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Screening of antibiogram, virulence factors, and biofilm production of *Staphylococcus aureus* and the bio-control role of some probiotics as alternative antibiotics

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Abstract

Background: Food safety is a serious challenge in the face of increasing population and diminishing resources. *Staphylococcus aureus* is a critical foodborne pathogen characterized by its capability to secret a diverse range of heat-resistant enterotoxins. Antibiotic usage in dairy herds resulted in the occurrence of antimicrobial resistance (AMR) patterns among bacterial species, which were consequently transmitted to humans via dairy products. Lactic acid bacteria (LAB) produce bacteriocins, which provide an excellent source of natural antimicrobials with the further advantage of being environmentally friendly and safe.

Aim: Detection of multidrug resistance (MDR) *S. aureus* isolates in concerned samples, molecular characteristics, biofilm production, and the inhibitory role of LAB against it.

Methods: Random samples of raw milk and other dairy products were analyzed for *S. aureus* isolation. Phenotypic and genotypic assessment of AMR was performed, in addition to detection of classical enterotoxin genes of *S. aureus*. Finally, evaluation of the antimicrobial action of some *Lactobacillus* strains against *S. aureus*.

Results: Incidence rates of presumptive *S. aureus* in raw milk, Kariesh cheese, and yogurt samples were 50%, 40%, and 60%, respectively. The highest resistance of *S. aureus* was to Kanamycin (100%) and Nalidixic acid (89.3%), respectively. (78.66%) of *S. aureus* were MDR. 11.1% of *S. aureus* carried *mecA* gene. In concern with enterotoxins genes, PCR showed that examined isolates harbored *sea* with a percentage of (22.2%), while *sed* was found in (11.1%) of isolates. Regarding biofilm production, (88.88%) of *S. aureus* were biofilm producers. Finally, agar well diffusion showed that *Lactobacillus acidophilus* had the strongest antimicrobial action against *S. aureus* with inhibition zone diameter ranging from 18 to 22 mm.

Conclusion: There is a widespread prevalence of MDR *S. aureus* in raw milk and dairy products. Production of staphylococcal enterotoxins, as well as biofilm production are responsible for public health risks. Therefore, installing proper hygienic routines and harsh food safety policies at food chain levels is substantial.

Keywords: *MecA* gene, Staphylococcal enterotoxins, Biofilm formation, Lactic acid bacteria.

Introduction

Milk is an essential nutrient for humans of all ages, especially children and teenagers, to promote their physiological functions and growth (Abunna *et al.*, 2019). Milk, in addition, is a great medium for pathogenic bacteria growth and development, chemical transfer, and the dissemination of other contaminants (Nirwal *et al.*, 2013).

Ingestion of unheated treated milk and dairy products has been associated with the incidence of foodborne illness outbreaks, owing to the development of pathogens such as *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus,* and *Bacillus cereus* (Biernbaum *et al.*, 2021).

Staphylococcus aureus is globally the third most common cause of foodborne illness and usually contaminates milk or its products via multiple pathways (Şanlıbaba, 2022). It serves as one of the most prevalent contributing factors of mastitis in dairy animals, with diseased animals usually releasing *S. aureus* into milk (Li *et al.*, 2017).

Antimicrobial resistance (AMR), biofilm formation, enterotoxin production, and other virulence characteristics, including nucleases, proteases, hyaluronidase, lipases, and collagenase production, are all associated with *S. aureus* pathogenicity (Shettigar and Murali, 2020).

Staphylococcal enterotoxins (SEs) are exotoxins that cause food poisoning in human beings (Balaban and

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Rasooly, 2000). Several classical SEs were incriminated in food poisoning as SEA, SEB, SEC, SED, and SEE (Bergdoll, 1983 and 1989). Recently, other types were identified as potential agents of food poisoning including SEG, SEH, SEI, SER, SES, and SET (Omoe *et al.*, 2004).

Nowadays, living organisms are facing an urgent threat from AMR due to increasing the distribution of antibiotic resistance genes (ARGs) among different populations (Van Boeckel *et al.*, 2015; Zhang *et al.*, 2022). Raw milk can be contaminated and transmit several types of these ARGs (T´oth *et al.*, 2020). On the other hand, the emergence of several ARGs within clinically relevant bacterial pathogens is a global issue that causes human death and provides massive economic costs (Mestrovic *et al.*, 2022).

Biofilms are structures of extracellular matrices of microbial communities characterized by heterogeneity that can easily colonize various substrates such as soil, water, and organic matter (Donlan and Costerton, 2002). Microorganisms with biofilms provide numerous advantages: improved metabolic cooperation between species (Shapiro, 1998), higher resistance to host immunological reactions, demanding larger dosages of antibiotics (Ceri *et al.*, 1999), and enhanced bacterial association capability (Hennequin *et al.*, 2012). The cycle of formation, maturation, and propagation is the primary reason for surface-to-food cross-contamination (Kumar *et al.*, 2017).

Lactobacilli are popularly recognized for their ability to protect food and prevent illnesses caused by food contamination (Adams, 1999). In the food industry, multiple *Lactobacillus* species and their metabolic products were granted a "generally regarded as safe" certification (Wells, 2011). In recent years, Lactobacilli strains have been used to replace synthetic preservatives in the food industry as natural antimicrobials due to the rising attention to healthy food, which has sparked curiosity among scientific organizations (Arena *et al.*, 2016).

The present study aims to investigate *S. aureus* prevalence in raw milk and some dairy product samples obtained from various markets in Zagazig city as well as to evaluate its phenotypic and genotypic antimicrobial susceptibility patterns, also to detect several virulence genes related to its pathogenicity. Furthermore, the ability for biofilm production and the antimicrobial effect of some *Lactobacillus* strains against this food-borne pathogen.

Materials and Methods

Samples collection

150 samples of raw milk and other dairy products (Kariesh cheese and plain yogurt), (50 of each) were obtained from various shops in Zagazig city, under optimum conditions of hygiene and delivered without delay for a microbiological assessment.

Isolation and identification of S. aureus

Isolation was performed following ISO 6888–1:1999 +A1:2003 guidelines protocol (ISO, 2003) on Baired Parker agar plates after enrichment, Incubation of cultures was performed at 37°C and examined for two successive days, and further biochemical identification was done.

Antibiotic sensitivity testing

All bacterial isolates were evaluated for their antibiotics' susceptibility *in vitro*, according to the guidelines of the Clinical and Laboratory Standards Institute, by Kirby–Bauer disk diffusion method CLSI (2020). Against the discs of Ampicillin (AM), Oxacillin (OX), Azithromycin (AZ), Erythromycin (E), Tetracycline (T), Cefotaxime (CF), Kanamycin (K), Gentamicin (G), Nalidixic acid (NA), Ciprofloxacin (CP), Imipenem (IPM), Clindamycin (CL), and Sulphamethoxazol (SXT). The diameters of inhibition zones were recorded in millimeters, and the measurements were classified as sensitive, moderate, or resistant based on the interpretative manual of CLSI (2020). In addition, *S. aureus* isolates were tested using the broth microdilution method to determine the minimum inhibitory concentrations (MICs) of Vancomycin. Then, according to CLSI (2020) interpretation, Vancomycin susceptible *S. aureus* isolates had MICs ≤2 mg/ml, VISA isolates had MICs of 4–8 mg/ml, and Vancomycin resistant S. aureus (VRSA) isolates had MICs > 16 mg/ml. The formula of Singh *et al.* (2010) was used to measure the multiple antibiotic resistance (MAR) index. Moreover, the multidrug resistance (MDR) isolates were that showed resistance to ≥3 classes of antimicrobial tested (Magiorakos *et al.*, 2012).

Multiplex polymerase chain reaction technique

Detection of *S. aureus* enterotoxins and resistance genes

Identification of ARGs represented by methicillin-resistant "MRSA" *(mecA),* E *(ermA),* and vancomycin (*vanA*) of *S. aureus* was applied using primers in Table 1. Specific primers of SEs (*sea*, *seb*, *sec,* and *sed*) genes were listed in Table 2.

DNA extraction

DNA extraction was done for isolates by Qiagene DNA extraction kits (QIAGEN, GmbH, Hilden, Germany) according to manufacturers' recommendations.

PCR amplification and analysis of PCR products

By using purified bacterial DNA, the resistance genes were identified by multiplex PCR according to Perez-Roth *et al.* (2001) (Table 1). Also, PCR procedures were based on Rall *et al.* (2008) to identify the classical enterotoxins.

Biofilm formation by microtiter plate assay (MTP)

The method MTP was performed on a 96-well, flat-bottomed, sterile MTP to examine the biofilm formation ability of *S. aureus* isolates. 200 µl of brain heart infusion broth were placed into wells of polystyrene microplate and 20 µl of each strain culture were dispersed in triplicate into the wells. Then, the

Table 1. Primer sequences of ***mec***A gene and other resistance genes of ***S. aureus.***

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Cycling condition	References
mecA (F)	**5′** AAAATCGATGGTAAAGGTTGGC**′3**	533	An initial denaturation at 94°C for 5 minutes was continued by 10 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 45 seconds and 25 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 1 minutes finishing with a final extension at 72°C for 10 minutes.	Buhlmann *et al.* (2008)
mecA (R)	**5′** AGTTCTGGAGTACCGGATTTGC**′3**			
ermA (F)	**5′** TATCTTATCGTTGAGAAGGGATT **′3**	139		Martineau *et al.* (2000)
ermA (R)	**5′** CTACACTTGGCTTAGGATGAAA **′3**			
vanA (F)	**5′**CATGAATAGAATAAAAGTTGCAATA**′3**	1,030		Clark *et al.* (1993)
vanA (R)	**5′** CCCCTTTAACGCTAATACGATCAA **′3**			

Table 2. Primer sequences of SE genes.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Annealing temp.	References
sea (F)	**5′** TTGGAAACGGTTAAAACGAA**′3**	120	50°C	Johnson *et al.* (1991)
sea (R)	**5′** GAACCTTCCCATCAAAAACA **′3**			
seb (F)	**5′** TCGCATCAAACTGACAAACG **′3**	478	50°C	
seb (R)	**5′** GCGGTACTCTATAAGTGCC **′3**			
sec (F)	**5′** GACATAAAAGCTAGGAATTT **′3**	257	50°C	
sec (R)	**5′** AAATCGGATTAACATTATCC **′3**			
sed (F)	**5′** CTAGTTTGGTAATATCTCCT **′3**	317	50°C	
sed (R)	**5′** TAATGCTATATCTTATAGGG **′3**			

Table 3. Prevalence of ***S. aureus*** in raw milk and dairy products.

Type of samples	NO. of samples	Positive presumptive *S. aureus*	
		NO.	%
Raw milk	50	25	50
Kariesh cheese	50	20	40
Yoghurt	50	30	60
Total	150	75	50

microplate was incubated overnight at 37°C in aerobic conditions. The plate was drained and properly washed with phosphate buffer saline three times. Afterward, 200 µl of methanol was transferred to each well to fix those attached cells. The adhering bacterial cells were stained with 200 µl of 0.5% (*w*/*v*) crystal violet for 10 minutes. Subsequent to staining, phosphate buffer saline (PBS) was used to wash the wells appropriately then dried aerobically before being resolved with 250 µl 33% glacial acetic acid. The optical density of the stained adherent bacteria was measured at 570 nm using a microplate reader. Following the formulas developed by Stepanovic´ *et al.* (2004), the bacterial strains were classified as non-producers, weak, moderate, and strong biofilm producers.

Agar well diffusion assay

Cell-free supernatant (CFS) preparation of *Lactobacillus* strains

Lacticaseibacillus rhamnosus LMG23522, *Lacticaseibacillus plantarum* MK 806485, and *Lacticaseibacillus acidophillus* MK850930 strains were used to measure the inhibitory effect against *S. aureus*. Inoculation of probiotic strains in MRS broth was performed and then incubated at 37°C in a CO_2 incubator for 2 days. After that, the bacterial suspension was centrifuged at 4,000 rpm for 10 minutes and CFS was extracted aseptically then sterilized with a 0.20-µm pore size filter and utilized freshly.

Antimicrobial activity

The agar well diffusion method was applied by loading the wells of 6 mm diameter with CFS from *Lactobacillus* strains (100 µl/well). After one night of incubation at 37°C, a digital caliper was used to record the inhibition zone diameters. Then, *Lactobacillus* strains were categorized into 4 groups as follows: inhibition zone of <11 mm diameter was regarded as negative (−), 11–16 mm as moderate (+), 17–22 mm as strong (++), and >23 mm as extremely strong (+++), respectively (Rammelsberg and Radler, 1990).

Results

Isolation and identification of S. aureus

Data presented in Table 3 showed that the highest incidence of presumptive *S. aureus* was in yogurt

Table 4. Antimicrobial susceptibility of ***S. aureus*** isolates (***n*** = 75).

Antimicrobial class	Antimicrobial agent	Conc. (µg)	Sensitive		Intermediate		Resistant	
			NO.	%	NO.	%	NO.	%
β-Lactams	AM	10	32	42.7	-	-	43	57.3
	OX	1	50	66.7	15	20	10	13.3
Macrolides	AZ	15	16	21.3	8	10.7	51	68
	E	15	22	29.3	18	24	35	46.7
Ts	T	30	40	53.3	-	-	35	46.7
Cephalosporins	CF	30	24	32	-	-	51	68
Aminoglycosides	K	30	-	-	-	-	75	100
	G	10	31	41.3	9	12	35	46.7
Quinolone	NA	30	-	-	8	10.7	67	89.3
Fluroquinolone	CP	5	40	53.3	8	10.7	27	36
Carbapenem	IPM	10	40	53.3	16	21.3	19	25.4
Lincosamide	CL	10	-	-	15	20	60	80
Sulfonamide	SXT	25	8	10.7	7	9.3	60	80
Glycopeptides	Vancomycin[a]	0.25–256 µg/ml	55	73.4	1	1.3	19	25.3

[a]Determined by broth micro dilution method.

Table 5. Multiple antibiotic resistance phenotypes of ***S. aureus*** strains (***n*** =75).

NO. of isolates	Antimicrobial resistance profile	No. of antibiotics	MAR index	MDR isolates NO. (%)
10	K, NA, CL, SXT, AZ, CF, AM, E, G, T, CP, IPM, V, OX	14	1	
9	K, NA, CL, SXT, AZ, CF, AM, E, G, T, CP, IPM, V	13	0.92	
8	K, NA, CL, SXT, AZ, CF, AM, E, G, T, CP	11	0.78	
8	K, NA, CL, SXT, AZ, CF, AM, E, G, T	10	0.71	
8	K, NA, CL, SXT, AZ, CF, AM	7	0.50	59 (78.66%)
8	K, NA, CL, SXT, AZ, CF	6	0.42	
8	K, NA, CL, SXT	4	0.28	
8	K, NA	2	0.14	
8	K	1	0.07	

OX: Oxacillin; E: Erythromycin; V: Vancomycin; NA: Nalidixic acid; AM: Ampicillin; T: Tetracycline; CF: Cefotaxime; CL: Clindamycin; CP: Ciprofloxacin; K: Kanamycin; G: Gentamicin; SXT: Sulphamethoxazol; IPM: Imipenem; AZ: Azithromycin.

samples, followed by raw milk samples, then Kariesh cheese samples at 60%, 50%, and 40%, respectively.

Antibiotic sensitivity testing

Antimicrobial susceptibility testing of *S. aureus* isolates showed that the highest resistance was to K and NA by percentages being (100%) and (89.3%), respectively, as mentioned in Table 4.

MDR and MAR index

Data in Table 5 declared that 59 out of total 75 *S. aureus* isolates showed MDR by a percentage of (78.66%).

MAR ranged from 0.07 to 1 and (78.66%) of total isolates had a MAR index ≥0.2.

Multiplex PCR assay

ARGs of *S. aureus*

Figure 1 and Table 6 revealed the incidence of *mecA*, *ermA,* and *vanA* genes in nine strains of *S. aureus* where *vanA* and *mecA* genes presented a percentage of (11.1%) of each. While *ermA* gene appeared in four out of nine strains (44.4%). The incidence rate of MRSA was (11.1%).

Staphylococcal enterotoxins

Figure 2 and Table 6 showed that *sea* was detected in 2 out of 9 (22.2%) of *S. aureus* strains while *sed* in 1/9 (11.1%). However, *seb* and *sec* failed to be detected in any isolate.

Ability to form biofilm

Table 7 represents the ability of *S. aureus* strains to produce biofilm. 8 out of 9 (88.88%) of *S. aureus* were biofilm producers as follows: 5 out of 9 *S. aureus* isolates (55.6%) were moderate biofilm producers, 2/9 (22.2%) were strong biofilm producers, while only one isolate was weak biofilm producer and so only one isolate was non-biofilm producer.

Antibacterial activity of *Lactobacillus* strains against *S. aureus*

Table 8 assessed the antimicrobial activity of three *Lactobacillus* strains against the most virulent strain of *S. aureus* (MRSA strain). *Lactobacillus acidophilus* MK850930 had the strongest antimicrobial action against *S. aureus* with inhibition zone diameter ranging from 18 to 22 mm.

Discussion

Improper personal hygiene practices, environmental contamination with wastes of infected animals, cross-contamination, and faulty handling during shipping to milk collecting centers may all contribute to the high occurrence of *S. aureus* in raw milk and investigated dairy products (Addis *et al.*, 2011). The predominant way of *Staphylococcus* pathogen into milk is through shedding from diseased mammary tissues (Rahimi, 2013). It is worth noting that, if some strains of this organism multiply heavily in foods, they can secrete food-poisoning enterotoxins (Pereira *et al.*, 2009).

The findings revealed that the highest prevalence of presumptive *S. aureus* was in yogurt, then raw milk and Kariesh cheese with percentages of 60%, 50%, and 40%, respectively (Table 3). These results agreed with Ahmed *et al.* (2019) results, who isolated *S. aureus* by a percentage of (42%) from raw milk samples, while greater percentages were detected by both Ibrahim *et al.* (2015) and Kandil *et al.* (2018), where they isolated *S. aureus* from (100%) and (80%) raw milk samples, respectively. Zeinhom and Abed (2020) obtained a lower incidence rate in raw milk (13%).

Regarding Kariesh cheese, Ibrahim *et al.* (2015) agreed with our results as *S. aureus* reported in (66%) of Kareish cheese samples. However, extremely higher results were detected by Kandil *et al.* (2018) where (92%) of Kariesh cheese samples were contaminated with *S. aureus*.

Our results were higher than those obtained by Ahmed *et al.* (2019) and Zeinhom and Abed (2020) who identified (38%) and (18%) prevalence of *S. aureus* in Kariesh cheese samples, respectively.

Yogurt had the highest incidence rate (60%). Several studies detected lower incidence rates of *S. aureus* in yogurt as El-Ansary (2014), Ahmed *et al.* (2019), and Kandil *et al.* (2018) found *S. aureus* in (42%), (40%), and (36%) of yogurt samples, respectively.

Ingestion of inadequate or non-heat-treated dairy products leads to infection with antibiotic-resistant bacteria, which develops a major public health threat. Inappropriate utilization of antibiotics in the treatment of mastitis can end up in the propagation of resistant strains serving as crucial public health issues (De Jong *et al.*, 2018).

Table 4 demonstrated the resistance rates of *S. aureus* against examined discs of antimicrobials and showed that the highest resistance was to K, NA, CL, and SXT

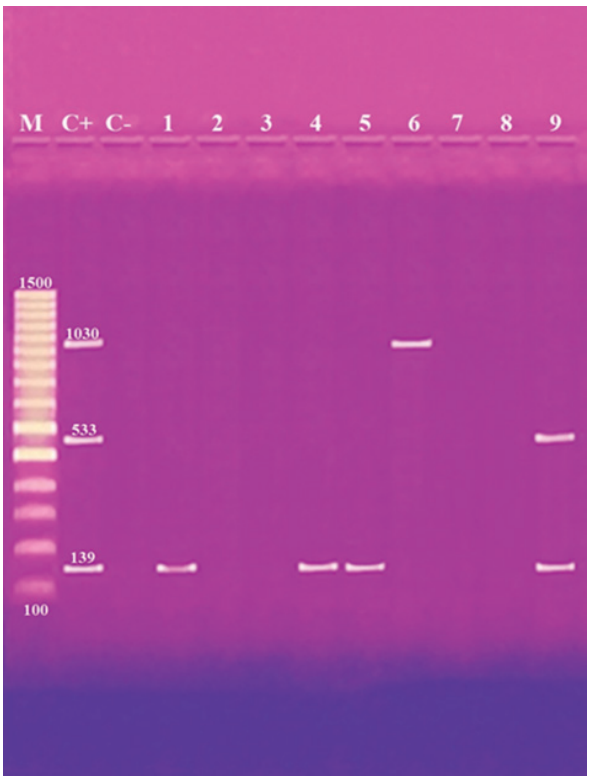

Fig. 1. Multiplex PCR of *ermA* (139 bp), *mecA* (533 bp) and *vanA* (1,030 bp) ARGs of *S. aureus*. Lane M: 100 bp ladder. Lane C+: Control positive for *ermA*, *mecA,* and *vanA* genes. Lane C-: Control negative. Lanes 1, 4 & 5: Positive *S. aureus* strains for *ermA* gene. Lanes 2, 3, 7 & 8: Negative strains for *ermA*, *mecA* and *vanA* genes. Lane 6: Positive *S. aureus* strain for *vanA* genes. Lane 9: Positive *S. aureus* strain for *ermA* and *mecA* genes.

Table 6. Distribution of SEs and some ARGs.

Genes NO.	Virulence genes								ARGs					
	Sea		*Seb*		*Sec*		*Sed*		*mecA*		*ermA*		*vanA*	
Staphylococcus aureus (n = 9)	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
	2	22.2	-	-	-	-	1	11.1	1	11.1	4	44.4	1	11.1

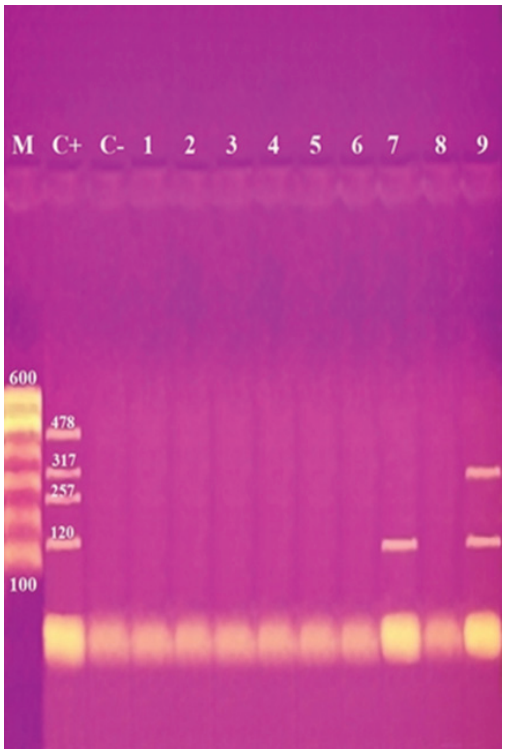

Fig. 2. Multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp), and *sed* (317 bp) as enterotoxin genes. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *sea, seb, sec,* and *sed* genes. Lane C-: Control negative. Lane 7: Positive *S. aureus* strain for *sea* gene. Lane 9: Positive *S. aureus* strain for *sea* & *sed* genes. Lanes from 1 to 6 and Lane 8: Negative *S. aureus* for *sea, seb, sec,* and *sed* genes

by percentages being (100%), (89.3%), (80%), and (80%), respectively. Moderate resistance to AM (57.3%) followed by E, T, and G by a percentage of (46.7%) of each. Low resistance to Vancomycin (25.3%) where 20 out of 75 *S. aureus* isolates had MIC values between 16 and 256 mg/ml. Similarly, Alnakip *et al.* (2023) declared that examined *S. aureus* isolates resisted AM by a percentage of (46.66%), T (44.45%), and E (40%), respectively. Nearly similar results reported by Thaker *et al.* (2013) showed low resistance to AM (40%) of *S. aureus* isolates. Higher results were detected by Gundogan and Avci (2014) who revealed that isolates of *S. aureus* showed resistance to AM (92.6%) and T (54.3%), respectively. Contrary to our findings, Feyissa *et al.* (2023) demonstrated that (100%) of *S. aureus* isolates were T resistant. While Bissong and Ateba (2020) reported high resistance to Vancomycin (83.1%) among *S. aureus* isolates.

Table 5 recorded that (78.66%) 59 out of 75 *S. aureus* isolates were MDR to at least 3 different classes of antibiotics. Furthermore, Abd El Halem (2019) detected that the MDR in *S. aureus* isolates had a percentage of (39.5%), while (95.5%) of isolates were multidrug resistant as mentioned by Samaha *et al.* (2012).

To achieve reliable, sensitive, and specific determination of MDR *S. aureus* strains, molecular diagnostics like PCR must be applied.

Data in Figure 1 and Table 6 revealed that both *mecA* and *vanA* were detected in 1/9 (11.1%) of *S. aureus* isolates while *ermA* in 4/9 (44.4%). Therefore, MRSA was detected by a percentage of (11.1%). Our results agreed with results obtained by Saka and Terzi Gulel (2018) who demonstrated that 9% of *S. aureus* isolates were methicillin-resistant (*mecA* gene positive). On the contrary, Nam *et al.* (2011) declared that 17/402 of *S. aureus* isolates were identified genotypically as MRSA (carrying *mecA* gene) by a percentage of (4.2%). However, Zhao *et al.* (2021) found that the prevalence of MRSA was (0.7%) which was *mecA* positive. In concern with *ermA* and *vanA* genes, Ning *et al.* (2023) revealed that none of *S. aureus* isolates harbored *vanA* or *ermA*. Kou *et al.* (2021) identified that 2 out of 62 (3.22%) isolates carried *ermA* gene while 11/62 (17.7%) carried *vanA* gene in *S. aureus* isolates. Furthermore, Pajohesh *et al.* (2019) determined that the prevalence of *mecA and ermA* was (22.22%) and (13.33%), respectively, in *S. aureus* isolates.

SEs are extremely firm and withstand against high temperatures and proteases such as pepsin and trypsin (Clarisse *et al.*, 2013). Universally, staphylococcal food poisoning is commonly caused by SEA, followed by SED and SEB (Argudin *et al.*, 2010).

As shown in Figure 2 and Table 6, 2 out of 9 strains (22.2%) harbored *sea* gene and 1/9 (11.1%) harbored *sed* gene. Unfortunately, the detection of *seb* and *sec* was failed. In line with our results, Saka and Terzi Gulel (2018) mentioned that 12 out of 99 *S. aureus* isolates were positive for SEs (harbored one or two genes) by a percentage of (12%). Among them, 5/12 (41.6%) were positive for *sea*, 1/12 (8.3%) carried *sed* and no strain carried *seb.* In contrast to our study, Zhao *et al.* (2021) detected the presence of *sed* gene in 16/121, followed by *sec* in 10/121 and *seb* in 8/121 while, no isolate harbored *sea* gene. Pajohesh *et al.* (2019) declared that *sed* was reported in 15 isolates (33.3%), *sea* in 8

Table 7. Ability of ***S. aureus*** strains to form biofilm.

Bacterial strain	Degree of biofilm formation							
	None		Weak		Moderate		Strong	
	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus (9)	1	11.1	1	11.1	5	55.6	2	22.2

Table 8. Antimicrobial activity of some *Lactobacillus* strains against *S. aureus.*

Lactobacillus strains **Bacterial strains**	Mean of inhibition zone diameter (mm)		
	Lacticaseibacillus rhamnosus	***Lacticaseibacillus plantarum***	***Lacticaseibacillus acidophillus***
Staphylococcus aureus (MRSA strain)	+	+	++

Diameters of inhibition zone are the mean of three replicates. Interpretation of zone diameter of inhibition: (−) less than 11 mm diameter, (+) 11–16 mm, (++) 17–22 mm, and more than 23 mm as very strong (+++).

isolates (17.7%), *sec* in 7 isolates (15.5%), and *seb* in 4 isolates (8.8%), respectively.

The existence of bacteria capable of creating a biofilm is critically important in the food industry. A microbiological biofilm developed on the equipment and machinery in food sector plants not only threatens the hygienic quality of the processed food items but also additionally represents a risk to consumer health since the structure of the biofilm could incorporate several food-borne pathogens (Van Houdt and Michiels, 2010). Data in Table 7 represents the biofilm production ability of *S. aureus* strains. Moderate biofilm production was detected in five out of nine with the highest percentage (55.6%), followed by strong producers in two out of nine (22.2%), then weak and non-producers in one out of nine (11.1%) of each, respectively. Several investigations evaluated the biofilm production ability in *S. aureus*. Ballah *et al.* (2022) examined isolates of *S. aureus* and found that (97%) of isolates were able to form biofilm. In addition, 20 *S. aureus* isolates were identified as strong biofilm formers. Pajohesh *et al.* (2019) revealed that biofilm formation was shown in 35 strains (77.77%) of *S. aureus*, 20 out of 35 showed strong biofilm formation (44.44%), 15 out of 35 were weak producers (33.33%) and 10 out of 35 (22.22%) had no ability to produce biofilms. Bissong and Ateba (2020) illustrated that (90%) of *S. aureus* isolates were biofilm producers, out of which the majority 66 out of 70 (94.3%) were detected as strong producers.

Organic acids, acetoin, diacetyl, bacteriocins, and hydrogen peroxide are among the antimicrobial metabolites synthesized by probiotics. These compounds help to minimize microbiological risk by suppressing pathogenic bacteria and restricting the growth of other microorganisms (Pyar and Peh, 2014). Table 8 assessed the antimicrobial activity of three *Lactobacillus* strains against the most virulent strain of *S. aureus* (MRSA strain). *Lactobacillus acidophillus* MK850930 had the strongest antimicrobial action against *S. aureus* with inhibition zone diameter ranged from 18 to 22 mm. While *L. plantarum* MK806485 and *L. rhamnosus* LMG23522 showed moderate action against *S. aureus* (14–16 mm) and (12–16 mm), respectively. Anas *et al.* (2008) stated that *L. plantarum* strain gives an inhibition diameter of 20 mm for *S. aureus.* While the antagonistic activity of different *Lactobacillus* (CFS) assessed by Koohestani *et al.* (2018) mentioned that the highest inhibition zone diameter (16 mm) against *S. aureus* was recorded with CFS of *L. acidophilus* LA5.

Conclusion

The findings of this investigation revealed that *S. aureus* was excessively dispersed in the investigated specimens and contributed to life-threatening situations for consumers. The elevated incidence of *S. aureus* showing MDR highlights the serious issue of AMR in the dairy sector that threatens public health by traveling through the food chain. Moreover, the formation of biofilms is a worrisome issue due to the high survival rate in the environment. Therefore, it is crucial to apply firm measures that achieve the hygienic quality of milk and its products at all levels of production to minimize their cross-contamination hazards. In addition, this study provides evidence that the screened *Lactobacillus* strains possess a significant ability to suppress the growth of *S. aureus* under *in vitro* conditions. However, *in-vivo* trials are additionally required to assess whether they operate as probiotics in real-life conditions for human health benefits.

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