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ORIGINAL ARTICLE

Prevalence, Characterization, and Control of *Staphylococcus aureus* Isolated from Raw Milk and Egyptian Soft Cheese

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Abstract
The present study aimed to detect the prevalence of *Staphylococcus aureus*) S. *aureus*) in raw milk, and soft cheese samples in Beni-Suef Governorate, Egypt, and to characterize some resistance and virulence related genes in the recovered *S. aureus* isolates. An additional objective was to evaluate the effectiveness of thyme oil for controlling *S. aureus* in cheese. A total of 200 samples of raw milk and cheese, including 100 samples of raw milk and 100 samples of two different types of cheese were used. *S. aureus* was isolated from 12.5% (25/200) of the raw milk and cheese samples. The highest prevalence was detected in Kareish cheese (18%), followed by raw milk samples (13%) and Talaga cheese (6%). S. aureus isolates showed high resistance to ampicillin (72%), and tetracycline (60%). PCR was applied on 6 multidrug resistant (MDR) *S. aureus* for detection of mecA and icaA genes which were detected in 66.7% and 33.3% of *S. aureus* isolates, respectively. As milk and dairy products are good substrates for *S. aureus* that causes staphylococcal food poisoning, reducing the level of such pathogen in milk and dairy products will lower the risk to consumers. Thyme oil 1% showed moderate inhibition against *S. aureus,* while using thyme oil 2% showed significant inhibition of *S. aureus* in Kareish cheese. Therefore, the use of thyme oil in preserving white soft Kareish cheese might be a promising approach to increase the shelf life and safety of the cheese.

Keywords Antimicrobial resistance, Cheese, Control, Raw milk, *S. aureus*, Thyme oil

1. Introduction
Access to healthy and safe food is a concern of both food manufacturers and consumers. Milk and its products are considered one of the basic meals for humans from birth to senility all over the world, including Egypt because they contain many ingredients that make them highly nutritious food for mammals; however, these benefits make them a good environment for the growth of many microbes **(Kandpal et al. 2012).** Milk and milk products can be contaminated with harmful bacteria through mastitis, polluted air, or storage and transportation equipment **(Baylis 2009).**

Kareish cheese is known to be one of Egypt's most common local forms of fresh soft cheese that is made from raw buffalo or cow's milk, which is often of low microbiological quality due to the high microbial load in raw milk and unsatisfactory conditions. Egyptian consumers' growing demand is largely due to its high protein, calcium, phosphorus and vitamin content as well as its low price. Moreover, the conventional

cheese production method offers many possibilities for microbial contamination. **(El Bagoury and Mosaad 2002).**

Foodborne diseases are a widespread global problem. Many outbreaks occur due to consuming contaminated dairy products, which appeared to have a natural taste and aroma but unfortunately contaminated with many harmful bacteria **(CDC 2009).** *S. aureus* is a Gram-positive, coagulase-positive ubiquitous organism. It is considered one of the most common causes of disease in the world **(Pereira et al. 2009).** Milk and milk products are known to be a source of *S. aureus* contamination whether they are collected from cows suffering from mastitis or from food handlers carrying the microbe because of poor personal hygiene **(Bingol et al. 2012).** The 16S rRNA PCR assay has been introduced as an effective tool that successfully could identify and classify multiple types of bacteria including staphylococci in multiple sample types **(Johnson et al. 2016).** Moreover, *S. aureus* produces an extracellular thermostable nuclease that encoded by *nuc* gene; one of the most successful distinguishing characteristics for *S. aureus* from other *Staphylo-coccus* spp. Therefore, *nuc* gene was suggested as a specific marker gene. PCR is currently considered as an effective and useful method for identifying *S. aureus* harbored this gene **(Sahebnasagh et al. 2014).**

Some strains of *S. aureus* are equipped with numerous virulence factors causing serious infections; they can produce food-poisoning enterotoxins if they grow in large numbers in foods **(Pereira et al. 2009)** as well as having biofilm-forming related genes including *ica* genes; especially *ica*A and *ica*D (**Melake et al. 2017).** Symptoms of *S. aureus* food poisoning typically appear fast, usually within two to four hours, and often include vomiting, abdominal cramps, nausea, and diarrhea **(Hennekinne et al. 2012).**

Antimicrobial resistance is one of the greatest threats for the global health and food security due to the continuous increasing of antimicrobial resistance of many pathogens against different antimicrobial agents **(Abed et al. 2018).** Moreover, antimicrobial resistance is implicated in hospital and community infections **(Friedrich 2019).** The frequent and improper use of antibiotics both in human and veterinary medicine, for several years, has led to the development of multidrug resistant (MDR) strains of *S. aureus*, as well as of other pathogens **(Hardy et al. 2004).** Mechanism of staphylococcal antimicrobial resistance and genotypic detection of resistance genes have been investigated for long time. Updating such knowledge may help in control programs, e.g. methicillin resistance gene (*mec*A) that encoded alternative penicillin binding protein, PBP2a, causing reduced binding to β-lactams antibiotics **(Abed et al. 2018).** This requires seeking to find alternative natural controls that are safe and healthy, such as the use of natural compounds with antimicrobial properties **(Holley and Patel 2005).**

Recently there has been a special focus on the uses of essential oils (EOs), which contain many natural, biologically active ingredients that have antimicrobial and antioxidant properties **(Yousefi Asli et al. 2017; Hanif et al. 2019).** Using EOs improve the nutritional value, organoleptic features and also the hygienic status of lactic acid products **(Amirdivani and Baba 2011).** Thyme oil is one of the EOs whose doses have been approved by the Food and Drug Administration (FDA) for safe use in food **(deCarvalho et al. 2015).** The antimicrobial effect of thyme oil inhibiting microbes in foods has been reported in several previous studies as supported by **deCarvalho et al. (2015); Kohiyama et al. (2015); Ben Jemaa et al. (2017).**

Therefore, it was important to direct the aims of the present work to determine the prevalence of *S. aureus* in raw milk and cheese in Beni-Suef Governorate, Egypt, as well as assessing their antibiotic resistance profile and assessing the survival of *S. aureus* in laboratory manufactured Kareish cheese with the addition of thyme essential oil.

2. Materials and methods

2.1. Samples collection and preparation

A total of 200 samples, including 100 raw milk, 50 Talaga cheese (made from pasteurized milk) and 50 Kareish cheese (made from raw milk) samples were collected from local markets, dairy shops and supermarkets widely distributed across Beni-Suef Governorate, Egypt. Kareish cheese (an unpasteurized white, soft homemade cheese) was collected from farmer's houses from different localities of Beni-Suef Governorate in Egypt over a period of 6 months during 2020. Samples were transferred to the laboratory in an icebox (2–5°C) within 1 h from purchase for laboratory examination **(APHA 2015).**
A well-mixed milk sample (10 ml) was added to 90 ml of 0.1% sterile peptone water (Oxoid, Ltd, Basingstoke, UK) to make a dilution of 1/10 from which 10-fold serial dilutions were made. For the

cheese samples, 25 g of cheese were added to 225 ml of 0.1% sterile peptone water solution and then mixed using a Lab-blender 400 (Stomacher; Inter science, France) for 2-4 min. Ten-fold serial dilutions were performed for the samples **(APHA 2015).**

2.2. Isolation and identification of *S. aureus*

From the appropriate dilutions and the original milk sample, 100 µl were evenly spread onto a dry surface of Baird-Parker agar plates (Oxoid, Basingstoke, UK). All the plates as well as the control ones were incubated at 37 ± 1ºC for 24-48 h. Suspected colonies of *S. aureus* (black, shiny appearance and surrounded by a clear zone) were counted. Bacterial films stained with Gram's stain were made from the suspected colonies to confirm being Staphylococci. suspected colonies were subjected to further biochemical identification, using catalase test, coagulase test, citrate utilization, oxidase test, urease production, mannitol fermentation tests and hemolysis on 5% sheep blood agar **(Singh and Prakash 2008; APHA 2015).** Isolates collected from raw milk and cheese samples and identified as *S. aureus* were subjected to PCR for further identification.

2.3. Antimicrobial susceptibility testing

All *S. aureus* isolates were tested for their antimicrobial susceptibility (AMS) using various classes of antimicrobials used in veterinary field.; amoxicillin-clavulanic (30µg), clindamycin (2µg), tetracycline (30µg), ampicillin (10 µg), streptomycin (10µg), cefoxitin (30µg), cefotaxime (30µg), florfenicol (30µg), sulfamethoxazole-trimethoprim (30µg) and imipenem (10µg). Disk diffusion assays were performed (in triplicate) on Muller Hinton agar (Oxoid, Ltd, Basingstoke, Hampshire, UK) according to **CLSI (2016).** The AMS based on the induced inhibition zones according to breakpoints available in the Clinical and Laboratory Standards Institute (CLSI-2016, https://clsi.org/). Resistance to two or more antimicrobials of different classes was considered as multidrug resistant (MDR) **(Chandran et al. 2008).**

2.4. The use of PCR for confirmation and screening some resistance and virulence genes of *S. aureus* isolates

PCR was applied on all *S. aureus* isolates for confirmative diagnosis using 16S rRNA and *nuc* genes. Moreover, PCR was applied on MDR isolates resistant to cefoxitin (n=6) for screening the presence of both of *mec*A resistance gene and *ica*A virulence genes. DNA Extraction from samples was processed by using QIAamp DNA mini kit instructions (Cat. No. 51304) (Qiagen, Germany, GmbH). The sequences and specificities of the primers (Metabion, Germany), as well as size of amplified products, temperature and time conditions of the primers, were illustrated in **Table (1).**

2.5. Impact of thyme oil on the behavior of *S. aureus* during manufacture and storage of Kareish cheese

The thyme EO concentration used was selected according to previous studies **(Ben Jemaa et al. 2017)** to determine its effect against *S. aureus*. The thyme oil was obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA) and stored in tightly closed glass bottles at 4°C until use. All volumes of thyme oil were diluted, prior to inoculation using 2 mL of Tween 20 as a safe food emulsifier (Sigma–Aldrich, Steinheim, Germany) to facilitate its dissolution. Tween 20 showed no inhibitory effect on the inoculated *S. aureus.*

2.6. Laboratory manufactured Kareish cheese and viability and control experiment

Kareish cheese was manufactured in the laboratory using skimmed milk following the method described by **Hamad (2015).** The milk was pasteurized at 63 ºC for 30 min. After the milk was cooled to 40 °C, calcium chloride and sodium chloride were added at levels of 0.02 and 3% w|w respectively. A fresh culture of MDR *S. aureus* isolate (selected randomly from MDR isolates harboring both *mec*A and *ica*A genes) was added to the pasteurized skimmed milk to give an initial count of approximately 1.75×10^5 CFU/ml (5.24 $\log_{10}$ CFU/ml). Rennet at a concentration of 1.5g/100 kg milk (Chr. Hansen, Hamilton, New Zealand) was added. At this point the milk was equally divided into three portions; the first was the control, the second was treated with 1% thyme oil, and the third was treated with 2% thyme oil. All portions were incubated at 40ºC for 2-3 h for curd formation. The formed cheese was stored in the refrigerator at 4ºC for 30 days. Counts of *S aureus* were achieved from day zero, then day 1, 3, 7, 14, 21 and 28 using the standard plate technique. Tenfold serial dilution of Kareish cheese samples (25 g) were prepared and streaked onto Baird-Parker agar plates (Oxoid, Basingstoke, UK) and incubated at 37ºC for 24 h. **(APHA 2015).**

2.7. Statistical Analysis

Statistical analysis of the data was carried out using SPSS software version 20 (SPSS, Chicago, IL). For all treatments, data are the means ± the standard deviation of the results. Significant differences between the samples were evaluated using the one-way analysis of variance (ANOVA) method at the 5% significance level.

3. Results

3.1. Prevalence of *S. aureus* in raw milk and cheese

S. aureus was isolated from the raw milk and cheese samples with an overall prevalence of 12.5% (25/200) **(Table 2).** Out of the examined 100 market raw milk samples, 13% were found to be positive for *S. aureus* with a count ranged from <1.00 to 4.89 $\log_{10}$ CFU/ml with a mean count of 4.30±3.04 $\log_{10}$ CFU/ml. Meanwhile, 3 out of 50 (6%) Talaga cheese samples showed positive *S. aureus* with a count ranged from <2.00 to 4.20 $\log_{10}$ CFU/g with a mean count of 3.18±2.43 $\log_{10}$ CFU/g. Moreover, *S. aureus* was detected in Kareish cheese samples in a proportion of 18% with a count ranged from <2.00 to 5.78 $\log_{10}$ CFU/g and a mean value of 5.32±4.11 $\log_{10}$ CFU/g.

3.2. Antimicrobial profile of *S. aureus* isolates

Results presented in **Table (3),** showed that *S. aureus* isolates were highly resistant to ampicillin (72%) and tetracycline (60%) while a moderate resistance was recorded against clindamycin (46%). On the other hand, they showed high sensitivity to streptomycin (96%) followed by sulfamethoxazole-trimethoprim (84%), florfenicol and cefoxitin (76% for each), imipenem (72%) and amoxicillin-clavulanic (68%). Fourteen isolates were MDR (56%).

3.3. The use of PCR for confirmation and screening some resistance and virulence genes of *S. aureus* isolates.

Confirmation of the results was performed using PCR through detection of 16S rRNA and *nuc* genes. All the tested *S. aureus* isolates harbored the two tested genes (100%).

Moreover, out of 6 cefoxitin resistant MDR *S. aureus* isolates subjected to PCR, 4 isolates (66.7%) harbored *mec*A gene of which 2 isolates (33.3%) harbored *ica*A gene.

3.4. Behavior of *S. aureus* during manufacture and storage of Kareish cheese

Results illustrated in **Table (4)** showed that *S. aureus* could survive in Kareish cheese for up to 28 days. Approximately 5.24 ±.0.15 $\log_{10}$ CFU/g was detected on day zero of the experiment. The *S. aureus* count continued to increase until it reached 8.77±.0.12 $\log_{10}$ CFU/g on day 28, with an increased rate estimated at about 3 log increments. Thyme oil at a concentration of 1% diminished *S. aureus* on day 14; around 2 log reduction (3.27±.0.34 $\log_{10}$ CFU/g), but was able to survive until the end of the experiment.

Interestingly, the increase in the concentration of thyme oil to 2% was more effective, as the number of *S. aureus* was reduced by a decrease of about 3 log reduction on the third day (2.52±.0.42 $\log_{10}$ CFU/g), and completely disappeared on the 7th day.

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene		Primers sequences (5'-3')	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extends	Reference
					Secondary denaturation	Annealing	Extension		
16S rRNA	F	*GTAGGTGGCAAGCGTTATCC*	228	95˚C/ 5 min	95˚C/ 1 min	54˚C/ 1 min	72˚C/ 1 min	72˚C/ 7 min	Lovseth et al. (2004)
	R	*CGCACATCAGCGTCAG*							
nuc	F	*GCGATTGATGGTGATACGGTT*	270	95˚C/ 5 min	95˚C/ 1 min	54˚C/ 1 min	72˚C/ 1 min	72˚C/ 7 min	Oliveira et al. (2016)
	R	*AGCCAAGCCTTGACGAACTAAAGC*							
mecA	F	*GTA GAA ATG ACT GAA CGT CCG ATA A*	310	94˚C/ 5 min	94˚C/ 30 sec	50˚C/ 30 sec	72˚C/ 30 sec	72˚C/ 7 min	McClure et al. (2006)
	R	*CCA ATT CCA CAT TGT TTC GGT CTA A*							
icaA	F	*CCT AAC TAA CGA AAG GTA G*	1315	94˚C/ 5 min	94˚C/ 30 sec	49˚C/ 1 min	72˚C/ 1 min	72˚C/ 12 min	Ciftci et al. (2009)
	R	*AAG ATA TAG CGATAA GTG C*							

Table 2. Prevalence and count of *S. aureus* in the examined milk and cheese samples.

Type of samples	No. of samples	Positive	*S. aureus* count (Log_{10} CFU/ml)			No. of samples above E.S. value	
		No. (%)	Minimum	Maximum	Mean ± STDV*	No	%
Raw milk	100	13 (13.0)	<1.00	4.89	4.30±3.04	13	13
Talaga cheese	50	3 (6.0)	<2.00	4.20	3.18±2.43	3	6
Kareish cheese	50	9 (18.0)	<2.00	5.78	5.32±4.11	9	18
Total	200	25 (12.5)				25	12.5

* STDV: standard deviation of the mean

Table 3. Antimicrobial susceptibility pattern of *S. aureus* isolates.

Class	Antimicrobial agent	conc. (µg)	% of *S. aureus* isolates (n=25)		
			R	I	S
Penicillins	Amoxicillin-clavulanic	30	20	12	68
	Ampicillin	10	72	0	28
Lincosamides	Clindamycin	2	46	12	42
Tetracyclines	Tetracycline	30	60	16	24
Aminoglycosides	Streptomycin	10	4	0	96
Cephalosporines	Cefoxitin	30	24	0	76
	Cefotaxime	30	16	56	28
Chloramphenicol	Florfenicol	30	0	24	76
Potentiated sulfonamides	Sulfamethoxazole-trimethoprim	25	4	12	84
Carbapenems	Imipenem	10	12	16	72

R=Resistant, S=Sensitive, I=intermediate, %: were calculated according to the No. of tested isolates (n=25).

Table 4. Viability and control of *S. aureus* using thyme oil during the manufacturing of Kareish cheese.

Storage days (4ºC)	*S. aureus*	*S. aureus+ thyme 1%*	*S. aureus+ thyme 2 %*
Zero	$5.24 \pm .0.15^{a}$	$5.24 \pm .0.15^{a}$	$5.24 \pm .0.15^{a}$
1	$5.76 \pm .0.14^{a}$	$5.37 \pm .0.07^{a}$	$4.16 \pm .0.15^{b}$
3	$6.59 \pm .0.08^{a}$	$5.07 \pm .0.07^{b}$	$2.52 \pm .0.42^{c}$
7	$7.76 \pm .0.08^{a}$	$4.26 \pm .0.66^{b}$	ND
14	$8.44 \pm .0.09^{a}$	$3.27 \pm .0.34^{c}$	ND
21	$8.71 \pm .0.05^{a}$	$2.68 \pm .0.15^{c}$	ND
28	$8.77 \pm .0.12^{a}$	$2.15 \pm .0.21^{c}$	ND

N.B. results expressed as log10CFU/g ± standard deviation. ND; means not detected. a, b, c significantly different at $P < 0.05$

4. Discussion

Contaminated food is well known as the key source of transmission of pathogenic bacteria to humans. It is the main cause of most diseases in developed countries, contributing to mortality and morbidity in many instances **(Gunasegaran et al. 2011).** In the present study, we found that 13% of raw milk samples exceeded the permissible limits according to the Egyptian Standards **(ES 2005)** which reported that the number of *S. aureus* in raw milk samples must not exceed 100 CFU/ml. Nearly similar results of *S. aureus* prevalence in raw milk were reported by **Ahmed et al. (2019).** Moreover, a high prevalence was obtained by **Ibrahim et al. (2015).**

The prevalence rate of *S. aureus* in Talaga and Kareish cheese samples was 6% and 18% respectively with a mean log value of 3.18±2.43 and 5.32±4.11 CFU/g. These results agreed with those reported by **Ibrahim et al. (2015)** and **Ahmed et al. (2019).** Our results revealed that 6% and 18% of the examined Talaga and Kareish cheese samples were above the permissible limits suggested by the Egyptian Organization for Standardization and Quality Control

(ES 2005), which stated that cheese should be free from *S. aureus* or their toxins.

The microbiological quality of raw milk, Talaga, and Kareish cheese in the present study indicates a lack of sanitation during manufacture and the high count of *S. aureus* in the examined products is considered as an index of probable enterotoxin production. In previous studies, it was mentioned that there is a possibility of toxin secretion from *S. aureus* if the bacterial count exceeds 10^3 CFU/ml or g **(Zeinhom et al. 2015).** The high incidence in raw milk could be attributed to environmental pollution, cross-contamination 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 **(Addis et al. 2011; Rahimi 2013).**

Talaga cheese was prepared from pasteurized milk; therefore, the contamination of cheese with *S. aureus* may be the result of post-pasteurization contamination **(Quero et al. 2014).** Kareish cheese is a home-made unpasteurized dairy product that is consumed regularly by the Egyptian society; because of the conventional methods of preparation, it is liable to contamination by various types of microorganisms. Importantly, it was noted that the hygienic quality of soft white cheeses sold in different regions of Beni-Suef Governorate, Egypt was poor and lacks the adequate public health assurance. Food poisoning outbreaks as a result of consumption of fresh soft cheese containing enterotoxins have been reported **(Carmo et al. 2002; Johler et al. 2015).** These findings emphasize the need to apply stricter hygienic practices to mitigate microbial contamination, especially in traditional cheese production.

The current study approved that the 25 tested *S. aureus* strains recovered from the examined market milk, Talaga and Kareish cheese samples contained both 16S rRNA gene and *nuc* gene.

The spread of antibiotic-resistant pathogens continues to challenge sustainable treatment options, with severe public health consequences. *S. aureus* isolates from raw milk and cheese were susceptible to streptomycin, sulfamethoxazole-trimethoprim, florfenicol and cefoxitin, but showed resistance to ampicillin (72%) and tetracyclines (60%). In line with our results, **Gundogan and Avci (2014),** reported that *S. aureus* isolated from raw milk and cheese samples was susceptible cefotaxime, chloramphenicol and ciprofloxacin while resistant to penicillin (97.1%) and ampicillin (92.6%). On contrary, a study conducted in Egypt reported that 78.57% of *S. aureus* strains were sensitive to amoxicillin/clavulanic **(Algammal et al. 2020).** Additionally, **Chao et al (2007)** reported that *S. aureus* isolated from food was most commonly resistant to tetracycline but with variable degrees. The risk of transmission of drug-resistant pathogens may increase as a result of the overuse of antibiotics in herd animals, either as a food supplement or for prevention and treatment of infectious diseases. High resistance of *S. aureus* isolated from milk and dairy samples to ampicillin and tetracyclines were reported **(Algammal et al. 2020; Chao et al 2007).**

The current results revealed the presence of resistance to many antimicrobials, emphasizing the need for new natural anti-microbial agents to treat *S. aureus* infection. Including a step of good hygiene practices with good heating of milk and its products is sufficient to control the microbe in milk and cheese.

The 16S rRNA PCR assay can successfully identify and classify many bacteria including staphylococci in different samples **(Johnson et al. 2016).** Also**,** *S. aureus* can be easily identified by PCR amplification of *nuc* gene; therefore, *nuc* gene has been used for the detection of *S. aureus* by many researchers **(Tang et al. 2008; Kilic et al. 2010).** The diagnostic values for detection of *nuc* gene by PCR based method were 93.3% sensitivity and 89.6% specificity **(Sahebnasagh et al. 2014).** Our results supported that as 100% of the tested *S. aureus* isolates harbored both 16S rRNA and *nuc* genes.

The *mec*A is an inducible 76-kDa penicillin binding protein carried on a mobile genetic component termed Staphylococcal Cassette Chromosomes (SCCs) which encoded alternative penicillin binding protein, PBP2a, which shows a reduced binding to β-lactams antibiotics. Therefore, presence of *mec*A promotes staphylococcal resistance to methicillin and other β-lactams antibiotics **(Abed et al. 2018).** The existence of *mec*A in MDR *S. aureus* have been reported worldwide in many previous studies **(Kreausukon et al. 2012; Awad et al. 2017; Abed et al. 2018).** High incidence of methicillin resistant *S. aureus* (MRSA) are very characteristic in notorious *S. aureus* which is clearly highlighting the potential risk of further lateral transfer of MRSA and other resistance genes among other staphylococci leading to limit therapeutic options, and successful antimicrobial therapy **(Abed**

et al. 2018). On the other hand, *ica*A is one of *ica* genes encoding for biofilm-forming ability **(Melake et al. 2017).** Our results revealed the presence of *mec*A gene in 66.7% of MDR *S. aureus* isolates; including cefoxitin. Moreover, *ica*A gene was found in 33.3% of these isolates.

Results illustrated in table (4) showed that *S. aureus* could survive in Kareish cheese for up to 28 days reaching a mean count of 8.77±.0.12 $\log_{10}$ CFU/g, which constitutes a public health hazard. The presence of *S. aureus* of up to 10^5 CFU/g or more is dangerous due to the potential for secretion of toxins **(Hennekinne et al. 2012).** Parallel findings were described by **Meshref et al. (2019)** who were able to detect the viability of *S. aureus* in white soft Kareish cheese for up to 30 days.

The ability of *S. aureus* to survive in cheese all this period with such huge number in addition to the antibiotic microbial resistance and the urgent need for products free of antibiotics have arisen the need to explore for alternative natural materials to combat foodborne pathogens. Recently, due to the growing concerns about the safety of synthetic chemicals and emerging antibiotic resistance in bacteria, the use of natural compounds has gained interest; one of these alternatives is the essential oils **(Salamci et al. 2007).** Thyme oil at a concentration of 1% showed a moderate reduction in the count of *S. aureus* in artificially manufactured Kareish cheese; only one log reduction in *S. aureus* count after seven days of storage. In a previous study conducted by Amatiste et al. (2014), they declared that *Thymus vulgaris* L and *Origanum vulgare* L. EOs had no effect on *S. aureus* count in cheese during 7 days of storage and explained this due to the interaction of active substances of EOs with cheese components.

Interestingly a very promising suppressive effect has been demonstrated and proved in the current study by increasing thyme concentration to 2% that was able to eradicate *S. aureus* at the seventh day of storage of Kareish cheese ($P < 0.05$). Therefore, 2% thyme EO significantly reduced ($P < 0.05$) *S. aureus* growth in laboratory manufactured Kareish cheese during cold storage at 4 ºC for 28 days. Nearly comparable results were reported by **deCarvalho et al. (2015),** who mentioned that increasing the concentration of thyme oil from 1.25 µl/ml to 2.5 µl/ml showed a higher inhibitory effect against *S. aureus* in Coalho cheese, where Thymol (43.19%), *p*-cymene (28.55%), γ-terpinene (6.36%), linalool (5.57%), carvacrol (3.14%) were the main active ingredients present. As well, **Ben Jemaa et al. (2017)** declared that thymus EO or its emulsion showed a good capacity to control bacterial growth including *S. aureus* and also was able to protect the milk from deterioration and extending its shelf life. Moreover, **Gammariello et al. (2008)** stated that thyme oil was able to inhibit the growth of microorganisms incorporated in milk deterioration without affecting the microflora of the milk. Several publications approved that the application of thyme oil and other EOs in cheese and other dairy foods displayed a good antimicrobial effect without affecting the taste and smell of the product that was accepted by the consumers **(Gammariello et al. 2008; Hayaloglu and Fox 2008).** Therefore, and because of its proved antimicrobial activity as well as prolonging the shelf life of cheese, thyme essential oil is highly recommended as a useful replacement for chemical preservatives and or antibiotic supplements.

5. Conclusion

A high prevalence of *S. aureus* in milk and Kareish cheese sold in Beni-Suef Governorate, Egypt is considered a public health hazard and might be involved as a cause of high prevalence of human food poisoning. *S. aureus* was able to tolerate salt and able to survive in soft cheese. It can be concluded that the two employed concentrations of thyme essential oil don't act in the same manner. Thyme essential oil at a concentration of 2% (v/v) is a promising natural antimicrobial agent that inhibits *S. aureus* contamination and/or deterioration of cheeses and prolongs the shelf-life. Future approaches involving the incorporation of probiotic bacteria with thyme essential oils or incorporation of other essential oils with thyme oils as a way for prevention of *S. aureus* growth and prolonging the shelf life of cheese should be taken in consideration in future directions.

6. Conflict of interest

No conflicts of interest

7. References

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