Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Assessment of Antibiotic Resistance and Toxin Production Capabilities among *S. aureus* Isolates Recovered from Food Samples

Dalia I. S. Abo El-ghar[1*], Ahmed F. Azmy[1], Nahla Abu El Roos[2] and Mohamed Sebak[1]

[1] *Department of Pharmaceutical Microbiology and Immunology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.*

[2] *Food Hygiene Department, Animal Health Research Institute, Shibin El Kom branch, Agriculture Research Center, Egypt.*

Abstract

Staphylococcus aureus (*S. aureus*) is a prevalent contributor to foodborne illnesses as a result of its capacity to create a variety of heat-stable enterotoxins. This study aimed to investigate the prevalence of *S. aureus* isolates that are both multiple antibiotic-resistant and toxin-producing in beef and chicken meat samples obtained from various restaurants in Egypt between January 2021 and March 2023. Seventy-four percent of *S. aureus* isolates were recovered from chicken meat, whereas 26% were obtained from beef meat. The antibiotic susceptibility testing for *S. aureus* isolates revealed that they were highly resistant to cefoxitin (86%) and penicillin (100%) but had low resistance to doxycycline (8%), moxifloxacin (4%), and norfloxacin (2%). When *S. aureus* isolates were genetically investigated to detect several enterotoxin-encoding genes, the *SEB* gene was detected in 26% of them. In comparison, none of the isolates possessed any of the other analyzed enterotoxin genes. Finally, the influence of various meal types on the expression of the *SEB* was assessed using the real-time PCR method. The findings highlighted that meat was the most influential factor in *SEB* gene expression, followed by milk and then, coshary (an Egyptian carbohydrate-based cuisine). To sum up, enterotoxins production and microbial resistance could contribute to the widespread of *S. aureus* infections, creating a risk to public health. Thus, stringent hygienic procedures must be taken to prevent or mitigate food contamination and subsequently food intoxication by *S. aureus*. These methods require quality control of raw materials, adequate handling, Personal hands sanitization, environmental hygiene and equipment disinfection.

Keywords: Antibiotic resistance; Enterotoxins; Food pathogens; Real-time PCR; *Staphylococcus aureus*.

Introduction

Eating or drinking water or meals contaminated by bacteria, fungi, parasites, or even non-microbial sources can cause food poisoning. [1]**.** Among food-related illnesses, staphylococcal food poisoning is one of the most common worldwide, and it can spread through tainted food that contains preformed *S. aureus* enterotoxins [2, 3]. *Staphylococcus aureus* is an opportunistic microorganism can cause a variety of infections ranging from minor skin conditions to severe, sometimes fatal invasive illnesses [2]**.** *Staphylococcus aureus* is dangerous microorganism due to its invasiveness, toxin production, and it may be drug resistance [4]. Unlike most other toxins that *S. aureus* produces, modest doses of *Staphylococcal* enterotoxins (*SE*s) are toxic to humans. *SE*s are also highly resistant to harsh environmental conditions including highly acidic environments, heating [5], and proteolytic enzymes [6]. Enterotoxins are virulence proteins produced by *S. aureus* that have a strong super antigenic activity that impairs adaptive immunity and shares structural and functional characteristics. *Staphylococcal* enterotoxins are classified into three major groups: 1) the first group (traditional), which includes *SEN*, *SEP*, *SEJ*, *SED*, *SEE*, *SEO*, and *SEA*; 2) the second group, which contains *SER*, *SEU*, *SEG*, *SEC*, and *SEB*; 3) the third group (non-classical), which involves the enterotoxins such as *SEQ*, *SEL*, *SEI*, *SEK*, and *SEM* [7]. Among them, *SEB* is a possible bioterrorism agent [8]. Interestingly, *S. aureus* possesses numerous genes encoding enterotoxins,

*Corresponding authors: Dalia I. S. Abo El-ghar, E-mail: dalia2019ibrahim@gmail.com Tel.: +201119012891
(Received 28 September 2025, accepted 07 November 2025)
DOI: 10.21608/ejvs.2025.427708.3156

which affect their potential production and, as a result, food-borne diseases. [9].

The objective of this study was to investigate the prevalence of *S. aureus* in diverse food samples. and to assess their ability to produce enterotoxins, particularly when cultivated in the existence of diverse types of food.

Material and Methods

Sample Collection

Over the period spanning January 2021 to March 2023, 112 food samples were collected from various restaurants and hospital food providers in Egypt's Menoufia Governorate. Sixty samples were raw chicken, while fifty-two were from raw meat. Under aseptic conditions, each sample was stored separately in sterile plastic bags kept in an ice box for refrigeration, then transported straight to the laboratory.

Isolation of Staphylococcal Isolates

Sample preparation was carried out as previously described [10]. Twenty-five grams of meat samples were put into a sterile blender containing two hundred and twenty-five milliliters of 0.1% sterile peptone water and mixed well for 1.5 min. One milliliter of each created dilution was applied to the surface of the Baird Parker agar plate using a sterilized bent glass spreader [11]. The lustrous black colonies were counted after the inoculated plates had been inverted and incubated for 48 h at 37°C, *S. aureus* isolates were eventually morphologically examined [12] and biochemically identified [13], [14].

Antibiotic Susceptibility Testing and MIC Determination

A modified Kirby-Bauer disk-diffusion method was utilized to determine the antibiotic susceptibility of isolated *S. aureus* strains [15] against penicillin, ampicillin, cefoxitin, linezolid, doxycycline, azithromycin, norfloxacin, moxifloxacin, ciprofloxacin, clindamycin, gentamycin, and amikacin (Oxoid, UK). In contrast, the minimum inhibitory concentrations (MICs) of both vancomycin and teicoplanin were measured using the agar dilution technique on Mueller-Hinton agar. [15]. While, *S. aureus* isolates' multiple antibiotic resistances (MAR) index was established using the equation: the count of antibiotics with resistance patterns / the overall number of antibiotics tested [16].

PCR Analysis of Enterotoxins-Encoding Genes

Following the manufacturer's instructions, a genomic DNA isolation kit (Metabion, Germany) was employed to extract DNA from *S. aureus* isolates. Then, two nanograms of the extracted DNA were used as a template in the PCR amplification. The primers utilized to detect the genes for the *SE*s (*SEB, SEC, SED, and SEA*) are provided in Table (1). Briefly, PCR-grade water, template DNA (2 ng) and master mix were added to the reaction mixture. Next, the reaction mixture was supplemented with primers at a concentration of 0.5 µM. In a PTC-100 thermal cycler (MJ Research, Inc.), PCR amplification was conducted using the following settings showed in Table (2). Finally, the products of PCR were tested on a 1.5% agarose gel (Sigma-Aldrich) against a 50 bp DNA ladder (Amersham-Pharmacia Biotech).

Effect of Different Foods on Staphylococcal Enterotoxin Gene Expression

The impact of various foods on the expression level of enterotoxin-encoding *SEB* gene was carried out using real-time PCR (RT-PCR). Four *S. aureus* isolates with the *SEB* gene were subjected to RT-PCR, whereas the average fold changes in the expression level of the four strains were calculated.

Eighty grams of meat and coshary were added to 50 mL of sterilized water in a flask (3 flasks for each) and then sterilized by autoclaving. After being enriched in brain heart infusion agar overnight at 37°C, two colonies of *S. aureus* were transferred into 10 mL of sterile saline. Next, one milliliter was added to the sterilized flasks of food and vigorously shaken, followed by incubation for 16 to 18 h at 37°C.

After that, 2 mL of milk broth was centrifuged for 10 min at 5000 rpm to collect the culture pellets. The supernatant was then discarded, and the pellets were rinsed with sterile saline and centrifuged once again for 5 min at 5000 rpm. Regarding meat and coshary, the flask was filled with 50 mL of sterile saline and shaken vigorously. Two milliliters were then centrifuged at 500 rpm for 2 min to precipitate the large food particles. The suspended bacteria were then collected through centrifugation at 5000 rpm for 5 min.

As instructed, the RNA of *S. aureus* isolates was extracted using the RNeasy Mini Kit (Qiagen Inc., Montreal, ON, Canada). Based on the information available in the literature, the primer sequences for the *Staphylococcal* enterotoxin *SEB* gene and a housekeeping gene (16S RNA) are shown in Table (3). The RT-PCR reaction mixture contains 10 ng of template RNA, 1 µl of primers (800 nM), 5 µl of SYBR Green Master Mix (Thermo Fischer Scientific, Waltham, MA, USA), and double distilled water up to 10 µl final volume. The RT-PCR amplification's thermal cycle conditions listed in Table (4).

Gene expression was assessed using a previously established mathematical model [18]. Based on the threshold set by the real-time PCR software, expression levels were interpreted as follows: a significant increase was defined as a value > 2, and a significant decrease as a value ≤ 0.5.

The Strata Gene MX3005P software has been employed to analyzes cycle threshold (Ct) values and amplification curves. For investigating how the gene expression was influenced by different types of food, the ΔΔCt method was employed to assess the effect of different foods on gene expression by comparing CT of each food sample to untreated control sample [19]**.** Excluding the false positive results was carried out by comparing the samples using the dissociation curves. Data analysis was conducted using IBM SPSS software version 20.0 (Armonk, NY: IBM Corp.). The quantitative data included descriptions of the range, mean, and standard deviation. The results were assessed for significance at the 5% level. F-test (ANOVA) and the chi-square test were the tests used.

Results

Isolation of S. aureus strains

A total of fifty *S. aureus* isolates were recovered from raw chicken samples (37) and raw beef meat (13). *S. aureus* isolates were identified via colonial and morphological characterization in addition to their biochemical testing (Figure 1A-E). The colonies of *S. aureus* had a smooth, opaque, golden-yellow appearance after incubation overnight on nutrient agar. Microscopic examination of *S. aureus* isolates revealed grape-like clusters. The isolates showed yellow color on mannitol salt agar plates, whereas their colonies were black on the Baird Parker agar medium. Moreover, they showed positive catalase activity and complete blood hemolysis on blood agar media, while they demonstrated positive coagulase activity on citrated rabbit plasma.

Antimicrobial susceptibility testing and MIC determination

Antimicrobial susceptibility testing of *S. aureus* isolates revealed a very high resistance pattern against both penicillin (100%) and cefoxitin (86%), while quite a low percentage of resistance was recorded against ampicillin (20%), clindamycin (12%), and azithromycin (10%). On the other hand, very low resistance was demonstrated against doxycycline (8%), moxifloxacin (4%), and norfloxacin (2%), whereas the isolates did not show any resistance to the remaining assessed antibiotics Table (5) and Figure 2 and 3A-B). The MAR index amongst the *S. aureus* isolates fell between 0.071 and 0.57 with a mean of 0.32 as shown Table (6) and Figure 4. Notably, both teicoplanin and vancomycin displayed MIC values of less than 2 µg/mL against all 50 *S. aureus* isolates, indicating microbial susceptibility for both antibiotics.

Detection of enterotoxins-encoding genes via PCR

Out of 50 *S. aureus* isolates, 13 isolates (26%) displayed positive existence of the *SEB* enterotoxin gene distributed between raw chicken meat and raw beef meat as 9 (18%) and 4 (8%), respectively (Figure 5), while *SED*, *SEA* and *SEC* genes were notin any isolate.

All SEB-positive *S. aureus* strains were resistant to at least two antibiotic classes, and nearly half (6 of 13) showed multi-drug resistance (MDRO), to at least three classes Table (7).

It is noteworthy that 6 isolates from these 13 *S. aureus* isolates were multi-drug-resistant organisms (MDRO) with resistance to antibiotics from at least three different antibiotic classes Table (7).

Effect of Different Foods on Staphylococcal Enterotoxin Gene Expression

Remarkably, the meat exhibited the greatest effect on *SEB* gene expression (13.11 ± 2.29), followed by milk (8.35 ± 1.0), and then, coshary (4.56 ± 1.41), as demonstrated in Table (8) and Figure 6.

Discussion

Food-borne illness is one of the most common health problems. *S. aureus* contamination may increase due to unsanitary conditions during the processing of meat and poultry [21, 22].

Current findings revealed 61.7% contamination of chicken meat, which is consistent with (70%) [23], (68.53%) [24] and (53.8%) [25]. Conversely, other studies reported lower prevalence rates, such as (25%) [26], (23.4%) [27] and (18.18%) [28].

Intriguingly, Wu et al. [29] reported that *S. aureus* isolates exhibited high significant resistance to ampicillin (85.4%) and penicillin (84.6%), while Abdalrahman et al. [25] stated that *S. aureus* isolates exhibited high resistance to doxycycline (62.5%), ampicillin (94.6%), and penicillin (70.8%). Also, Waters et al. [30] found that fifty-two percent of *S. aureus* isolates that contaminated meat and poultry were resistant to multiple antibiotics. These reports are partially similar to our antibiogram results. Furthermore, all isolates in this study were susceptible to gentamicin, amikacin, teicoplanin, linezolid, or vancomycin. Close findings were documented by several other researchers [25, 27, 31].

In this study, the *SEB* gene was detected in 26% of *S. aureus* strains, which aligns with the reported rates of 19.6% [32], 24% [31], and 21.6% [33]. Interestingly, Baz et al. (2021) reported that the *SEE*, *SEC*, and *SED* genes were absent in all the isolates they screened. Conversely, Karmi [23] detected that the *SEA* gene and *SEC* gene were 53.6% and 40.6% respectively. However, the *SEB* gene was absent in the *S. aureus* isolates obtained from the examined beef and chicken samples. Also, Baz et al. [34] found the *SEA* gene 32.6%), *SEB* gene (4.3%), and both the *SEA* and *SEB* genes (2.1%) in examined *S. aureus* isolates.

Furthermore, Meat showed the greatest gene expression, followed by milk and then coshary. Expression of toxins may be influenced by components of the food matrix [35, 36]. Also, Wang et al. [37] manifest that vitamin B2, a key nutritional component of chicken meat, motivate the expression of enterotoxin genes in S. aureus

Accordingly, milk had a lower impact on *SEB* gene expression than meat, which may be attributed to the presence of lactic acid in milk. Consistent with Lin et al.'s [38] findings, which identified sodium lactate as a significant inhibitor of *S. aureus* growth and *SEA* enterotoxin production. Additionally, it is comparable to the findings of Abd El Ghany et al. [39], who found that the expression of *SEA* decreased with increasing lactic acid concentration. It is noteworthy that Homsombat et al. [40] found that milk had much greater levels of *SEC* and *SEA* expression than *SEE* and *SEB*.

Conclusion

The present study concluded that food poisoning with *S. aureus* is a great alarming health threat, as the high percentage of collected samples revealed the presence of *S. aureus* as a food borne pathogen. This problem should be highlighted because of the presence of MDR and enterotoxin-producing *S. aureus* isolates (Table 3). This problem may be attributed to environmental contamination of food and unhygienic measures in the handling and processing of food. Enterotoxin genes and antibiotic resistance may contribute to the expansion of *S. aureus* infections, creating a risk to public health.

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 (ethics approval number; 49/11/2023).

TABLE 1. List of primers used for PCR screening of *Staphylococcal* enterotoxins with respective amplicon sizes.

Enterotoxin Gene	Primer Sequences	Amplicon Size (bp)	Reference
SEA	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102 bp	[17]
SEB	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164 bp	
SEC	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451 bp	
SED	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278 bp	

TABLE 2. Cycling conditions of the different primers during cPCR

Target	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. aureus	enterotoxins	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

TABLE 3. Oligonucleotide primers used in the detection level of gene expression of Enterotoxin *SEB* under the effect of various foods.

Gene	Primer sequence (5'-3')	Amplicon size (bp)	Reference
16S rRNA (Standard)	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	179 bp	[20]
SEB	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164 bp	[17]

TABLE 4. Cycling conditions for RT PCR:

Target gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
16S rRNA	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 40 sec.	72˚C 40 sec.	94˚C 1 min.	55˚C 1 min.	94˚C 1 min.
Seb				57˚C 30 sec.			57˚C 1 min.	

TABLE 5. Percentages of Antimicrobial susceptibility of S. aureus strains isolated from the examined samples of raw chicken meat and raw meat. (n=50).

Antimicrobial agent	S		I		R	
	No.	%	No.	%	No.	%
penicillin (P)	-	-	-	-	50	100
cefoxitin (Fox)	7	14	-	-	43	86
ampicillin (Amp)	40	80	-	-	10	20
clindamycin (CD)	41	82	-	-	6	12
azithromycin (AZM)	43	86	2	4	5	10
doxycycline (DO)	44	88	2	4	4	8
Moxifloxacin (MO)	48	96	-	-	2	4
norfloxacin (NX)	48	96	1	2	1	2
ciprofloxacin (CP)	47	94	3	6	-	-
amikacin (AK)	50	100	-	-	-	-
gentamicin (G)	50	100	-	-	-	-
teicoplanin (TEI)	50	100	-	-	-	-
linezolid (LZ)	50	100	-	-	-	-
vancomycin (V)	50	100	-	-	-	-

TABLE 6. Antimicrobial resistance profile of S. aureus strains isolated from the examined samples of raw chicken and raw meat. (n=50).

	Number of isolate	Antimicrobial resistance profile	MAR index
S. aureus	1	P, FOX, AMP, CD, AZM, DO, MO, NX	0.57
	1	P, FOX, AMP, CD, AZM, DO, MO,	0.50
	2	P, FOX, AMP, CD, AZM, DO	0.428
	1	P, FOX, AMP, CD, AZM	0.357
	1	P, FOX, AMP, CD	0.285
	4	P, FOX, AMP	0.214
	33	P, FOX	0.143
	7	P	0.071

TABLE 7. The resistance pattern for *S.aureus* isolates that harbor the enterotoxin *SEB* gene. Highlighted cells in gray are for MDRO. P= Penicillin, FOX= cefoxitin, AMP= ampicillin, CD= clindamycin, AZM=azithromycin, DO= doxycycline, MO= moxifloxacin, NX= norfloxacin

Strain ID	Source of Food	Antimicrobial Resistance Profile
S.A1	Raw Chicken	*P, FOX, AMP, CD, AZM, DO, MO, NX*
S.A2	Raw Chicken	*P, FOX, AMP, CD, AZM, DO, MO,*
4	Raw Chicken	*P, FOX, AMP, CD, AZM, DO*
C	Raw Chicken	*P, FOX, AMP, CD, AZM, DO*
16	Raw Chicken	*P, FOX, AMP, CD, AZM*
B	Raw Meat	*P, FOX, AMP, CD*
NA	Raw Chicken	*P, FOX, AMP*
AA	Raw Meat	*P, FOX, AMP*
L3	Raw Meat	*P, FOX, AMP*
G1	Raw Meat	*P, FOX, AMP*
C1	Raw Chicken	*P, FOX*
T	Raw Chicken	*P, FOX*
H2	Raw Chicken	*P, FOX*

TABLE 8. Comparison between the different tested foods with their impact on the expression of the *SEB* gene.

Expression of *SEB* gene	**Milk (n = 4)**	**Meat (n = 4)**	**Coshary (n = 4)**	**Control (n = 4)**	**F**	
Min. – Max.	7.31 – 9.71	10.27 – 15.35	3.20 – 6.54	1.0 – 1.0	52.345*	<0.001*
Mean ± SD	8.35 ± 1.0	13.11 ± 2.29	4.56 ± 1.41	1.0 ± 0.0		
p_0	<0.001*	<0.001*	0.020*			
Significance between groups	p_1=0.003*, p_2=0.013*, p_3<0.001*					

F: F for the One-way ANOVA test; a Post Hoc Test (Tukey) was used to compare each two groups.
p_0: p-value for comparing **control** and each other group.
p_1: p-value for comparing **Milk** and **Meat**
p_2: p-value for comparing **Milk** and **Coshary**
p_3: p-value for comparing **Meat** and **Coshary**
*: Statistically significant at $p \leq 0.05$

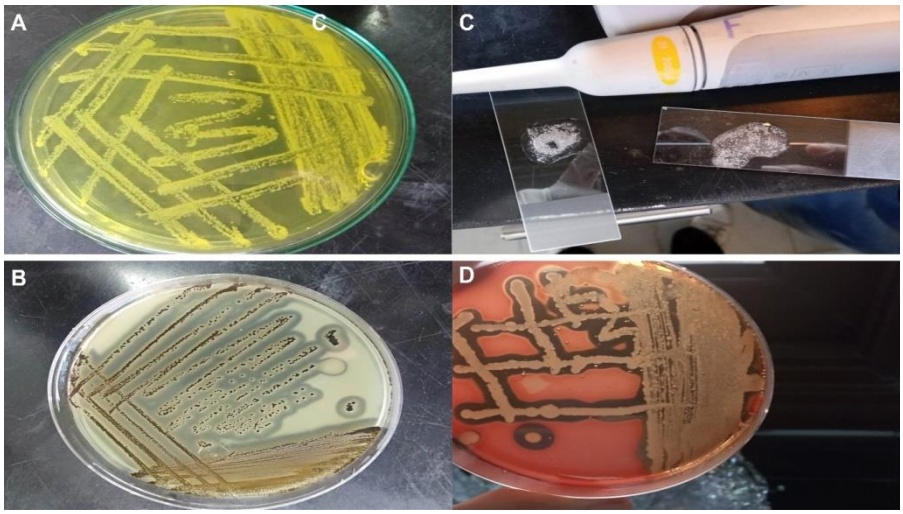

Fig. 1. Examples of culture characteristics and biochemical identification tests of *S. aureus* food isolates. A) Growth on mannitol salt agar B) Growth on Baird Parker agar C) Catalase test D) Blood hemolysis test.

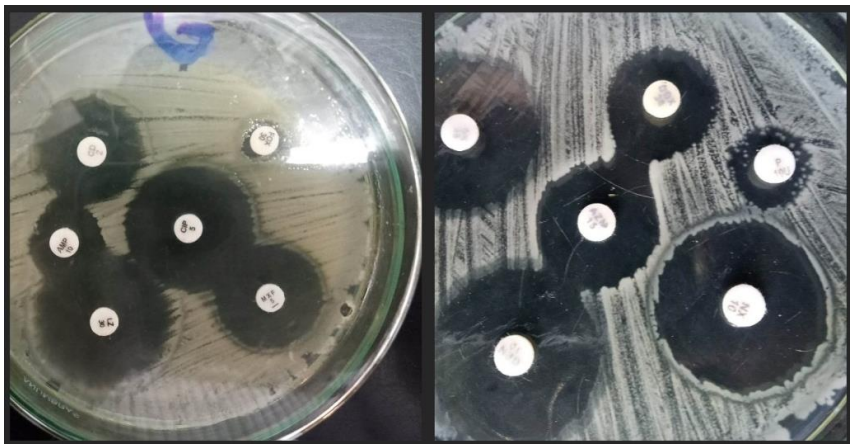

Fig. 2. Antibiogram of *S. aureus* against various antibiotics using agar disk diffusion method.

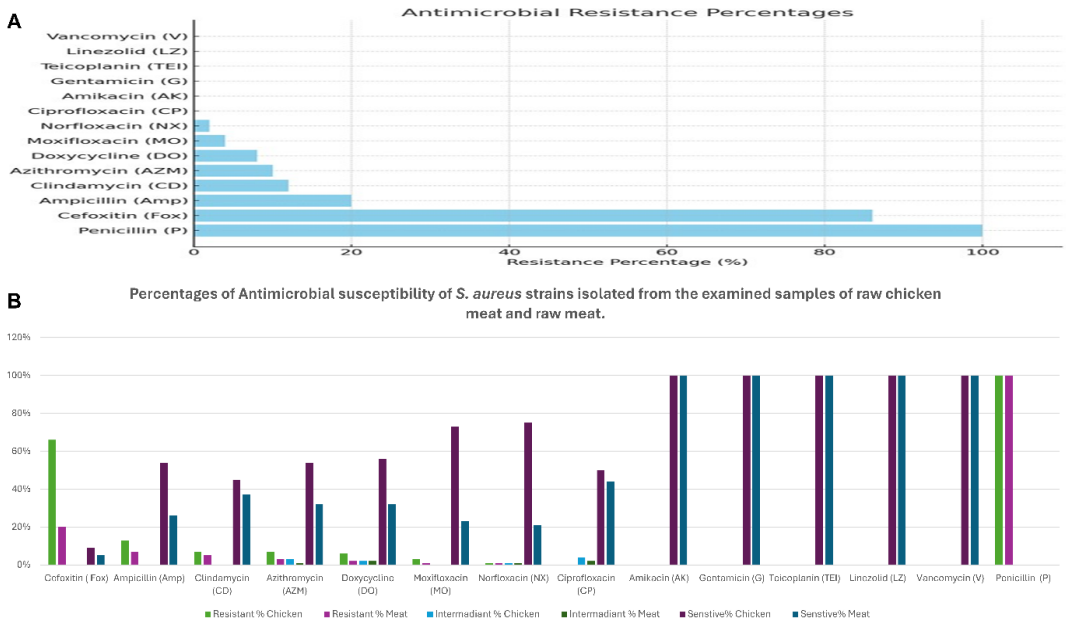

Fig. 3. Result of antimicrobial susceptibility testing of food *S. aureus* strains in the current study. A) Percentage of resistance pattern of isolates to different tested antibiotics B) Detailed result of antimicrobial susceptibility testing of recovered food isolates from raw chicken and raw meat.

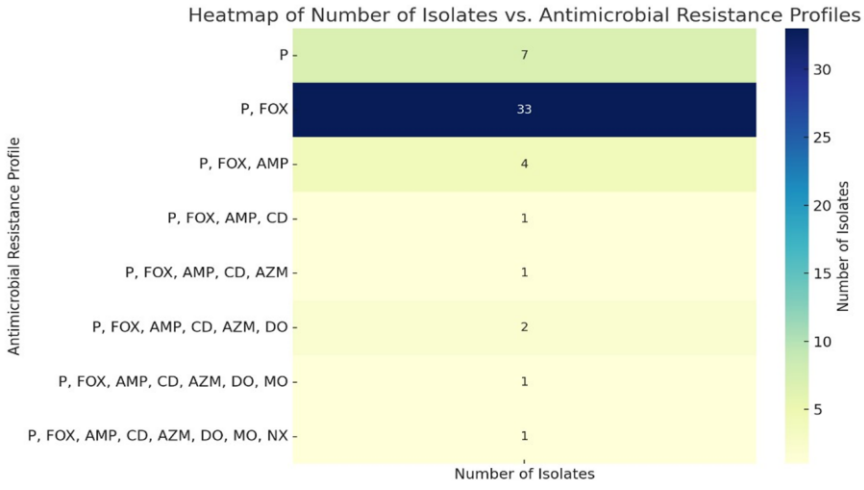

Fig.4. A heatmap illustrates the relationship between the *number of isolates* and their corresponding *antimicrobial resistance profiles.*

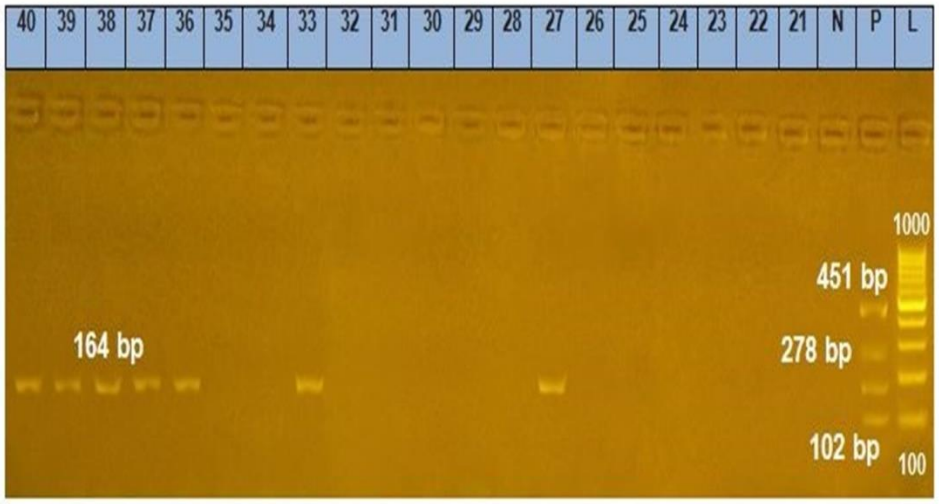

Fig. 5. PCR amplification products of the *SEB* gene at 164 bp, using agarose gel electrophoresis.
Lane L: 100 bp ladder as a molecular size DNA marker.
Lane P: positive control for *SEC* (451), *SED* (278), *SEB* (164), and *SEA* (102) genes,
Lane N: negative control.
Lanes 27, 33, 36, 37, 38, 39, and 40 Positive *S. aureus* strains for the *SEB* gene.

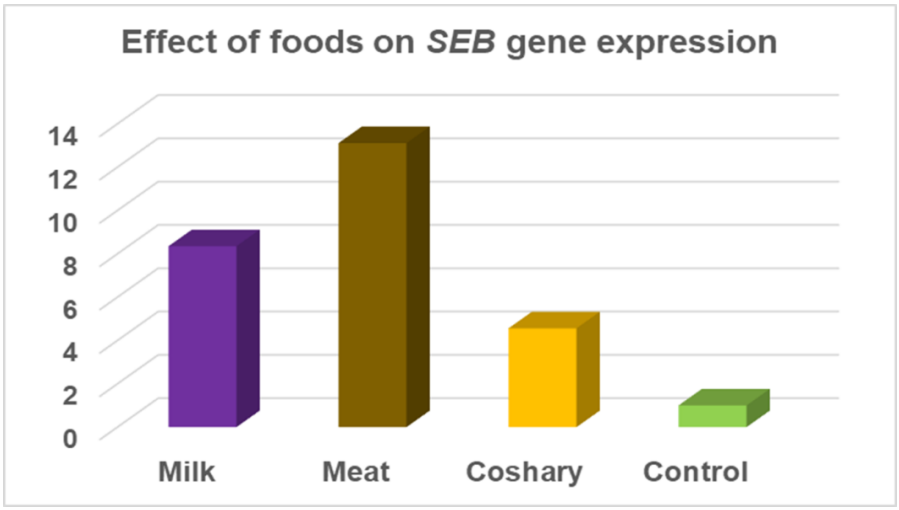

Fig. 6. Effect of different foods on the expression level of the enterotoxin *SEB* gene using RT-PCR.

References

1. Bhardwaj, N., Bhardwaj, S.K., Nayak, M.K., Mehta, J., Kim, K.H. and Deep, A. Fluorescent nanobiosensors for the targeted detection of foodborne bacteria. *TrAC Trends Anal. Chem.*, **97**, 120-135 (2017).
2. Kadariya, J., Smith, T.C. and Thapaliya, D. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. *Biomed. Res. Int.*, **2014**, 827965 (2014).
3. Etter, D., Schelin, J., Schuppler, M. and Johler, S. Staphylococcal enterotoxin C—An update on SEC variants, their structure and properties, and their role in foodborne intoxications. *Toxins*, **12**(9), 84 (2020).
4. Chaibenjawong, P. and Foster, S.J. Desiccation tolerance in Staphylococcus aureus. *Arch. Microbiol.*, **193**(2), 125-135 (2010).
5. Asao, T., Kumeda, Y., Kawai, T., Shibata, T., Oda, H., Haruki, K., Nakazawa, H. and Kozaki, S. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: Estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiol. Infect.*, **130** (1), 33-40 (2003).
6. Regenthal, P., Hansen, J.S., André, I. and Lindkvist-Petersson, K. Thermal stability and structural changes in bacterial toxins responsible for food poisoning. *PLoS ONE*, **12**(4), 0175989 (2017).
7. Uchiyama, T., Imanishi, K., Miyoshi-Akiyama, T. and Kato, H. Staphylococcal superantigens and the diseases they cause. In, *Comp. Sourcebook Bact. Protein Toxins*, Elsevier, 830-843 (2006).
8. Madsen, L.J.M. Toxins as weapons of mass destruction: A comparison and contrast with biological-warfare and chemical-warfare agents. *Clin. Lab. Med.*, **21**(3), 593-606 (2001).

9. Hennekinne, J.A., De Buyser, M.L. and Dragacci, S. Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiol. Rev.*, **36**(4), 815-836 (2012).

10. American Public Health Association (APHA). *Compendium of methods for the microbiological examination of food*, APHA Press, Washington DC (2011).

11. Food and Drug Administration (FDA). *Bacteriological Analytical Manual Online*, FDA, USA (2001).

12. Cruickshank, R. Medical Microbiology. *The Practice of Medical Microbiology*, Volume 2, Churchill Livingstone, London (1975).

13. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed., Lippincott Williams and Wilkins, Philadelphia (2000).

14. Lachica, R.V., Genigeorgis, C. and Hoeprich, P.D. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. *Appl. Microbiol.*, **21**(4), 585-587 (1971).

15. Clinical and Laboratory Standards Institute (CLSI), *Performance standards for antimicrobial susceptibility testing, CLSI supplement M100*, 30th ed., CLSI, Wayne, PA (2021).

16. Singh, A., Yadav, S., Singh, S. and Bharti, P. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Res. Int.*, **43**, 2027-2030 (2010).

17. Mehrotra, M., Wang, G. and Johnson, W.M. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J. Clin. Microbiol.*, **38**(3), 1032-1035 (2000).

18. Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.*, **29**(9), e45 (2001).

19. Yuan, J.S., Reed, A., Chen, F. and Stewart, C.N. Statistical analysis of real-time PCR data. *BMC Bioinformatics*, **7**, 85 (2006).

20. Mason, W.J., Blevins, J.S., Beenken, K., Wibowo, N., Ojha, N. and Smeltzer, M.S. Multiplex PCR protocol for the diagnosis of staphylococcal infection. *J. Clin. Microbiol.*, **39**(9), 3332-3338 (2001).

21. Gundogan, N., Citak, S., Yucel, N. and Devren, A. A note on the incidence and antibiotic resistance of Staphylococcus aureus isolated from meat and chicken samples. *Meat Sci.*, **69**(4), 807-810 (2005).

22. Gündoğan, N., Citak, S. and Turan, E. Slime production, DNase activity, and antibiotic resistance of Staphylococcus aureus isolated from raw milk, pasteurized milk, and ice cream samples. *Food Control*, **17**(5), 389-392 (2006).

23. Karmi, M. Food poisoning ability of Staphylococcus aureus isolated from meat products and poultry meat. *Assiut Vet. Med. J.*, **65**(162), 7-13 (2019).

24. Islam, N.N., Akter, M., Farzana, Z., Kader, A.J.B., Uddin, I., Siddiki, A.M.A.M.Z. and Kamaruddin, K.M. Detection of Staphylococcus aureus in frozen chicken rinse through bacteriological and nuc gene-specific PCR methods and their drug resistance patterns in Southern Chittagong, Bangladesh. *Res. J. Microbiol.*, **9**(5), 251-264 (2014).

25. Abdalrahman, L.S., Stanley, A., Wells, H. and Fakhr, M.K. Isolation, virulence, and antimicrobial resistance of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) strains from Oklahoma retail poultry meats. *Int. J. Environ. Res. Public Health*, **12**(6), 6148-6161 (2015).

26. Bhargava, K., Wang, X., Donabedian, S., Zervos, M., da Rocha, L. and Zhang, Y. Methicillin-resistant Staphylococcus aureus in retail meat, Detroit, Michigan, USA. *Emerg. Infect. Dis.*, **17**(6), 1135-1137 (2011).

27. Abd El Tawab, A., El-Hofy, F., Maarouf, A. and Mousa, D. Bacteriological and molecular studies on methicillin-resistant Staphylococcus aureus (MRSA) isolated from chicken meat and its products in Kaliobia Governorate. *Benha Vet. Med. J.*, **31**(1), 64-72 (2016).

28. Akbar, A. and Anal, A.K. Prevalence and antibiogram study of Salmonella and Staphylococcus aureus in poultry meat. *Asian Pac. J. Trop. Biomed.*, **3**(2), 163-168 (2013).

29. Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X. and Xue, L. Staphylococcus aureus isolated from retail meat and meat products in China: Incidence, antibiotic resistance, and genetic diversity. *Front. Microbiol.*, **9**, 2767 (2018).

30. Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K. and Price, L.B. Multidrug-resistant Staphylococcus aureus in US meat and poultry. *Clin. Infect. Dis.*, **52**(10), 1227-1230 (2011).

31. Sabouni, F., Mahmoudi, S., Bahador, A., Pourakbari, B., Sadeghi, R.H., Ashtiani, M.T.H., Nikmanesh, B. and Mamishi, S. Virulence factors of Staphylococcus aureus isolates in an Iranian referral children's hospital. *Osong Public Health Res. Perspect.*, **5**(2), 96-100 (2014).

32. Al-Jumaily, E.F., Saeed, N.M., Hussain, H. and Khanaka, H. Detection of enterotoxin types produced by coagulase-positive Staphylococcus species isolated from mastitis in dairy cows in Sulaimaniyah region. *Appl. Sci. Rep.*, **2**(1), 19-26 (2014).

33. Fooladi, A.I., Tavakoli, H.R. and Naderi, A. Detection of enterotoxigenic Staphylococcus aureus isolates in domestic dairy products. *Iran. J. Microbiol.*, **2**(3), 137 (2010).

34. Baz, A.A., Bakhiet, E.K., Abdul-Raouf, U. and Abdelkhalek, A. Prevalence of enterotoxin genes (SEA to SEE) and antibacterial resistant pattern of Staphylococcus aureus isolated from clinical specimens in Assiut city of Egypt. *Egypt. J. Med. Hum. Genet.*, **22**, 84 (2021).

35. Sihto, H.M., Tasara, T., Stephan, R. and Johler, S. Temporal expression of the staphylococcal enterotoxin D gene under NaCl stress conditions. *FEMS Microbiol. Lett.*, **362**(6), fnv024 (2015).

36. Budi Susilo, Y., Sihto, H.M., Rådström, P., Stephan, R., Johler, S. and Schelin, J. Reduced enterotoxin D formation on boiled ham in Staphylococcus aureus Δagr mutant. *Toxins*, **9**(9), 263 (2017).

37. Wang, H., Cai, L., Wang, H., Xu, X. and Zhou, G. Influence of protein and vitamin B2 as nutrients of chicken meat on staphylococcal enterotoxin genes expression via virulence regulators. *LWT*, **111**, 688-693 (2019).

38. Lin, L., Hu, J., Wu, Y., Chen, M., Ou, J. and Yan, W. Assessment of the inhibitory effects of sodium nitrite, nisin, potassium sorbate, and sodium lactate on Staphylococcus aureus growth and staphylococcal enterotoxin A production in cooked pork sausage using a predictive growth model. *Food Sci. Hum. Wellness*, **7**(1), 83-90 (2018).

39. Abd El Ghany, H.A.A., Zaki, M.B.A.H., Tolba, K.S. and Yassin, N.A. Staphylococcus aureus and enterotoxin A relative gene expression in beef meat after lactic acid treatment and storage at different temperatures. *Bulg. J. Vet. Med.*, **23**(3), 378-388 (2020).

40. Homsombat, T., Boonyayatra, S., Awaiwanont, N. and Pichpol, D. Effect of temperature on the expression of classical enterotoxin genes among staphylococci associated with bovine mastitis. *Pathogens*, **10**(8), 975 (2021).

تقييم مقاومة المضادات الحيوية وقدرات إنتاج السموم بين معزولات ستاف اوريوس المفصولة من عينات الأغذية

داليا إبراهيم شعبان [1]، احمد فرج عزمي [1]، نهلة أحمد شوقي أبوالروس[2] و محمد أحمد سباق محمد [1]

[1].قسم المكروبيولوجيا والمناعة كلية الصيدلة، جامعة بني سويف، مصر.

[2] قسم مراقبة الأغذية، مركز بحوث صحة الحيوان، مركز البحوث الزراعية فرع شبين الكوم، مصر.

الملخص

تعد معزولات ستاف اوريوس من أهم مسببات الأمراض المنقولة بالغذاء نظرًا لقدرته على إنتاج مجموعة واسعة من السموم المعوية المقاومة للحرارة. هدفت هذه الدراسة إلى استقصاء مدى انتشار العزلات المقاومة للمضادات الحيوية والمنتجة للسموم، والمعزولة من عينات لحوم الأبقار والدواجن المجمعة من مطاعم مختلفة في مصر خلال الفترة من يناير 2021 حتى مارس 2023 .أظهرت النتائج أن أغلب المعزولات (74%) تم الحصول عليها من لحوم الدواجن مقابل 26% من لحوم الأبقار. وقد بيّنت اختبارات الحساسية للمضادات الحيوية مقاومة عالية تجاه السيفوكسيتين (86%) والبنسلين (100%)، بينما كانت المقاومة منخفضة تجاه الدوكسيسيكلين (8%)، والمكسيفلوكساسين (4%)، والنورفلوكساسين (2%). كما كشفت الفحوص الجزيئية عن وجود جين **SEB** في 26% من العزلات، في حين لم يُرصد وجود الجينات الأخرى محل الدراسة مثل **SEC** و **SED** و **SEA.** وعند تقييم تأثير أنواع مختلفة من الأغذية على التعبير الجيني للسم المعوي **SEB** باستخدام تقنية تفاعل البلمرة المتسلسل في الزمن الحقيقى **(RT-PCR)**، تبين أن اللحوم كانت الأكثر تأثيرًا على مستوى التعبير الجيني، تلاها الحليب ثم طبق الكشري (أحد الأطباق المصرية التقليدية الغنية بالكربوهيدرات) .تخلص الدراسة إلى أن إنتاج السموم المعوية والمقاومة للمضادات الحيوية لدى ***S. aureus*** قد يمثل خطورة بالغة على الصحة العامة وصحة المستهلك. وعليه، فإن تطبيق الممارسات التصنيعية والصحية الصارمة، بما في ذلك الرقابة على المواد الخام، الممارسات التصنيعية والصحية للأغذية، والتطهير الجيد والامن للمعدات والادوات والاسطح الملامسة للغذاء، والاهتمام بالنظافة العامة والشخصية يُعَد أمرًا ضروريًا للحد من التلوث ومنع حدوث حالات التسمم بميكروبات ستاف اوريوس.

الكلمات الدالة: مقاومة المضادات الحيوية، السموم المعوية، مسببات الأمراض الغذائية، تفاعل البلمرة المتسلسل في الزمن الحقيقى، المكورات العنقودية الذهبية.