3$ CFU/g in the of examined rabbit meat samples from shoulder, ribs, loin, and thigh regions, respectively. In terms of food poisoning bacteria, 6 (30 %), 7 (35 %), 6 (30 %) and 4 (20 %) out of 20 rabbit cuts tested positive for *Salmonella*e in the shoulder, ribs loin, and thigh regions, respectively. However, *E. coli* was found in 18 (90%), 16 (80%), 15 (75%) and 19 (95%) of the examined rabbit meat samples, respectively. The obtained results suggested that fresh rabbit meat cuts may carry numerus microorganisms from different sources, emphasizing the importance of stringent sanitary precautions during the slaughtering, handling and cooking procedures.

KEYWORDS
Bacteriological evaluation, *E. coli*, Mould count, *Salmonella*, Rabbit meat.

INTRODUCTION

The consumption of rabbit meat primarily high in Mediterranean and middle eastern countries (Dalle Zotte, 2002; Morshdy *et al*., 2021). Rabbits are commonly reared in tiny colonies in backyards by Egyptian housewives to supplement their family's income but in recent decades, rabbit breeding has gained prominence as a source of meat production. Because of their short gestation period, short life cycle, and high feed conversion rate, rabbits are regarded as good meat-producing animal. It is characterized by lower production cost and breeding space (Lebas *et al*., 1997; Nasr *et al*., 2017). The consumption of rabbit meat would benefit human health since it includes lean meat with high quality protein, as well as a high level of unsaturated fat, phosphorous, vitamin B and a low content of cholesterol and sodium (Gergis, 2004; Hernández and Gondert, 2006).

Microbiological contamination is one of the most serious issues affecting meat quality (including rabbit) and public health. Numerous factors, including storage conditions and slaughterhouse cleanliness, may influence the microbiological quality of rabbit meat (Koutsoumanis and Sofos, 2004; Plym and Wierup, 2006). Contamination of muscular tissue during slaughter may be induced by a wide range of microorganisms, notably after skinning and evisceration, resulting in a rise in the microbial count of gastrointestinal origin (Nakyinsige *et al*., 2015). Nutrition also has a substantial impact on the level of microbes since specific feed elements can slow the growth rate of microorganisms (Cwiková and Pytel, 2017; Miteva *et al*., 2020).

Rabbit meat may be contaminated with several forms of food spoilage and food poisoning microorganisms that get access to carcasses from living animals during slaughter, evisceration, and subsequent processing (Rouger *et al*., 2017). The degree of carcass contamination depends on received rabbit cleanliness, slaughter facility design, slaughter technology, sanitation and disinfection system, and hygiene (Zweifel *et al*., 2014). Microorganisms present on carcass surfaces may have originated from the rabbit itself, particularly from the fur or skin, the feet, the digestive tract and fecal contamination (Hulot and Ouhayoun, 1999). Furthermore, carcasses can be contaminated during the whole process from transportation, animal slaughtering till consumption, resulting in an elevated initial microbial count and subsequently lower the keeping quality of meat (Badr, 2004).

In Egypt, rabbit carcasses are sold chilled and the assessment of microbiological quality of rabbit carcasses has not been studied as thoroughly as that of other meats, thus it is critical for public health to determine the quantity and types of microor-

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ganisms contaminating the animal carcass to judge the efficacy of sanitary measures during processing. Therefore, the current study was conducted to evaluate the microbiological profile of rabbit carcasses marketed in Zagazig city by determining of aerobic plate count (APC), *Enterobacteriaceae*, and total mould and yeast count, as well as isolating and identifying of *E. coli* and *Salmonella* species.

MATERIALS AND METHODS

Collection of samples

From January to June 2020, twenty healthy domestic rabbits (10 weeks of age, around 2 kg live weight) were obtained from different rabbit markets in Zagazig City, Sharkia province, Egypt and slaughtered manually under hygienic conditions. The samples were transferred in an ice box as quickly as possible to the lab of Meat Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt. A total of 80 random samples of fresh rabbit meat cuts from shoulder, loin, ribs, and thigh regions (20 of each) were collected for further microbiological evaluation.

Microbiological analyses

Rabbit meat samples and serial dilutions were prepared in accordance with ISO 6887-2 (2003). In brief, twenty-five grams of each sample were aseptically homogenised in 225 ml of 0.1 % buffered peptone water (BPW HIMEDIA, M614) in a stomacher (Colworth, 400) for 2 min at room temperature (25ºC) and then left to stand for 5 min to produce a homogenate that represents the dilution of (10-1). One ml of the homogenate was placed into a sterile test tube containing 9 ml of 0.1% BPW, and tenfold serial dilutions were made. According to ISO 4833-1 (2013), APC was performed using plate count agar (PCA HIMEDIA M091) and incubated for 48 h at 37°C. Enumeration of *Enterobacteriaceae* was carried out using violet red bile glucose ager (VRBGA HIMEDIA M581) in accordance with ISO 21528-2 (2004) and incubated at 37°C for 48 h. Total mould and yeast count was carried out using Sabouraud Dextrose agar medium plates (SDA HIMEDIA M063) according to ISO 21527-1 (2008) and then incubated for 48 h at 25±0.5˚C.

However, *E. coli* isolation was done using EMB agar (HIMEDIA M317). The inoculated plates were incubated at 37°C for 24 h. Colonies with a metallic sheen were isolated for further identification according to the method reported by AOAC (1995). Suspected isolates of *E. coli* were identified based on morphological characters either microscopical, biochemical, and serological examinations. Pre-enrichment of *Salmonella* was performed by incubating the original suspension (10-1) at 37°C for 24 h. After that, 1 ml of pre-enriched peptone water was enriched in Rappaport Vassiliadis broth (HIMEDIA M880) for 24 h at 41.5±0.5 °C. Following that, a loopful of enriched broth was streaked on XLD agar (HIMEDIA M031) and incubated for 24 h at 37°C, and red colonies with a black center were subjected to further identification using biochemical, and serological tests (ISO 6579-1, 2017).

Statistical analysis

All values of microbiological analysis are reported as means ± standard error (SE). One-way analysis of variance (ANOVA) was done by using the statistical package for social sciences (SPSS-14; Chicago, IL, USA) with post hoc tukey-kramer honestly correction to estimate the differences in microbial counts. P-values less than 0.05 were considered statistically significant.

RESULTS

The results presented in Table 1 showed the APC in different cuts of the examined rabbit meat samples. The highest APC was found in thigh samples ($1.2x10^6$) and the lowest count was found in ribs samples ($9.6x10^5$). While the highest *Enterobacteriaceae* count was reported in thigh samples ($6.0x10^4$) and the lowest count was reported in shoulder samples ($4.7x10^4$) (Table 2).

The results in Tables 3 demonstrated that the total mould and yeast count in different cuts of the examined rabbit meat samples. The highest count of mould and yeast was found in thigh samples ($9.8x10^4$), and the lowest count ($8.2x10^4$) was observed in ribs samples. As regard to the incidence of isolated pathogens from examined rabbit carcasses, data in Table 4 showed that 19

Table 1. Statistical analytical results of APC at 37˚C in fresh rabbit meat cuts of examined rabbit carcasses (CFU/g).

Rabbit meat cuts	Positive Samples*		Minimum	Maximum	Mean	SE
	NO	%				
Shoulder	20	100	$4.4x10^5$	$1.8x10^6$	$1.1x10^{6\,A}$	$1.1x10^5$
Ribs	20	100	$4.5x10^5$	$1.7x10^6$	$9.6x10^{5\,A}$	$9.0x10^4$
Loin	20	100	$2.9x10^5$	$1.8x10^6$	$1.0x10^{6\,A}$	$1.2x10^5$
Thigh	20	100	$5.8x10^5$	$2.4x10^6$	$1.2x10^{6\,A}$	$1.4x10^5$

* Number of examined samples (n=20). SE: Standard error of mean.
Means within the same column carrying different superscripts are significantly different ($p< 0.05$).

Table 2. Statistical analytical results of *Enterobacteriaceae* in fresh rabbit meat cuts of examined rabbit carcasses (CFU/g).

Rabbit meat cuts	Positive Samples*		Minimum	Maximum	Mean	SE
	No	%				
Shoulder	20	100	$5.0x10^3$	$9.2x10^4$	$4.7x10^{4\,A}$	$7.1x10^3$
Ribs	20	100	$1.3x10^4$	$9.0x10^4$	$5.7x10^{4\,A}$	$5.9x10^3$
Loin	18	90	$1.1x10^4$	$9.5x10^4$	$5.1x10^{4\,A}$	$6.5x10^3$
Thigh	20	100	$3.0x10^3$	$1.3x10^5$	$6.0x10^{4\,A}$	$9.2x10^3$

* Number of examined samples (n=20). SE: Standard error of mean.
Means within the same column carrying different superscripts are significantly different ($p< 0.05$).

Table 3. Statistical analytical results of Mould and Yeast in fresh rabbit meat cuts of examined rabbit carcasses (CFU/g).

Rabbit meat cuts	Positive Samples*		Minimum	Maximum	Mean	SE
	No	%				
Shoulder	20	100	$2.6x10^4$	$1.7x10^5$	$9.7x10^{4\,A}$	$1.1x10^4$
Ribs	20	100	$2.9x10^4$	$1.8x10^5$	$8.2x10^{4\,A}$	$9.9x10^3$
Loin	20	100	$3.2x10^4$	$1.9x10^5$	$9.5x10^{4\,A}$	$1.4x10^4$
Thigh	20	100	$3.5x10^4$	$1.5x10^5$	$9.8x10^{4\,A}$	$8.5x10^3$

* Number of examined samples (n=20). SE: Standard error of mean.
Means within the same column carrying different superscripts are significantly different ($p< 0.05$).

Table 4. Incidence of *Salmonella* and *E. coli* isolated from fresh rabbit meat cuts of examined rabbit carcasses (CFU/g).

Rabbit meat cuts	*Salmonella* (n=20)		*E. coli* (n=20)	
	No	%	No	%
Shoulder	6	30	18	90
Ribs	7	35	16	80
Loin	6	30	15	75
Thigh	4	20	19	95

(95 %) 18 (90 %), 16 (80 %), 15 (75 %) contained *E. coli* in thigh, shoulder, ribs, loin regions, respectively. Meanwhile, 6 (30%), 7 (35%), 6 (30%) and 4 (20%) out of 20 rabbit meat samples contained *Salmonella* spp. in shoulder, ribs, loin, and thigh regions, respectively.

DISCUSSION

Microbial contamination limits the safety and shelf life of meat. The slaughtering procedure may cause extensive contamination of muscle tissue with a wide variety of microorganisms. Concerning levels of aerobic plant counts in different examined rabbit meat samples, the results in Table 1, revealed that the mean values of APC varied from 9.6×10^5 to 1.2×10^6 CFU/g. Ali *et al*. (2016) had almost identical results in Egypt, with mean APC of 1.5×10^6, 1.2×10^6, 1.1×10^6 and 5.7×10^5 CFU/g in shoulder, ribs, loin, and thigh regions, respectively. On the other hand, higher APC values of 2.7×10^6 and 4.2×10^6 in the fore and hind quarters, respectively were reported in Egypt (Abd-Allah and Abd-Elaziz, 2018), and 3.9×10^6 in Spain by Rodríguez-Calleja *et al*. (2004). Furthermore, Comin *et al*. (2008) assessed the microbial quality of rabbit meat from 4 abattoirs and discovered that most carcasses had APC of less than 10^5 CFU/g. Similarly, Nakyinsige *et al*. (2014) and Swami *et al*. (2014) found that the mean value for APC in rabbit meat samples was lower than 10^5 CFU/g. Lower results noticed by Kone *et al*. (2016) who found that the mean total aerobic mesophilic count was 0.14×10^3 CFU/g in thighs of rabbit carcasses.

In terms of permissible limits, all 20 analyzed rabbit meat samples from the shoulder, ribs, loin, and thigh regions surpassed the maximum allowable limits established by the Egyptian Standards (ES, 2005) for aerobic plate count in chicken and rabbits (10^5 CFU/g flesh). The high levels of APC discovered in this study might be attributed to poor hygiene practices observed during slaughtering, processing, and handling of carcasses at retail marketplaces (Morshdy *et al*., 2022). According to Gill (2005), water, air, equipment, and workers are all sources of microbial contamination of meat. Rabbit carcasses have a low microbial count when slaughtered under sanitary conditions, however the number of microorganisms increased dramatically during handling and processing. These findings support the conclusion of Khalafalla (1993) that slaughtered rabbit carcasses from the grocery shops had greater bacterial counts than home slaughtered ones. Based on the data presented, it is possible to conclude that unsanitary handling of rabbit carcasses during slaughtering, evisceration, dressing, storage, and marketing is the primary source of a greater overall bacterial population.

The presence of *Enterobacteriaceae* in meat is an indicator of fecal contamination and inadequate hygiene during processing and storage (Steinhauser, 1995; Görner and Valík, 2004). In this investigation, the mean values of *Enterobacteriaceae* counts in the shoulder, ribs, loin, and thigh regions of analyzed rabbit carcasses were 4.7×10^4, 5.7×10^4, 5.1×10^4 and 6.0×10^4 CFU/g, with no significant difference ($P>0.05$) between them. Similarly, Badr (2004) discovered *Enterobacteriaceae* count of 6.2×10^4 CFU/g in raw rabbit meat samples. Abd-Allah and Abd-Elaziz (2018) observed higher *Enterobacteriaceae* count with mean values of 3.56×10^5 and 9.53×10^5 in the fore and hind quarters, respectively in Egypt. However, Cwiková and Pytel (2017) recorded lower *Enterobacteriaceae* counts of 8.1×10^2, 0.30×10^2, 0.23×10^2 CFU/g for butcher shops, domestic slaughtered carcasses, and frozen rabbit carcasses, respectively. In addition, Comin *et al*. (2008) discovered that the mean *Enterobacteriaceae* count was less than 103 CFU/g in the examined rabbit meat samples, and Khalafalla (1993) reported *Enterobacteriaceae* count of 6×10^2 CFU/g in the freshly slaughtered rabbits.

Mould and yeast can survive in surrounding environment even under very unfavorable conditions, they are among the most prevalent causes of food spoilage (Vlková *et al*., 2009). There was no significant difference between the mean values of mold and yeast counts of the examined rabbit meat cuts ($P > 0.05$). The variation in counts might be due to inadequate handling and hygienic standards during processing and storage. The results in the current study were nearly similar to those reported by Badr (2004), who found that the mean Mould and yeast value in raw rabbit meat samples was 6.2×10^4 CFU/g. However, Abd-Allah and Abd-Elaziz (2018) observed higher Mould ($1.29x10^5$) and Yeast ($4.60x10^6$) counts in examined rabbit carcasses from Egypt. Lower results were obtained by Cwiková and Pytel (2017) who recorded Mould and yeast count of $9.33x10^2$ CFU/g in rabbit meat samples from butcher shops. Additionally, Rodríguez - Calleja *et al*. (2005) reported lower mould and yeast counts of $2.9x10^3$ CFU/g in chilled rabbit meat, while Chabela *et al*. (1999) discovered that the mean mould and yeast count was $5.75x10^3$ CFU/g in the examined rabbit meat samples.

E. coli is an emerging cause of food poisoning and currently is recognized as a significant human pathogen. The isolation of *E. coli* from food samples is critical for public health since consuming of contaminated food can cause infantile diarrhea and gastroenteritis in adults (Sapna-Kumari *et al*., 2001). In this study,

E. coli was found in varied percentages in different cuts of examined rabbit meat samples. The obtained incidences of *E. coli* indicate that positive samples are not acceptable according to the rabbit meat guidelines established by Egyptian standards (ES, 2005), which specify that rabbit meat must be free from *E. coli*. similar results were reported by Ali *et al*. (2016) who found *E. coli* in 11 (44 %), 15 (60 %), 8 (32 %) and 10 (40 %) out of 25 rabbit samples from the shoulder, ribs, loin, and thigh regions, respectively. However, Khalafalla (1993), Abou-Taleb (1995), and Comin *et al*. (2008) all found lower incidences of *E. coli*. Likewise, Rodríguez -Calleja *et al.* (2004), Nakyinsige *et al.* (2014), and Swami *et al*. (2014) found lower *E. coli* counts that was less than10^3 CFU/g. In contrary, Rodríguez-Calleja *et al*. (2006) could not detect *E. coli* in any of the examined rabbit carcasses, but Abd-Allah and Abd-Elaziz (2018) could isolate *E. coli* from only one hind quarter sample.

Salmonella species remain a major cause of foodborne illness in humans worldwide. According to the guidelines established by the Egyptian standards (ES, 2005), the presence of *Salmonella* species in rabbit meat is not allowed. In the present study, *Salmonella*e were detected in rabbit meat cuts with a prevalence ranged from 20% to 35%, which is coincided with the findings of Abd-Allah and Abd-Elaziz (2018), who discovered *Salmonella* spp. in rabbit carcasses at an incidence of 23.3% and 20% in fore and hind quarters, respectively. However, lower rates of *Salmonella* spp. (8 to 12%), and 5% (*Salmonella* Typhimurium) were detected in retail rabbit carcasses from Egypt by Ali *et al*. (2016) and Khalafalla (1993), respectively. Also, Comin *et al*. (2008) identified *Salmonella* spp. (0.5 to 11.9%) in rabbit meat samples. On the other hand, Rodríguez-Calleja *et al*. (2006), Kpodékon *et al*. (2008) and Swami *et al*. (2015) found no *Salmonella*e in any of the examined rabbit carcasses. Likewise, Morshdy *et al*. (2022) were also unable to isolate *Salmonella* from slaughtered carcasses surfaces, but they found *Salmonella* spp. with varying percentages (25% to 30%) in the investigated abattoir samples (Wall, floor, and abattoir effluents). Additionally, our findings were comparable with those of Molla *et al*. (2003) who isolated *Salmonella*e from slaughtered animals and slaughterhouse personnel with a percentage of 4.2% and 6.0%, respectively. They attributed the contamination of slaughtered rabbit carcasses by *Salmonella* spp. to inadequate personnel hygiene and/or unsanitary practices during carcass processing.

CONCLUSION

According to the findings of this study, the major reason of the high total microbial populations is improper handling of rabbits during slaughtering, dressing, evisceration, storage, and marketing. The existence of substantial amounts of *Enterobacteriaceae* and *E. coli* in investigated rabbit meat cuts indicates fecal contamination of carcasses due to either poor evisceration or an unsanitary environment during market preparation. The presence of *Salmonella* spp. and *E. coli* in investigated rabbit cuts might be associated with insufficient evisceration and a lack of staff cleanliness, posing public health risks to consumers. Thus, further future research on the application of natural decontaminants such as essential oils or others antibacterial agents is required to minimize such foodborne pathogens in rabbit meats.

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CONFLICT OF INTEREST

The authors declare they don't have conflict of interest.

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