Menoufia Veterinary Medical Journal
(MVMJ)

Menoufia Veterinary Medical Journal
https://vmmj.journals.ekb.eg

MVMJ. Vol. ١ (١): … September ٢٠٢٥

Menoufia University

Prevalence of coagulase positive pathogenic *Staphylococcus aureus* in milk and dairy products

Radwa H.Negm[١], Ashraf A. Abd El Tawab [٢] ,Ahmed S. Alfayomy [٣], Doaa M.Sharaf[٤], and Marwa A.Saad [٥]

[١] Bacteriology, Immunology and Mycology Department Faculty Veterinary Medicine, Sadat city University, Egypt. [٢] Bacteriology, Immunology and Mycology Department Faculty of Veterinary Medicine, Benha University, Egypt. [٣] Anatomy Department Faculty of Veterinary Medicine Shebin El-com Menofia University, Egypt. [٤] Animal Health Research Institute, Tanta Branch, Egypt. [٥] Food Hygiene and control Shebin El-com Menofia University, Egypt

Key words:
Staphylococcus aureus; coagulase; PCR

*Correspondence to
e-mail@e-mail.com

Article History
Received: ٠٩ Jan ٢٠٢٥.
Accepted: ١٥ Apr ٢٠٢٥.

ABSTRACT

Coagulase positive *Staphylococcus aureus* (*S. aureus*) is one of the most common etiological pathogens, causing intramammary infection in dairy herds leading to severe economic losses in dairy industry,So this study aimed for the sampling, isolation ,biochemical and genotypic characterization of coagulase positive *Staphylococcus aureus* from raw milk (cow and buffalo)and dairy products . A total of ١٠٠ random samples of raw milk(cow and buffalo), kareish cheese, baladi yogurt (٢٥ of each) were collected from different shops and supermarkets , all samples examined bacteriologically for characterization of coagulase positive *Staphylococcus aureus.* A total of ٥٢ isolates of *S.aureus* was recovered ٢١/٢٥ (٨٤٪), ١٧/٢٥ (٦٨٪), ٨/٢٥ (٣٢٪), ٦/٢٥ (٢٤٪)from raw cow milk, raw buffalo milk, kareish cheese, baladi yogurt , respectively, then the positive isolates examined for their coagulase activity and for their resistance to different types of antibiotics and results of some strains were highly resistant to penicillin (٨٠٪) followed by clindamycin (٦٠٪) ,

erythromycin (٥٣٪) and other strains were highly sensitive for gentamicin(١٠٠٪) ,norfloxacin (٩٣٪) ,vancomycin (٨٦٪) and linezolid (٦٠٪). Then Polymerase chain reaction (PCR) was applied on positive strains of *S.aureus* to detect their virulence genes *staphylococcal* protein A (*SpA)* and coagulase gene (*coa*) which were detected with percentage (١٠٠٪)of tested isolates, and beta- lactam resistance gene (*blaZ*) was found in (٣٣٪) of examined *s.aureus* strains.

١.INTRODUCTION

Staphylococcus aureus is one of the most common facultative pathogenic bacterium that has long been recognized as a challenge in both human and veterinary medicine[١] , organism of bovine mastitis ,which is a common ,complicated and economically unbearable disease in dairy animals worldwide. *Staphylococci* can have a wide variety of virulence factors which allow the bacteria to avoid the immune system and contribute to increased severity of infections. Most of these factors have been initially founded in *S. aureus* and include surface proteins (Protein A, clumping factor, fibronectin binding proteins or iron regulated surface determinants), capsular polysaccharides, molecules involved in biofilm formation (for example polysaccharide intercellular adhesin) or toxins (pore-forming toxins, toxins that act as superantigens). Cell wall adhesins that recognize extracellular matrix proteins and Some enzymes (coagulase, staphylokinase and proteases) also contribute to immune evasion and host tissue penetration [٣],[٤],[٥],[٦].

Coagulase substance is seen as the fundamental danger factor that coagulation plasma and coats the bacterial cell, so prevent phagocytosis[٧]through enables staphylococci to got into a fibrin meshwork, spread and go against opsonophagocytic instrument of host safe cells [٨]. Production of coagulase is a crucial phenotypic feature and the major determinant factor for identifying *S. aureus* strains, the variability at the ٣′ end coding region of the *coa* gene has been used for genotyping of *S. aureus* strains from humans and animals [٩]. Staphylococcal protein A (spA) is a layer bound exoprotein of bacterial cell wall that consider a critical destructiveness factor which ruin opsonization by serum supplement and phagocytosis of Polymorphonuclear leukocytes through confining to FC locale of immunoglobulins[١٠]. *S.aureus* cultivated a high protected against a wide grouping of hostile to contamination specialists which increase their danger and inconvenience in treatment [١١]. The most important antibiotic resistant genes of *S.aureus* strains was *blaZ* gene which coded for *β*-lactamase important virulence factor that impair

opsonization by serum complement and phagocytosis of Polymorphonuclear leukocytes through binding to FC region of immunoglobulins[١٢].

Presence of *S. aureus* in milk and dairy items even in low numbers has been viewed as a general wellbeing danger since it has been laid out that *Staphylococcus* enterotoxins continue for longer lengths in the polluted milk and dairy items, even after the microorganisms lose its viability[١٣].

Improper food handling either in the home or food industry constitutes a major factor leading to *Staphylococcus* food poisoning outbreaks. Only few outbreaks can be traced directly to contamination during food processing [١٤].

The proper heat treatment followed by the refrigeration can minimize the chance of contamination with *S. aureus*. In our country it is commonly noticed that during heat treatment of milk, the temperature not rise up to the boiling point many a time or even if it reaches, consumers do not boil it enough. [١٥].

١. MATERIALS AND METHODS

٢.١ Samples collection

A total of ١٠٠ random samples of raw cow milk, raw buffalo milk, kareish cheese, baladi yogurt (٢٥ of each) were collected from different shops and supermarkets at Menofia and Gharbia Governorate **.** The collected samples were transferred directly to the animal health research laboratory (Tanta branch) in an ice box under complete aseptic conditions for bacteriological examination .

٢.٢. Bacteriological examination[١٦],[١٧].

Samples were homogenized in ٠.١٪ peptone water, maintained for ١ h at ٢٥ ◦C then samples were pre-enriched into nutrient broth and incubated at ٣٧°C for ٢٤ hrs. It was used for the growth and propagation of isolates before platting under aerobic condition. A loopful from incubated nutrient broth was streaked on Baired parker agar plates (oxoid) and incubated at ٣٧oC for ٢٤-٤٨ hrs Positive samples (showed black shiny colonies with clear halo zone around colonies and opaque zone of precipitation). Also, a loopful from incubated nutrient broth was streaked on Mannitol salt agar (Oxoid) and incubated at ٣٧ oC for ٢٤ -٤٨ hrs Positive samples (yellow colonies and turned media to colorless). These colonies were kept in Brain Heart Infusion broth for biochemical identification and PCR examination

٢.٢.١. Morphological examination [١٨].

The suspected *s.aureus* isolates were stained by Gram stain for morphological examination .

٢.٢.٢. Biochemical identification [١٩].

Indol test, catalase test , urease test, oxidase test ,coagulase test and β – hemolysis test .

٢.٢.٣. Antibiotic sensitivity test:

The obtained bacterial isolates were tested in vitro for their susceptibility to the following antimicrobial discs: pencillin (P) ١٠ IU, gentamicin (CN) ١٠ mcg, erythromycin (E) ١٥ mcg, vancomycin (VA) ٣٠ ml , clindamycin(DA) ٢ mcg, norfloxacin (NOR) ١٠

mcg and linezolid (LNZ) ٣٠ mcg. According to [٢٠] and the level of sensitivitywas deciphered agreeing [٢١].

٢.٢.٤. Molecular detection of Coagulase Positive *S.aureus* :

DNA was taken out from the bound S.aureus using QIAamp DNA limited scope unit. It was applied on ٦ sporadic limits. PCR Master Mix and cycling conditions of the foundations during PCR was prepared according to Emerald Amp GT PCR mastermix (Takara) pack. Oligonucleotide foundations used in PCR have express gathering and improve a specific thing as shown in (table, ١) . DNA tests were upgraded in an amount of ٢٥µl as follows: ١٢.٥ µl of Emerald Amp GT PCR expert mix, ١ µl of each and every preparation of ٢٠ pmol centers, ٤.٥ µl of water and ٦ µl of format DNA. The reaction was acted in a Biometra warm cycler. Temperature and time conditions of the starters during PCR were applied .Aliquots of upgraded PCR things were electrophoresed in ١.٥ % agarose gel (ABgene) in ١x TBE pad at room temperature. For gel assessment, ١٥ µl of PCR things were stacked in each gel opening . A ١٠٠ bp DNA ladder (QIAGEN Inc, Valencia, CA, USA) was used to conclude the piece sizes. The gel was caught by a gel documentation structure and the data was inspected through PC programming.

Table (١): Sequence and amplicon size of primers used for detection *S. aureus* virulence and resistance genes :

.

Target gene	Sequence	Amplified product	Reference
S. aureus ٢٣S *rRNA*	AC GGAGTTACAAAGGACGAC	١٢٥٠ bp	**[٢٢]**
	AGCTCAGCCTTAACGAGTAC		
Coa	ATA GAG ATG CTG GTA CAG G	Four different types of bands may be detected ٣٥٠ bp ٤٣٠ bp ٥٧٠ bp ٦٣٠ bp	**[٢٣]**
	GCT TCC GAT TGT TCG ATG C		
Spa	TCA ACA AAG AAC AAC AAA ATG C	٢٢٦ bp	**[٢٤]**
	GCT TTC GGT GCT TGA GAT TC		
blaZ	TACAACTGTAATATCGGAGGG	٨٣٣ bp	**[٢٥]**
	CATTACACTCTTGGCGGTTTC		

Table (٢): Cycling conditions of the primers during cPCR:

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. aureus ٢٣S *rRNA*	٩٤°C ٥ min.	٩٤°C ٣٠ sec.	٥٥°C ٤٠ sec.	٧٢°C ١.٢ min.	٣٥	٧٢°C ١٢ min.
Coa	٩٤°C	٩٤°C	٥٥°C	٧٢°C	٣٥	٧٢°C

	٥ min.	٣٠ sec.	٤٠ sec.	٤٥ sec.		١٠ min.
Spa	٩٤°C ٥ min.	٩٤°C ٣٠ sec.	٥٥°C ٣٠ sec.	٧٢°C ٣٠ sec.	٣٥	٧٢°C ٧ min.
blaZ	٩٤°C ٥ min.	٩٤°C ٣٠ sec.	٥٠°C ٤٠ sec	٧٢°C ٥٠ sec	٣٥	٧٢°C ١٠ in.

٣.RESULTS

٣.١. Incidence of *S. aureus* isolated from examined samples.

١. A total of ٥٢ isolates of *S. aureus* were recovered from ١٠٠ Samples represented as ٢١/٢٥ (٨٤٪), ١٧/٢٥ (٦٨٪), ٨/٢٥ (٣٢٪), ٦/٢٥ (٢٤٪) from of raw cow milk, raw buffalo milk, kareish cheese, baladi yogurt , respectively, (Table ٣).

٢. **Table (٣): Incidence *S. aureus* isolated from examined samples:**

Samples	Number of examined samples	Number of positive samples	% of positive samples
Raw cow milk	٢٥	٢١	٨٤٪
Raw buffalo milk	٢٥	١٧	٦٨٪
Kareish cheese	٢٥	٨	٣٢٪
Baladi yogurt	٢٥	٦	٢٤٪
Total No	١٠٠	٥٢	٥٢٪

٢.٣.Phenotypic Identification of *Staphylococcus aureus*:

In this study *Staphylococcus aureus* were confirmed through Gram staining, *S.aureus* is non-motile facultative anaerobic Gram-positive cocci.graps likr cluster (Figure ١).

The isolated strains were cultured, on Baired-Parker agar: showed black shiny colonies with clear halo zone around colonies and opaque zone of precipitation figure (٢), on Mannitol salt agar: showed (yellow colonies and turned media to colorless) figure (٣).

Biochemically, Negative results were recorded on Indol test, Oxidase test and positive results were recorded on coagulase test (fig ٤) and β – hemolysis test as show in (fig٥)

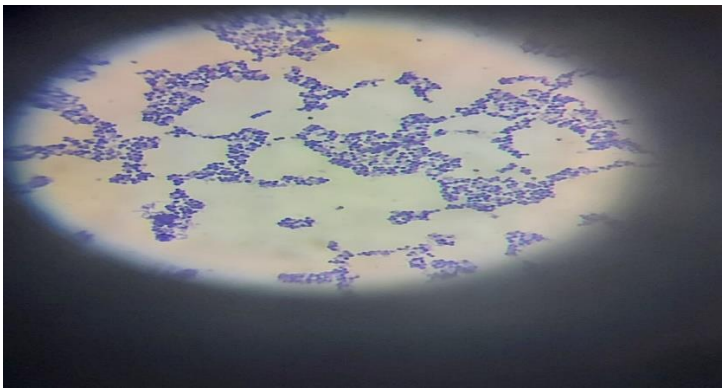


Fig (١) *S.aureus* under microscope after Gram's staining appear as grape like cluster

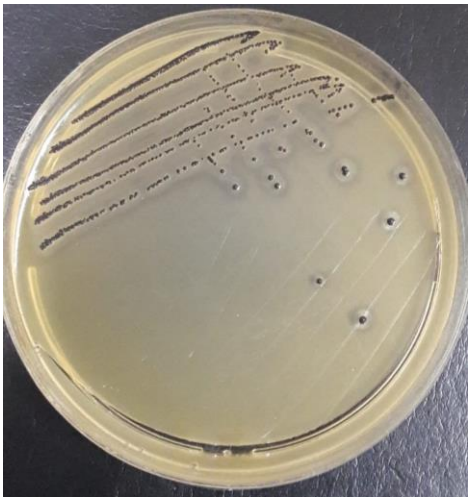


Fig (٢) Colonies of *S.aureus* on baired parker agar media.

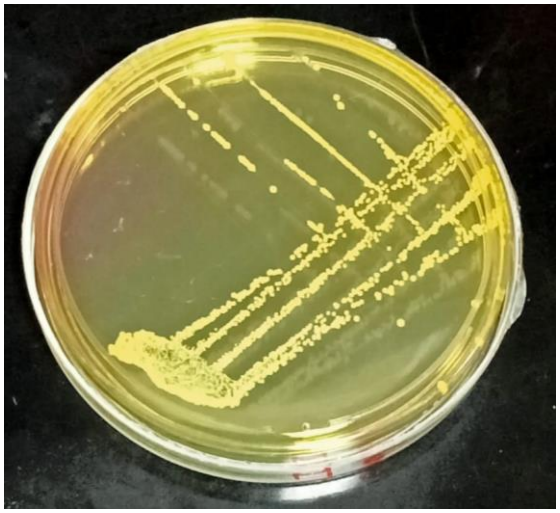


Fig (٣) *S.aureus* on mannitol salt agar.

٣.٣.Prevalence of Coagulase-positive

Samples that showed phenotypically positive growth on Baird–Parker medium investigated for their coagulase activity ,The prevalence of Coagulase-positive *S.aureus* was ١٤/٢١(٦٦٪) ,٧/١٧(٤١٪) ,٦/٨(٧٥٪) ,٣/٦(٥٠٪) from raw cow milk, raw buffalo milk, kareish cheese, baladi yogurt , respectively.

Table(٤) :results of coagulase test:

samples	No, of examined samples	No. of s.aureus isolates	No. of coagulase positive isolates	% of coagulase positive isolates	No. of coagulase negative isolates	% of coagulase negative isolates
Raw cow milk	٢٥	١	٤	٦٦٪	٧	٣٤٪
Raw buffalo milk	٢٥	٧		٤١٪	١٠	٥٩٪
Kareish cheese	٢٥			٧٥٪	٢	٢٥٪
Baladi yogurt	٢٥			٥٠٪	٣	٥٠٪
Total	١٠٠	٢	٠	–	٢٢	–

Fig (٤) coagulase test show that clot form when bacterial cell are incubated with plasma.

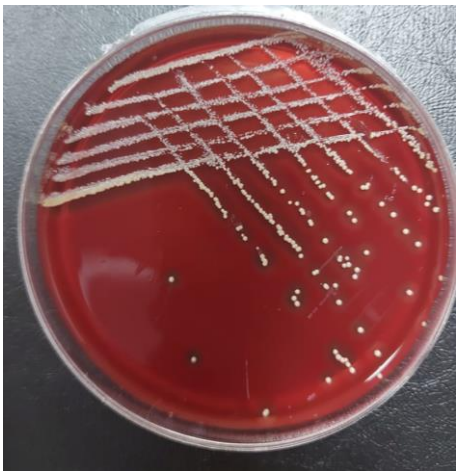


Fig (٥) *S.aureus* showed β – hemolysis on blood agar media.

٣.٤. Antibiotic sensitivity test results:

Based on CLSI breakpoints, Results of antibiotic sensitivity test on ١٥ isolates of *S. aureus* recovered from raw cow milk, raw buffalo milk, kareish cheese, baladi yogurt were highly resistant for penicillin (٨٠٪) followed by clindamycin (٦٠٪) , erythromycin (٥٣٪) and highly sensitive for gentamicin(١٠٠٪) ,norfloxacin (٩٣٪) ,vancomycin (٨٦٪) and linezolid(٦٠٪). Figure (٦).

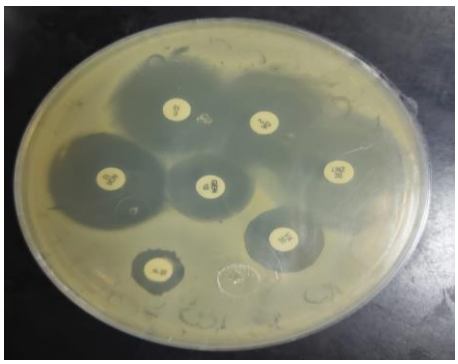


Fig (٦) Antibiotic sensitivity test of *S.aureus*.

Table (٥): Invitro anti-microbial sensitivity test for isolated *S.aureus*

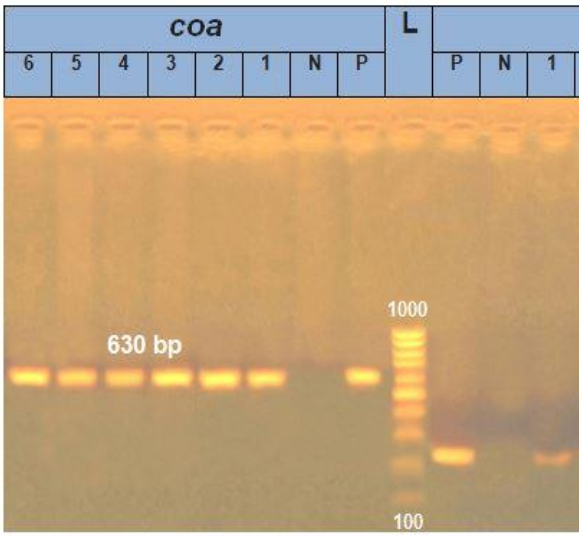
Antimicrobial disc	Code	Disk concentration	Sensitive		Intermediate		Resistant		AA
			No.	%	No.	%	No.	%	
Penicillin	P	١٠ IU	٣	٢٠	٠	٠.٠	١٢	٨٠	R
Clindamycin	DA	٢٠ µg	٦	٤٠	٠	٠.٠	٩	٦٠	R
Linezolid	LNZ	٣٠ µg	٩	٦٠	٠	٠.٠	٦	٤٠	S
Erythromycin	E	١٥ µg	٦	٤٠	١	٦.٦	٨	٥٣	R
Gentamicin	CN	١٠ µg	١٥	١٠٠	٠	٠.٠	٠	٠.٠	S
Vancomycin	VA	٣٠ µg	١٣	٨٦	٠	٠.٠	٢	١٤	S
Norfloxacin	NOR	١٠ µg	١٤	٩٣	١	٦.٦	٠	٠.٠	S

AA: Antibiogram activity

% Percentage in relation to isolates of *S. aureus* no=١٥.

٣.٥. PCR results.

Detection of virulence and resistant genes of *S.aureus.* PCR using primers fragments for detection of two virulence genes ,These genes were *staphylococcal spA* and coagulase (*coa*). It was applied on six isolates of *S. aureus*. all tested isolates harbored these genes(١٠٠٪),Also these isolates were tested for detection of one resistance gene beta- lactam resistance gene (*bla*Z) by uniplex PCR.the results revealed that (٣٣٪)of tested isolates harbored this gene .



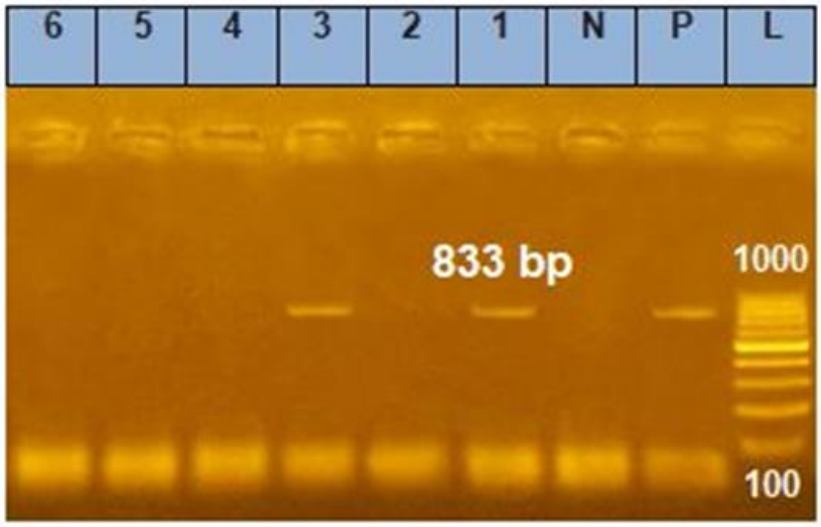


Fig (٧): Agarose gel electrophoresis pattern of PCR for detection of s*taphylococcal* spa and coagulase (coa) genes at٢٢٦ and ٦٣٠bp respectively.

L: Ladder from ١٠٠ bp to ١٠٠٠ bp

Pos: Positive control: *S.aureus* ATCC ٢٥٩٢٣.

Neg: Negative control: Field withdraw that were attempted and attested to be negative by PCR for the associated characteristics in R.L.Q.P

Way ١, ٢, ٣, ٤, ٥,٦: positive upgrade of staphylococcal virulance characteristics (spa and coagulase characteristics).

Fig. (٨): Agarose gel electrophoresis pattern of PCR for detection of *blaZ* gene of *S. aureus* at ٨٣٣ bp.

L: Stepping stool from ١٠٠ bp to ١٠٠٠ bp.

Pos: Positive control: *S.aureus* ATCC ٢٥٩٢٣.

Neg: Negative control: Field confine that were tried and affirmed to be negative by PCR for the connected qualities in R.L.Q.P

Path ١, ٣: Positive enhancement of blaZ quality at ٨٣٣ bp

٤.DISCUSSION

S. aureus can convey heat stable enterotoxins achieving food intoxication in human with fluctuating power El-Makarem and Amer[٢٦] along these lines, the ongoing audit was planned to perceive and depict coagulase positive S. aureus.

In this study ٥٢ bounds of *S. aureus* (٥٢ %) were recovered from ١٠٠ models from unrefined milk and its things, which nearly agreed with the eventual outcomes of Lílian et al., (٢٠١١) who reported higher event of *S.aureus* in rough milk (٦٨٪) But it went against Ahmadi [٢٧] and El-Sayed [٢٩] who bound *S.aureus* at recurrence rate (١٤٪). Moreover,this is higher prevalence at whatever point diverged from results of Zeinhom [٣٠] who uncovered that *S. aureus* was isolated from the rough milk tests with an overall inescapability of ١٢.٥٪ (٢٥/٢٠٠) and saw that as ١٣٪ of unrefined milk tests outperformed beyond what many would consider possible as demonstrated by the Egyptian Standards [٣١].

The high incidence in raw milk could be attributed to environmental pollution, crosscontamination between the milk and each other and poor handling during transportation or in milk collection centers, besides, shedding of *S. aureus* from infected animals is another cause of contamination of milk and dairy food.

Positive isolates tested for their coagulase activity and the results were ٥٦٪ ,٢٨٪,٢٤٪,١٢٪ for raw cow milk, raw buffalo milk, kareish cheese and baladi yogurt respectively.

According to microbiological standards set out by the Egyptian authority, w cheese must be free from *S. aureus* .

In this study the regularity of *S. aureus* in Karish chese was (٣٢٪).which nearly agreed with the eventual outcomes of Mousa [٣٢] and Elmaghraby [٣٣] who nitty gritty that inescapability of ٣٠٪ and ٢٤٪ in Karish cheese in Egypt. these results were seen as low when appeared differently in relation to other Egyptian examinations of El - Malt [١٥] and Sallam [٣٤] that uncovered S. aureus transcendence speeds of ٩٣٪ and ٩٠٪ from Karish cheese .

Variable reported incidence rates could be influenced by many factors such as different techniques of cheese production, storage conditions, type of cheese either manufactured from raw or pasteurized milk, and also related to personal hygiene of workers and unhygienic measures during production.

The bacterium (*S. aureus*) is liable to inactivation and elimination by heating, but as the cheese manufacturer in Egypt use raw milk which is most likely not subject to heating [٣٥].

The prevalence of *s.aureus* in baladi yogurt was ٢٤٪ then the positive isolates tested for coagulase activity and the coagulase positive isolates was ١٢٪ .

This consider low recurrence when diverged from delayed consequences of [٣٦]

who reported that % of positive *s.aureus* isolates was ٧٢٪ and ٤٨٪ for plain and fruit yogurt respectively with Prevalence of Coagulase-positive *S. aureus* in the examined fermented dairy product samples ٣٥٪ and ٢٠٪ for plain and fruit yogurt respectively.

The spread of antibiotic-resistant pathogens continues to challenge sustainable treatment options, with severe public health consequences.

The most common mechanism of resistance to antibiotics of coagulase-positive *staphylococci* is the synthesis of beta lactamases that inactivate penicillinase sensitive antibiotics. Resistance to beta-lactam antibiotics in coagulase- positive staphylococci is increasing and following resistance in human isolates. Some studies show that penicillin disk diffusion method showes deviation in the case of sensitive beta-lactam antibiotics in relation to the dilution method. [٣٧].

Fifteen *S. aureus* detaches were moreover investigated for their shortcoming to antimicrobials ,concerning the results of serum poison weakness testing, by far most of *S.aureus* strains separated were impenetrable to penicillin G (٨٠٪),clindamycin(٦٠٪) ,erythromycin (٥٣٪) sensitive showed most raised responsiveness towards gentamicin (١٠٠٪) ,followed by norfloxacin (٩٣٪) ,vancomycin (٨٠٪) and linezolid (٦٠٪) .

higher security from penicillin scraped by Abera [٣٨] and Shi [٣٩] in a degree of (٩٤.٤٪) and (٨٧.٦٪) independently.

Practically identical results got by Begum [٤٠] who revealed that S. aureus was ٨٢.٨٦٪ and ٣٧.١٤٪ impenetrable to Penicillin-G and Amoxicillin, independently;

In like manner nearly to eventual outcomes of Abdeen [٤١] The MRSA strains showed assurance from penicillin (٩٧.١%), cefoxitin (٨٥.٧٪), and erythromycin (٥٢.٨٪)

The development of PCR-based methods provides a promising option for the rapid identification of bacteria. With this method, identification of bacterial pathogens can be made in hours, rather than days, as conventional cultural methods require. PCR can also improve the level of detection due to its high sensitivity. Theoretically, only a few cells of pathogen are necessary to yield a positive diagnosis [٤٢]. The molecular identification of the *coa* gene in *S. aureus* strains was performed through amplification of the *coa* gene at (٦٣٠) bp. The use of the coa gene to detect *S. aureus* strains from milk origin was previously reported in two studies o [١٥] and [٤٣] which improved the coa quality from mastitic milk and dairy things at ٦٣٠ and

٧٥٠ bp. Moreover, Javid [٤٤] perceived the coa quality at ٥١٤ bp, ٥٩٥ bp, ٧٥٧ bp, and ٨٠٢ bp.

The findings of the present investigation showed that ١٠٠٪ the examined *S. aureus* isolates (٦/٦) harbored *coa* gene. Similar result obtained by [٣٧]

who reported that detection of the *coa* gene from milk samples could help to assess the microbiological safety of raw milk intended for direct use in the dairy industry. The *coa* gene was detected in all isolates with the primers used, even though one of them was considered coagulase-negative both by the tube coagulase test and by the clumping factor assay.

The *SpA* gene is a major important surface protein of bacterial cell wall, which binds with FC region of immunoglobulin G, so the decreasing in *SpA* on cell surface of *S. aureus* resulted in increasing number of free receptor sites for complement and enhance phagocytosis [٤٥].

In the ongoing audit, ٦/٦(١٠٠٪) of attempted *s.aureus* withdraws clutched SpA quality and it upgraded at ٢٢٦ bp that like the examination of [٤٣] that showed strengthening of SpA nature of *S.aureus* at ٢٢٩ bp.and El-Sayed [٢٩] who recognized SpA quality Positive in ٩٠٪ of isolates and heightened at ٢٢٦ bp).

[٤٦] reported that *SpA* gene can be used for typing the isolates of *S.aureus*. in addition to [٤٥] point by point that the revelation of genetic polymorphisms in the X district of the SpA quality can be used for making out of *S. aureus.* In like manner Karahan [٤٧] gathered that ID of SpA quality polymorphisms with Coa-PCR is proposed as a fair illustrative method for making out of *S. aureus* limits which gave critical results to practical control of staphylococcal mastitis.

Practically like eventual outcomes of El-Sayed [٢٩] who declared that Spa quality and Coa quality were perceived in (٩٠٪) and (٨٠٪) of the attempted *S.aureus* strains

The resistance to β-lactam antibiotics occur through hydrolyzing the β-lactam ring and convert to inactive form, so the application of PCR for detection of *blaZ* gene is recommended in veterinary laboratories for detection of the resistant strains of *S. aureus* [٤٨] . In this study, ٢/٦ (٣٣,٣٪) limits of *S. aureus* presented to PCR were positive for blaZ quality and gave a single amplicon at ٨٣٣ bp. At any rate special result got by [٤٩] and [٤٣] recognized a singular amplicon of ١٧٣ bp of blaZ quality. [٥٠]

noticed that all positive strains to *mecA* were also positive for gene and the presence of both genes was correlated to phenotypic beta-lactam resistance of S. aureus strains .

In this study, ٢\٦ of tested *S. aureus* isolates harbored *blaz* gene which disagreed with the result of [٥١]

who founnd that all S. aureus isolates harbored *blaZ* gene associated with penicillin

٥.CONCLUSIONS

Staphylococcal food poisoning is of major concern in public health programs worldwide. S. aureus may be present in milk and milk products as a result of milk collected from the animal suffering from disease condition and excreting S. aureus in milk or due to unhygienic conditions during production, processing, storage and handling of milk products, which are the main causes of food borne diseases. Results clearly indicated that milk and milkbased products available in the market were contaminated with S. aureus, posing a high risk of food poisoning. Thus more hygienic preventive measures are required to reduce the bacterial contamination, so as to increase the wholesomeness of these milk and milk based products.

Study concluded that S. aureus was isolated from milk and milk products, and raw milk was highly contaminated with the bacterium than fermented milk products (cheese and yoghurt). However, none of the dairy products was devoid of the isolates.

٦.ACKNOWLEDGEMENTS

deepest thanks to supervisor Prof. Dr. Ashraf Awad Abd El – Tawab, Prof. Dr. Ahmed Saber ELfayoumy, Dr. Marwa Awad Meawad Mohamed and to Dr. Doaa Mostaafa Ragheb sharaf

Publication consent
Each author has demonstrated their consent for the publication of the current manuscript.
Data and material availability:
All data of this study is provided.
Conflict of interests.
All authors have stated the absence of any conflicts of interest.
Funding.
This research did not receive funding from any specific grant.
Authors' contributions.
K.M.S: Conceptualization, Formal Analysis, Investigation, Supervision, Resources, Writing – original draft
A.R.S: Data collection, Formal Analysis, Project administration, Resources, Writing – review and editing.
M.M.Z: Conceptualization, Data curation, Formal Analysis, Resources, Supervision, , Writing – review and editing.

REFERENCES

١.Nemeghaire, S., Argudin, M. A., Haesebrouck, F., and Butaye, P. (٢٠١٤): Epidemiology and molecular characterization of methicillin-resistant *Staphylococcus aureus* nasal carriage isolates from bovines. *BMC Vet. Res.* ١٠:١٥٣. doi: ١٠.١١٨٦/١٧٤٦-٦١٤٨-١٠-١٥٣.

٢.Wyder, A. B., Boss, R., Naskova, J., Kaufmann, T., Steiner, A., and Graber, H. U. (٢٠١١): *Streptococcus* spp. and related bacteria: their identification and their pathogenic potential for chronic mastitis – *a molecular approach. Res. Vet. Sci.* ٩١, ٣٤٩–٣٥٧. doi: ١٠.١٠١٦/j.rvsc.٢٠١٠.٠٩.٠٠٦

٣.Zecconi A, Scali F. (٢٠١٣): Staphylococcus aureus virulence factors in evasion from innate immune defenses in

human and animal diseases. Immunol Lett [Internet]. ١٥٠(١–٢):١٢–٢٢.

٤.Scali F, Camussone C, Calvinho LF, Cipolla M, Zecconi A.(٢٠١٥): Which are important targets in development of S. aureus mastitis vaccine? Res Vet Sci. ١٠٠:٨٨–٩٩.

٥.Ballhausen B, Kriegeskorte A, van Alen S, Jung P, Köck R, Peters G, Bischoff M, Becker K. (٢٠١٧): The pathogenicity and host adaptation of livestock-associated MRSA CC٣٩٨. Vet Microbiol. ٢٠٠:٣٩–٤٥.

٦.Seilie ES, Bubeck Wardenburg J. (٢٠١٧): Staphylococcus aureus pore-forming toxins: The interface of pathogen and host complexity. Semin Cell Dev Biol. ٧٢:١٠١–١١٦.

٧.Panizzi, P.; Friedrich, R.; Fuentes-Prior, P.; Bode, W. and Bock, P.E. (٢٠٠٤): The staphylocoagulase family of zymogen activator and adhesion proteins. Cell Mol Life Sci ٦١: ٢٧٩٣-٢٧٩٨.

٨.Mcadow, M.; Missiakas, D. M. and Schneewind, O. (٢٠١٢): S.aureus secretes Coagulase and von willebrand factor binding protein to modify the coagulation cascade and establish host infections. Journal of Innate immune, ٤(٢):١٤١-١٤٨.

٩.Dallal, M.M.S.; Khoramizadeh, M.R.; Amiri, S.A.; Yaraghi, A.A.S.; Fard, R.M.N(٢٠١٦): Coagulase gene polymorphism of Staphylococcus aureus isolates: A study on dairy food products and other foods in Tehran, Iran. *Food Sci. Hum. Wellness*. ٥, ١٨٦–١٩٠.

١٠.Alonso, D.O.V. and Daggett, V.(٢٠٠٠): Staphylococcal Protein A: Unfolding pathways, unfolded states, and differences between the B and E domains. Proc. Natl Acad. Sci. USA ٩٧:١٣٣-١٣٨.

١١.Gentilini, E.; Denamiel, G.; Llorente, P.; Godaly, S.; Rebueto, M. and De Gregorio, O. (٢٠٠٠): Antimicrobial susceptibility testing of Staphylococcus aureus isolated from bovine mastitis in Argentina. J. Dairy Sci., ٨٣: ١٢٢٤-١٢٢٧. http://dx.doi.org/١٠.٣١٦٨/jds.S٠٠٢٢ - ٠٣٠٢(٠٠)٧٤٩٨٨-٥.

١٢.Taponen, S. and Pyorala , S.(٢٠٠٩): Coagulase-negative Staphylococci as cause of bovine mastitis: not so different from Staphylococcus aureus? Vet Microbiol ١٣٤:٢٩–٣٦.

١٣.Lindqvist R, Sylven SI, Vagsholm I (٢٠٠٢): Quantitative microbial risk assessment exemplified by S. aureus in unripened cheese made from raw milk. International Journal Food Microbiology, ٧٨:١٥٥-١٧٠.

١٤.Tamarapu S, McKillip JL, Drake M (٢٠٠١): Development of a multiplex polymerase chain reaction assay for detection and differentiation of S. aureus in dairy products. J. Food Protect. ٦٤:٦٦٤-٦٦٨.

١٥.El-Malt LM, Hameed KGA, Mohammed AS (٢٠١٣): Microbiological evaluation of yoghurt products in Qena city, Egypt. Veterinary World, ٦: ٤٠٠-٤٠٤. doi:١٠.٥٤٥٥/vetworld.٢٠١٣.٤٠٠-٤٠٤.

١٦.APHA(American Public HealthAssociation)(٢٠٠١): Compendium of methods for the microbiological examination of foods, ٤th edition. American Public Health Association (APHA). Washington, DC USA.

١٧.Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C.; Leonard, F.C. and Maguire, D. (٢٠٠٢): Veterinary Microbiology and Microbial Disease, ٢nd Edition. WileyBlackwell.

١٨.Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (١٩٧٥): Medical Microbiology. ١٢th Ed. Churchill Livingstone Edinburgh, London and New York

١٩.MacFaddin, J. F. (٢٠٠٠): Biochemical tests for identification medical bacteria. Warery Press Inc,

٢٠.Bauer, A.; Kirby, W.; Sherris, J. and Turck, M. (١٩٦٦): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., ٤٥ (٤): ٤٩٣ – ٤٩٦

٢١.Clinical Laboratory Standards Institute (CLSI) (٢٠٢١): Performance standards for antimicrobial Susceptibility Testing; M١٠٠ ٣١th Edition. Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing.

٢٢.Bhati, T.; Nathawat, P.; Sharma, S.K.; Yadav, R.; Bishnoi, J. and Kataria, A.K. (٢٠١٦): Polymorphism in *spa* gene of *Staphylococcus aureus* from bovine subclinical mastitis. Veterinary World, ٢٠١٦;٩:٤٢١-٤٢٤.

٢٣.Iyer, A.P. and Kumosani, T.A. (٢٠١١): PCR based detection of nosocomial infection causing MRSA (Methicillin resistant Staphylococcus aureus). ٢٠١١ ٢*nd*

International Conference on Biotechnology and Food Science IPCBEE vol. ٧(٢٠١١) © (٢٠١١) IACSIT Press, Singapore.

٢٤.**Wada, M.; Lkhagvadorj, E.; Bian, L.; Wang, C.; Chiba, Y.; Nagata, S.; Shimizu, T.; Yamashiro, Y.; Asahara, T. and Nomoto, K. (٢٠١٠):** Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus. Journal of Applied Microbiology ١٠٨ (٢٠١٠) ٧٧٩–٧٨٨.

٢٥.**Bagcigil, A.B.; Taponen, S.; Koort, J.; Bengtsson, B.; Myllyniemi, A, and Pyörälä, S. (٢٠١٢):** Genetic basis of penicillin resistance of S. aureus isolated in bovine mastitis. Acta Veterinaria Scandinavica ٢٠١٢, ٥٤:٦٩.

٢٦.**El-Makarem, H. S. A. and A. Amer (٢٠١٨):** "Multiplex PCR and sandwich ELISA for identification of enterotoxigenic Staphylococcus aureus isolated from Egyptian soft cheese." Egyptian Journal of Food Safety ٢(١).

٢٧.**Lílian, P. O.; Ludmilla, S. S.E. B.; Valdir, C. S. and Marina, G. C. (٢٠١١):** Study of Staphylococcus aureus in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. J. Food Process Technol., ٢(٦): ١:٥

٢٨.**Ahmadi, M., Rohani, S. M. R., & Ayremlou, N. (٢٠١٠):**Detection of Staphylococcus aureus in milk by PCR. *Comparative Clinical Pathology*, ١٩, ٩١-٩٤.

٢٩.**El-Sayed, M., et al. (٢٠١٩):** "Prevalence and genetic characterization of S. aureus strains isolated from raw milk and its products." Suez Canal Veterinary Medical Journal. SCVMJ ٢٤(٢): ٢٤٥-٢٥٦.

٣٠.**Zeinhom, M. and A. Abed (٢٠٢٠):** "Prevalence, characterization, and control of Staphylococcus aureus isolated from raw milk and Egyptian soft cheese." Journal of Veterinary Medical Research ٢٧(٢): ١٥٢-١٦٠.

٣١.**ES (Egyptian Standards) (٢٠٠٥):** Egyptian Organization of Standardization and Quality Control. Issued by ministry of industry and technological development. Egypt.

٣٢.**Mousa, W.S.; Abdeen, E.; Hussein, H.; Hadad, G.(٢٠١٧):**Prevalence and multiplex PCR for enterotoxin genes of Staphylococcus aureus isolates from subclinical mastitis

and Kareish cheese. *J. Anc. Dis. Prev. Remedies* ٢٠١٧, ٥

٣٣.Elmaghraby, M.; Abdeen, E.; AbouElros, N.; Bagory,(٢٠١٨): A. Molecular identification of virulence genes of Staphylococcus aureus isolated from milk products. *Alex. J. Vet. Sci.*, ٥٩, ٥٧.

٣٤.Sallam, K. I.; Abd-Elghany, S. M.; Elhadidy, M. and Tamura, T. (٢٠١٥): Molecular Characterization and Antimicrobial Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* in Retail Chicken. Journal of Food Protection, ٧٨: (١٠) ١٨٧٩–١٨٨٤ .

٣٥.Paulin S, Horn B, Hudson JA (٢٠١١): Factors influencing staphylococcal enterotoxin production in dairy products. MPI Technical, ٢٠١٢/٠٧.

٣٦.Abd El, R. A. E. M., et al. (٢٠٢٢).:"Prevalence and Characterization of Some Pathogenic Bacteria in Fermented Milk Products and Mish Cheese in Dakahalia Governorate, Egypt." Journal of Advanced Veterinary Research ١٢(٤): ٤٤٦-٤٥٠.

٣٧.Linage, B., Rodríguez-Calleja, J. M., Otero, A., García-López, M. L., & Santos, J. A. (٢٠١٢): Characterization of coagulase-positive staphylococci isolated from tank and silo ewe milk. *Journal of dairy science*, ٩٥(٤), ١٦٣٩-١٦٤٤.

٣٨.Abera, M., Demie, B., Aragaw, K., Regassa, F., and Regassa, A. (٢٠١٠): Isolation and identification of staphylococcus aureus from bovine mastitic milk and their drug resistance patterns in adama town, ethiopia. Journal of Veterinary Medicine and Animal Health, ٢(٣), ٢٩-٣٤.

٣٩.Shi, D., Hao, Y., Zhang, A., Wulan, B., and Fan, X. (٢٠١٠): Antimicrobial resistance of staphylococcus aureus isolated from bovine mastitis in china. Transboundary and Emerging Diseases, ٥٧(٤), ٢٢١-٢٢٤.

٤٠.Begum HA, Uddin MS, Islam MJ, Nazir KHMNH, Islam MA, Islam MT (٢٠٠٧): Detection of biofilm producing coagulase positive Staphylococcus aureus from bovine mastitis, their pigment production, hemolytic activity and antibiotic sensitivity pattern. Journal of Bangladesh Society for Agricultural Science and Technology, ٤: ٩٧-١٠٠.

٤١.Abdeen, E. E., et al. (٢٠٢١):"Prevalence and characterization of coagulase positive Staphylococci from food products and human specimens in Egypt." Antibiotics ١٠(١): ٧٥

٤٢.Phuektes P, Mansell PD, Browning GF (٢٠٠١): Multiplex polymerase chain reaction assay for simultaneous detection of Staphylococcus aureus and Streptococcal causes of bovine mastitis. J Dairy Sci ٨٤:١١٤٠–١١٤٨

٤٣.Abdeen E., E.; Walid, M.; Hussien, H.; Roshdy, S. (٢٠١٥):PCR for detection of virulence and antibiotic resistance genes of coagulase positive staphylococcus aureus from clinical mastitis in Egypt. *Int. J. Basic Appl. Sci.*, ٤, ٣١٥.

٤٤.Javid, F.; Taku, A.; Bhat, M.A.; Badroo, G.A.; Mudasir, M.; Sofi, T.A. (٢٠١١): Molecular typing of Staphylococcus aureus based on coagulase gene. *Vet. World* ٢٠١٨, ١١, ٤٢٣–٤٣٠.

٤٥.GAO, J. and Stewart, G.C. (٢٠٠٤): Regulatory elements of the Staphylococcus aureus protein A (Spa) promoter. J Bacteriol, ١٨٦: ٣٧٣٨-٣٧٤٨.

٤٦.Gholamreza Feizi, M.A.H.; Zarrini, G. and Tahmasebi, M. (٢٠١٣): Evaluation of Protein a Gene Tandem Repeat Polymorphism of Staphylococcus aureus isolated From Bovine Mastitis in Tabriz. Jundishapur Journal of Microbiology. Vol ٦(٦): e٧٩٣٤.

٤٧.Karahan, M.; Acik, M. N. and Cetinkaya, B. (٢٠١١): Investigation of virulence genes by PCR in Stapylococcus aureus isolates originated from subclinical bovine mastitis in Turkey. Pak Vet J, ٣١(٣): ٢٤٩-٢٥٣.

٤٨.Kernodle, D. S. (٢٠٠٠): Mechanism of resistance to Beta-lactam antibiotics in Gram-positive Pathogens. American Society of Microbiology, Washington, DC, USA.Pp.٦٠٩-٦٢٠.

٤٩.Chandrasekaran, D.; Venkatesan, P.; Tirumurugaan, K.G.; Subapriya, S.; Nambi, A.P; Thirunavukkarasu, P.S. And Vairamuthu, S. (٢٠١٤): Comparison of tests for detection of lactamase staphylococcus aureus in mastitis cows: International Journal of Science, Environment and Technology, Vol. ٣, No: ١٥٢٥ – ١٥٢٧

٥٠.Soares, L.C.; Pereira, I.A.; Pribul, B.R.; Oliva, M.S.; Coelho, S.M.O. and Souza, M.M.S. (٢٠١٢): Antimicrobial resistance and detection of mecA and blaZ genes in coagulase-negative Staphylococcus isolated from bovine mastitis. Pesq. Vet. Bras. ٣٢(٨):٦٩٢-٦٩٦

٥١.**Ahmed, W., et al. (٢٠٢٠).:**Characterization of staphylococci and streptococci isolated from milk of bovides with mastitis in Egypt." Pathogens ٩(٥): ٣٨١.