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Prevalence, Molecular Characterization, Antibiotics Resistance of *Staphylococcus aureus* Isolated from Egyptian Soft Cheese, and Effect of Rosemary Essential Oil on its Viability

Amira H. El-Baz
Department of Food Hygiene and control, Faculty of Veterinary Medicine, Mansoura University, 35516, Egypt

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***Correspondence to:**
amirahusseinel-baz@mans.edu.eg

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ABSTRACT

Staphylococcus aureus can yield heat stable enterotoxins resulting in food intoxication in human with variable intensity. So, the objectives of this research are to investigate the incidence of *Staphylococcus aureus* in Egyptian Soft Cheese (Talaga cheese), molecular identification of *nuc* and *mecA genes* in isolated strains by Multiplex PCR, assess the antimicrobial resistance profile using 16 different antibiotics as well as evaluate the antimicrobial activity of rosemary oil on the viability of *S. aureus.* Sixty random samples of Talaga soft cheese were collected from different marketplaces and dairy shops in various districts of Mansoura town, Dakahlia governorate. Egypt. *S. aureus* was discovered in 30% (18/60) from examined samples where 4 isolates (22.2%) were proved to be enterotoxigenic strains. All *S. aureus* isolates contained on *nuc* gene, however the *mecA* gene was found only in 5 *S. aureus* isolates (27.7%). *S. aureus* isolates showed a higher multiple antibiotic resistance (MAR) where the average MAR index was 0.479 and this leads to an increase in the public health hazard. The antimicrobial properties of rosemary essential oil as a natural antimicrobial agent were evaluated against *S. aureus* strain (ATCC 25923). It could be concluded that using rosemary essential oil with 2% concentration was highly effective in reducing and inhibiting *S. aureus*.

1. INTRODUCTION:

Dairy products have extensively been documented as a significant food for human growth **(**Pereira, 2014). But it may have pathogenic organisms (Dhanashekar et al. 2012). *S. aureus* is considered one of the greatest microbes obtained from milk. It exists in milk because of contamination during milking or from the milk gained from diseased dairy animals by subclinical mastitis or because of post milking pollution (Mekonnen et al. 2018 and Grispoldi et al. 2019).

S. aureus is colonizing then infect humans and animals together besides to pollute food too (Vitale, et al. 2015). It is one the greatest common microbial infections in persons and causes numerous human diseases as bacteremia, endocarditis, skin diseases, septic arthritis, osteomyelitis, pulmonary infections, prosthetic device infections, meningitis, gastroenteritis, toxic shock syndrome, and urinary tract diseases (Tong et al. 2015).

The appearance of numerous virulence agents that induce adhesion problems besides avoidance of the human immunologic replies is linked to the pathogenicity of *S. aureus*, also definite toxins are powerfully related to diseases, and a great mortality ratio (Grumann et al. 2014).

S. aureus yields a thermostable extracellular nuclease which coded by *nuc* genetic factor that is a single of the greatest popular unique features for *S. aureus* from other *Staphylococcus* strains. So nuc gene was recommended as a precise marker gene. Currently PCR is a real and useful way for recognizing *S. aureus* contained this gene (Sahebnasagh et al. 2014).

Milk that is contaminated through *S. aureus* causes severe illness in addition to *staphylococcal* related toxins (Grispoldi et al. 2021). Cure of

animals have mastitis (clinical and subclinical) through antibiotics is commonly occurred to diminish the economic as well as health problems of mastitis in dairy animal. But this failed by presence of great number of resistant microbes to definite antimicrobials because of their unsuitable use. Additionally, antibiotics-resistant isolates besides related resistant genetic factors may be transported to humans via the food chain, giving extra public health problems (Hammad et al. 2012).

S. aureus frequently appears antibiotic-resistant. *S. aureus* that was not affected by single or numerous- antibiotics is named methicillin-resistant *S. aureus* (MRSA) isolates have been continually isolated from the surroundings, food, and hospitals (Gould et al. 2012 and Rasigade et al. 2014). From all *S. aureus* resistance types, MRSA is the greatest significant, as MRSA strains are virulent strains (Dweba et al. 2018 and Gajdács, 2019), and resistant to most usually recommended class of beta lactam drugs [Lee et al. 2018] as methicillin, oxacillin, nafcillin, and cephalosporins (CDC, 2003; Rasigade and Vandenesch 2014) due to these strains have a *mec* gene on its chromosome (CDC, 2003), which codes the protein penicillin-binding protein 2a leading to decrease linking to β-lactams drugs or encodes vital microbial enzyme which induces the manufacture of the peptidoglycan in the microbial wall so these strains can grow in the existence of numerous antibiotics, and become resistant to numerous antibiotics so it are a serious microbes in both hospital and community locations (Chambers 2005, Boucher and Corey 2008 and Abed et al. 2018). These needs looking for discovery substitute natural methods which are harmless besides healthy, as the usage of natural materials have antibacterial characters (Holley and Patel 2005). Oils that are extracted from plants are beneficial sources of antimicrobial agents (Friedman et al. 2002). Essential oils (EOs comprise of numerous natural, active elements that have antimicrobial besides antioxidant belongings (Yousefi Asli et al. 2017; Hanif et al. 2019).

Rosemary essential oil has numerous uses particularly in the food treating besides preservative manufacturing because of its normal antioxidant besides antibacterial influence (Abdel-Massih and Abraham, 2014).

So, this study was carried out to (a) investigate the prevalence of *Staphylococcus aureus* in soft cheese (Talaga) in Mansoura city, Egypt, (b) serologically detection and typing of enterotoxin, (c) detect virulence genes *(nuc* and *mecA)* of isolated strains, (d) apply antimicrobial resistance profile using 16 different antibiotics (e) perform experiment on the antimicrobial activity of rosemary essential oil on the viability of *S. aureus.*

2. MATERIALS AND METHODS:

Ethics statement

The accumulating of specimens that were used in this research monitored the rules of Mansoura University moreover the procedure of this research was accepted through the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (code R/ 116).

The procedures that practical for samples collecting were recommended by American Public Health Association (A.P.H.A. 1992). Sixty samples of fresh soft cheese (Talaga)) (each 250gm) randomly collected from different marketplaces and dairy shops in various areas of Mansoura town, Dakahlia governorate. Egypt. The samples reserved in a secure ice box (4±1 ºC) to be transport to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University for a microbiological check-up. Biosafety measures through samples handing and applying microbiological check in the laboratory were practical agreeing to guidelines of WHO (2004).

2.1 Preparation of samples:

Twenty-five gram of every cheese specimen was additional to 225 ml of broth (tryptone soya) and then homogenized. The ready homogenate incubated at 37 ºC for 24 h **(**Robert et al. 1995).

2.2 Isolation then identification of *Staphylococcus aureus*:

Baird- Parker agar (Oxoid, CM 0275) added via potassium tellurite besides egg yolk mixture was employed for *S. aureus* isolation. Next the incubation period (24-48 h) at 37°C, the BPA petri dishes were examined for the existence of *S. aureus* that appeared as black with clear area colonies. Five probable colonies from every plate will select then subculture onto nutrient agar plates and incubated at37 ºC for 18-24 h. The golden yellow purified colonies were then transported onto slants then incubated at 37 ºC for 18-24 h for additional confirmatory tests. **(**Robert et al. 1995).

Confirmation of the suspected colonies was examined microscopically (ISO, 2013), biochemically (MacFaddin, 2000) and serologically

discovery of enterotoxins A, B, C besides D agreeing to Shingaki et al., 1981.

Molecular identification of thermonuclease (*nuc*) besides Methicillin Resistant *S. aureus* "MRSA" (mecA) virulence genetic factor was done basically by primers (Pharmacia Biotech) as exposed in Table, 1. Commonly DNA Extraction by QIA amp kit was performed agreeing to Shah et al. 2009 then the supernatant having the DNA was transmitted to new tube then kept at -20°C till usage. The amplification *of nuc* and *mecA* virulence genetic factors was achieved agreeing to Cho et al., 2007.

2.3 Antibiotics Resistance profile of *S. aureus* strains:

Antimicrobial vulnerability was established through the single diffusion way agreeing to Deresse et al. (2012) for *S. aureus* strains. The vulnerability of the microbial isolates was detected by using different concentrations discs (Oxoid Limited, Basingstoke, Hampshire, UK).

Agar petri dish technique was practical by the nutrient agar for growing of the examined microbe for its drug resistance. The isolate was evenly extent on the agar. After that the drug discs were positioned above the superficial of this petri dish. Then, keep the dish at suitable temperature 35±2°C for two days then tested for the development of the *S. aureus* round the discs. The highest inhibition region for the development of bacteria is supposed to this drug owned extreme influence on the bacteria growing.

So, the antibacterial vulnerability examination was practical agreeing to the rules specified through National Committee for Clinical Laboratory Standards "NCCLS" (2001). Consequently, the antibiotics discs besides their concentrations in addition to the widths of the areas of suppression for the examined isolates are shown in the Table, 2.

The examined isolates were assessed to resistant, susceptible besides intermediate. Multiple Antibiotic Resistance (MAR) index for every isolate was calculated agreeing to the method specified through Singh et al. (2010):

MAR index= Number of resistance (strains categorized as intermediate were counted susceptible for MAR index) / Whole Number of examined drugs.

2.4 Experimental part:

This experiment was done five independent trials. The influence of the rosemary essential oil for reduction of the growth and proliferation of *S. aureus* (ATCC 25923), that obtained from the Food Analysis Center, Faculty of Veterinary Medicine, Benha University, experimentally inoculated into soft cheese through manufacture was studied as follow:

2.4.1 Preparation of soft cheese samples (Youssef et al. 2016).

Fresh buffaloes' milk was gained from the private farm. Milk was pasteurized at 63ºC/30 min in water bath then left for warming at 40-45ºC. Marketable well grade salt of El-Nasr Salines Corporation, Egypt (0.5%) besides $CaCl_2$ (0.2g/L) from Sigma Chemical Corporation, Str. Louis, USA, were added. The coagulant Hannilase rennet powder obtained from Christian Hansen's Laboratory, Copenhagen, Denmark (0.2g/L) was used through the fermentation procedure. The ready-made rosemary oil (Rosmarinus officinalis) was obtained from the Food Analysis Center in the Faculty of Veterinary Medicine, Benha University.

Consequently, 4 groups of soft cheeses were ready as follows. The 1st group was untreated control, while the other 3 groups were treated directly before adding the rennet via rosemary essential oil at concentrations of 1%, 1.5% and 2%.

All milk groups were mixed fully by homogenizer, and then keep till coagulating. NaCl (0.5%, w/v of milk) was additional among cheeses layers then allow to whey drainage into minor cheese moulds at room temperature. Then, all sets (both control or treated) were exposed to Bacteriological valuation for enumeration of *S. aureus* at day zero (in 2 hours subsequently the treatment) then kept at fridge and examined at times after 2, 4 and 6 days.

2.4.2 Preparation of bacterial suspension:

The antimicrobial character of rosemary oil was detected against reference strain of *S. aureus* (ATCC 25923). *S. aureus* isolate was cultured in Brain Heart Infusion broth (BHI) (Fluka, Sigma-Aldrich Chemie GmbH) for 24 h at 37ºC. One ml of the cultivated microbial suspension was diluted in peptone water (0.1%, w/v) (Merck, Darmstadt, Germany).

The viable count of *S. aureus* was happened agreeing to the plate count way (the culture broth matching to almost 3×10^6 of *S. aureus* was centrifuged (500 rpm, 15 minutes at 5ºC) and the microbial pellets were washed two times by deionized water (Eom et al., 2015).

2.4.3 Binding assay

The microbial pellets were pending in of milk mix directly before addition of the rennet necessary for cheese curdling. The mix was adjusted to have

definitive concentration of 3×10^6 bacteria agreeing to Halttunen et al. (2008).

2.5. Statistical Analysis:

All calculations were applied based on Statistical Package for the Social Sciences (SPSS) statistical package (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY). Data represented are the means of five trails ±standard deviation of means through using of the Analysis of Variance (ANOVA) exam agreeing to Feldman et al. (2003).

3. RESULTS:

3.1 Isolation, identification, and enterotoxin production of *Staph. aureus:*

In this study 18 *Staph aureus* were isolated from Talaga soft white cheese samples with prevalence of 30% (Table, 3)

Results in Table (4) exposed that out of 18 *S. aureus* bacterial isolates, 4 (22.2%) were toxigenic isolates which 2 isolates were belonging to enterotoxin A, one isolate was belonging to enterotoxin D, and the last enterotoxigenic isolate can be yield together A besides C enterotoxins.

3.2 Discovery of *nuc* and *mecA* genes of *S. aureus* strains

Confirmation of the results was performed using PCR through detection of *nuc* genes. All the tested *S. aureus* isolates harbored this gene (100%). Also, all *S. aureus* strains were exposed to PCR for discovery of *mecA* virulence gene, 5 isolates (27.7%) harbored *mec*A gene **(**Table, 5 and Figure, 1)

3.3 Antibiotics resistance profile of the *Staph aureus* isolates

The antimicrobial drug vulnerability outlines for the 18 isolated *Staph. aureus* from Talaga soft white cheese is exposed in table 6 as 100% of *Staph aureus* isolates show resistance versus Kanamycin (K) followed by Penicillin G (P) (88.9%), Nalidixic acid (NA) / Ampicillin (AM) (83.3%), Sulphamethoxazol (SXT) (66.7%), Tetracycline (T) (61.1%), Clindamycin (CL) / Erythromycin (E) (44.4%), Levofloxacin (L) (38.9%), Cefazolin (CZ) / Oxacillin (OX) (33.3%), and Meropenem (M) (27.8%). On the other hand, they showed high sensitivity to Amikacin (AK) (88.9%) followed by Gentamicin (G) (83.3%), and Ciprofloxacin (CP) / Ipipenem (IPM) (72.2%). Also, there is a higher multiple antibiotic resistance between *Staph aureus* isolates in table 7 as the average multiple antibiotic resistance index (MAR) was 0.479 and it reached to 1 in one strain as this strain was resistant to all used antibiotics followed by 0.937 in second and third strain.

3.4 Effect of Rosemary essential oil on the viability of *Staph aureus*

Results demonstrated in **Table (8)** showed that *S. aureus* remain viable in Talaga soft white cheese for up to 6 days. Approximately $3.0\times10^6 \pm 0.2\times10^6$CFU/g was detected on day zero of the experiment. The *S. aureus* count slightly continued to decrease until it reached to $2.6\times10^6\pm0.2\times10^6$CFU/g on day 6 with a reduction rate of 13.3%. Using the Rosemary oil with 1% leads to decrease the count of *S. aureus* till reach to $1.3\times10^6\pm 0.1\times10^6$on the day 6 with a reduction rate of 56.7%.

Interestingly, the increase in the concentration of rosemary oil to 1.5 % and 2% was more effective, as by using the oil with 1.5% concentration the reduction rate of *Staph aureus* reached to 73.7% on the day 6 with a count of $7.9\times10^5\pm 0.8\times10^{5.}$ While by increasing the concentration of oil to 2% the reduction rate reached to 85% on the day 6 with a count of$4.5\times10^5\pm 0.4\times10^5$.

Table (1): Target genes, primers sequences and amplicon sizes.

Target gene	Oligonucleotide sequence (5′ → 3′)http://www.ncbi.nlm.nih.gov/pmc/articles/PMC140333/table/t2/ - t2fn1	Product size (bp)	References
nuc (F)	5′ GCGATTGATGGTGATACGGTT ′3	270	**Brakstad et al. (1992)**
nuc (R)	5′ AGCCAAGCCTTGACGAACTAAAGC ′3		
mecA (F)	5′ TAGAAATGACTGAAC GTCCG ′3	533	**Jukes et al. (2004)**
***mecA* (R)**	**5′ TTGCGATCA ATGTTACCGTAG ′3**		

Table (2): Antimicrobial discs, concentration, and interpretation of their action on the isolated *S. aureus* strains.

Antimicrobial agent	Sensitivity disc content (ug)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Tetracycline (T)	30	14 or less	15-18	19 or more
Ampicillin (AM)	10	13 or less	14-17	18 or more
Cefazolin (CZ)	30	10 or less	11-14	15 or more
Ciprofloxacin (CP)	5	15 or less	15-19	20 or more
Erythromycin (E)	15	13 or less	14-22	23 or more
Penicillin G (P)	10 IU	20 or less	21-28	29 or more
Gentamicin (G)	10	12 or less	13-14	15 or more
Meropenem (M)	10	9 or less	10-12	13 or more
Amikacin (AK)	30	12 or less	13-15	16 or more
Ipipenem (IPM)	10	18 or less	19-21	22 or more
Oxacillin (OX)	1	10 or less	11-12	13 or more
Levofloxacin (L)	5	18 or less	19-21	22 or more
Nalidixic acid (NA)	30	13 or less	14-18	19 or more
Clindamycin (CL)	10	13 or less	14-16	17 or more
Kanamycin (K)	30	13 or less	14-17	18 or more
Sulphamethoxazol (SXT)	25	10 or less	11-15	16 or more

Table (3): Prevalence of *S. aureus* in samples.

Type of samples	Number of examined samples	No.	%
Soft cheese (Talaga)	**60**	**18**	**30**

Table (4): Occurrence and distribution of enterotoxigenic *S. aureus* strains isolated from Talaga cheese samples. (n= 18 strains).

No. of the examined strains	Positive enterotoxigenic strains		Types of the enterotoxin				
	No.	%	A	B	C	D	A&C
18	**4**	**22.2**	**2**	**_**	**_**	**1**	**1**

Table (5): Occurrence of *nuc* and *mecA* genes of *S. aureus* isolates obtained from the inspected Talaga cheese samples (n= 18 strains).

Target genes	No. of examined strains	No. of positive strains	% of positive strains
***nuc* gene**	**18**	**18**	**100**
***mecA* gene**	**18**	**5**	**27.7**

***nuc*: Thermonuclease gene.** ***mecA*: Methicillin Resistant *S.aureus* "MRSA" gene.**

Table (6): Antimicrobial susceptibility of the *S. aureus* isolates (n=18)

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Kanamycin (K)	-	-	-	-	18	100
Penicillin G (P)	-	-	2	11.1	16	88.9
Nalidixic acid (NA)	-	-	3	16.7	15	83.3
Ampicillin (AM)	2	11.1	1	5.6	15	83.3
Sulphamethoxazol (SXT)	5	27.8	1	5.6	12	66.7
Tetracycline (T)	5	27.8	2	11.1	11	61.1
Clindamycin (CL)	7	38.9	3	16.7	8	44.4
Erythromycin (E)	10	55.6	-	-	8	44.4
Levofloxacin (L)	9	50.0	2	11.1	7	38.9
Cefazolin (CZ)	11	61.1	1	5.6	6	33.3
Oxacillin (OX)	12	66.7	-	-	6	33.3
Meropenem (M)	12	66.7	1	5.6	5	27.8
Ipipenem (IPM)	13	72.2	1	5.6	4	22.2
Ciprofloxacin (CP)	13	72.2	2	11.1	3	16.7
Gentamicin (G)	15	83.3	-	-	3	16.7
Amikacin (AK)	16	88.9	1	5.6	1	5.6

Table (7): Antimicrobial resistance profile of *S. aureus* isolates (n=18)

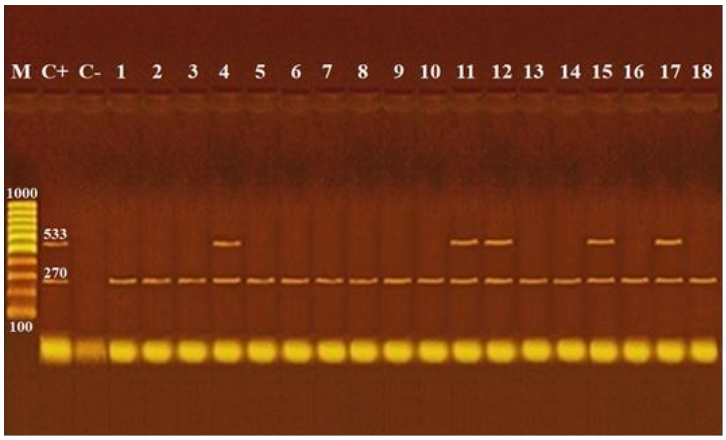
NO	Strains	Antimicrobial resistance profile	MAR index
1	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX, M, IPM, CP, G, AK	1
2	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX, M, IPM, CP, G	0.937
3	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX, M, IPM, CP, G	0.937
4	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX, M, IPM	0.812
5	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX, M	0.750
6	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L, CZ, OX	0.688
7	*S. aureus*	K, P, NA, AM, SXT, T, CL, E, L	0.563
8	*S. aureus*	K, P, NA, AM, SXT, T, CL, E	0.500
9	*S. aureus*	K, P, NA, AM, SXT, T	0.375
10	*S. aureus*	K, P, NA, AM, SXT, T	0.375
11	*S. aureus*	K, P, NA, AM, SXT, T	0.375
12	*S. aureus*	K, P, NA, AM, SXT	0.312
13	*S. aureus*	K, P, NA, AM	0.250
14	*S. aureus*	K, P, NA, AM	0.250
15	*S. aureus*	K, P, NA, AM	0.250
16	*S. aureus*	K, P	0.125
17	*S. aureus*	K	0.062
18	*S. aureus*	K	0.062
		Average	0.479

K: Kanamycin P: Penicillin G NA: Nalidixic acid AM: Ampicillin SXT: Sulphamethoxazol T: Tetracycline CL: Clindamycin E: Erythromycin L: Levofloxacin CZ: Cefazolin OX: Oxacillin M: Meropenem IMP: Ipipenem CP: Ciprofloxacin G: Gentamicin AK: Amikacin

Table (8): Antimicrobial activity of rosemary essential oil on viability of *S. aureus* inoculated into soft cheese by intensity of 3.0×10^6 (Trails numbers=5).

Concentration	Control		1%		1.5%		2 %	
	Count	R %*	Count	R %	Count	R %	Count	R %
Storage time								
Zero time	$3.0\times10^6\pm 0.2\times10^6$	------	$3.0\times10^6\pm 0.2\times10^6$	------	$3.0\times10^6\pm 0.2\times10^6$	------	$3.0\times10^6\pm 0.2\times10^6$	------
2 days	$2.9\times10^6\pm 0.2\times10^{6\,A}$	3.3	$2.4\times10^6\pm 0.1\times10^{6\,B}$	20	$2.2\times10^6\pm 0.1\times10^{6\,C}$	26.7	$1.9\times10^6\pm 0.1\times10^{6\,D}$	36.7
4 days	$2.7\times10^6\pm 0.1\times10^{6\,A}$	10	$1.7\times10^6\pm 0.1\times10^{6\,B}$	43.3	$1.1\times10^6\pm 0.1\times10^{6\,C}$	63.3	$9.2\times10^5\pm 1.3\times10^{5\,D}$	69.3
6 days	$2.6\times10^6\pm 0.2\times10^{6\,A}$	13.3	$1.3\times10^6\pm 0.1\times10^{6\,B}$	56.7	$7.9\times10^5\pm 0.8\times10^{5\,C}$	73.7	$4.5\times10^5\pm 0.4\times10^{5\,D}$	85

*Means ± standard deviation with various capital letters in the same row are significantly different (P<0.05).



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *nuc* (270bp) and *mecA* (533bp) virulence genes of *S. aureus.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *nuc* and *mecA* genes. Lane C-: Control negative. Lanes from 1 to 18: Positive *S. aureus* strains for *nuc* gene .Lanes 4, 11, 12, 15 & 17: Positive *S. aureus* strains for *nuc* and *mecA* genes.

4. DISCUSSION:

Cheeses are considerably consumed because of their great nutritional value (Drake and Delahunty 2017). Foodborne diseases that are linked with cheese were described in numerous areas universally. Poisoning from cheeses resulted from Milk having *S. aureus* in great quantities and is treated lacking pasteurization, insufficient starter action, after-pasteurization pollution, besides unsuitable settings for processing and storing of the cheeses (Part 2006).

The current study identified that 30% (18) of the total examined Talaga soft white cheese that collected from different districts in Mansoura city were contaminated with *Staph. aureus* (Table, 3). Closely similar outcomes (26.66%) were stated by Eid and Eltalawy 2014 in Egypt. Lower results were recorded by Zeinhom and Abed 2021(6%) in Egypt, however higher outcomes were described in Egypt by Younis et al. 2020 (68%) and Kasem et al. 2021 (60%).

The existence of this bacterium in the cheese establishes a probable human risk as numerous isolates of *S. aureus* bacteria yield heat-stable enterotoxins that reason of food poisoning if consumed and demonstrating a sanitary risk (Jablonski and Bohach, 1997). Eid and Eltalawy 2014 also showed that there were 4 enterotoxigenic *S. aureus* in their applied study where 2 isolates (20%) were belonging to enterotoxin A and the other 2 enterotoxigenic isolates (20%) were belonging to both A and C. Food poisoning occurrences due to ingesting of fresh soft cheese having enterotoxins have been stated (Carmo et al. 2002; Johler et al. 2015). These results underline the necessity to apply severer hygienic applies to diminish microbial contamination, particularly in traditional cheese manufacture.

This study approved that the total 100% (18) examined *S. aureus* isolates that are obtained from Talaga white soft cheese samples were contained on the *nuc* gene (Table, 5 and Figure, 1). Many investigators as Tang et al. 2008 in addition to Kilic et al. 2010 were used the *nuc* gene for the discovery of *S. aureus* as the analytical values for finding the *nuc* genetic factor through PCR established way were 89.6% specificity besides 93.3% sensitivity (Sahebnasagh et al. 2014).

Our results exposed that the existence of *mecA* gene in five isolates (27.7) (Table, 5 and Figure, 1) of 4 MDR *S. aureus* isolates, and one Kanamycin resistant isolate. Also, numerous preceding studies (Kreausukon et al. 2012; Al-Ashmawy et al. 2016; Awad et al. 2017; Abed et al. 2018; Zeinhom and Abed 2021) recorded that the presence of *mecA* in MDR *S. aureus.*

The *mecA* gene is established in MRSA isolates. It codes an extra penicillin-binding protein (PBP2a) that shows a little attraction to totally ß-lactam drugs. So, the existence of *mec*A encourages the resistance of bacterium to methicillin in addition to further β-lactams antibiotics (Lindsey et al. 2008; Khosravi et al. 2012 and Abed et al. 2018).

Also, oxacillin was used as the indicator drug to discover MRSA (CLSI, 2013), however, this study revealed that 4 positive *mecA* isolates don't show resistant against oxacillin disc and one positive *mecA* isolate has resistant against oxacillin disc. On the contrary, although there are 5 isolates give resistant to oxacillin disc in antimicrobial resistance test, but not show positive *mec A* gene in PCR. So, there are further intrinsic causes that can vie with *mecA* gene in creating the resistance of MRSA (Elhassan et al. 2015). Alternatively, the lack of *mec*A gene inside resistant *staphylococcal* strains was registered previously (Hawraa, et al. 2014). Moreover, intermediate methicillin confrontation was detected in the strains that do not have the *mecA* genetic factor (Ligozzi et al. 1991, and Hiramatsu et al. 1992). Also, an earlier study in Nigeria stated the whole lack of *mecA* gene in addition to the genetic factor yield of PBP2a in the strains that were described MRSA phenotypically proposing a possibility of hyper creation of β-lactamase a reason for the occurrence [Olayinka, et al. 2009]. Moreover, lately Ba et al. 2014 stated specific changes in various amino acids existent in the protein-bound proteins series that can be the main cause of the resistance. These discoveries provided a strong indication that there are further mechanisms than the existence of the *mecA* genetic factor that led to resistance of MRSA to beta-lactam drugs, therefore using molecular ways only are not sufficient for approving the existence of MRSA strain, a topic that must be down attention by a reference research laboratory.

Additionally, according to table 6 all the *S. aureus* strains revealed 100% resistant to Kanamycin followed with pencillin. Simillar results were recorded by Kasem et al. 2021; while their result in case of the resistance against ampicllin (30%), nalidixic acid (60%), sulfamethoxazole (60%) and tetracycline (40%) were lower than my result. On the other hand, they recorded higher resistance results than me in case of erythromycin (50%), gentamicin (40%), amikacin (20%) and ciprofloxacin (30%), and also in case of the resistant against ampicillin and tetracyclines nearly similar results were recorded by Zeinhom and Abed 2021. In addition to Gundogan and Avci 2014 and Can et al. 2017 were showed that their *staph aureus* isolates exposed higher resistance against penicillin and ampicillin (97.1%, 92.6%, 95% and 92.5%, respectively). Also, Chao et al 2007 and Algammal et al. 2020 were recorded that *S. aureus* that isolated from dairy samples had high resistance to ampicillin and tetracyclines. Regarding to oxacillin and gentamicin 66.7% and 83.3% from my isolates showed susceptibility to these antibiotics, respectively, while 88.9% of my isolates showed susceptibility against amikacin. Can et al. 2017 stated that the wholly ***S. aureus*** bacteria strains exposed susceptibility to gentamicin, vancomycin, and oxacillin.

Staph aureus isolates in this study showed higher multiple antibiotic resistances as the MAR index was extended from 0.062 to 1 with a mean of 0.479 (table, 7). When MAR index is more than 0.2 this means the greater contamination danger and greater usage of antibiotics in the field (Rotchell and Paul 2016), perhaps due to the ill usage of antibiotics in the treatment of animals and individuals besides addition its by method of growth supporter for animals emerging severe health ills (Olaimat et al. 2018).

Due to the worldwide spreading of multiple drug-resistant isolates, there is a need to find out novel antibacterial agents (Puvača and de Llanos Frutos 2021). Numerous aromatic, therapeutic plants, spices besides herbs have been planned as an important basis of natural antibacterial as a substitute to artificial drugs against bacterial diseases (Puvača et al. 2020).

Table 8 showed that using of rosemary essential natural oil is very effective against *Staph aureus* especially when increasing its concentration to 2% as the reduction rate reached to 85% with a mean count of 4.5×10^5 at the day number 6 from

starting the experiment. This result was corresponding with Burt 2004; Stojanović-Radić et al. 2010; Hafez et al. 2011; Reham 2013, and Hassanien et al. 2016. This reduction on *Staph aureus* count can be explained by Rosemary oil shows antibacterial action through crossing over the cell wall then the cytoplasm membranes of this bacteria then troublesome their composition by way of a characteristic lipophilic material (Stojanović-Radić et al., 2010), moreover Hafez et al. (2011); Helmy (2012); Reham (2013) and Hassanien et al. 2016 revealed that the top sensual (physical) feature was gained at maximum ratio of rosemary oil (2%), while minor development quality was observed in the samples that were preserved by 1% rosemary oil.

5. CONCLUSION:

Staph aureus is widely dispersed in nature. A high prevalence of multiple antibiotic resistances *Staph aureus* in Talaga cheese sold in Mansoura city, Egypt is acting as a public health hazard which leads to food poisoning and many dangerous health problems in humans. Hygienic measures through processing and handling the milk and dairy products should be applied. Also effective pasteurization of milk before the manufacture of cheese is very important. Rosemary oil at the concentration of 2% is very effective in food preservation and *Staph aureus* inhibition.

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CONFLICT OF INTEREST:

The author stated no probable conflicts of interest with respect to this research.

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