Slov Vet Res 2023; 60 (Suppl 25): 439–45
DOI 10.26873/SVR-1674-2023

Original Research Article
Veterinary Medicine and The One Health Concept

RABBIT MEAT AS A POTENTIAL SOURCE OF *Staphylococcus aureus* AND *Salmonella spp.*

Alaa Eldin M. A. Morshdy[1], Abdullah F. Alsayeqh[2], Mohammed F. Aljasir[2,3], Hassan Mohieldeen[4], Shymaa Gamal El-Abody[4]*, Mohamed Elsayed Mohamed[5], Wageh Sobhy Darwish[1]

[1]Food Control Department, [5]Zoonoses Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, [2]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qas-sim University, Buraidah 51452, Qassim, [3]Ministry of Environment, Water, and Agriculture, Buuraydah, 52355, Kingdom of Saudi Arabia, [4]Animal Health Research Institute (AHRI), Zagazig Province Laboratory, Zagazig, Egypt

*Corresponding author, E-mail: shym.gamal.com@gmail.com

Abstract: Rabbit meat and offal are considered valuable sources of high biological value animal protein. Rabbit meat is rich in essential amino acids, low in cholesterol, and contains considerable amounts of trace elements such as calcium, magnesium, and zinc. However, the potential contribution of rabbit meat and offal in the transmission of foodborne pathogens such as *Staphylococcus aureus* (*S. aureus*) and *Salmonella* spp. is neglected. Therefore, this study was conducted first to investigate the prevalence rates of *S. aureus* and *Salmonella* spp. in the retailed rabbit meat at Sharkia Governorate, Egypt. Second, serological identification of the isolated bacteria was followed. Detection of *S. aureus*-enterotoxins and Salmonella-virulence-associated genes was also done using PCR. Antimicrobial susceptibility testing of the recovered bacterial isolates was additionally examined. The acquired results showed that 17% of the investigated samples of rabbit meat contained *S. aureus*. Where *S. aureus* was isolated from the investigated rabbit's breast, thigh, liver, and kidney at 20%, 24%, 12%, and 12% of each, respectively. *Salmonella* spp. was isolated at 13%. *Salmonella* spp. was isolated from the investigated rabbit thigh, liver, breast, and kidney at 16%, 16%, 12%, and 8%, respectively. Four different strains of *Salmonella* spp namely, *S.* Typhimurium, *S.* Kentucky, *S.* Virchow, and *S.* Infantis were recovered in the current study. The recovered *S. aureus* and *Salmonella* spp. harbored enterotoxins and virulence attributes with multidrug resistance. Therefore, strict hygienic measures should be followed during the processing and handling of rabbit meat and offal.

Key words: rabbit meat; offal; *Staphylococcus aureus*; *Salmonella spp.*; Egypt

Introduction

Most Mediterranean countries produce a significant amount of rabbit meat, and many others have a thriving rabbit meat business. Good muscle-to-bone ratios, quick growth rates, high feed efficiency, early marketing age, and a low land area demand are all characteristics of rabbits (1). This suggests that the rabbit has a real future in large-scale livestock production. Studies have shown that rabbit meat is a great food for humans to consume because it is high in protein and low in sodium, fat, and cholesterol (2).

Rabbits have historically been bred in Egypt in small colonies in backyards to augment the family income, but in more recent years, the practice has emerged as a distinctive source of meat. Due to their rapid feed conversion rates, brief life cycles, and short gestation periods, rabbits are regarded as the best animals for producing meat. Low manufacturing costs and a

Received: 1 January 2023
Accepted for publication: 7 January 2022

constrained breeding area set it apart. Similar to how rabbits make excellent research animals for experiments and gain economically. Because it contains lean meat with high biological value, as well as high quantities of unsaturated fat and low cholesterol content, consuming rabbit meat would be beneficial to human health (3). Rabbit meat is rich in amino acids content as recently reported in the New Zealand White rabbit in the following order: lysine > isoleucine > valine > methionine > threonine > histidine > phenylalanine > leucine, such amino acids in California rabbits were in the following order: lysine > leucine > valine > threonine > isoleucine > phenylalanine > histidine > methionine (4). Even though rabbit meat has a high nutritional value, it is also linked to the spread of various foodborne infections, which can have serious negative health effects (5, 6).

Microbes can contaminate rabbit meat at any point during the production process, including the slaughtering procedure, dressing, evisceration, and storage. As a result, tainted rabbit meat has been linked to the spread of microorganisms that cause food poisoning, such as *Salmonella* spp. and *Staphylococcus aureus* (*S. aureus*) (7).

Consuming foods contaminated with *S. aureus*-enterotoxins is the primary cause of foodborne poisoning cases. These enterotoxins are well-known for their rapid onset (between one- and six hours), nausea, vomiting, stomach cramps, and diarrhea (8). *S. aureus* was isolated from both rabbit meat and offal sold in Egypt (9, 10) and Spain (6).

Salmonella spp. causes a serious foodborne illness brought on by eating items tainted with these bacteria. The illness is distinguished by an incubation period of 12 to 36 hours following the consumption of infected foods, as well as by nausea, vomiting, fever, severe diarrhea, abdominal cramps, and malaise (11).

Antimicrobials are widely used in the meat industry as preservatives, as feed additives to increase feed conversion ratios, and in rabbit farms for the prevention and management of bacterial infections (12). But uncontrolled use of such antibiotics had resulted in the emergence of drug-resistant foodborne bacteria (13).

In the sight of the previous facts, the current study was undertaken to investigate the prevalence of *S. aureus* and *Salmonella* spp. in rabbit meat retailed in Egypt. Virulence attributes and antimicrobial resistances among the recovered isolates were additionally screened.

Material and methods

Sampling and samples preparation:

A hundred rabbit meat parts including 25 each of breast, thigh, liver, and kidney were collected from rabbit butchery shops at Sharkia Governorate, Egypt. The samples were transferred and cooled without delay to Animal Health Research Institute, Zagazig branch for bacteriological examination.

The procedure outlined by APHA (14) was adhered to while preparing samples for bacteriological analysis. In brief, 90 mL of sterile peptone water 1% (Oxoid CM9, UK) were blended for 3 min at 3000 rpm with 10 g of each sample after being aseptically homogenized. Each sample's serial dilution was also prepared.

Count, isolation, and identification of S. aureus:

S. aureus was isolated and identified using APHA's (14) technique. In short, 0.1 mL of each generated serial dilution was added using the surface spreading method to a Baird Parker agar (Oxoid, UK) plate that had been supplemented with an egg yolk emulsion. 48 hours were spent with the plates inverted in the incubator at 37°C. The *S. aureus* colonies were spherical, shiny, and black, with a thin white edge, and they were encircled by a transparent zone that extended into the opaque medium. Total *S. aureus* (TSC) was counted according to the following equation:

TSC/g = average No. of colonies × reciprocal of the dilution

Counted colonies expressed as log 10 cfu/g.

Five potential *S. aureus* colonies were selected from each cultivated plate and purified on nutrient agar slopes. Utilizing morphological, biochemical, and serological traits, *S. aureus* colonies were identified.

Isolation and identification of Salmonella spp.:

During the *Salmonella* spp. isolation and identification processes, the ISO 6579 (15) method was used. Pre-enrichment was carried out by

incubating ten mL of each of the previously produced meat homogenates for 18 ± 2 hours at 37 °C. As a selective enrichment medium, Rappaport Vassiliadis soy broth (Oxoid, UK) was employed. For 24 ± 2 hours, enriched cultures were incubated at 41.5°C. A loopful of that enriched culture was inoculated using the surface streaking technique on the xylose lysine desoxycholate (XLD) agar (Oxoid, UK) plate, and it was then incubated at 37°C for 24 ± 2 hours. Purified and sub-cultured suspected colonies (red with or without black centers) were placed on nutrient agar slopes, and they were then incubated at 37°C for 24 hours. The purified colonies underwent morphological, biochemical, serological, and genetic analyses.

Molecular identification of Staphylococcal enterotoxins and Salmonella-associated virulence genes:

According to the manufacturer's recommendations, genomic DNA was extracted from the recovered bacterial isolates. The oligonucleotide pairs for the enterotoxin genes (*sea,* and *seb*) specific to *S. aureus* and the virulence-associated genes (*hilA, invA*, and *stn*) specific to *Salmonella* spp. were purchased from Metabion International, Gmbh, Germany, and were prepared according to previous reports (16-18). PCR amplifications were carried out on a Thermal Cycler (Master cycler, Eppendorf, Germany) following the previously described method (17). The initial denaturation at 95°C for two minutes was followed by 35 amplification cycles, each of which included a denaturation step at 95°C for 15 seconds, an annealing step at 50°C or 60°C for targets of *S. aureus* or *Salmonella* spp., and an extension step at 72°C for one minute. Following a final extension for seven minutes at 72°C, a holding period at 4°C was followed. The resulting PCR products were electrophoresed on a 1.5% agarose gel in 1x Tris Borate EDTA (TBE) buffer and stained with ethidium bromide (AppliChem, GmbH, Germany). With the use of a UV transilluminator, PCR products were visualized.

Antimicrobial sensitivity testing:

Antimicrobial susceptibility of the recovered isolates to the most commonly used antimicrobials in the rabbit farms was performed following the Clinical and Laboratory Standards Institute's standards (19).

Statistical analysis:

All microbial counts were converted into log 10 cfu/g for statistical analysis. The data were presented as means ± SE. One-way ANOVA was used to evaluate the data, and Tukey's multiple comparison tests were performed (SPSS Inc., Chicago, Illinois, USA), with a P-value of 0.05.

Results

The obtained results indicated the isolation of *S. aureus* from the examined rabbit meat samples at 17% (17 out of 100 samples). Where *S. aureus* was isolated at 20%, 24%, 12%, and 12% from the examined rabbit breast, thigh, liver, and kidney, respectively. The average *S. aureus* counts (log 10 cfu/g) in the examined samples were 2.76 ± 0.09, 2.64 ± 0.10, 2.54 ± 0.11, and 2.39 ± 0.06 in the examined breast, kidney, liver, and thigh respectively (Table 1). The overall isolation rate of *Salmonella* spp. was 13% (13 out of 100 samples). Where *Salmonella* spp. was isolated at 16%, 16%, 12%, and 8% from the examined rabbit thigh, liver, breast, and kidney, respectively (Table 2). Four *Salmonella* species were recovered from the examined rabbit samples namely, *S.* Typhimurium, *S.* Infantis, S. Virchow, and *S.* Kentucky. *S.* Typhimurium was isolated from the liver and thigh at 50% and 25%, respectively. *S.* Kentucky was isolated from kidney, liver, and thigh samples at 50%, 25%, and 25%, respectively. S. Virchow was isolated from the breast and thigh samples at 66.66%, and 25%, respectively. *S.* Infantis was isolated from kidney, breast, liver, and thigh samples at 50%, 33.33%, 25%, and 25%, respectively. (Table 3).

PCR testing of randomly selected colonies revealed the detection of *S. aureus* enterotoxins *sea, seb*, and salmonella virulence attributes at variable rates (data are not shown).

Antimicrobial susceptibility testing of the recovered *S. aureus* isolates towards the most used antimicrobials for treatment of *S. aureus* infections revealed resistance of the recovered isolates to cefotaxime (100%), chloramphenicol (88.2%), erythromycin (82.4%), norfloxacin (29.4%), cipro-

floxacin (17.6%), and doxycycline (5.9%) (Table 4). While the recovered Salmonella isolates showed resistance to ampicillin (76.9%), tetracycline (76.9%), azithromycin (69.2%), sulfamethoxazole/ trimethoprim (61.5%), ciprofloxacin (38.5%), chloramphenicol (38.5%), gentamicin (7.7%), and Meropenem (7.7%) (Table 5).

Table 1: Prevalence and total *S. aureus* count (log 10 cfu/g) in the examined rabbit samples

	Positive samples		Min.	Max.	Mean ± SE
	No.	%			
Breast	5	20	2.00	3.40	± 0.09[a]2.76
Thigh	6	24	1.70	2.90	2.39 ± 0.06[a]
Liver	3	12	1.30	3.48	2.54 ± 0.11[a]
Kidney	3	12	2.00	3.60	2.64 ± 0.10[a]

Values within the same column carrying the same superscript letter are not significantly different at P< 0.05.

Table 2: Prevalence of *Salmonella* spp. in the examined rabbit samples

Sample	Positive samples	
	No.	%
Breast	3	12
Thigh	4	16
Liver	4	16
Kidney	2	8

Table 3: Serological identification of the recovered *Salmonella* spp.

***Salmonella* serotypes**	**Breast (n=3)**		**Thigh (n=4)**		**Liver (n= 4)**		**Kidney (n= 2)**		**Antigenic Structure**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**O**	**H**
***S.* Typhimurium**	-	-	1	25	2	50	-	-	1,4,5,12	i: 1,2
***S.* Kentucky**	-	-	1	25	1	25	1	50	8,20	i: Z6
***S.* Virchow**	2	66.66	1	25	-	-	-	-	6,7,14	r: 1,2
***S.* Infantis**	1	33.33	1	25	1	25	1	50	6,7,14	r: 1,5
Total	2	100	4	100	2	100	2	100		

Table 4: Antimicrobial susceptibility of the recovered *S. aureus* isolates (N=17

Antimicrobial agent	**S**		**I**		**R**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Cefotaxime (CXT)	-	-	-	-	17	100
Chloramphenicol (C)	2	11.8	-	-	15	88.2
Ciprofloxacin (Cip)	11	64.7	3	17.6	3	17.6
Doxycycline (DO)	9	52.9	7	41.2	1	5.9
Erythromycin(E)	3	17.6	-	-	14	82.4
Gatifloxacin (GAT)	16	94.1	1	5.9	-	-
Nitrofurantion (F)	12	70.6	5	29.4	-	-
Norfloxacin (NOR)	12	70.6	-	-	5	29.4
Ofloxacin (OFX)	13	76.5	4	23.5	-	-

Table 5: Antimicrobial susceptibility of the recovered *Salmonella* spp. isolates (N=13)

Antimicrobial agent	S		I		R	
	No.	%	No.	%	No.	%
Amoxicillin (AMC)	11	84.6	1	7.7	1	7.7
Ampicillin (AM)	3	23.1	-	-	10	76.9
Azithromycin (AZM)	4	30.8	-	-	9	69.2
Cefoxitine (FOX)	12	92.4	1	7.7	-	-
Chloramphenicol (C)	7	53.8	1	7.7	5	38.5
Ciprofloxacin (Cip)	5	38.5	3	23.1	5	38.5
Gentamycin (CN)	8	61.6	4	30.8	1	7.7
Meropenem (MEM)	12	92.3	-	-	1	7.7
Sulfamethoxazole trimethoprim (SXT)	5	38.5	-	-	8	61.5
Tetracycline (TE)	1	7.7	2	15.38	10	76.9

Discussion

There is no doubt that each year, foodborne pathogenic bacteria lead to significant human and financial costs due to the numerous illnesses and fatalities they cause. The microbiological quality of red meat, poultry, and their products has been the subject of numerous research in Egypt (8, 11, 20, 21). However, there is some question as to the microbiological quality of rabbit meat, which, like many other raw meals of animal origin, might be contaminated with a vast array of microorganisms including food poisoning organisms such as *S. aureus* and *Salmonella* spp. The obtained results of the present study demonstrated isolation and identification of enterotoxigenic *S. aureus*, and *Salmonella* spp. that harbor several virulence attributes. In agreement with the current study *S. aureus* and *Salmonella* spp. were isolated from processed rabbit carcasses retailed in grocery stores in Beni-Suef city, Egypt (9). Initial counts of Enterobacteriaceae, psychrophilic bacteria, aerobic mesophilic bacteria, molds, and yeasts were found to be relatively high in rabbit meat samples, with mean log10 counts of 6.021, 5.888, 4.785, and 4.886 cfu/g, respectively. This represents the potential for cross-contamination during slaughter, which significantly affects the bacterial status of the carcass (22). Additionally, the rabbit's cooled muscles contained Enterobacteriaceae (5.72 ± 0.26 log10 cfu/g), Staphylococcus (5.32 ± 0.24 log10 cfu/g), and aerobic plate count (7.82 ± 0.34 log10 cfu/g) (10). Besides, Mahmoud et al. (23) recently examined the microbiological condition of rabbit carcasses sold at retail in Zagazig City, Sharkia Governorate, Egypt. They collected 80 random samples of fresh chops of rabbit meat (shoulder, loin, ribs, and thigh regions). Aerobic plate count (APC), Enterobacteriaceae, as well as the isolation and identification of *E. coli* and *Salmonella* Spp., were examined. The mean APC and Enterobacteriaceae values for the shoulders were $1.1x10^6$ and $4.7x10^4$ cfu/g, respectively. The values for the ribs were $9.6x10^5$ and $5.7x10^4$ cfu/g, the loin samples were $1.0x10^6$ and $5.1x10^4$ cfu/g, and the thigh samples were $1.2x10^6$ and $6.0x10^4$ cfu/g. Salmonellae were isolated from the shoulder, ribs, loin, and thigh regions at 6 (30%), 7 (35%), 6 (30%), and 4 (20%), respectively. On the other hand, *E. coli* was isolated at 18 (90%), 16 (80%), 15 (75%), and 19 (95%) of the aforementioned samples. The findings highlighted the significance of stringent sanitary procedures during slaughtering and revealed that fresh rabbit meat might contain a diversity of spoilage and food poisoning bacteria. Globally, Enterotoxigenic *S. aureus* and *Salmonella* spp. were isolated from rabbit carcasses retailed in Spain (24).

The uncontrolled usage of antimicrobials in rabbit farming is a major reason for the emergence of multidrug-resistant foodborne pathogens as was apparent in the current study. Therefore, strict observation of the withdrawal times of the antimicrobials, besides the use of antimicrobials at the minimum requirement is of significance to avoid the development of drug resistance.

Conclusion

The current study revealed the isolation of multidrug-resistant *S. aureus* and *Salmonella*

spp. from rabbit meat and offal, suggesting that these products might act as potential sources for transmission of such foodborne pathogens.

Acknowledegments

The authors would like to thank the Deanship of Scientific Research, Qassim University for supporting the publication of this project.

Authors declare that they have no conflict of interest.

This study was done according to the guidelines of Zagazig University, Egypt. This study did not use any experimental animals or human subjects.

References

1. Cambero MI, de la Hoz L, Sanz B, et al. Lipid ~ and fatty acid composition of rabbit meat: Part 1 - A polar fraction. Meat Sci 1991; 29: 153–66.

2. Fernandez-Espl MD, O'Neill E. Lipid oxidation in rabbit meat under different storage conditions. J Food Sci 1993; 58(6): 1262–4.

3. Abd-Allah SMS, Abd-Elaziz DM. Nutritional value and quality profile of fresh rabbit meat in Assiut city, Egypt. Int J Res Agric Food Sci 2018; 4: 1–15.

4. Morshdy AEM, Darwish WS, El Sayed S, et al. Amino acid profile of rabbit meat: dietary intake and the effect of freezing on the amino acid composition. Thai J Vet Med 2022; 52(2): 251–8.

5. Nakyinsige K, Sazili AQ, Aghwan ZA, et al. Development of microbial spoilage and lipid and protein oxidation in rabbit meat. Meat Sci 2015; 108: 125–31.

6. Rodríguez-Calleja JM, Santos JA, Otero A, Garcia-Lopez ML. Microbiological quality of rabbit meat. J Food Prot 2004; 67(5): 966–71.

7. Morshdy AE, Mohieldeen H, Shymaa G, Mohamed ME, Darwish W. Microbiological Quality of Rabbit Meat in Egypt and Worldwide: A Review. J Adv Vet Res 2022; 12(6): 807–10.

8. Darwish WS, Atia AS, Reda LM, et al. Chicken giblets and wastewater samples as possible sources of methicillin-resistant Staphylococcus aureus: Prevalence, enterotoxin production, and antibiotic susceptibility. J Food Safety 2018; e12478.

9. Khalafalla FA. Microbiological status of rabbit carcases in Egypt. Zeitschrift für Lebensmittel-Untersuchung und Forschung 1993; 196(3): 233–5.

10. Morshdy AM, Al Ashkar AT, Mahmoud AFA. Improving the quality and shelf life of rabbit meat during chilled storage using lemongrass and black seed oils. J Anim Health Prod 2021; 9: 56–61.

11. Morshdy AMA, Darwish WS, El-Dien WMS, et al. Prevalence of multidrug-resistant Staphylococcus aureus and Salmonella Enteritidis in meat products retailed in Zagazig city, Egypt. Slov Vet Res 2018; 55(Suppl 20): pp.295–300.

12. Darwish WS, Eldaly EA, El-Abbasy MT, et al. Antibiotic residues in food: the African scenario. Jpn J Vet Res 2013; 61(Supplement): S13–22.

13. Alsayeqh AF, Baz AHA, Darwish WS. Antimicrobial-resistant foodborne pathogens in the Middle East: A systematic review. Environ Sci Pollut Res 2021; 28(48): 68111–33.

14. American Public Health Association (APHA) Compendium of methods for the microbiological examination of food, 4th Ed., Washington. 2001.

15. ISO (International Standard Organization) 6579: 4th ed. Microbiology - General guidance on methods for the detection of Salmonella, International Organization for Standardization, Geneve, Switzerland. 2002(E).

16. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000; 38 (3): 1032–5.

17. Cardona-Castro N, Restrepo-Pineda E, Correa-Ochoa M. Detection of hilA Gene Sequences in Serovars of Salmonella enterica Subspecies Enterica. Mem Inst Oswaldo Cruz 2002; 97(8): 1153–6.

18. Murugkar HV, Rahman H, Dutta PK. Distribution of virulence genes in Salmonella serovars isolated from man & animals. Indian J Med Res 2003; 117: 66–70

19. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 12th ed. Wayne, PA: Clinical and Laboratory Standards Institute. 2015.

20. El-Ghareeb WR, Smulders FJM, Morshdy AMA, et al. Microbiological condition and shelf life of meat from hunted game birds. Europ J Wildlife Res 2009; 55(4): 317–23.

21. Saleh E, Morshdy AE, El-Manakhly E, et al. Effects of olive leaf extracts as natural preservative on retailed poultry meat quality. Foods 2020; 9(8): 1017.

22. Badr HM. Use of irradiation to control foodborne pathogens and extend the refrigerated market life of rabbit meat. Meat Sci 2004; 67(4): 541–8.

23. Mahmoud AFA, Hafez AESE, Abdullatif AF, et al. Microbiological Evaluation of Fresh Retail Rabbit Meat Cuts from Zagazig City, Egypt. J Adv Vet Res 2022; 12(4): 466–70.

24. Rodríguez-Calleja JM, García-López ML, Santos JA, et al. Development of the aerobic spoilage flora of chilled rabbit meat. Meat Sci 2005; 70(2): 389–94.