10.21608/avmj.2025.338696.1488 Assiut University web-site: www.aun.edu.eg

COMPREHENSIVE ASSESSMENT OF *STAPHYLOCOCCUS AUREUS* CONTAMINATION IN RAW MILK: OCCURRENCE, ANTIBIOTIC RESISTANCE MECHANISMS, ENTEROTOXIN GENE MARKERS AND PUBLIC HEALTH RISKS

PASSANT A. ELHENAWY[1*], ADEL M. SAUDI[1], SAMAH F. DARWISH[2], TAREK N. SOLIMAN[3] AND ZEINAB I. ALI[1]

[1]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

[2]Biotechnology Research Unit, Animal Reproduction Research Institute (ARRI), Agricultural Research Center (ARC), Giza, 65221, Egypt

[3] Dairy Department, Food Industries and Nutrition Research Institute, National Research Center, El-Buhouth St., Dokki, Cairo 12622, Egypt

*Corresponding Author:Passant Adel, Phone: 01002943312; E-Mail: Passantadel259@gmail.com

Received: 10 December 2024; **Accepted:** 12 January 2025

ABSTRACT

Staphylococcus aureus has evolved as one of the most outstanding foodborne pathogens. As a member of the ESKAPE group, it can "escape" the antimicrobial effects of biocidal agents, posing a global public health threat. Hence, the current research was designed to investigate the presence of *Staph. aureus* isolated from raw milk samples and evaluated their antibiotic resistance and enterotoxin genes. Fifty raw milk samples were randomly purchased from dairy farms, small holders, dairy shops and street vendors located in Cairo, Giza and Qalyubia governorates, Egypt to highlight the contamination at farm level and after distribution. *Staph. aureus* isolates were identified using biochemical and molecular methods. Antibiotic resistance was determined against 10 antibiotics, using the phenotypic disc diffusion assay and genotypically. Furthermore, 2 staphylococcal enterotoxin genes (*SEA* and *SEC*) were screened. The obtained results indicated that the mean counts of *Staph. aureus* was 1.9±0.2, 2.2±0.3, 1.6±0.3 and 2.9±0.2 $\log_{10}$ cfu/ml, among the raw milk samples from dairy farms, small holders, dairy shops and street vendors, respectively. Additionally, Multi-drug resistance (MDR) was exhibited in 50% of the isolates, and 78.95% were methicillin-resistant *Staph. aureus* (MRSA). Moreover, high resistance to penicillin (62.5%), cefoxitin (59.38%) and tetracycline (56.25%) was observed. A percentage of 28.1٣% of *Staph. aureus* strains were having enterotoxin genes, out of them 21.9% and 6.25% harbored *SEA* and *SEC* genes, respectively. Concurrently, the obtained findings advocate for the implementation of surveillance programs to ensure effective preventive measures starting at the farm level.

Keywords: *Staph. aureus***,** antibiotics resistance, enterotoxins, farms, MRSA.

INTRODUCTION

Milk plays an essential role in providing humans with their nutrient requirements, as it is rich in nutritional components, such as proteins, lactose, fat, minerals, vitamins and water. Milk, thus, provides an optimal environment for the proliferation of a wide range of spoilage and pathogenic microorganisms (Gajewska *et al.*, 2023; Hassan and Ali, 2024).

Staph. aureus is considered a versatile virulent opportunistic Gram-positive pathogen. It constitutes a part of normal microflora of mucous membrane and skin

Corresponding author: PASSANT A. ELHENAWY
E-mail address: Passantadel259@gmail.com
Present address: Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt

of humans and animals (Naz *et al.,* 2020). This pathogen induces a diversity of infections that range from mild superficial skin lesions to severe life-threatening illness. It is regarded as a significant cause of mastitis in dairy animals and causes prominent economic losses to the dairy sector globally. It necessitates a particular focus from farmers living in low-income nations, attributable to numerous losses, including diminished milk output, milk condemnation owing to antibiotic residues, veterinary expenses, and the culling of chronically diseased animals (Fenta *et al.,* 2023). Additionally, *Staph. aureus* is a prevalent cause of bacterial food poisoning outbreaks marked by abrupt onset of symptoms, including vomiting, stoma-chache and nausea (Rajkovic *et al.,* 2020). The presence of *Staph. aureus* in raw milk poses a threat to food consumers (Pierezan *et al.,* 2022). The existence of such bacteria may be attributed to insufficient heat treatment, poor handling practices, contaminated equipment, bad storage and transport conditions (Chimuti *et al.,* 2024).

The aptitude of the bacteria to cause an array of infections is most likely attributed to the production of various enzymes and toxins as virulence factors, such as Staphylococcal enterotoxins (SEs), Panton-Valentine leukocidin (PVL), hemolysins, toxic shock syndrome toxin-1 (TSST-1) and exfoliative toxins. Staphylococcal enterotoxins (SEs) are known to induce 95% of *Staph. aureus*-associated food poisoning (SFP) in humans. They are categorized into 5 major standard types: *SEA, SEB, SEC, SED* and *SEE*. The other 5% of the outbreaks are caused by recently recognized SEs (*SEG-SEI, SEIJ, SER-SET, SEIQ* and *SEIU-SEIV*). They are stable and exhibit significant resistance to elevated temperatures and most proteolytic enz-ymes, including pepsin and trypsin. The minimal infective dose of SE is 1 µg, which occurs when *S. aureus* levels in food exceed 10^5 cfu/ml or g. Ingesting 100-200 ng of SE may induce SFP in vulnerable individuals (Ahmed *et al.,* 2019a; Zhao *et al.,* 2021; Aguiar *et al.,* 2024).

As *S. aureus* acts as the principal cause of mastitis (Ghimpețeanu *et al.,* 2022; Bacanlı, 2024). Antibiotics have been the mainstay of treatment for decades. However, the extensive and empirical use of antibiotics, in addition to unawareness of the advisable withdrawal periods, will result in the alterations of the gut microbiota and expose milk and dairy products to pollution by antimicrobial drug residues, which results in the emergence of antimicrobial resistant (AMR) bacterial strains (Ghimpețeanu *et al.,* 2022; Bacanlı, 2024).

Antimicrobial resistance (AMR) represents a substantial challenge to global public health and development. In 2019, bacterial antimicrobial resistance was linked to approximately 1.27 million deaths world-wide and contributing to an additional 4.95 million fatalities. Projections indicate that by 2050, this number could escalate to 20 million, with an economic impact exceed-ing $2.9 trillion (WHO, 2023). The emergence of resistance is a multifaceted and intricate process, involving the gradual development of increasingly effective resistance genes via genetic alterations, frequently accompanied by their dissemination across populations through various mobile genetic elements (Christaki *et al.,* 2020).

Prior to the discovery of penicillin in the early 1940s, patients had severe staphylococcal infections. However, several years later, the first penicillin-resistant strain of *S. aureus* was identified (Lade and Kim, 2023). Shortly after, multiple resistant strains were discovered, and in the ensuing years, there has been an increasing incidence of different *S. aureus* strains exhibiting resistance to multiple drugs, including methicillin-resistant S. aureus (MRSA) (Larsen et al., 2022). Determining anti-microbial resistance genes (ARGs) becomes useful to observe

and evaluate the pathogenic capacity of *Staph. aureus* (Hodille *et al.*, 2017). Moreover, less harmful bacteria harboring ARGs can be disseminated to other pathogens by horizontal gene transfer (HGT) which raises the latter group's antibiotic resistance. The occurrence of HGT relies on multiple circumstances, nonetheless, the physical proximity of bacteria always elevates the likelihood. Additionally, HGT is even more likely if ARGs are situated on mobile genetic elements (as integrative conjugative elements (ICEs) or plasmids) (Tóth *et al.*, 2020).

Recently, minimizing the food safety risks associated with raw milk considered mandatory through the existence of a comprehensive system of control measures commencing with safe animal feed, encompassing effective farming methods and on-farm controls, extending to good manufacturing practices (GMPs), consumer safety awareness, and effective implementation of food safety manag-ement systems across the supply chain (Owusu-Kwarteng *et al.*, 2020).

Thus, the current research intended to assess the incidence and count of *Staph. aureus* in raw milk collected from dairy farms, small holders, dairy shops and street vendors located in Cairo, Giza and Qalyubia to highlight the contamination at farm level and after distribution. Additionally, antibiotic resistance was assessed both phenotypically and genotypically to evaluate the prevalence of MRSA and MDR *Staph. Aureus*; in addition to the genotypic identification of enterotoxigenic *Staph. aureus* strains and their enterotoxins.

MATERIALS AND METHODS

Samples collection:

A total of 50 raw milk samples were randomly purchased from Cairo, Giza and Qalyubia governorates, Egypt, under complete sanitary condition from different sources, including dairy farms, small holders, dairy shops, and street vendors, and transported to the laboratory in an insulated icebox (4^{o} C) with minimum delay to be immediately examined.

Guaiac test:

Guaiac test was performed to ensure that the raw milk samples were not subjected to any heat treatments above 80^{o} C (Schonberg, 1956).

Enumeration and characterization of *Staph. aureus:*

Preparation of serial dilutions of the milk samples were performed according to ISO 6887-5 (2020). Total Staphylococci count was enumerated according to the standard method (ISO 6888-1:2021). Briefly, 0.1 ml of the previously prepared serial dilutions was inoculated on Baird Parker agar media (HiMedia, MU043) and incubated at 35° C±2° C for 48 h. Typical morphological colonies of *Staph. Aureus had a black* and shiny appearance with narrow white margins and surrounded by a clear zone. They were isolated and identified by conventional methods, which involved Gram-staining and biochemical reactions (coagulase, DNase agar, heat stable nuclease, catalase, mannitol fermentation, oxidase, urease, nitrate reduction, hemolysis, fibrinolysin & hyaluronidase) according to Vos *et al.* (2011).

Antibiotic susceptibility testing (Phenotypic by Disc Diffusion Assay (DDA):

All the biochemicals identified *Staph. aureus* isolates (32 isolates) were evaluated for antibiotic susceptibility to 10 antibiotics utilizing the Kirby-Bauer disc diffusion method, as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). The subsequent antibiotic discs (Oxoid, UK); Gentamycin (10 µg/disc), Chloramphenicol (30 µg/disc), Penicillin G (10 units/disc), Meropenem ((10 µg/disc), Cefoxitine (30

µg/disc), Clindamycin (2 µg/disc), Erythromycin (15 µg/disc), Linezolid (30 µg/disc), Levofloxacine (5 µg/disc) and Tetracycline (30 µg/disc) were placed on Müller-Hinton agar (Oxoid, UK) inoculated with the isolates of *Staph. aureus* after matching bacterial suspension to 0.5 MacFarlands standard, then the plates were incubated at 37^{o} C for 18-24 h. The results were represented as resistant, intermediate and sensitive depending on the zone of inhibition diameter recorded by the CLSI guidelines (CLSI, 2020).

Genotypic analysis (Identification of antibiotic-resistance and enterotoxin genes):

According to Darwish and Asfour (2013), a quick boiling process was used to produce crude DNA from bacterial strains. The *Staph. aureus* isolates were taken off the mannitol salt agar plate (HiMedia, M118) and dispersed in 200 µl of lysis buffer (1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH 8.0), and one mM EDTA). To remove bacterial debris, the solution was centrifuged for 5 min after boiling for 10 min. Five microliters of the supernatant were immediately utilized for PCR amplification after being aspirated.

First, the isolates were verified using the specific 16s rRNA gene. Then, the confirmed isolates were screened for the presence of antibiotic resistance genes (*blaZ, mecA, ermA, ermC, aac (6')-aph (2')* and *tetM*) and *Staph. aureus* enterotoxin genes (*SEA* and *SEC*) (Table A). PCR was carried out in a 20 µl reaction volume containing 5 µl of template DNA, 20 picomol of each primer, and 1X of PCR master mix (Dream Taq Green PCR Master Mix, Fermentas Life Science).

Table A: Primers used in the genotypic analysis.

PCR	Genes	Target gene	Microbial strain	Primer sequence 5'-3'	PCR product	References
mPCR I	*Staph. aureus* specific genes	16S rRNA gene	*Staph. aureus*	F: GTAGGTGGCAAGCGTTATCC R: CGCACATCAGCGTCAG	228	Monday and Bohach (1999)
		Nuc gene	*S. aureus*-specific sequence	F: GCGATTGATGGTGATACGGTT R:AGCCAAGCCTTGACGAACTAAAGC	279	Asfour and Darwish (2014)
mPCR II	Antibiotic resistance genes	*blaZ* gene	β-Lactamase *Staphylococci*	F: AAGAGATTTGCCTATGCTTC R: GCTTGACCACTTTTATCAGC	517	Vesterholm-Nielsen *et al.* (1999)
		mecA gene	Methicillin-resistant *Staphylococci*	F: GTGAAGATATACCAAGTGATT R: ATGCGCTATAGATTGAAAGGAT	147	Zhang *et al.* (2005)
mPCR III		*ermA* gene	Erythromycin resistant *Staphylococci*	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190	Strommenger *et al.* (2003)
		ermC gene	Erythromycin resistant *Staphylococci*	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299	
mPCR IV		*aac (6')-aph (2')* gene	Gentamycin resistant *Staphylococci*	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	
		tetM gene	Tetracycline resistant *Staphylococci*	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	
mPCR V	Enterotoxin genes	*SEA* gene	Staphylococcal enterotoxin A	F: TAAGGAGGTGGTGCCTATGG R: CATCGAAACCAGCCAAAGTT	120	Cremonesi *et al.* (2005)
		SEC gene	Staphylococcal enterotoxin C	F: GACATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	257	da Cunha *et al.* (2006)

Initial denaturation occurred at 94° C for 4 min, succeeded by 35 cycles of 94° C for 60 s, 55° C for 60 s, and 72° C for 60 s. A final extension step of 10 min at 72° C was completed. Amplification products were electrophoresed at 70 volts for 70 min in a 1.5% agarose gel containing 0.5X TBE and examined under ultraviolet light.

Statistical analysis:

The statistical analysis was conducted using the Statistical Program for Social Science (SPSS Inc., Chicago, IL, USA), version 27. Chi square was used to evaluate the correlation between phenotypic and genotypic antimicrobial resistance. The percentages of phenotypic and genotypic discrepancies were calculated with the formula given by Rasheed *et al.* (2023). All experiments were duplicated, and results were demonstrated as mean ±SEM. A *P*-value < 0.05 was deemed statistically significant.

RESULTS

Table ١: *Staphylococci* count in the examined raw milk samples (N=50).

Samples source	Total *Staphylococci* count (log_{10} cfu/ml)			*Staph. aureus* count (log_{10} cfu/ml)			No. of acceptable samples based on E.S. (2005)	No. of *Staph.* isolates	No. of +ve Coagulase, +ve TNase *S. aureus* isolates
	Positive samples		Mean ±SEM	Positive samples		Mean ±SEM			
	No.	%		No.	%				
Dairy farms	15	100%	4.8±0.2*	7	46.7 %	1.9±0.2*	8 (53.3%)	45	6 (13.3%)
Smallholders	10	100%	5.4±0.1*	6	60%	2.2±0.3*	4 (40%)	45	11 (24.4%)
Dairy shops	15	100%	4.6±0.2*	4	26.7 %	1.6±0.3*	11 (73.3%)	45	3 (6.7%)
Street vendors	10	100%	6.02±0.1*	8	80%	2.9±0.2*	2 (20%)	45	12 (26.7%)
Total	**50**	**100 %**		**25**	**50%**		**25 (50%)**	**180**	**32 (17.78%)**

N: Total number of the samples; No.: Number of the positive samples; SEM: Standard error of mean; E.S.: **Egyptian Standards (ES: 154-1/2005)**; *: Indicates presence of significant difference (*P*<0.05).

Table ٢: Phenotypic antimicrobial resistance analysis of *Staph. aureus* isolates (N=32).

Antimicrobial class	Antimicrobial agent	Dose	R	I	S
			(N)%	(N)%	(N)%
Aminoglycoside	Gentamycin	10 µg	(6)18.75	(6)18.75	(20)62.5
Amphenicol	Chloramphenicol	30 µg	(5)15.6	0	(27)84.4
Penicillin (β-lactam)	Penicillin	10 units	(20)62.5	0	(12)37.5
Carbapenems	Meropenem	10 µg	(7)21.9	0	(25)78.1
Cephalosporin (β-lactam)	Cefoxitine	30 µg	(19)59.38	(4)12.5	(9)28.12
Lincosadmide	Clindamycin	2 µg	(2)6.25	(2)6.25	(28)87.5
Macrolides	Erythromycin	15 µg	(8)25	(6)18.75	(18)56.25
Oxazolidinones	Linezolid	30 µg	0	0	(32)100
Quinolons	Levofloxacine	5 µg	(2)6.25	0	(30)93.75
Tetracyclines	Tetracycline	30 µg	(18)56.25	(5)15.63	(9)28.12

R: Resistant; I: Intermediate; S: Sensitive; N: Number of isolates.

Table 3: Occurrence of MDR* and XDR** among *Staph. aureus* isolates.

	***Staph. aureus* isolates (N=32)**	**MRSA (N=19)**
	(N)%	**(N)%**
MDR	(16)50	(15)78.95
XDR	(1)3.13	0

*MDR: multidrug-resistant isolates which showed resistance to a minimum of 3 antibiotic classes; **XDR: extensively drug-resistant isolates which were resistant to 9 antibiotics tested; MRSA: Methicillin-resistant *Staph. aureus*; N: Number of isolates.

Table 4: Correlation between the antimicrobial resistance phenotypes and genotypes among the *Staph. aureus* isolates.

Antimicrobial agent	**Targeted gene**	**n-Pr[a]**	**n-Gp[b]**	**P+/G-[c]**	**P-/G+[d]**	**Phi coefficient[e]**	***P* value[f]**
Penicillin	*blaZ*	20	19	1	0	0.936	**<0.001**
Cefoxitin	*mecA*	19	5	15	1	0.181	0.307
Erythromycin	*ermA*	8	11	5	3	0.698	**<0.001**
	ermC	8	13	3	5	0.798	**<0.001**
Tetracycline	*tetM*	18	14	4	0	0.778	**<0.001**
Gentamycin	*aac (6')-aph (2')*	6	12	0	6	0.620	**<0.001**

[a]n-Pr: Number of isolates showing resistance to the specified antimicrobial agent; [b]n-Gp: Number of isolates harboring the specific resistance gene; [c]P+/G−: Number of isolates that are phenotypically resistant (P+) but do not possess the resistance gene (G−) for the antimicrobial; [d]P−/G+: The number of phenotypically susceptible isolates (P−) that carry one or more resistance genes (G+); [e]Phi coefficient value of 1 indicates a strong positive correlation, while 0 suggests no correlation, the highest correlation observed is marked in **bold**; [f]P values < 0.05 indicate significant correlation are highlighted in **bold**.

Table 5: Discrepancies between phenotypic and genotypic identification methods of MRSA and MSSA* strains.

***Staph. aureus* strains**	**Targeted gene**	**Resistance pattern N(%)**			**Discrepancies%**		**Ratio[c]**
		Phenotypic (n=32)	**Genotypic**		**Phenotypic**	**Genotypic**	
			P[a]	**N[b]**			
MRSA	*mecA*	19 (59.38)	4 (12.5)	15 (46.88)	78.9	7.6	10.4
MSSA		9 (28.12)	1 (3.13)	8 (25)			

*MSSA: methicillin-sensitive *Staph. aureus*; [a]P: Genotypic positive isolates out of phenotypic resistant/sensitive *Staph. aureus* isolates; [b]N: Genotypic negative isolates out of phenotypic resistant/sensitive *Staph. aureus* isolates; [c]Ratio = Phenotypic discrepancies / Genotypic discrepancies.

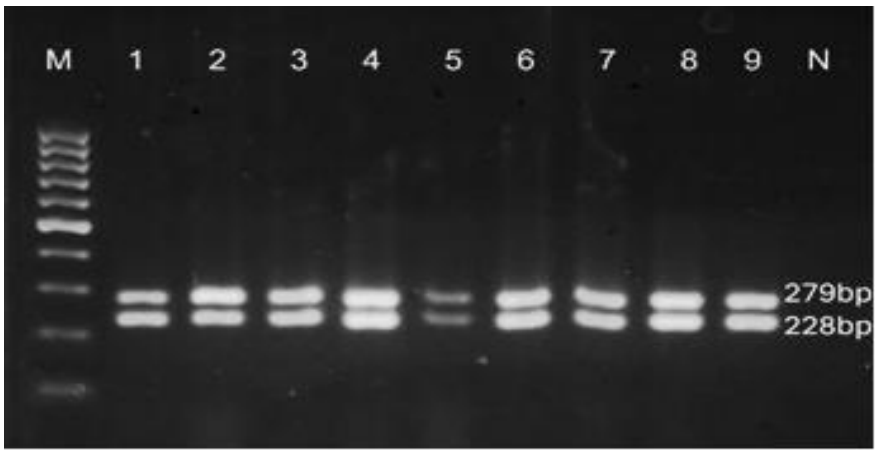

Fig. 1: Agarose gel electrophoresis of PCR amplification for 16srRNA gene (228 bp) and *Nuc* gene (279 bp) to identify *Staph. aureus.*
Lane M: 100 bp DNA ladder.
Lanes 1 to 8: Positive isolates for 16srRNA and *Nuc* genes.
Lane 9: Control positive.
Lane N: Control negative.

A **B**

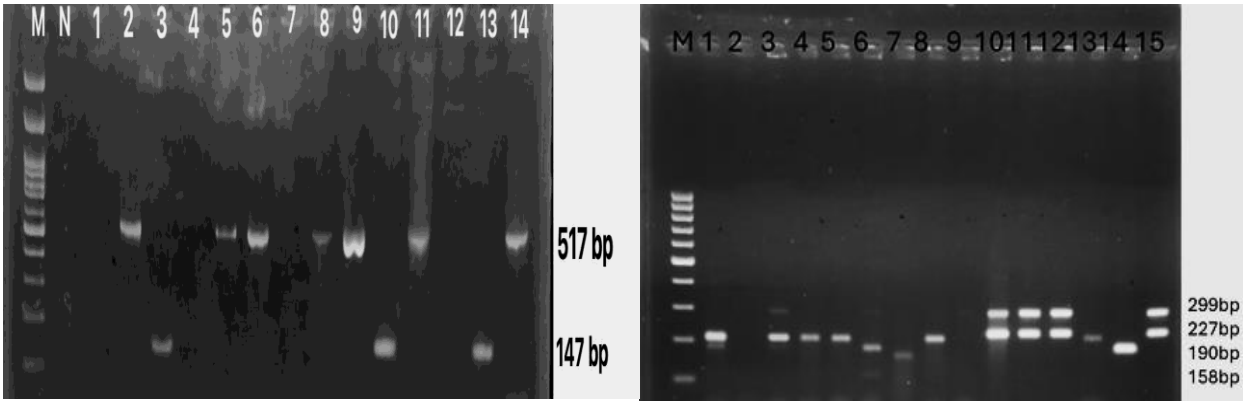

Fig. 2: Agarose gel electrophoresis for antibiotic resistance genes.
(A) Multiplex PCR for *blaZ* (517 bp) and *mecA* (147 bp):
Lane M: 100 bp DNA ladder.
Lane N: Control negative.
Lanes 1, 4, 7 and 12: Negative isolates.
Lanes 2, 5, 6, 8, 9, 11 and 14: Positive isolates for *blaZ* gene.
Lanes 3, 10 and 13: Positive isolates for *mecA* gene.

(B) Multiplex PCR for *ermC* (299 bp), *aac (6')-aph (2')* (227 bp), *ermA* (190 bp) and *tetM* (158 bp):
Lane M: 100 bp DNA ladder.
Lane 1, 4, 5, 8 and 13: Positive isolates for *aac (6')-aph (2')* gene.
Lane 2: Control negative.
Lane 3: Positive isolates for *ermC* and *aac (6')-aph (2')* genes.
Lane 6 and 14: Positive isolates for *ermA* gene.
Lane 7: Positive isolate for *tetM* gene.
Lanes 9: Negative isolate.
Lane 10, 11, 12 and 15: Positive isolates for *ermC* and *aac (6')-aph (2')* genes.

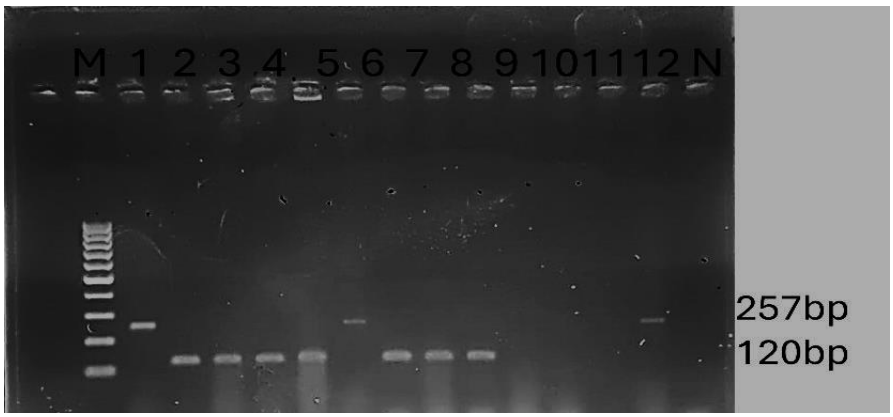

Fig. 3: Agarose gel electrophoresis of PCR amplification for enterotoxin genes, *SEA* gene (120 bp) and *SEC* gene (257 bp).
Lane M: 100 bp DNA ladder.
Lane N: Negative control.
Lanes 1, 6 and 12: Positive isolates for *SEC* gene.
Lanes 2, 3, 4, 5, 7, 8 and 9: Positive isolates for *SEA* gene.
Lanes 10 and 11: Negative isolates.

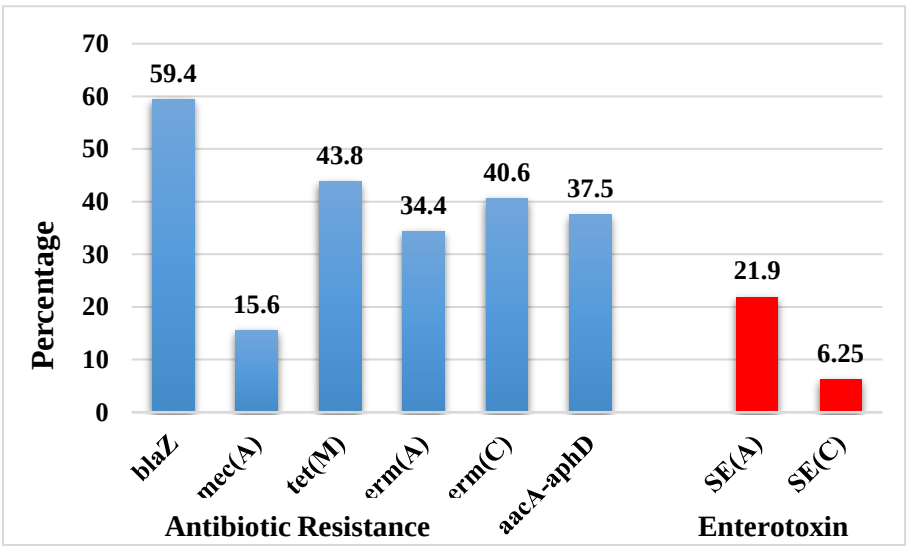

Fig. 4: Genotypic prevalence of virulent genes

DISCUSSION

Staph. aureus is a prominent cause of foodborne diseases worldwide. Milk and dairy products are recognized as potential sources of *Staph. aureus* contamination, originating from cows afflicted with mastitis or food handlers carrying the bacteria because of inadequate personal hygiene. Consequently, assessing the prevalence of staphylococci and *Staph. aureus* is essential to prevent their transmission through the food chain by applying suitable preventative measures (Sadat *et al.*, 2022).

In the current study, the collected raw milk samples from dairy farms, small holders, dairy shops, and street vendors were tested for presence of *Staph. aureus*. The obtained results in Table ١ indicated that all the examined samples were contaminated with staphylococcal species, where the milk samples collected from street vendors recorded the highest mean (6.02±0.1 $\log_{10}$ cfu/ml). The significant contamination of samples is likely

attributed to unclean practices during milking and improper storage and handling. However, this could also be attributed to bacteria shedding from infected mammary glands of dairy animals. Disparities between countries and even between regions in the same country can arise from different sample sizes and hygiene standards.

The *Staph. aureus* counts varied across different sources, including dairy farms, smallholders, dairy shops and street vendors, with mean counts of 1.9±0.2, 2.2±0.3, 1.6±0.3 and 2.9±0.2 $\log_{10}$ cfu/ml, respectively, with a significant difference (P <0.05) (Table 1). In the dairy farms, 53.3% of the samples complied with the Egyptian standard (E.S. 2005), while smallholders showed a lower compliance rate of 40%. The examined dairy shops exhibited the highest compliance at 73.3%, whereas street vendors had the lowest, with only 20% of the samples meeting the Egyptian Standard (2005) (Table 1). This contrasts with the findings of Abdel-Wahab *et al.* (2024), who reported that all the tested samples exceeded the Egyptian standard. The 32 biochemically identified *Staph. aureus* isolates (Table 1) from different sampling sources possessed the *nuc* gene (Fig. 1) were tested to evaluate their susceptibility against a panel of 10 antimicrobial agents (Table 2).

Table (3) demonstrated that 16 strains (50%) showed resistance to at least 3 antibiotic classes and categorized as MDR and 1 strain (1/32, 3.13%) showed resistance to 9 antibiotics tested and categorized as extensively drug-resistant (XDR) (Magiorakos *et al.,* 2012; Shahzad *et al.,* 2024). Moreover, 78.95% of the MRSA isolates (15/19) were MDR. The elevated levels of antibiotic resistance, particularly in the MRSA isolates, were corroborated by other studies (El-Ashker *et al.,* 2020; Ali *et al.,* 2021), which highlighted the potential public health threat posed by MRSA in milk and emphasized the clinical significance of antibiotic susceptibility testing and ongoing monitoring of MRSA in dairy farms.

As per CLSI guidelines (CLSI, 2020), any *Staph. aureus* isolate exhibiting resistance to penicillinase-stable penicillins (e.g. oxacillin) or carrying the *mecA* gene must be classified as methicillin (oxacillin)-resistant *Staph. aureus* and deemed resistant to further β-lactam drugs. Cefoxitin is utilized to identify methicillin-resistance; nevertheless, the gold standard is the molecular identification of the *mecA* gene. For *mecA* screening, only isolates pocess a cefoxitin inhibition zone smaller than 22 mm were considered. Table (2) shows that Staph. aureus isolates were highly resistant to penicillin, cefoxitin and tetracycline, accounting for 62.5%, 59.38% and 56.25%, respectively (CLSI, 2020). These results were similarly consistent with findings of Algammal *et al.* (2020) and Abdeen *et al.* (2021). On the contrary, a study reported that all *Staph. aureus* isolates were susceptible to cefoxitin (Zeinhom and Abed, 2021).

Furthermore, it was discovered that all *Staph. aureus* strains were resistant to a minimum of one β-lactam drug. One possible cause of the elevated rate of β-lactams resistance in small milk-producing units is the extensive use of β-lactams; in addition to lack of veterinary supervision in these units. Consequently, β-lactams are no longer effective in curing *Staph. aureus* infections. Moreover, these units are typically excluded from regular national veterinary monitoring programs (Abebe and Birhanu, 2023).

The resistance of *Staph. aureus* isolates to erythromycin and clindamycin were 25% and 6.25%, respectively (Table ٢). These results concur with the findings obtained by Diab *et al.* (2021) and Fikry *et al.* (2021). However, a higher resistance for erythromycin (56.7%) was detected by Taalat *et al.* (2023). Additionally, Abdeen *et al.* (2021) found that 23.5% of isolates showed resistance to gentamycin, which is

consistent with the obtained result as 18.75%.

The present findings found that 15.6% of the isolates were resistant to chloramphenicol (Table 2). The results matched those of Abd El-Halem *et al.* (2019), who found that 13.8% of isolates were resistant to chloramphenicol. On the other hand, Abo-Shama *et al.* (2022) reported higher resistance to chloramphenicol (59.7%).

The current study indicates 93.75% of the *Staph. aureus* isolates were susceptible to levofloxacin, nearly like Sallam *et al.* (2023), who reported 100% sensitivity to levofloxacin. The current study's finding on linezolid sensitivity was consistent with Abd El-Halem *et al.* (2019). On the contrary, 50% of resistance was detected by Ahmed *et al.* (2020).

The results of the present study underlined the need for strict implementation of good hygienic practices during milking and proper storage of raw milk to control the spread of resistant *Staph. aureus*, particularly MRSA, which pose a significant public health threat.

The initial identified resistance mechanism of *Staph. aureus* to penicillin was carried out by the *blaZ* gene, which encodes β-lactamase (penicillinase). Production of this primarily extracellular enzyme occurs when staphylococci interact with β-lactam antibiotics, hydrolyze the β-lactam ring, thus inactivating the β-lactam (Ivanovic *et al.*, 2023).

Methicillin-resistance was acquired by incorporating a movable genetic element, staphylococcal chromosomal cassette *mec* (*SCCmec*). This genetic element carries the *mec* genes *mecA* (*mecA*-MRSA) and *mecC* (*mecC*-MRSA), which encode a surrogate penicillin-binding protein (PBP2a). The *mec* genes confer resistance to most β-lactam antibiotics in MRSA strains (Venla *et al.*, 2023).

In the present investigation, a genotypic analysis was performed to confirm the resistance profile of the isolates. Antibiotic resistance genes, including *blaZ* (59.4%), *tetM* (43.8%), *ermA* (34.4%), *ermC* (40.6%) and *aac (6')-aph (2')* (37.5%), were identified in consistency with the phenotypic profile (Qu *et al.*, 2019; El-Ashker *et al.*, 2020; Abdeen *et al.*, 2021).

According to the results obtained in Table (4), only 5 out of the 19 (26.32%) of the phenotypically identified MRSA isolates (5 out of the 32 of the total isolates, 15.63%) harbor the mecA gene. This may be ascribed to methicillin-resistance being expressed phenotypically in these isolates by other resistance genes that weren't examined in the current study. This finding agreed with a study by Ahmed *et al.* (2019)[b], who proposed minimal burden of the *mecA* gene in MRSA isolates. Slightly higher results were observed by Selim *et al.* (2022), who identified 35.7% MRSA in bovine milk in the PCR assay targeting the *mecA* gene. However, Taalat *et al.* (2023) found all strains carried the mecA gene.

Table 4 cleared the correlation between phenotypic and genotypic antimicrobial resistance. Significant correlations (Phi coefficient near 1, *P* value <0.05) were found between most antibiotic-resistant phenotypes and genotypes among the investigated *Staph. aureus* isolates. Stronger correlation was observed between penicillin antibiotic resistance and *blaZ* gene, erythromycin and *erm* genes, tetracycline and *tetM* genes and finally gentamycin and *aac (6')-aph (2')* genes with *P* value <0.001, On the other hand, no significant correlation was found between cefoxitin antibiotic resistance and *mecA* gene. Discrepancies between phenotypic and genotypic identification methods of MRSA and MSSA strains were represented in Table (5). A high discrepancy ratio (10.4%) between phenotypic and genotypic discrepancies indicated that multiple genes and

resistance mechanisms contributed to the resistance observed in the isolates (Ballhausen *et al.*, 2014; Rasheed *et al.*, 2023). Discordance between phenotypic and molecular detection of methicillin-resistance was previously reported, and may be attributed to many reasons, including the presence of other intrinsic factors that may contend with the *mecA* gene in producing resistance phenomena, such as the *mecC* gene, a homolog of the *mecA* gene, was recently recognized. Moreover, the production of modified intrinsic PBPs with altered affinity for methicillin, increasing the production of β-lactamase, and the drawback of disk diffusion assays to identify *mecA*-negative strains as resistant results (Ahmed *et al.*, 2019[b]; Darwish and Bakheet, 2019).

For deep insights into the virulence of *Staph. aureus* strains, the results detected the genes encoding staphylococcal enterotoxins. *SEA* is the enterotoxin most frequently associated with SFP and produced by strains of human origin (human strains), while *SEC* is associated with animal contamination (Şanlıbaba, 2022). The obtained findings (Fig. 4) revealed that 9 out of the 32 (28.13%) identified *Staph. aureus* isolates from the examined milk samples were enterotoxic. The *SEA* and *SEC* genes were detected in 21.9% and 6.25% of *Staph. aureus* isolates, respectively. Similar results were obtained by Algammal *et al.* (2020), who found 26.6% of strains were positive for the *SEA* gene and 6.7% were positive for the *SEC* gene, while Ahmed *et al.* (2019a) did not detect the SEA gene in market milk samples.

The obtained results indicated the significant importance of profiling *Staph. aureus* virulence. Multiple resistances to antibiotics highlighted the urgent need for novel natural antimicrobial agents to combat *Staph. aureus* infections. The presence of enterotoxigenic *Staph. aureus* also poses a public health risk. Thus, implementing proper hygienic measures and thoroughly heating of milk and dairy products can effectively control this pathogen.

CONCLUSION

The achieved results concluded that the copious majority of the isolates had a concerning antibiotic resistance pattern, particularly MRSA isolates, which were multi-drug resistant. In addition to the presence of a large proportion of antibiotic resistance genes, whose incidence of transmission to other pathogens via horizontal gene transfer can be studied through further investigations. Therefore, it is recommended to raise the awareness about the essential practices for maintaining hygienic dairy production, which is vital for minimizing contamination levels in Egypt's dairy sector. Moreover, conducting routine MRSA screenings is crucial to lowering the prevalence of multidrug-resistance, along with exercising great caution in the use of antibiotics.

REFERENCES

Abd El-Halem, S.G., Attia, I.A., El-Dera, H.B., and El-Seedy, A.S. (2019): Prevalence and Antibiotic Resistance of *Staphylococcus aureus* Isolated From Raw Milk and Dairy Products Collected From Alexandria, Egypt. *Alexandria Journal of Food Science & Technology*, 17-26.

Abdel-wahab, R.M., Saad, N.M., Amin, W.F., Shaker, E.M. and Elsherif, W. (2024): Antibiotic Residues and Their Corresponding Resistance Genes of *Staphylococcus aureus* in Raw Milk. *Assiut Veterinary Medical Journal*, 70(182), 220-239.

Abdeen, E.E., Mousa, W.S., Abdel-Tawab, A.A., El-Faramawy, R. and Abo-Shama, U.H. (2021): Phenotypic, Genotypic and Antibiogram among *Staphylococcus aureus* Isolated from Bovine. *Pakistan Veterinary Journal*, 41, 289-293.

Abebe, A.A. and Birhanu, A.G. (2023): Methicillin resistant Staphylococcus aureus: molecular mechanisms underlying drug resistance development and novel strategies to combat. *Infection and drug resistance*, 7641-7662.

Abo-Shama, U.H., Hassan, W.H., Fawy, A.S. and Sayed, H.H. (2022): Phenotypic and Genotypic Characterization of *Staphylococcus aureus* Isolated from Raw Cow's Milk at Sohag Governorate, Egypt. *Journal of Current Veterinary Research*, 4(1), 93-105. .

Aguiar, R.C., Ferreira, F.A., Cieza, M.R., Silva, N.C., Miotto, M., Carvalho, M.M. and Lindner, J.D. (2024): Staphylococcus aureus Isolated From Traditional Artisanal Raw Milk Cheese from Southern Brazil: Diversity, Virulence, and Antimicrobial Resistance Profile,. *Journal of Food Protection,, 87*(6).

Ahmed, A.A., Maharik, N.M., Valero, A. and Kamal, S.M. (2019)[a]: Incidence of enterotoxigenic *Staphylococcus aureus* in milk and Egyptian artisanal dairy products. *Food Control*, 20-27.

Ahmed, S.H., Tolba, S.T. and El-Zawahry, Y.A. (2019)[b]: Evaluation of the Role Of bla Genes in Beta Lactam and Methicillin Resistant Staphylococcus aureus. *Egyptian Journal of Botany*, 29-38.

Ahmed, W., Neubauer, H., Tomaso, H., Elhofy, F.I., Monecke, S., Abdeltawab, A.A. and Hotzel, H. (2020): Characterization of Staphylococci and Streptococci Isolated from Milk of Bovides with Mastitis in Egypt. *Pathogens*, 9,381.

Algammal, A.M., Enany, M.E., El-tarabili, R.M., Ghobashy, M.O. and Helmy, Y.A. (2020): Antimicrobial Resistance Profiles, Virulence and Enterotoxins-Determinant Genes of MRSA Isolated from Subclinical Bovine Mastitis in Egypt. *Pathogens*, 9(5):362.

Ali, H.R., Koraney, N.F., Hefny, E.G., Ali, S.F., Abdel Mawgoud, S.R. and EltouKhy, E.I. (2021): Phenotypic and genotypic profiling of Methicillin-resistant *Staphylococcus aureus* isolates from human and bovine milk. *Egyptian Journal of Agricultural Research*, 190-196.

Asfour, H.A. and Darwish, S.F. (2014): Evaluation of phenotypic methods versus molecular methods for differentiation of Coagulase positive staphylococci causing bovine mastitis with a special reference to atypical *Staphylococcus aureus International Journal of Current Microbiology and Applied Sciences* 3**,** 543-58.

Bacanlı, M.G. (2024): The two faces of antibiotics: an overview of the effects of antibiotic residues in foodstuffs. *Archives of Toxicology 98*, 1717–1725.

Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G. and Becker, K. (2014): The *mecA* homolog mecC confers resistance against β-lactams in *Staphylococcus aureus* irrespective of the genetic strain background. *Antimicrobial agents and chemotherapy*, *58*(7), 3791-3798.

Chimuti, S., Mugadza, D.T., Ntuli, V. and Njage, P.M. (2024): Contribution of Farm-level Hygiene and Handling Practices to Microbial Safety Profiles in the Informal Dairy Sector in Zimbabwe,. *Journal of Food Protection, 87*(8), 100313.

Christaki, E., Marcou, M. and Tofarides, A. (2020): Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. *Journal of molecular evolution, 88,(1)*, 26-40.

Clinical and Laboratory Standards Institute (CLSI) (2020): Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement., CLSI document M100-S25, Wayne, PA.

Cremonesi, P., Luzzana, M., Brasca, M., Morandi, S., Lodi, R., Vimercati, C., Agnellini, D., Caramenti, G., Moroni, P. and Castiglioni, B. (2005): Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. *Molecular and Cellular Probes* 19(5): 299–305.

Da Cunha, M.R., Eliana Peresi, Regina Adriana Oliveira Calsolari., João Pessoa Araújo Júnior. (2006): Detection of enterotoxins genes in coagulase-negative staphylococci isolated from foods. *Brazilian Journal of Microbiology* 37 (1) :70-74

Darwish, S.F. and Asfour, H.A. (2013): Investigation of biofilm forming ability in Staphylococci causing bovine mastitis using phenotypic and genotypic assays. *Scientific World Journal*, 2013**,** 378492

Darwish, S.F. and Bakheet, A.A. (2019): Prevelence of Methicillin–Resistant *Staphylococcus aureus* (MRSA)in Layer Chickens with Reference to its Ability to Form Biofilm and Enterotoxins. *Alexandria Journal of Veterinary Sciences*, 63 (2).

Diab, M.S., Ibrahim, N.A., Elnaker, Y.F., Zidan, S.A. and Saad, M.A. (2021): Molecular detection of *Staphylococcus aureus* enterotoxin genes isolated from mastitic milk and humans in El-Behira, Egypt. *International Journal of One Health*, 7(1): 70-77.

Egyptian standards (ES: 154-1/2005): Milk and milk products. Part 1: Raw milk. Egyptian Organization for Standardization and Quality.

El-Ashker, M., Gwida, M., Monecke, S., El-Gohary, F., Ehricht, R., Elsayed, M. and Maurischat, S. (2020): Antimicrobial resistance pattern and virulence profile of *Staph. aureus* isolated from household cattle and buffalo with mastitis in Egypt,. *Veterinary Microbiology*, 108535.

Fenta, M.D., Tafere, F.A., Mebratu, A.S. and Malede, B.A. (2023): Quarter-wise proportion and beta-lactam resistance rate of bovine mastitis associated- *Staphylococcus aureus* among infectious episodes in Ethiopia: Systematic review and meta-Analysis. *Heliyon, 9*(7).

Fikry, A., Ahmed, A.T., Samir, A., El-Yazeed, H.A., El-Amry, K. and Naim, H. (2021): Bacteriological and Molecular Comparative Study between Staphylococcus aureus Isolated from Animals and Human. *Journal of Applied Veterinary Sciences*, 6(2), 50-58.

Gajewska, J., Zakrzewski, A., Wioleta, C.-W. and Zadernowska, A. (2023): Meta-analysis of the global occurrence of Staph. aureus in raw cattle milk and artisanal cheeses. *Food Control, Volume 147*, 109603,ISSN 0956-7135.

Ghimpețeanu, O.M., Pogurschi, E.N., Popa, D.C., Dragomir, N., Drăgotoiu, T., Mihai, O.D. and Petcu, C.D. (2022): Antibiotic Use in Livestock and Residues in Food—A Public Health Threat: A Review. *Foods (Basel, Switzerland), 11(10), 1430.*

Hassan, H.A. and Ali, Z.I. (2024): Assesment of *Listeria monocytogenes* presence in raw milk samples and certain cheese varieties. *Assiut Veterinary Medical Journal*, 183.

Hodille, E., Rose, W., Diep, B.A., Goutelle, S., Lina, G. and Dumitrescu, O. (2017): The Role of Antibiotics in Modulating Virulence in Staphylococcus aureus. *Clinical microbiology reviews, 30(4), 887–917.*

Ivanovic, I., Boss, R., Romanò, A., Guédon, E., Le-Loir, Y., Luini, M. and Graber, H.U. (2023). Penicillin resistance in bovine Staphylococcus aureus: Genomic evaluation of the discrepancy between phenotypic and

molecular test methods. *Journal of Dairy Science, 106*(1), 462-475.

ISO 6887-5 (2020): Microbiology of food chain preparation of test Samples, Initial Suspension, and decimal dilutions for microbiological Examination - Part 5: Specific rules for the preparation of milk and milk products. 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. 2nd edition. ICS07.100.30 Food microbiology. International Organization for Standardization

Lade, H. and Kim, J.S. (2023): Molecular Determinants of β-Lactam Resistance in Methicillin-Resistant Staphylococcus aureus (MRSA): An Updated Review. *Antibiotics, 12(9), 1362*.

Larsen, J., Raisen, L.C., Ba, X. et al. (2022): Emergence of methicillin resistance predates the clinical use of antibiotics. . *Nature 602*, 135–141.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G. and Monnet, D.L. (2012): Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.

Monday, S.R. and Bohach, G.A. (1999): Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in Staphylococcal isolates. *Journal of Clinical Microbiology,* 37**,** 3411-4.

Naz, K., Ullah, N., Zaheer, T., Shehroz, M., Naz, A. and Ali, A. (2020): Chapter 9 - Pan-genomics of model bacteria and their outcomes. In D. Barh, S. Soares, S. Tiwari, & V. Azevedo, *Pan-genomics: Applications, Challenges, and Future Prospects* (pp. 189-201). Academic Press.

Owusu-Kwarteng, J., Akabanda, F., Agyei, D. and Jespersen, L. (2020): Microbial Safety of Milk Production and Fermented Dairy Products in Africa. *Microorganisms*, 8(5), 752.

Pierezan, M.D., Maran, B.M., Maran, E.M., Verruck, S., Pimentel, C.T. and Cruz, A.G. (2022): Chapter Six - Relevant safety aspects of raw milk for dairy foods processing. In F. Toldrá, *Advances in Food and Nutrition Research* (pp. 211-264). Academic Press.

Qu, Y., Zhao, H., Nobrega, D., Cobo, E.R., Han, B., Zhao, Z. and Gao, J. (2019): Molecular epidemiology and distribution of antimicrobial resistance genes of Staphylococcus species isolated from Chinese dairy cows with clinical mastitis. *Journal of Dairy Science, 102*(2), 1571-1583.

Rajkovic, A., Jovanovic, J., Monteiro, S., Decleer, M., Andjelkovic, M., Foubert, A. and Uyttendaele, M. (2020): Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria. *COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY, 19*(4), 1605-1657.

Rasheed, H., Ijaz, M., Ahmed, A., Javed, M.U., Shah, S.F.A. and Anwaar, F. (2023): Discrepancies between phenotypic and genotypic identification methods of antibiotic resistant genes harboring *Staphylococcus aureus. Microbial Pathogenesis*, *184*, 106342.

Sadat, A., Shata, R.R., Farag, A.M., Ramadan, H., Alkhedaide, A., Soliman, M.M. and Awad, A. (2022): Prevalence and characterization of PVL-positive *Staphylococcus aureus* isolated from raw cow's milk. . *Toxins*, 14(2), 97.

Sallam, M.G., Khalifa, N.O., Barakat, A.M. and Salem, L.M. (2023): Occurrence of Staphylococcus aureus in dairy

farms and humans with reference to antimicrobial profile in Qalyobia governorate. *Benha Veterinary Medical Journal*, 40-45.

Şanlıbaba, P. (2022): Prevalence, antibiotic resistance, and enterotoxin production of *Staphylococcus aureus* isolated from retail raw beef, sheep, and lamb meat in Turkey. *International Journal of Food Microbiology*, 109461.

Schonberg, F. (1956): Milch-Kunde and Milch hygiene., 7. Auffage, Verlarg M. and H. Scheber, Hannover.

Selim, A., Kelis, K., AlKahtani, M.D. et al. (2022): Prevalence, antimicrobial susceptibilities and risk factors of Methicillin resistant *Staphylococcus aureus* (MRSA) in dairy bovines. *BMC Veterinary Research 18*, 293.

Shahzad, M.A., Yousaf, A., Ahsan, A., Irshad, H., Riaz, A., Khan, A. and Javed, S. (2024): Virulence and resistance profiling of Staphylococcus aureus isolated from subclinical bovine mastitis in the Pakistani Pothohar region. *Scientific Reports, 14*(1), 14569.

Strommenger, B., Kettlitz, C., Werner, G., and Witte, W. (2003): Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. *Journal of Clinical Microbiology,* 41**,** 4089-94.

Taalat, H., El-beskawy, M., Atwa, S., Eissa, M., Mahmmod, Y., Elkady, M. and El-Diasty, M. (2023): Prevalence and Antibiogram of *Staphylococcus aureus* in Clinical and Subclinical Mastitis in Holstein Dairy Cows in Egypt. *Zagazig Veterinary Journal*, 51(1).

Tóth, A.G., Csabai, I., Krikó, E. al., e. (2020): Antimicrobial resistance genes in raw milk for human consumption. *Scientific Reports 10*, 7464.

Venla, J., Ahmad, A.-M., Viivi, H., Laura, L., Saara, S., Sanna, S. and Annamari, H. (2023): Clonal dissemination of successful emerging clone mecA-MRSA t304/ST6 among humans and hedgehogs in the Helsinki metropolitan area in Finland. *One Health, Volume 16*, 100516.

Vesterholm-Nielsen, M., Ølholm Larsen, M., Elmerdahl Olsen, J. and Møller Aarestrup, (1999). Forekomst af blaZ genet i penicillinresistente *Staphylococcus aureus* isoleret fra bovin mastitis i Danmark. *Acta Veterinaria Scandinavica*. 40**,** 279-286.

Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A. and Whitman, W.B. (Eds.). (2011): Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes (Vol. 3). Springer Science & Business Media.

WHO (2023): https://www.who.int/news-room/fact-sheets/ detail/ antimicrobial-resistance. World Health Organization, 21 November 2023.

Zeinhom, M.M. and Abed, A.H. (2021): Prevalence, Characterization, and Control of Staphylococcus aureus Isolated from Raw Milk and Egyptian Soft Cheese. *Journal of Veterinary Medical Research*, 152-160.

Zhang, K., Mcclure, J.A., Elsayed, S., Louie, T. and Conly, J.M. (2005): Novel multiplex PCR assay for characterization and concomitant subtyping of Staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 43**,** 5026-33.

Zhao, X., Yuan, X., Hu, M., Zhang, Y., Li, L., Zhang, Q. and Liu, Y. (2021): Prevalence and characterization of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus isolated from bulk tank milk in Shandong dairy farms. *Food Control, Volume 125*.

تقييم شامل لتلوث اللبن الخام بالمكورات العنقودية الذهبية: تواجدها، مقاومتها للمضادات الحيوية، جينات السموم المعوية، ومخاطرها علي الصحة العامة

بسنت الحناوي ، عادل سعودي ، سماح درويش ، طارق سليمان ، زينب علي

Email: Passantadel259@gmail.com Assiut University web-site: www.aun.edu.eg

لقد اصبحت بكتريا المكورات العنقودية الذهبية أحد أهم مسببات الأمراض المنقولة بالغذاء، وبإعتبارها عضوًا في مجموعة ESKAPE يمكنها "الإفلات" من التأثيرات المضادة للميكروبات لعوامل المبيدات الحيوية، مما يشكل تهديدًا عالميًا للصحة العامة. لذلك، صممت هذه الدراسة للكشف عن إنتشار المكورات العنقودية الذهبية في عينات اللبن الخام وتقييم مقاومتها للمضادات الحيوية ووجود جينات السموم المعوية. تم شراء 50 عينة من اللبن الخام بشكل عشوائي من مزارع الألبان وصغار المربين ومحلات الألبان والباعة المتجولين في محافظات القاهرة والجيزة والقليوبية، مصر، وذلك لتسليط الضوء على التلوث في المزرعة ومن ثم التوزيع. وقد تم التاكد من المعزولات بإستخدام الطرق البيوكيميائية والجزيئية، كما تم الكشف عن مقاومة المعزولات لعشرة مضادات حيوية مظهريا بإستخدام إختبار الأقراص والإختبار الجيني. علاوة على ذلك، تم فحص ٢ من جينات السموم المعوية للمكورات العنقودية. وقد أشارت نتائج هذه الدراسة إلى أن متوسط الأعداد لبكتيريا المكورات العنقودية الذهبية كانت 0.2±1.9, 0.3±2.2, 0.3±1.6, 0.2±2.9 في عينات اللبن الخام من مزارع الألبان وصغار المربين ومحلات الألبان والباعة المتجولين، على التوالي. بالإضافة إلى ذلك، تم إظهار مقاومة مضادات متعددة في 50% من العزلات ونسبة 78.95% منها كانت مكورات عنقودية ذهبية مقاومة للميثيسيلين. بالإضافة لذلك، لوحظت مقاومة عالية للبنسلين (62.5%)، السيفوكسيتين (59.38%) والتتراسيكلين (56.25%). ووُجد أن 28.13% من سلالات المكورات العنقودية الذهبية كانت إيجابية لجينات السموم المعوية، ووجد أن 21.9% و 6.25% تحمل جينات *SEA* و *SEC* على التوالى. ولهذا فإن النتائج المتحصل عليها تحذر من التهديد الكامن لبكتيريا المكورات العنقودية الذهبية وخاصة تلك المقاومة للميثيسيلين في اللبن الخام وتقترح الحاجة الملحة إلى تنفيذ برامج مراقبة لضمان إتخاذ تدابير وقائية فعالة تبدأ من المزرعة.