

Phenotypic and Genotypic Characterization of *Staphylococcus aureus* Isolated from Raw cow's Milk at Sohag Governorate, Egypt.

Usama Hassan Abo-Shama¹, Walid Hamdy Hassan², Ahmed Sapit Fawy¹ and Haitham Helmy Sayed^{*1}

(1) Department of Microbiology, Faculty of Veterinary Medicine, Sohag University, Sohag, 82524, Egypt.

(2) Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, 62511, Egypt.

*Corresponding author: vet_haitham@yahoo.com Received: 24/12/2021 Accepted: 11/1/2022

ABSTRACT

Staphylococcus aureus (*S. aureus*) is one of the most pathogenic bacteria isolated from milk and is one of the main causes of food poisoning outbreaks worldwide. This study was performed to investigate prevalence of *S. aureus* in raw cow's milk sold in dairy shops at Sohag Governorate, Egypt, determine some virulence factors and antimicrobial susceptibility of the isolates and to examine some Methicillin-resistant *S. aureus* (MRSA) isolates by PCR for presence of some antibiotic resistance and virulence genes including *coa* (coagulase), *nuc* (thermonuclease), *spa* (*Staphylococcal* protein A), *mecA* (a determinant of methicillin resistance) and *tetK* (a determinant of tetracycline resistance) genes. Therefore, a total of 300 samples of raw cow's milk were randomly collected from dairy shops in three different cities at Sohag Governorate, Egypt (El Balyana, Sohag and Tahta cities) during the period from November 2020 to August 2021. Bacteriological examination revealed that 67 samples were positive for *S. aureus* with percentage of (22.3%) and that (68.7%) and (31.3%) of the isolates exhibited β -hemolysis and α -hemolysis on blood agar, respectively. Also, it revealed that (46.3%) and (58.2%) of the isolates had Congo red binding (CRB) activity and DNase activity, respectively. Antimicrobial susceptibility testing for *S. aureus* isolates (n=67) against 9 different antibiotics revealed that they were sensitive to vancomycin (100%), gentamycin (100%), amoxicillin/clavulanic acid (97%), ciprofloxacin (92.5%) and erythromycin (82.1%) while they showed highest resistance to oxacillin (70.1%) followed by tetracycline (67.2%), chloramphenicol (59.7%) and penicillin (47.8%). Furthermore, it revealed that (43.4%) of the isolates were resistant to 3 antibiotics or more and that (79.3%) of these multiple drug resistance (MDR) isolates were MRSA. Screening of 10 randomly selected MRSA isolates for presence of *coa*, *nuc*, *spa*, *mecA* and *tetK* genes by PCR revealed that all of them harbor *coa*, *nuc* and *spa* genes, while only (80%) and (70%) of them harbor *mecA* and *tetK* genes, respectively. In conclusion, our results indicated presence of a high prevalence of MDR *S. aureus* especially MRSA in raw cow's milk which represents a major threat to public health, therefore more restrictive hygienic measures must be applied during all stages of milk production.

Keywords: Prevalence, Antibiotic resistance, *Staphylococcus aureus*, MRSA, Sohag.

INTRODUCTION

Milk and dairy products represent one of the most important foods for all age categories worldwide due to its biological components (Pereira, 2014). But unfortunately, they can harbor a variety of microorganisms and be a major source of foodborne pathogens to human (Oliver *et al.*, 2005) which poses a serious health threat to human and causes serious economic problems in dairy industry (Gwida and EL-Gohary, 2013). In Egypt, direct consumption of raw milk is more popular than consumption of the pasteurized milk. Furthermore, milk is mainly produced in small farms lacking the proper sanitary measures and may be either consumed directly, manufactured into dairy products or sold in the retail markets (Meshref *et al.*, 2019), therefore utilization of raw milk and these products has been frequently associated with food-borne diseases (Gwida and EL-Gohary, 2013).

S. aureus is contagious pathogenic bacteria can cause many diseases in human and animals (Lemma *et al.*, 2021). It is ubiquitous in nature and presents in the nasal passages and throat, and on skin of 50% or more of the healthy individuals (Samir *et al.*, 2018). It represents one of the major causes of mastitis in dairy cattle (Al-Ashmawy *et al.*, 2016). *S. aureus* can reach milk through direct excretion from the infected udders or contamination of milk during the milking process (Samir *et al.*, 2018).

S. aureus is one of the most important causes of foodborne illnesses all over the world (Gwida and EL-Gohary, 2013). *Staphylococcal* food poisoning occurs due to consumption of contaminated food by performed *Staphylococcal* enterotoxins and it is characterized by appearance of nausea, vomiting, diarrhea, general malaise and weakness within 1-8 hours after consumption of such food (Abo-Shama, 2014). Several virulence factors are implicated in pathogenesis of *S. aureus* infections. These include surface components (capsule, peptidoglycan, teichoic acid, protein A, cell attachment protein), enzymes (coagulase, lipases, esterases, proteases, hyaluronidase, deoxyribonuclease, catalase, beta-lactamase, and staphylokinase), and various toxins (Hasan *et al.*, 2014).

Antimicrobial resistance is an issue of increasing global concern and it is associated

mainly with the uncontrolled usage of antimicrobials (Barber *et al.*, 2003). *S. aureus* has been reported frequently show MDR thereby rendering the antibiotic treatment ineffective (Abo-Shama, 2014). MRSA has spread worldwide, being among the most isolated antibiotic resistant bacteria worldwide (Nkwelang *et al.*, 2009). It always exhibits resistance to multiple antimicrobial agents including β -lactam antibiotics, quinolones, aminoglycosides, macrolides, lincosamides, tetracyclines and chloramphenicol, so it is considered life-threatening bacteria for both human and animals (Algammal *et al.*, 2020). Multiple antibiotic resistant *S. aureus* and MRSA strains have been isolated from milk in many parts of the world (Abo-Shama, 2014 and Al-Ashmawy *et al.*, 2016) which represents great threat to public health through transmission of such MDR pathogens and antibiotic resistance genes via consumption of the contaminated food (Lemma *et al.*, 2021).

Limited data are available about prevalence of *S. aureus* in raw cow's milk sold in Sohag Governorate, Egypt and their virulence factors and antimicrobial susceptibility. Since such data could serve as a tool for assessing the sanitary conditions implemented in milk production and the health risks to human and they are also essential to guide the correct control and prevention measures and the appropriate use of antimicrobials in both human and animals. Therefore, the present study aimed to investigate prevalence of *S. aureus* in raw cow's milk sold in dairy shops at Sohag Governorate, Egypt, determine some virulence factors and antimicrobial susceptibility of the isolates and to examine some MRSA isolates by PCR for presence of some antibiotic resistance and virulence genes including *coa*, *spa*, *mecA* and *tetK* genes.

MATERIALS AND METHODS

1. Sampling:

A total of 300 samples of raw cow's milk were randomly collected from dairy shops in three different cities at Sohag Governorate, Egypt (El Balyana, Sohag and Tahta cities) during the period from November 2020 to August 2021. The samples were collected under aseptic conditions and were placed in an insulated ice box containing ice bags and they were immediately transported to the laboratory where they were immediately examined.

2- Isolation and identification of *S. aureus*:

For isolation of *S. aureus*, milk samples were centrifuged at 3000 rpm for 10 minutes then sediments were cultured on Baird-Parker agar (BPA) (Oxoid, England) and Mannitol salt agar (MSA) (Oxoid, England) and were incubated at 37°C under aerobic condition for 24-48 hours. The isolates were identified as *S. aureus* according to their morphological characteristics, Gram-staining, coagulase test and catalase test (APHA, 1992).

3- Determination some virulence factors of *S. aureus* isolates:

In this study, *S. aureus* isolates were investigated for hemolytic activity, DNase activity and for biofilm formation. Hemolytic activity was determined as described by Boerlin *et al.* (2003). β -hemolysis is indicated by appearance of a clear zone around the colonies, α -hemolysis is indicated by appearance of a green or brown discoloration in the medium while γ -hemolysis is indicated by absence of hemolysis (Buxton, 2005).

DNase activity was determined as described by Kateete *et al.* (2010) and DNA degradation is indicated by appearance of a clear zone around the colonies. Ability of the isolates for biofilm formation was determined by Congo red agar (CRA) test as described by Freeman *et al.* (1989) and production of rough black colonies indicates slime producing strains.

4- Antimicrobial susceptibility testing of *S. aureus* isolates:

S. aureus isolates were tested by using a Kirby-Bauer disk diffusion method against 9 different antibiotics including Penicillin (P) (10 μ g), Amoxicillin/clavulanic acid (AMC) (30 μ g), Oxacillin (OX) (1 μ g), Vancomycin (VA) (30 μ g), Erythromycin (E) (15 μ g), Gentamicin (CN) (10 μ g), Ciprofloxacin (CIP) (5 μ g), Tetracycline (TE) (30 μ g) and Chloramphenicol (C) (30 μ g) (Oxoid, UK). An aliquot of suspension of each tested isolate (0.5 McFarland) was spread plated onto Mueller-Hinton agar (Himedia, India) then antibiotic disks were dispensed on the inoculated plate and it was incubated aerobically at 37°C for 24 hours. Thereafter, inhibition zones diameters were measured and interpreted according to CLSI (2017). Resistance to three antibiotics or more was considered as MDR (Dai *et al.*, 2019).

5 - Molecular detection of some antibiotic resistance and virulence genes in some MRSA isolates:

In this study, 10 phenotypically MRSA isolates were randomly selected and genotypically confirmed as *S. aureus* by PCR assay targeting 16s rRNA gene of *S. aureus*, then they were screened by PCR for presence of some antibiotic resistance and virulence genes including *coa*, *nuc*, *spa*, *mecA* and *tetK* genes.

Freshly grown typical *S. aureus* colonies were harvested and DNA extraction was performed using Patho Gene-SpinTM DNA/RNA Extraction kit (iNtRON Bio, Korea) according to the manufacturer's guidelines.

DNA amplification was performed in BIO-RAD S1000 thermocycler (USA) using Thermo ScientificTM PCR Master Mix (2X) (USA) under PCR conditions illustrated in Table (1) for each target gene. According to mastermix manufacturer's instructions, PCR reaction mixture was prepared in 50 μ l comprising Master Mix (25 μ l), forward and reverse primers (1 μ l from each), extracted DNA (2 μ l) and nuclease-free deionizer water (21 μ l). The used primers (Metabion, Steinkirchen, Germany) were illustrated in Table (1).

PCR products were electrophoresed on 1% agarose gel (Sigma, USA) in 1x TBE buffer using gradients of 1-5V/cm at room temperature and generuler 100 bp DNA ladder (Gene Direx, BIO-HELIX Co., LTD) was used for fragments size determination. Thereafter, the gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and data was analyzed by a computer software.

RESULTS***1- Prevalence of *S. aureus* among the examined raw cow's milk samples:***

Out of 300 samples examined, 67 samples were positive for *S. aureus* on MSA and BPA with percentage of (22.3%). *S. aureus* isolates produced yellow colonies on MSA while produced black colonies surrounded by opaque zone on BPA. They were catalase and coagulase positive and they appeared as gram positive cocci arranged in clusters resemble bunches of grapes on microscopical examination.

2- Results of determination some virulence factors of *S. aureus* isolates:

On blood agar media, (68.7%) of *S. aureus* isolates produced β -hemolysis while (31.3%) of them produced α -hemolysis. On the other hand, (46.3%) and (58.2%) of *S. aureus* isolates showed CRB activity and DNase activity, respectively.

3- Antimicrobial susceptibility testing and MDR profiles of *S. aureus* isolates:

Antimicrobial susceptibility testing of *S. aureus* isolates (n=67) revealed the highest sensitivity to vancomycin and gentamycin (100% for reach), followed by amoxicillin/clavulanic acid (97%), ciprofloxacin (92.5%) and erythromycin (82.1%), while the highest resistance to oxacillin (70.1%) followed by tetracycline (67.2%), chloramphenicol (59.7%) and penicillin (47.8%) (Table 2).

On the other hand, it was found that (43.4%)

of *S. aureus* isolates were resistant to 3 antibiotics or more (MDR) and that 23 (79.3%) of these MDR isolates were MRSA, while 6 (20.7%) of them were MSSA. MDR patterns of *S. aureus* isolates were illustrated in Table (3).

4- Molecular detection of some antibiotic resistance and virulence genes in some MRSA isolates:

All the examined MRSA isolates (n=10) were genotypically confirmed as *S. aureus* through detection of 16s rRNA gene of *S. aureus* as illustrated in Fig. (1).

Furthermore, it was found that all the examined MRSA isolates harbor *coa*, *nuc* and *spa* genes (Figs. 2-4 respectively), while only (80%) and (70%) of them harbor *mecA* and *tetK* genes (Figs. 5 & 6 respectively). Phenotypic and genotypic characters of the examined MRSA isolates were summarized in Table (4).

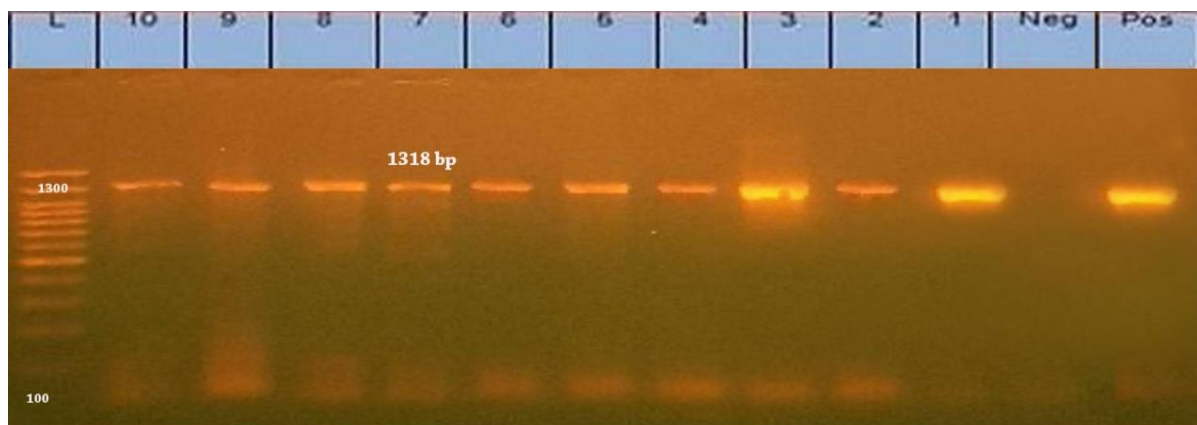


Fig. (1): Agar gel electrophoresis for PCR products using specific primers target 16s rRNA gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 1318-bp in all the examined isolates.

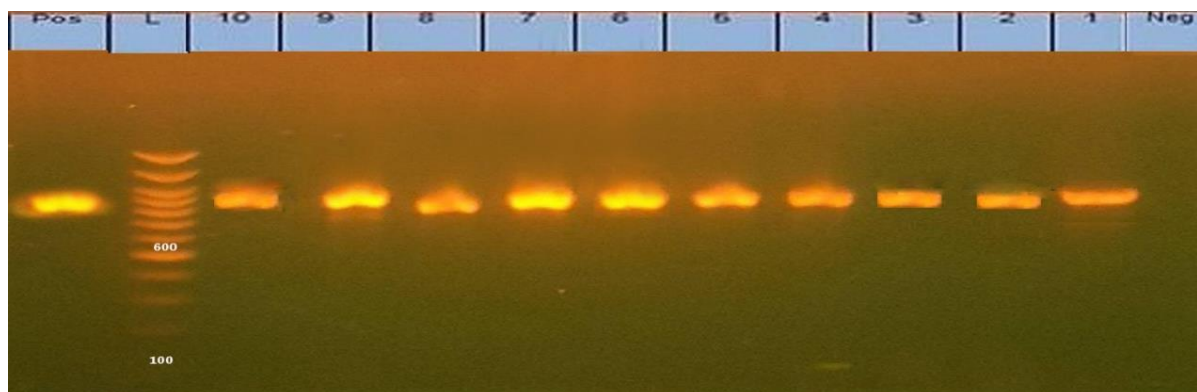


Fig. (2): Agar gel electrophoresis for PCR products using specific primers target *coa* gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 1000 bp in all the examined isolates.

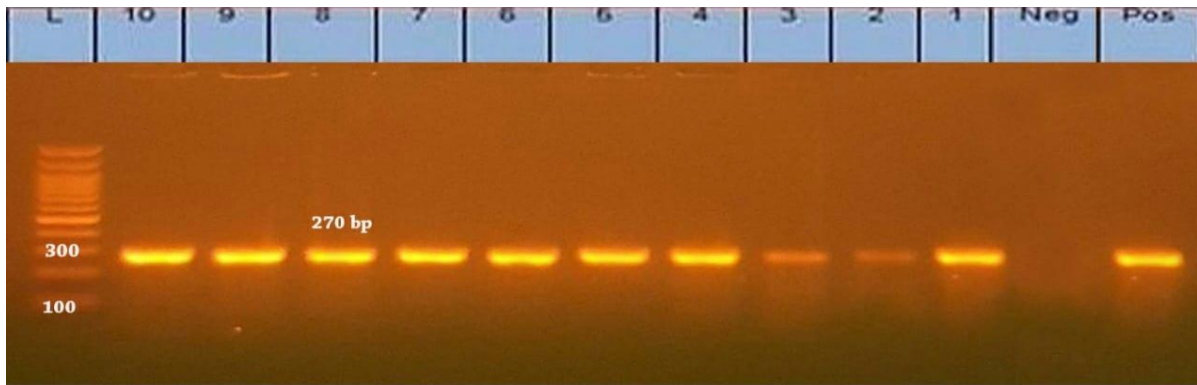


Fig. (3): Agar gel electrophoresis for PCR products using specific primers target *nuc* gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 270-bp in all the examined isolates.

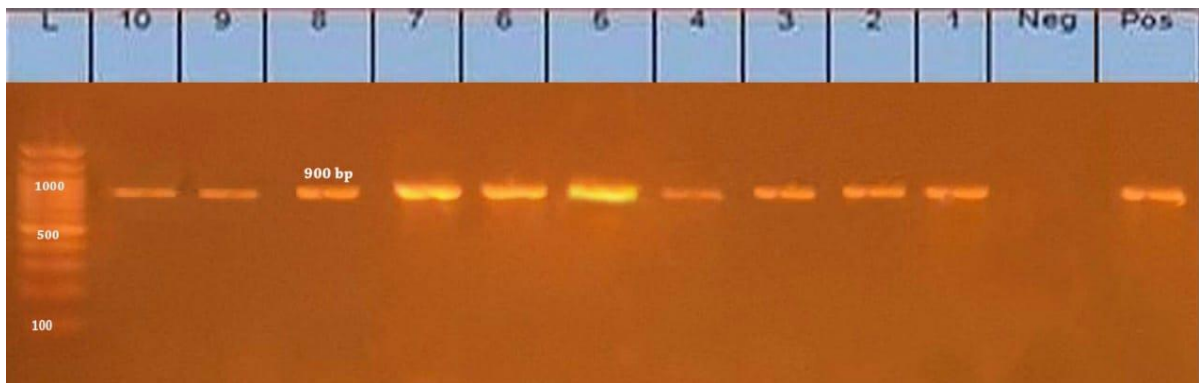


Fig. (4): Agar gel electrophoresis for PCR products using specific primers target *spa* gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 900 bp in all the examined isolates.

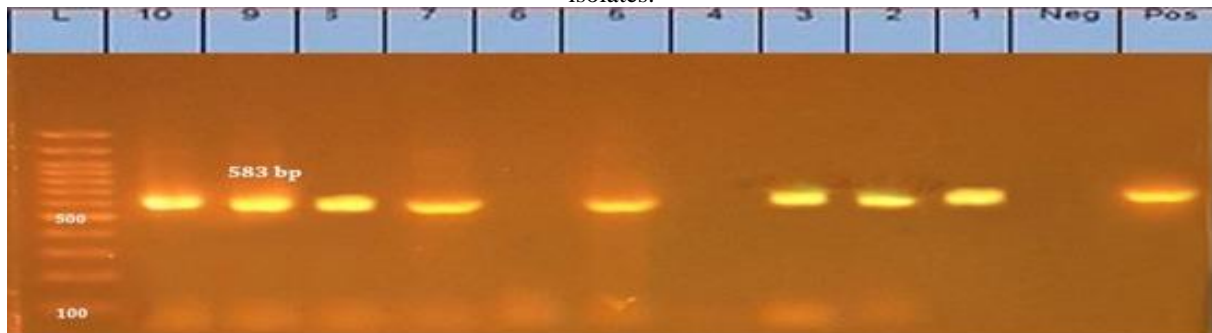


Fig. (5): Agar gel electrophoresis for PCR products using specific primers target *mecA* gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 583-bp in all the examined isolates except 4 and 6.

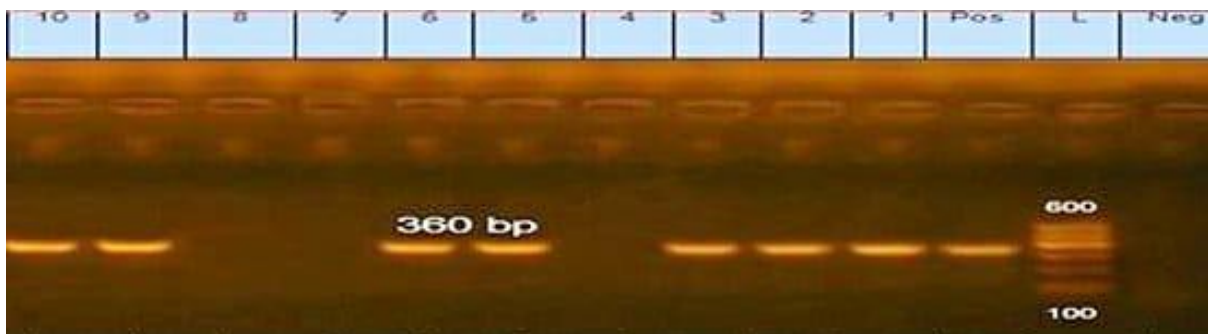


Fig. (6): Agar gel electrophoresis for PCR products using specific primers target *tetK* gene in the examined MRSA isolates. Lane L: 100 bp molecular weight marker, lane Pos: positive control, lane Neg: negative control and lanes 1-10: DNA extracted from the examined isolates (S1-S10, respectively) showing positive bands at 360-bp in all the examined isolates except 4, 7 and 8.

Table (1): Target genes in the study, primers sequences and PCR conditions used.

Target gene	Primers sequences (5`- 3`)	Product Size (bp)	PCR conditions				Reference	
			Primary denaturation	Number of PCR cycles				Final extension
	Denaturation	Annealing		Extension				
16srRNA	GGA CGA CAT TAG ACG AAT CA	1318	94°C 2 min.	35 cycles			72°C 10 min.	Riffon <i>et al.</i> (2001)
	CGG GCA CCT ATT TTC TAT CT			94°C 45 sec.	64°C 1 min.	72°C 2 min.		
<i>nuc</i>	GCGATTGATGGTGATACGGTT	270	94°C 5 min.	35 cycles			72°C 10 min.	Louie <i>et al.</i> (2002)
	AGCCAAGCCTTGACGAACTAA AGC			94°C 30 sec.	55°C 30 sec.	72°C 1 min.		
<i>mecA</i>	AGAAGATGGTATGTGGAAGTT AG	583	94°C 5 min.	40 cycles			72°C 5 min.	Azimian <i>et al.</i> (2012)
	ATGTATGTGCGATTGTATTGC			94°C 30 sec.	57°C 45 sec.	72°C 30 sec.		
<i>tetK</i>	GTAGCGACAATAGGTAATAGT	360	95°C 3 min.	30 cycles			72°C 4 min.	Duran <i>et al.</i> (2012)
	GTAGTGACAATAAACCTCCTA			95°C 30 sec.	54°C 30 sec.	72°C 30 sec.		
<i>coa</i>	ACCACAAGGTAAGTGAATCAAC G	600-1000	95°C 5 min.	30 cycles			72°C 10 min.	Aarestrup <i>et al.</i> (1995)
	TGCTTTCGATTGTTCGATGC			95°C 30 sec.	55°C 2 min.	72°C 4 min.		
<i>spa</i>	CACCTGCTGCAAATGCTGCG	900	94°C 10 min.	35 cycles			72°C 7 min.	Seki <i>et al.</i> (1998)
	GGCTTGTTGTTGTCTTCCTC			94°C 30 sec.	55°C 30 sec.	72°C 30 sec.		

Table (2): Results of antimicrobial susceptibility of *S. aureus* isolates.

Antibiotic	Disc conc.	Result					
		Sensitive		Intermediate		Resistance	
		NO.	%	NO.	%	NO.	%
Oxacillin	1 µg	20	29.9%	0	0.0%	47	70.1%
Penicillin	10 µg	35	52.2%	0	0.0%	32	47.8%
Amoxicillin / clavulanic acid	30 µg	65	97.0%	0	0.0%	2	3.0%
Tetracycline	30 µg	21	31.3%	1	1.5%	45	67.2%
Erythromycin	15 µg	37	55.2%	18	26.9%	12	17.9%
Ciprofloxacin	5 µg	57	85.1%	5	7.4%	5	7.5%
Chloramphenicol	30 µg	21	31.4%	6	8.9%	40	59.7%
Gentamycin	10 µg	65	97.0%	2	3.0%	0	0.0%
Vancomycin	30 µg	67	100.0%	0	0.0%	0	0.0%

Table (3): MDR patterns of *S. aureus* isolates.

MDR pattern	No. of resistance antibiotics	No. of resistance antibiotics classes	MDR isolates	
			No.	%*
OX-TE-C	3	3	4	6.0%
OX-TE-E	3	3	4	6.0%
P-TE-C	3	3	3	4.5%
OX-CIP-C	3	3	1	1.5%
P-E-CIP	3	3	1	1.5%
P-TE-E	3	3	1	1.5%
OX-P-TE-C	4	3	8	11.9%
OX-P-TE-E	4	3	3	4.5%
OX-AMC-TE-E	4	3	1	1.5%
OX-P-TE-CIP	4	3	1	1.5%
P-TE-E-C	4	4	1	1.5%
OX-P-TE-E-CIP	5	4	1	1.5%
Total			29	43.4%

* Percentage was calculated according to the total number of *S. aureus* isolates (n=67).

AMC=Amoxicillin/Clavulanic acid, C=Chloramphenicol, CIP=Ciprofloxacin, CN=Gentamycin, E=Erythromycin, OX=Oxacillin, P=Penicillin, TE=Tetracycline and VA=Vancomycin.

Table (4): Phenotypic and genotypic characters of the examined MRSA isolates by PCR.

Strain No.	Virulence factors				MDR pattern	Antibiotic resistance genes		Virulence genes		
	Coagulase	Haemolysin (β or α)	Biofilm activity	DNase activity		<i>mecA</i>	<i>tetK</i>	<i>coa</i>	<i>nuc</i>	<i>spa</i>
S1	+	+	+	+	OX- P-TE-C	+	+	+	+	+
S2	+	+	+	+	OX-P-TE-C	+	+	+	+	+
S3	+	+	+	+	OX-TE-E	+	+	+	+	+
S4	+	+	+	+	OX-P-TE-E-CIP	-	-	+	+	+
S5	+	+	+	+	OX-AMC-TE-E	+	+	+	+	+
S6	+	+	+	+	OX-P-TE-CIP	-	+	+	+	+
S7	+	+	+	+	OX-P-TE-E	+	-	+	+	+
S8	+	+	+	+	OX-TE	+	-	+	+	+
S9	+	+	+	+	OX-P-TE-C	+	+	+	+	+
S10	+	+	+	+	OX-P-TE	+	+	+	+	+

AMC=Amoxicillin/Clavulanic acid, C=Chloramphenicol, CIP=Ciprofloxacin, E=Erythromycin, OX=Oxacillin, P=Penicillin, and TE=Tetracycline.

DISCUSSION

In the present study, it was found that 67 samples from the examined samples were positive for *S. aureus* with percentage of (22.3%) which pose public health hazards to milk consumers. Our results agreed with findings of Meshref (2013) and Ayele *et al.* (2017) who isolated *S. aureus* with percentage of (23.7%) and (23.4%) from raw cow's milk samples collected from Beni-Suef Governorate, Egypt and Sebeta, Central Oromia, Ethiopia, respectively. While they disagreed with findings of Rall *et al.* (2008) and Abdeen *et al.* (2020) who isolated *S. aureus* with percentage of (70.4%) and (44%) from raw milk samples collected from west of São Paulo state, Brazil and Sadat city, Egypt, respectively. On the other hand, our results are higher than those that have been reported by Imani *et al.* (2010) and Jamali *et al.* (2015) who isolated *S. aureus* with percentage of (4%) and (12%) from raw cow's milk samples collected from Tehran and Mazandaran Province, Iran, respectively. These differences in prevalence of *S. aureus* in raw milk may be attributed to the differences in the geographical location, farm management practices and hygiene practices, animal breeds, sampling season and number and the difference in detection methods (El Seedy *et al.*,

2017).

In this study, it was found that (68.7%) and (31.3%) of *S. aureus* isolates were β -hemolytic and α -hemolytic, respectively. Our results nearly similar to those of Boerlin *et al.* (2003) who recorded that (71.6%) and (28.4%) of *S. aureus* isolates of bovine mastitis were β -hemolytic and α -hemolytic, respectively, while disagreed with those of Jahan *et al.* (2015) who recorded that (100%) of *S. aureus* isolated from raw cow's milk in Bangladesh were β -hemolytic.

Biofilm formation is one of the most important virulence factors of *S. aureus*. It is implicated in chronic infections in both human and animals (Cucarella *et al.*, 2002). In this study, (46.3%) of *S. aureus* isolates showed CRB activity indicating their virulence. Our results agreed with those of Dhanawade *et al.* (2010) who reported that (48.03%) of *S. aureus* isolates were biofilm producer on Congo red agar.

On the other hand, (58.2%) of *S. aureus* isolates showed DNase activity. Our results agreed with those of El-Faramaway *et al.* (2019) who reported that (54.4%) of *S. aureus* isolates showed DNase activity. While, El-Jakee *et al.* (2008) reported higher percent of DNase activity (67.9%) in *S. aureus* isolates recovered from dairy cows, also Abdeen *et al.* (2021) reported lower percent of DNase

activity (23.25%) in *S. aureus* isolates recovered from bovine mastitis.

The misuse of antimicrobials to combat mastitis had led to emerge of resistant strains of *Staphylococci* (Abdeen *et al.*, 2021). As shown in Table (2), antimicrobial susceptibility testing of *S. aureus* isolates revealed the highest sensitivity to vancomycin and gentamycin (100% for each), followed by amoxicillin/clavulanic acid (97%), ciprofloxacin (92.5%) and erythromycin (82.1%) while they showed highest resistance to oxacillin (70.1%) followed by tetracycline (67.2%), chloramphenicol (59.7%) and penicillin (47.8%). Our results agreed with findings of Abdeen *et al.* (2021) who reported that *S. aureus* isolates were sensitive to amoxicillin/clavulanic acid, vancomycin, gentamicin, and ciprofloxacin while they were resistant to penicillin, oxacillin, chloramphenicol and tetracycline. Our results were also nearly similar to those reported by Abo-Shama (2014) except in the sensitivity to chloramphenicol and tetracycline where he recorded that (93.3%) and (100%) of *S. aureus* isolated from milk in Sohag Governorate were sensitive to chloramphenicol and tetracycline respectively and this could be attributed to the differences in antimicrobial usage, management practices and time of examination where antimicrobial resistance is increasing over time (Ali *et al.*, 2017). The high resistance of *S. aureus* isolates found in this study against penicillin, oxacillin, chloramphenicol and tetracycline, indicates to the abuse of these antibiotics in dairy farms especially for treatment of mastitis (Liu *et al.*, 2017).

On the other hand, it was found that (43.4%) of *S. aureus* isolates were resistant to 3 antibiotics or more with presence of 12 different antibiotic resistance patterns (Table 3). These results may reflect the microbial adaptive response to use and overuse of antimicrobials and farm-level management (Al-Ashmawy *et al.*, 2016) which may reflect in difficulty of *S. aureus* infections treatment in animals especially mastitis in the future due to the limited therapeutic options especially from the antibiotics commonly used for its treatment, this in addition to their epidemiological and public health implications represented in transfer of these resistant bacteria especially MRSA and their resistance genes to human and animals. Consistent with

our results, several studies reported high percentages of MDR *S. aureus* in milk with presence of different antibiotic resistance patterns (Aarestrup *et al.*, 1995; Ammar *et al.*, 2016; Dai *et al.*, 2019 and Abdeen *et al.*, 2021).

MRSA represents a global health concern (Al-Ashmawy *et al.*, 2016). Antimicrobial susceptibility testing revealed that (70.1%) of *S. aureus* isolates were MRSA strains and that (79.3%) of these MRSA isolates were MDR. Our results came agreed with those of El-Faramawy *et al.* (2019) who reported that (67.39%) of *S. aureus* isolated from bovine mastitis in Egypt were MRSA strains and showed resistance to several classes of antibiotics. While, Silveira-Filho *et al.* (2014) and El monir *et al.* (2018) recorded lower prevalence of MRSA strains, (37.2%) and (9.1%) among *S. aureus* isolated from milk in Brazil and Egypt, respectively.

Molecular techniques represent an alternative tool for accurate identification and classification of *Staphylococcus* species (Hasan *et al.*, 2014). 16s rRNA gene is a molecular marker for identification of bacterial species (Srinivasan *et al.*, 2015). In this study, 10 randomly selected MRSA isolates were genotypically confirmed as *S. aureus* by PCR through targeting 16s rRNA gene where they produced amplicons of 1310 bp as shown in Fig. (1). Specificity of genotypic identification of *S. aureus* through 16s rRNA gene agreed with the results recorded by Abo-Shama *et al.* (2014) and Ali *et al.* (2014).

S. aureus produces numerous virulence factors that are involved pathogenesis and infection of the udder (El-Faramaway *et al.*, 2019). In this study, it was found that all the examined MRSA isolates harbor *coa*, *nuc* and *spa* genes as illustrated in Figs. (2), (3) and (4) respectively and Table (4). In accordance with our results, Abdeen *et al.* (2021) found that all the tested *S. aureus* isolates from bovine mastitis were positive for *coa*, *nuc* and *spa* genes, also El-Faramawy *et al.* (2019) found that all the tested *S. aureus* isolates from bovine mastitis carried both *coa* and *spa* genes.

Production of penicillin-binding protein 2a (PBP2a) encoded by *mecA* gene, is the most important cause of penicillin and methicillin resistance (Algammal *et al.*, 2020). On the other hand, Shrief *et al.* (2019)

reported that *tetK* and *tetM* genes were the common genetic basis of the resistance to tetracycline. In the current study, PCR revealed that (80%) and (70%) of the examined MRSA isolates harbor *mecA* and *tetK* genes as illustrated in Figs. (5) and (6) respectively and Table (4). Our results came agreed with findings of **Elsayed *et al.* (2019)** and **Abdeen *et al.* (2021)** who found that (75%) and (66.7%) of the examined *S. aureus* isolates harbor *mecA* and *tetK* genes respectively, while disagreed with findings of **Qu *et al.* (2019)** who found that *S. aureus* isolated from dairy farms in China harbor *mecA* and *tetK* genes with percentage of (16%) and (31%), respectively. On the other hand, it was noted that (20%) and (30%) of the examined isolates didn't harbor *mecA* and *tetK* genes although they were phenotypically resistance to oxacillin and tetracycline respectively, this may be attributed to that oxacillin and tetracycline resistance were expressed phenotypically in these isolates through another resistance genes aren't investigated in this study. The high prevalence of *mecA* gene in our *S. aureus* isolates could explain the MDR observed in these isolates where *mecA* gene complex allows the cross resistance to the other antibiotics because it carries insertion sites for mobile genetic elements that facilitate acquisition of resistance determinants to the other antibiotics (**Bakheet *et al.*, 2018**).

As shown in table (4), several MRSA isolates exhibited resistance to multi-classes of antibiotics and produced virulence factors including coagulase, haemolysin, DNase in addition to biofilm formation. They also carry antibiotic resistant and virulence genes including *coa*, *nuc*, *spa*, *mecA*, and *tetK* genes, representing serious threat of MRSA to both human and animals.

Conclusion

The current study revealed presence of a high prevalence of MDR *S. aureus* especially MRSA in the raw cow's milk sold at Sohag Governorate cities, Egypt representing a major threat to public health. Therefore, more restrictive hygienic measures must be applied in all stages of milk production till reaching the consumers. Moreover, strict government must be taken to the misuse of antibiotics in dairy farms.

Author's contribution

All authors contributed equally in this work. They read and approved the final manuscript.

Conflict of interest

The author declares that there is no conflict of interest.

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