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Incidence of *Staphylococcus aureus* and *Enterococcus faecalis* in Some Meat Products

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Abstract

A total of one hundred samples of domestically produced meat products, including frozen beef kofta, sausage, burger and luncheon (25 of each), were randomly collected from various shops in El-Menoufia governorate, Egypt. The samples underwent bacteriological examination using the conventional approach to detect the presence of *S. aureus* and *E. faecalis*. Additionally, 40% of these samples were subjected to PCR analysis. The test findings indicated the presence of *S. aureus* and *E. faecalis*. *S. aureus* was found in 68%, 60%, 48%, and 36% of the previously analyzed samples, respectively. *Enterococcus faecalis* was found also in 12%, 52%, 20%, and 40% of the previously analyzed samples, respectively. *S. aureus* was identified using PCR in 90% of each (frozen kofta samples, sausage samples, burger samples), and 80% of the luncheon samples. *E. faecalis* was found in 0%, 80%, 30%, and 50% of the same analyzed samples, respectively. The present study revealed that some meat products were contaminated with *S. aureus* and *E. faecalis*, especially beef kofta, burgers, sausage, and luncheon products. The unsanitary conditions in which these meat products were handled are the primary factors contributing to the elevated levels of *S. aureus* and *E. faecalis* seen in this investigation. Effective manufacturing protocols can help to minimize contamination.

Keywords: Burger; *E. faecalis*; Kofta; *S. aureus;* Sausage.

INTRODUCTION

When preparing high-quality food, it is important to take into account the management of microbial contamination in meat products. During the slaughtering process, the meat is susceptible to contamination from the surrounding environment, equipment and workers' hands. The sanitary condition of animals prior to, during and following the slaughtering process

significantly impacts the quality of the final product. Furthermore, while deboning process takes place, the flesh undergoes significant manipulation and is vulnerable to bacterial contamination, resulting in decay, a change in color, and the formation of unpleasant-smelling pigments (Shaltout et al., 2019). The meat becomes contaminated throughout the slaughtering process because of exposure to the surrounding atmosphere, contact with machines, and handling by workers (Armany et al., 2021).

Staphylococcus aureus is ranked as one of the most important foodborne pathogens by food safety agencies due to the high occurrence and seriousness of the diseases it causes (Saad et al., 2023). *Staphylococcus aureus* is responsible for a wide variety of foodborne intoxications. These encompass serious illnesses such as septicemia and endocarditis, which can result in significant fatality rates if not well managed (Thwala et al., 2021).

The main types of Enterococci are *Enterococcus faecalis* and *Enterococcus faecium*. These bacteria cause many illnesses in humans, including bacteremia, septicemia, endocarditis, wound infections, urinary tract infections, neonatal sepsis and meningitis (Younis et al., 2022). Enterococci have the ability to persist in the environment. Their significant heat resistance allows them to become prevalent in heat-treated foods, resulting in their dominance. Enterococci can serve as indications of fecal contamination (Ibrahim and Hassan, 2016).

One major task of food safety sector is to confirm safety of the meat products retailed at the market, therefore, this study aimed at investigation of the prevalence and molecular characterization of *S. aureus* and *E. faecalis* in the retailed meat products in Menoufia government, Egypt.

MATERIAL AND METHODS

Collection of samples

A total of one hundred random samples of locally produced meat products, including 25 each (frozen beef Kofta, sausage, burger, and luncheon), were collected from various shops and supermarkets in Menoufia government, Egypt. The collected samples were individually stored in a sterile plastic bag and promptly transported to the laboratory in an ice box, ensuring complete aseptic conditions. The samples underwent a bacteriological analysis to identify the presence of *Staphylococcus aureus* and *Enterococcus faecalis*.

Sample preparation (ISO, 2021)

In a sterile manner, a total of 25 grams of each sample under investigation were taken to a sterile blender jar. Subsequently, 225 ml of sterile buffered peptone water (0.1%) was added to the jar's contents. Next, the mixture was subjected to homogenization at a speed of 1500–2000 rpm for 2 minutes, resulting in the formation of a homogenate (1:10). This homogenate was then used to generate decimal serial dilutions up to 10^{-6}. The prepared samples were tested as follows:

S. aureus count (FDA, 2001 and ISO, 2021)

The colonies that grew on the Baird Parker agar plate after being kept at a temperature of 37 °C for 48 hours were

suspected to be *S. aureus*. The suspected colonies were picked up and purified on nutrient agar slopes, so they could be further identified using their morphology, biochemistry and serology.

E. faecalis count (Fernandes and Dhanashree, 2013)

The assessment of hemolysin production was conducted by utilizing blood agar plates containing 5% defibrinated sheep blood were used to assess hemolysin production. Following incubation at 37 °C for 24 hours, a distinct area of hemolysis surrounding enterococcal colonies was used to determine a positive result. The *Enterococcus faecalis* count per gram was enumerated and recorded. The colonies that were thought to be there were also picked up and purified on nutrient agar slopes, so that they could be analyzed further for morphological, biochemical and serological identification.

Molecular characterization of S. aureus and E. faecalis.

Table 1. Preparation of uniplex PCR Master Mix.

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	**12.5*µl***
PCR grade water	**5.5 *µl***
Forward primer (20 pmol)	**1 *µl***
Reverse primer (20 pmol)	**1 *µl***
Template DNA	**5 *µl***
Total	**25 *µl***

Table 2. Temperature and time conditions of the primers during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Staphylococcus 16S rRNA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
E. faecalis 16S rRNA	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.

A DNA molecular weight marker is a substance used to determine the size of DNA molecules based on their molecular weight. The ladder was agitated by pipetting in an upward and downward motion. Exactly 6 microliters of the necessary ladder were loaded directly. Agarose gel electrophoresis was performed according to the method described by Sambrook et al. (1989), with some modifications. A sterile flask containing 1.5 grams of electrophoresis-grade agarose was prepared in 100 milliliters of TBE buffer. The flask was then heated in a microwave with agitation until all the granules were dissolved. Afterward, the flask was allowed to cool to a temperature of 70 ^{o}C. Next, a solution containing 0.5 µg/mL of ethidium bromide was introduced and properly blended. The heated agarose solution was poured

straight into the gel casting device with the required comb in place and left to solidify at room temperature. Subsequently, the comb was extracted, and the electrophoresis tank was filled with TBE buffer. Twenty microliters of each individual PCR product were placed on the gel. The power supply had a voltage range of 1 to 5 volts per centimeter of the tank's length. The run was terminated after approximately 30 minutes, and the gel was thereafter moved to the UV cabinet. The gel was captured using a gel documentation method, and the data was analyzed using computer software.

Statistical analysis

It was done according to Peacock and Peacock (2020).

RESULTS

The results recorded in Table (3) clearly indicated that the examined samples of frozen beef Kofta, sausage, burger, and luncheon were found to be contaminated with *S. aureus*. The *Staphylococcus aureus* count (log cfu/g) ranged from 1.75 to 4.05 with a mean value 3.25 ± 0.02 for Kofta, 1.62 to 3.94 with a mean value 3.11 ± 0.08 for sausage, 1.51 to 3.28 with a mean value 2.55 ± 0.05 for burger, and from 1.4 to 3.01 with a mean value 2.04 ± 0.04 for luncheon. Table (4) indicated that 52%, 60%, 68%, and 76% of the samples met the Center for Food Safety (CFS) criteria for staphylococci counts. The prevalence of *Staphylococcus aureus* in the analyzed meat product samples is 68%, 60%, 48%, and 36%, as documented in Table (5).

Table 3. Statistical analysis of *S. aureus* (log cfu/g) in the examined meat product samples (n=25).

Products	Min. (log cfu/g)	Max. (log cfu/g)	Mean ± S.E* (log cfu/g)
Kofta	1.75	4.05	3.25 ± 0.02^{a}
Sausage	1.62	3.94	3.11 ± 0.08^{b}
Burger	1.51	3.28	2.55 ± 0.05^{c}
Luncheon	1.4	3.01	2.04 ± 0.04^{d}

Table 4. Acceptability of the examined meat product samples based on their *S. aureus* content according to CFS (2014) for ready-to-eat food in general and specific food items.

Products	***S. aureus* /g***	**Accepted**		**Unaccepted**	
		No.	**%**	**No.**	**%**
Kofta	$> 10^2$	13	52	12	48
Sausage	$> 10^2$	15	60	10	40
Burger	$> 10^2$	17	68	8	32
Luncheon	$> 10^2$	19	76	6	24

Table 5. The incident of *S. aureus* detected in examined meat product samples using traditional method (n=25).

pathogen / Products	*S. aureus*	
	No	%
Kofta	17	68
Sausage	15	60
Burger	12	48
Luncheon	9	36
Total	53	53

The results presented in Table (6) showed that the count of *E. faecalis* found in frozen beef kofta, sausage, burger, and luncheon samples ranged from 1.69 to 2.37 (log cfu/g), with a mean value 2.21 ± 0.01 for kofta, 2.17 to 3.39 with a mean value 3.11± 0.04 for sausage, 1.60 to 3.04 with a mean value 2.15 ± 0.04 for burger, and 1.39 to 2.95 with a mean value 2.01 ± 0.03 for luncheon. Table (7) showed that 88%, 48%, 80%, and 60% of the samples were acceptable for the enterococci count according to the "CFS" criteria. The prevalence of *E. faecalis* in the analyzed meat product samples is 12%, 52%, 20%, and 40%, as documented in Table (8).

Table 6. Statistical analysis of *E. faecalis* (log cfu/g) in the examined meat product samples (n=25).

Products	Min. (log cfu/g)	Max. (log cfu/g)	Mean ± S.E* (log cfu/g)
Kofta	1.69	2.37	2.21 ± 0.01^{a}
Sausage	2.17	3.39	3.11 ± 0.04^{b}
Burger	1.60	3.04	2.15 ± 0.04^{a}
Luncheon	1.39	2.95	2.01 ± 0.03^{d}

Table 7. Acceptability of the examined meat product samples based on their *E. faecalis* /g* according to CFS (2014) for ready-to-eat food in general and specific food items.

Products	*E. faecalis* /g*	Accepted		Unaccepted	
		No.	%	No.	%
Kofta	-ve	22	88	3	12
sausage	-ve	12	48	13	52
burger	-ve	20	80	5	20
luncheon	-ve	15	60	10	40

Table 8. The incidence of *E. faecalis* detected in the examined meat product samples using traditional method (n=25).

pathogen / Products	*Enterococcus faecalis*	
	No	%
Kofta	3	12
Sausage	13	52
Burger	5	20
Luncheon	10	40
Total	31	31

Based on the results shown in Figures (1) and (2), Agarose gel electrophoresis was used for the multiplex PCR assay to detect the *S. aureus* 16S rRNA gene, which is 791 base pairs. The obtained results revealed detection of such gene in the recovered isolates.

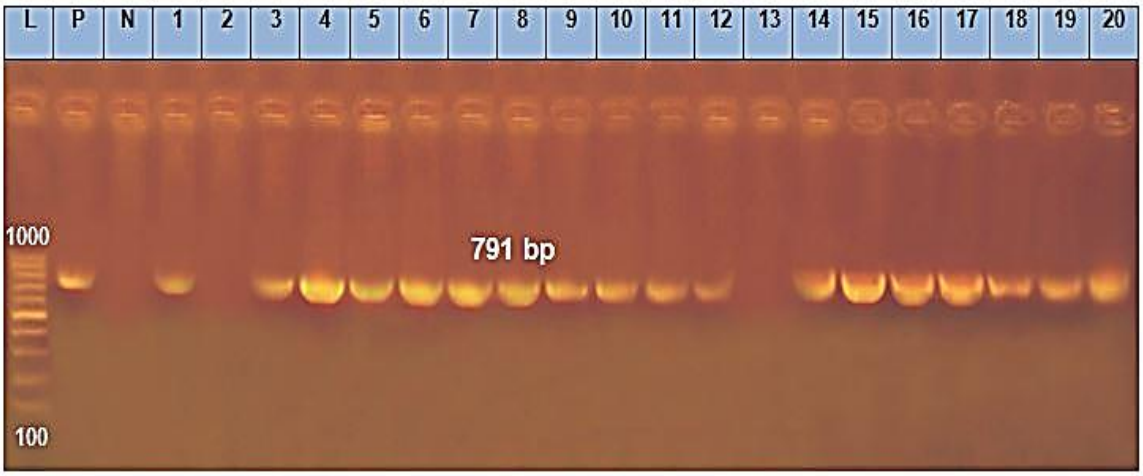

Figure 1. Agarose gel electrophoresis of PCR amplified products of *S. aureus* 16S rRNA gene (791bp).
Lane L: DNA molecular size marker (100 bp).
Lane P: positive control at 791 bp.
Lane N: negative control.
Lanes 1, 3, 4,5, 6, 7,8, 9 and 10 are positive for *S. aureus* from burger samples. Lanes 11, 12, 14, 15, 16, 17, 18, 19 and 20 are positive for *S. aureus* from sausage samples 791bp.

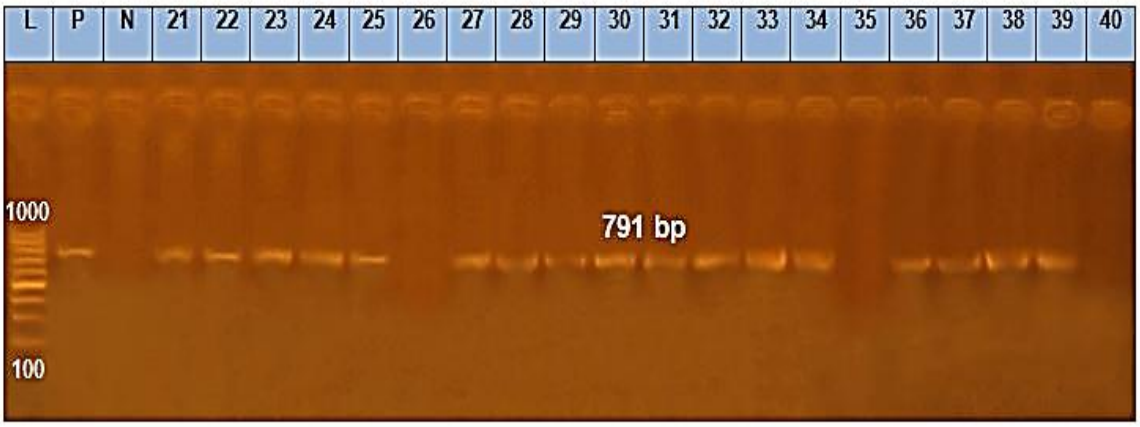

Figure 2. Agarose gel electrophoresis of PCR amplified products of *S. aureus* 16S rRNA gene (791bp).
Lane L: DNA molecular size marker (100 bp).
Lane P: positive control at 791 bp.
Lane N: negative control.
Lanes 21, 22, 23, 24, 25, 27, 28, 29 and 30 are positive for *S. aureus* from kofta samples.
Lanes 31, 32, 33, 34, 36, 37, 38 and 39 are positive for *S. aureus* from luncheon samples 791bp.

We used Agarose gel electrophoresis and multiplex PCR to find the 310-base

pair *E. faecalis* 16S rRNA gene, which could be detected in the recovered isolates (Figures 3, 4).

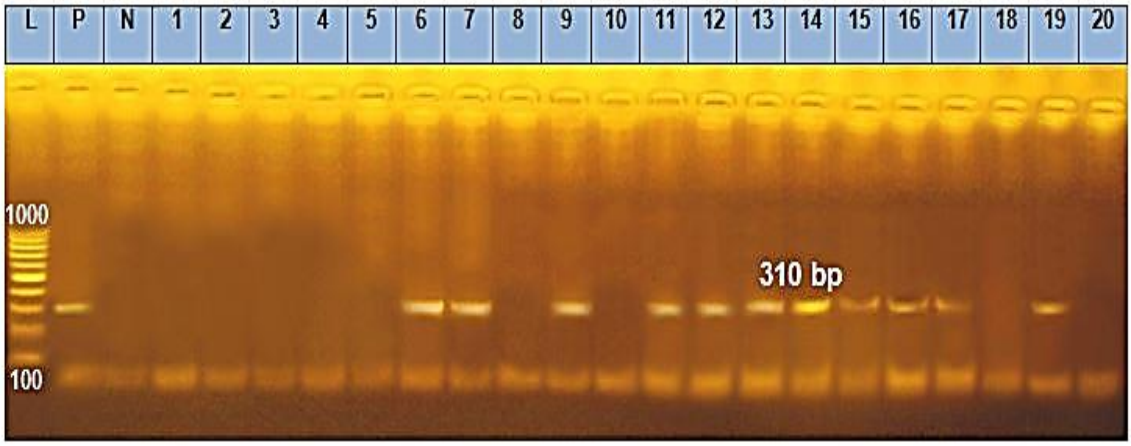

Figure 3. Agarose gel electrophoresis of PCR amplified products of *Enterococcus faecalis* 16S rRNA gene (310bp).
Lane L: DNA molecular size marker (100 bp).
Lane P: positive control at 310bp.
Lane N: negative control.
Lanes 6, 7, 9 are positive for *E. faecalis* from burger samples while,
Lanes 11, 12,13, 14, 15, 16, 17 and 19 are positive for *E. faecalis* from sausage samples at 310 bp.

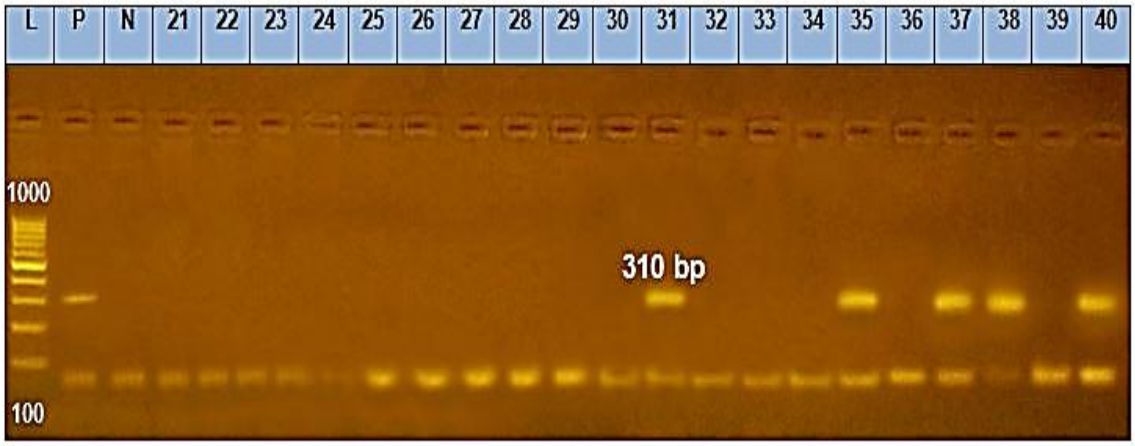

Figure 4. Agarose gel electrophoresis of PCR amplified products of *Enterococcus faecalis* 16S rRNA gene (310bp).
Lane L: DNA molecular size marker (100 bp).
Lane P: positive control at 310bp.
Lane N: negative control.
Lanes 31,35, 37, 38 and 40 are positive for *E. faecalis* from luncheon samples at 310 bp.
Lanes 21, 22, 23, 24, 25, 26, 27, 28, 29 and 30 are negative for *E. faecalis* from kofta samples at 310 bp.

DISCUSSION

Meat products are gaining popularity due to their affordability and convenience as quick meals. Additionally, they enable processors to blend several types of meat into a unified product. In contrast, disease-causing microbes are preserved in human and animal reservoirs and are introduced into the food chain via contamination from the affected humans. Furthermore, meat may become contaminated with foodborne pathogens during the entire slaughtering process, as well as subsequent stages such as food processing, distribution, marketing, storage, preparation, and serving (Alum et al., 2016).

The results were comparable with previous reports, in their study, Badr (2018) reported higher results than expected. They observed that the average count of staphylococci in sausage samples was $2.38\times10^4\pm 0.51\times10^4$ cfu/g, and in beef burger samples it was $4.07\times10^3 \pm 0.69\times10^3$ cfu/g. However, the results for kofta were virtually comparable, with an average count of $9.52 \times 10^3 \pm 2.14\times10^3$ cfu/g. In addition, Hassan et al. (2018) reported higher values in their study, they recorded that the staphylococcal count (cfu/g) varied between $7.95\times10^3 \pm 1.22\times10^3$ and $1.87x10^3 \pm 0.36x10^3$ for beef burger, $2.12\times10^4 \pm 0.48\times10^4$ and $3.72\times10^3 \pm 0.51\times10^3$ for kofta, and $9.06\times10^2 \pm 2.15\times10^2$ and $4.29\times10^2 \pm 0.67\times10^2$ for luncheon. The values for kofta samples reported by Abd El Satter (2016) ranged from 2×10^2 to 7.5×10^3, with a mean value of $3.05\times10^3 \pm 0.97\times10^3$. Heweidy (2017) documented a reduced average of *Staphylococcus count* of $1.9 \times 10 \pm 0.11x10^2$ for sausage samples. Hassan et al. (2018) reported that almost 64% of the samples suspected to contain *S. aureus* tested positive, with a bacterial count ranging from $3.72x10^3 \pm 0.51x10^3$ cfu/g. Similarly, Badr (2018) found that 46.67% of the tested samples were positive for suspected *S. aureus*, with a mean value of $3.10\times10^3 \pm 0.74\times10^3$ cfu/g.

People consuming food contaminated with *S. aureus* might practice enterointoxication. The predominant symptoms, including nausea, vomiting, abdominal cramping, and diarrhea, typically manifest within a time frame of 3 to 8 hours after ingestion (Hassanin et al., 2018). However, the findings in Table (5) indicated that *S. aureus* was present in 15 (60%) of the sausage samples that were analyzed, with an average value of 3.11 ± 0.08. The study conducted by El-Maghraby, (2014) reported higher results, with 94.4% of the samples testing positive for *S. aureus*. The mean count of *S. aureus* was found to be $1.79x10^4 \pm 7.1xl0^3$. Upon analysis, it was found that 11 out of the investigated sausage samples, accounting for 22% of the total, tested positive for the suspected *S. aureus*. The bacterial count in these samples varied from 1.00×10^2 to 1.00×10^4 cfu/g, with an average value of $3.03\times10^3 \pm 6.33\times10^2$, as reported by Ibrahim (2016). Almost similar findings were obtained, with 63.33% of the sausage samples testing positive. The mean value was determined to be $5.96\times10^3 \pm 0.88\times10^3$ (cfu/g) (Badr, 2018).

The findings presented in Table (5) indicate that *S. aureus* was found in 48%

of the beef burgers analyzed. The bacterial count (log cfu/g) ranged from 1.51 to 3.28, with an average value of 2.55 ± 0.05. The findings showed that 15 of the tested beef burger samples, accounting for 30% of the total, tested positive for the suspected *S. aureus*. Likely, the bacterial count in these samples ranged from 2.00×10^2 to 7.00×10^4 cfu/g, with an average value of $1.02\times10^4 \pm 2.53\times10^3$ (Ibrahim, 2016). The suspected *S. aureus* showed a similar outcome, with 40% of the samples testing positive. The count of *S. aureus* varied from 1.0×10^2 to 2.0×10^3 cfu/g, with an average value of $8.71\times10^2 \pm 1.49\times10^2$, as reported by Badr (2018).

Nine of the luncheon samples (36%) tested positive for the suspected *S. aureus* with an average count of $2.0^4 \pm 0.0^4$. Similar values were recorded by Eldaly et al. (2014). Additionally, 93.3% of the luncheon samples were positive for staphylococci, with counts ranging from 1.00 x 10^3 to 3.16 x 10^8 (cfu/g) Ibrahim (2016).

Enterococcus faecalis was isolated in 3 (12%) of the tested kofta samples. Unlikely, Younis et al. (2022) reported a higher prevalence of 22% positive samples for suspected Enterococci in the examined kofta samples. Furthermore, the study conducted by Ibrahim and Hassan (2016) reported higher counts for street vending and restaurant kofta sandwiches, with a mean of $11.9x10^5$ and $63.3x10^4$, respectively.

In sausage samples, *Enterococcus faecalis* was isolated at 52%. The bacterial count (log cfu/g) varied from 2.17 to 3.39, with an average value of 3.11± 0.04. The analysis showed that the sausage samples tested positive for the suspected Enterococcus bacteria. According to Ibrahim and Hassan (2016), the average value was 22.68 x 10^4 for street-vended sausage sandwiches and 28.63 x 10^4 for restaurant sausage sandwiches.

Furthermore, *Enterococcus faecalis* was identified in 5 out of the total number of the analyzed beef burgers, accounting for 20% of the samples. Similarly, the count ranged from 15.61 x 10^4 to 34.03 x 10^4 cfu/g for street vending and restaurant beef burger sandwiches, as reported by Ibrahim and Hassan (2016). Younis et al. (2022) recorded lower results, with 12% of the examined burger samples testing positive for suspected Enterococci.

CONCLUSION

The ongoing study has revealed that the examined meat products, namely beef kofta, sausage, burgers, and luncheon, have been contaminated with *S. aureus* and *E. faecalis*. The high prevalence of *S. aureus* and *E. faecalis* observed in this study can be attributed to the unhygienic handling and processing conditions of these beef products. Implementing appropriate manufacturing procedures helps reduce contamination.

REFERENCES

Abd El Satter, A.M. (2016). Incidence and importance of some pathogenic microorganisms contaminating meat products. M.V.Sc. Thesis (Meat Hygiene), Fac of Vet. Med. Benha Univ, Egypt.

Alum, E.A.U.; Ben, S.M.O. and Ahudic, M.C. (2016). Microbiological contamination of food: the

mechanisms, impacts, and prevention. Intern. J. scientific and technology research, 5: 65-78.

Armany, G.; Ibrahim, H.; Amin, R. and Hagag, N. (2021). Incidence and Duplex PCR for detection of *S. aureus* and *L. monocytogenes* in Meat Products. Benha Veterinary Medical Journal, 40 (2): 106-110.

Badr, S.M. (2018). Follow-up of *E. coli* and *Staphylococcus aureus* in some locally manufactured meat products. M.V.Sc., Thesis (Meat Hygiene), Fac. Vet. Med. Benha Univ. Egypt.

Center for Food Safety ″CFS″ (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Risk Assessment Section, Food and Environmental Hygiene Department43/F, Queensway Government Offices, 66 Queensway, Hong Kong.

Eldaly, E.A.; Nermeen, F.E. and Rasha, M.E. (2014). Detection of enterotoxigenic *S. aureus* genes prevalent in some meat products using Multiplex PCR. The 1st Int. Conf. On The Impact of Environmental Hazards on Food Safety, Zagazig University J., p 162-168.

El-Maghraby, M.S.M. (2014). Bacteriological evaluation of meat and some meat products at consumer level. M. V. Sc, Thesis (Meat Hygiene), Fac. Vet. Med., Sadat City University.

Fernandes, S.C. and Dhanashree, B. (2013). Drug resistance & virulence determinants in clinical isolates of *Enterococcus* species. Indian J. Med. Res., 137: 981–5.

Food and Drug Administration "FDA" (2001). *Staphylococcus aureus*. Bacteriological analytical manual. 8th Edition. Chapter 12. Academic Press, Gaithersburg, UK.

Hassan, M.A.; Amin, R.A.; Eleiwa, N.Z. and Gaafar, H.W. (2018). Detection of *Staphylococcus aureus* in some meat products using PCR technique. Benha veterinary medical journal, 34 (1): 392 – 403.

Hassanin, F.S.; Hassan, M.A.; Nada, S.M. and Badr, S.M. (2018). Staphylococci in some meat products. Benha Veterinary Medical Journal, 34(1): 1-9.

Heweidy, A.Y.A. (2017). Prevalence of some foodborne Micro-organisms in meat and meat products. M.V.Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Benha Univ.

Ibrahim, L.M. and Hassan, A.M. (2016). The role of fast food in transmitting some bacterial hazards to consumers. Assiut Vet. Med. J., 62(149): 110-117.

Ibrahim, S.M.A. (2016). Detection of *Staph. aureus* classic enterotoxin genes in some meat products using multiplex PCR. Ph.D. Thesis (Meat Hygiene), Fac. Vet. Med.Kafr El-Sheikh. Univ. Egypt.

International Organization for Standardization (ISO) (6888-1: 2021). Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1:

Method using Baird-Parker agar medium.

Peacock, J. and Peacock, P. (2020). Oxford Handbook of Medical Statistics (2nd ed.). Oxford University Press.

Saad, R.I.; Elsabagh, R.; Abo El-Roos, N.A.; Edris, S. and Sabeq, I. (2023). *Staphylococcus aureus* incidence in Egyptian meat outlets and butcheries, and their biofilm, antibiotic-resistance, and virulence capabilities. Benha Veterinary Medical Journal, 45(1): 136-140.

Sambrook, J.; Fritscgh, E.F. and Mentiates (1989). Molecular cloning. A laboratory manual.Vol !., Cold Spring Harbor Laboratory Press, New York.

Shaltout, F.A.; Saad, S.M.; Abou Elroos, N.A. and El-Nahas, S.B. (2019). Incidence of *Staphylococci* and *E. coli* in Meat and Some Meat Products. EC Nutrition J., 14(6).

Thwala, T.; Madoroba, E.; Basson, A. and Butaye, P. (2021). Prevalence and characteristics of *Staphylococcus aureus* associated with meat and meat products in African countries. https://www.mdpi.com/journal/antibiotics.

Younis, W.; Mohamed, A.A.; Abd Al-Azeem, M.W. and Mohamed, H.M.A. (2022). Isolation and identification of *Enterococcus faecium* from Meat products with special reference to some virulence and antibiotics resistance factors. SVU- International Journal of Veterinary Sciences, 5(1): 1-16.