

## MOLECULAR DETECTION OF ENTEROTOXIGENIC GENES FOR *STAPHYLOCOCCUS AUREUS* ORGANISM ISOLATED FROM RAW MILK AND SOME MILK PRODUCTS

SADEK, O.A.<sup>1</sup> AND KORIEEM, A.M.<sup>2</sup>

<sup>1</sup> Food Hygiene Department, Assiut Lab., Animal Health Research Institute, ARC, Egypt.

<sup>2</sup> Bacteriology Department, Assiut Lab., Animal Health Research Institute, ARC, Egypt.

**Received:** 14 September 2020; **Accepted:** 5 October 2020

This study aimed to determine *Staph. aureus* in raw milk and some milk products and study the correlation between *Staph. aureus* enterotoxin genes and its ability to resist different types of antibiotics. A total of 120 raw milk, kareish cheese and baladi yoghurt (40 samples, each) were collected from different dairy shops and street peddlers in Assiut city, Egypt and were bacteriologically examined for presence and count of *Staph. aureus*. The incidences of counted *Staph. aureus* in raw milk, kareish cheese and baladi yoghurt were 62.5, 27.5 and 0.0%, respectively, with average counts of 3.25 log, 4.13 log and < 1 log cfu/ml, respectively. All the isolated *Staph. aureus* strains were tested by Multiplex PCR assay for the presence of enterotoxigenic *sea*, *seb*, *sec* and *sed* genes and 94.44% of the tested strains harbored *sea* gene and 2.77% were positive for *sed* gene, while none of the tested strains was positive for *seb* and *sec* gene. The recovered organisms exhibited 52.78, 11.11, 77.78, 61.11, 11.11, 36.11, 63.89 and 16.67% resistance towards Oxacillin (Methicillin), Vancomycin, Amoxicillin, Ceftriaxone, Gentamicin, Tetracycline, Erythromycin, and Trimethoprim-Sulfamethoxazole, while, they exhibited 100% sensitivity towards Ciprofloxacin. The tested organisms showed multi-antibiotics resistance percentage of 55.56% and with average resistance index of 0.37. The correlation between *Staph. aureus* enterotoxin genes and its ability to resist different types of antibiotics revealed that, most of the enterotoxigenic strains were multi-antibiotics resistance and resist simultaneously to Amoxicillin, Ceftriaxone and Erythromycin. All the methicillin resistant *Staph. aureus* (MRSA) isolates harbored *sea* gene. The public health hazards of *Staph. aureus* in milk and its products as well as the suggestive control measures were discussed.

**Keywords :** *Staphylococcus aureus*, enterotoxigenic genes, raw milk, kareish cheese, yoghurt, PCR

### INTRODUCTION

In humans, *Staph. aureus* is considered a well-documented opportunistic pathogen. It may cause food poisoning, pneumonia, skin infections,

enterotoxemia, and septicemic infections; furthermore, it is important as the cause of toxic shock syndrome (TSS) (Sidhu *et al.*, 2007). With the emergence of methicillin-resistant *Staph. aureus* (MRSA) and its increasing resistance towards antibiotics of various groups such as penicillins, cephalosporins, fluoroquinolones, aminoglycosides and macrolides. Vancomycin has been the antibiotic of the last resort to treat the hospitalized patients

Corresponding author: SADEK, O.A.

E-mail address: [onsi\\_2000@yahoo.com](mailto:onsi_2000@yahoo.com)

Present address: Food Hygiene Department, Assiut Lab., Animal Health Research Institute, ARC, Egypt

critically infected with MRSA or other Gram-positive organisms like *Clostridium difficile*. While, widespread use of Vancomycin to treat infections caused by methicillin resistant *Staph. aureus* (MRSA) and other Gram-positive cocci has led to the emergence of Vancomycin resistance (VRSA). The large scale of spread of resistance to Vancomycin has been perceived as a fearsome threat to the already challenging therapy of MRSA (Moellering, 2008 and Abdel-Baky *et al.*, 2014). Antibiotic-resistant strains of bacteria cause humans to become more virulently ill for longer periods of time than their antibiotic susceptible counterparts. Treatment failure occurs as a consequence and there is the need for expensive and/or toxic alternative drugs which in most cases are more expensive (WHO, 2007).

Enterotoxigenic *Staph. aureus* is one of the major pathogens causing food poisoning cases worldwide. Staphylococcal enterotoxins (SE) are emetic toxins and are classified as members of the pyrogenic toxin superantigen family because of their biological activities and structural relatedness (Dinges *et al.*, 2000). Staphylococcal food poisoning is associated with contaminated foods of animal origin, such as milk and dairy products and other protein rich animal origin foods such as ice cream, meat, poultry and fish (Janštová *et al.*, 2012).

The staphylococcal food poisoning (SFP) is a mild intoxication occurring after the ingestion of food containing from 20 ng up to 1 µg of Staphylococcal enterotoxins (SEs) which is enough to induce symptoms in human beings (Normanno *et al.*, 2007). The SFP symptoms appear 1-6 h after ingestion of contaminated food, depending on individual and toxic dose ingested. They include nausea, abdominal cramps, diarrhea, general malaise, weakness and characteristic projectile vomiting. Clinical signs of SFP generally disappear within 24-48 h. Deaths occur rarely and specifically in

the very young or elderly (Jay *et al.*, 2005). The five classical enterotoxins (SEs type A, B, C, D and E) were known to be responsible for 95% of SFP cases, the rest of cases were due to the new types of SEs (SE G – SE O) (Wang *et al.*, 2012).

Milk; kareish cheese (traditional Egyptian cheese made from raw skimmed milk) and baladi yoghurt (small scale yoghurt) act as a good vehicle for *Staph. aureus* organisms and sometimes this organisms may be antibiotic resistant and enterotoxins producers. Therefore, the aim of this study was to determine the prevalence of *Staph. aureus* in raw milk, kareish cheese and baladi yoghurt sold in Assiut City, Egypt and testing them for antimicrobial sensitivity test for detection of MRSA and VRSA strains and application of multiplex PCR to detect some classical enterotoxins genes (*sea*, *seb*, *sec* and *sed*) in the isolated strains.

## MATERIALS AND METHODS

A total of 120 raw milk, kareish cheese and baladi yoghurt samples (40 samples, each) were collected from dairies and street peddlers in Assiut city, Egypt during the period from December 2018 to April 2019. The samples were collected in clean and sterile plastic bags in an ice-box and transferred rapidly as soon as possible to the laboratory for bacteriological examination.

### A) Preparation of samples:

The apparently normal raw milk samples were mixed thoroughly and tested for heat treatment by Storch test according to Lampert (1975) before being subjected to examination. Ten ml from liquid samples or 10g from the prepared solid samples were added individually to 90 ml of 0.1% sterile peptone water. Ten-fold serial dilutions from each sample were done up to 10<sup>6</sup> (A.P.H.A., 2001).

**B) Isolation and enumeration of *Staph. aureus* from the prepared samples:**

1) Enrichment procedure according to Lee (2003): 10ml milk or 10gm of each prepared cheese and yoghurt samples were inoculated into 10% NaCl nutrient broth and incubated at 35°C for 20h.

2) Isolation of *Staph. aureus* by selective plating on mannitol salt agar according to Finegold and Martin (1982): Loopfulls from the incubated 10% NaCl nutrient broth tubes were streaked on mannitol salt agar plates then incubated at 37°C for 48h. Suspected golden yellow colonies with yellow halo were picked up onto nutrient agar slant and incubated at 37°C for 24 hour before being subjected to identification.

3) Enumeration of *Staph. aureus*, using Baird-Parker agar, according to FDA (2001): From each dilutions, 1.0ml was spread onto large plate Baird-Parker agar with sterile bent glass rod and incubated at 37°C for 24 hours. Then the numbers of small black, shiny with narrow white margins and surrounded by clear zones extended into the opaque medium were calculated and typical colonies were picked into nutrient agar slants for biochemical identification.

**C) Identification of *Staph. aureus* organism.**

1) Gram's stain showed Gram positive cocci forming an irregular grape like clusters.

2) Catalase activity test according to FDA (2001) where the organism was catalase positive.

3) Tube coagulase test according to FDA (2001): Sterile rabbit plasma was diluted 10 folds with sterile normal saline. 0.5 ml of the diluted plasma was put in each 2 Wassermann tubes. Five drops of overnight incubated broth culture at 37°C were added to first tube, while the other was left to serve as a control. The

tubes were incubated at 37°C and then examined after one and 24 h incubation for coagulation.

4) Anaerobic utilization of mannitol according to FDA (2001): Pure culture of the isolated organisms were inoculated into peptone water containing mannitol (0.5%) and bromocresol purple as indicator and the surface of the medium were covered by sterile paraffin oil at least 25mm thick. The tubes were incubated for 5 days at 37°C. Acid was produced anaerobically; if the indicator is changed to yellow color throughout the tubes indicates the presence of *Staph. aureus*.

5) Detection of haemolysis according to Bailey and Scott (1994): Isolated strains were cultured on blood agar plates contained 10% sheep blood and incubated at 37°C for 24 h for detection the type of haemolysis.

**D) Antibiogram of *Staph. aureus* species according to CLSI (2011):** Each *Staph. aureus* was standardized using colony suspension methods and strain's suspension diluted with sterile saline and adjusted to 0.5 McFarland standards. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer disc diffusion technique (CLSI (2011) by swabbing onto Mueller-Hinton agar plates with the resultant saline suspension of each strain and the following (9) antibiotic discs were placed on the plate: Oxacillin (Methicillin) 1µg, Vancomycin 30 µg, Amoxicillin 10µg, Ceftriaxone 30µg, Gentamicin 10µg, Tetracycline 30µg, Erythromycin 15µg, Trimethoprim-Sulfamethoxazole 25µg and Ciprofloxacin 5µg. The plates containing the discs were allowed to stand for at least 30 min before incubated at 30°C for 24h so as to favor the growth of methicillin resistant strains (BSAC, 2002). The diameter of the zone of inhibition produced by each antibiotic

disc was measured and interpreted using the CLSI zone diameter interpretative standards.

Antibiotic resistance (ARI) index was calculated as a/b, where "a" represents the number of antibiotics to which the isolates were resistant and "b" represents the total number of antibiotics to which the isolate was exposed (Krumperman, 1983).

### E) Multiplex Polymerase Chain Reaction

**(m PCR):** for detection of *sea*, *seb*, *sec*, and *sed* genes in the isolated *Staph. aureus* strains which was done in Animal Reproduction Research Institute, El Haram, Giza, Egypt.

#### 1- DNA extraction:

A crude extraction method based on boiling procedure was used to prepare template

DNA from bacterial strains according to Reischl *et al.* (1994). Two to 5 loops of bacteria taken from the brain heart infusion agar plate were collected and suspended in 200 µl of lysis buffer comprised of 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH 8.0), and 1 mM EDTA. After boiling for 10 min, the suspension was centrifuged for 2 min to sediment bacterial debris. The supernatant was aspirated and kept at - 20°C as source for DNA to be used directly for PCR amplification.

#### 2- Primers:

Primers used for PCR amplification were synthesized in Bio Basic Inc. (Canada). Details of primer sequences, their specific target genes, references, annealing temperature and amplicon sizes are summarized in the following table:

Gene targeted	Primer sequence 5"-3"	references	Amplified segment (bp)	Primary denaturation	Amplified cycles			Final extension
					Secondary denaturation	annealing	Extension	
<i>sea</i>	TAAGGAGGTGGTGCCTATGG CATCGAAACCAGCCAAAGTT	Cremonesi <i>et al.</i> , 2005	180					
<i>seb</i>	TCGCATCAAACGACAAACG GCAGGTACTCTATAAGTGCC	Johnson <i>et al.</i> , 1991	478	94°C for 5 min	30 cycle at 94°C for 1 min	56°C for 1 min	68°C for 1 min	72°C for 7 min
<i>sec</i>	GACATAAAAGCTAGGAATTT AAATCGGATTAACATTATCC	Johnson <i>et al.</i> , 1991	257					
<i>sed</i>	CTAGTTTGGTAATATCTCCT TAATGCTATATCTTATAGGG	Johnson <i>et al.</i> , 1991	317					

#### 3- DNA amplification reaction:

Multiplex PCR assay was used that detect staphylococcal enterotoxins A, B, C and D genes. The amplification cycles were carried out in Nexus gradient Master cycler (Eppendorf, Germany). The reaction condition was optimized to be 94°C for 5min. as initial denaturation, followed by 30 cycles of 94°C for 60 seconds as secondary denaturation, annealing for 60 seconds at 56°C and extension at 68°C for

60 seconds. A final extension step at 72°C for 7min. was followed. PCR reaction was optimized using a total volume of 20µL reaction mixtures which contained 3µL of DNA as template, 20pmol of each primer, 1X of PCR master mix (Dream Taq Green PCR Master Mix, Fermentas Life Science). PCR products were analyzed by electrophoresis in 1.5% agarose gel in 0.5X TBE buffer containing ethidium bromide and visualized under a UV transilluminator.

## RESULTS

**Table 1:** Statistical analytical results of *Staph. aureus* counts in raw milk, kareish cheese and yoghurt samples (n = 40 of each).

Sample type	<i>S. aureus</i> count (cfu/ g)							
	Positive samples		Negative samples		Min.	Max.	Average	± SE
	No.	%	No.	%				
Milk	25	62.50	15	37.50	< 1 log	4.53 log	3.25 log	2.93 log
Kareish cheese	11	27.50	29	72.50	< 1 log	5.51 log	4.13 log	3.93 log
yoghurt	0	0.0	40	100	< 1 log	-	-	-

**Table 2:** Frequency distribution of *Staph. aureus* counts in the examined raw milk and kareish cheese samples (n = 40 of each).

Intervals	Raw milk		Kareish cheese	
	No.	%	No.	%
< 10 <sup>2</sup>	15	37.5	29	72.5
log 2 - < log 3	8	20	1	2.5
log 3 - < log 4	16	40	3	7.5
log 4 - < log 5	1	2.5	5	12.5
log 5 - < log 6	0	0.0	2	5
Total	40	100	40	100

**Table 3:** Antibiogram of *Staph. aureus* organisms isolated from raw milk and kareish cheese (n= 36).

Antibiotics	Resist		Sensitive	
	No./36	%	No./36	%
Oxacillin (Methicillin)	19	52.78	17	47.22
Vancomycin	4	11.11	32	88.89
Amoxicillin	28	77.78	8	22.22
Ceftriaxone	22	61.11	14	38.89
Gentamicin	4	11.11	32	88.89
Tetracycline	13	36.11	23	63.89
Erythromycin	23	63.89	13	36.11
Trimethoprim-Sulfamethoxazole	6	16.67	30	83.33
Ciprofloxacin	0	0.0	36	100

**Table 4:** Percentage of multi-antimicrobial resistant (MAR) of *Staph. aureus* organisms isolated from raw milk and kareish cheese (n = 36).

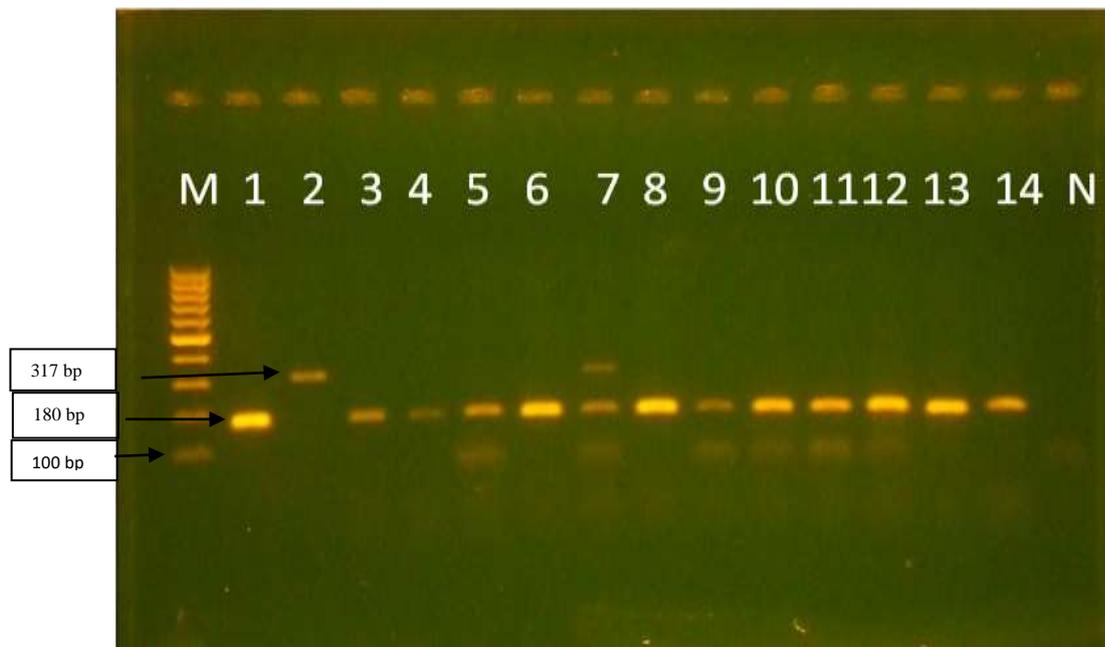
Multi-antimicrobial resistant <i>Staph. aureus</i> organisms		Average of RI
No./36	%	
20	55.56	0.37

**Table 5:** Frequency of antimicrobial resistant index (ARI) of *Staph. aureus* organisms isolated from raw milk and kareish cheese (n = 36).

ARI	Frequency	%
0.1	4	11.11
0.2	3	8.33
0.3	4	11.11
0.4	8	22.22
0.5	8	22.22
0.6	3	8.33
0.7	1	2.78
0.8	0	0
0.9	0	0
1	0	0

**Table 6:** Percentage of *Staph. aureus* strains positive for enterotoxigenic genes by using Multiplex PCR assay.

Gene targeted	No. of tested strains	Positive strains	
		No.	%
<i>sea</i>	36	34	94.44
<i>seb</i>	36	0	0.0
<i>sec</i>	36	0	0.0
<i>sed</i>	36	1	2.77



**Figure 1:** The electrophoresis pattern of Multiplex PCR amplicon on *Staph. aureus* strains for detection enterotoxigenic *sea*, *seb*, *sec* and *sed* genes.

M: 100 bp ladder DNA marker

Lane 1: *sea* control positive *Staph. aureus* (180 bp)

Lane 2: *sed* control positive *Staph. aureus* (317 bp)

Lane 3-6 and 8-14: *sea* positive isolates

Lane 7: *sea* and *sed* positive isolate

N: Negative control.

N.B.: control positive for *seb* and *sec* *Staph. aureus* strains were not available during performance of this study.

## DISCUSSION

Results illustrated in Table 1 revealed that, the prevalence of *Staph. aureus* in the examined raw milk samples was 62.50%, with counts ranging from  $< 1$  log to 4.53 log and with an average count of 3.25 log cfu/ml. Nearly similar result (64%) was revealed by Yildirim *et al.* (2019). Lower incidence of 16.3% was found by Mansour *et al.* (2017), whereas higher incidence (94%) was recorded by Kamal *et al.* (2013).

Table 2 revealed that, the highest frequency distribution of positive *Staph. aureus* count in raw milk samples was 40% and in the range of log 3 -  $< \log 4$  cfu/ml. Presence of *Staph. aureus* in raw milk in this study could be attributed to either indigenous

source due to *Staph. aureus* subclinical mastitis or exogenous source from infected handler's hands or from sneezing and cough. Also, contaminated utensils and infected flies and insects could be a source of contamination.

The prevalence of *Staph. aureus* in kareish cheese samples was 27.50%, with counts ranging from  $< 1$  log to 5.51 log and with an average count of 4.13 log cfu/g (Table 1). Relatively similar result of 24% was estimated by Elmaghraby *et al.* (2018). On the contrary, higher incidence (93%) was found by Kamal *et al.* (2013).

The highest frequencies distribution of positive *Staph. aureus* in kareish cheese samples was 12.5% and in the range log 4 -

< log 5 cfu/g (Table 2). The presence of *Staph. aureus* in kareish cheese is attributed to; this type of cheese is made from raw skimmed milk without any heat treatment and also, contaminated utensils and improper storage could be a sources of contamination such type of cheese with *Staph. aureus* and other public health important pathogens.

From the public health point of view, most of the isolated *Staph. aureus* strains from raw milk and kareish cheese in this study had count equal to or more than  $10^3$  cfu/ml (Table 2) and this count is sufficient for the organism to produce enterotoxins responsible for occurrence of food poisoning to consumers as Jablonski and Bohach (2001) have found *Staph. aureus* counts in the range from  $10^3$ - $10^5$  CFU/g to be able to produce enterotoxin in such high quantities that may pose a health risk to the consumers.

The results listed in Table 1 revealed that, the prevalence of *Staph. aureus* in baladi yoghurt samples was 0.0%, with a count of < 1 log cfu/g. This result coincided with Sadek *et al.* (2014a). Incidence of 14% was recorded by Zakary *et al.* (2011), while extremely higher incidence (88%) was revealed by Meshref *et al.* (2019). The absence of *Staph. aureus* in baladi yoghurt in this study could be attributed to either heat treatment of milk during yoghurt production or due to acidity of baladi yoghurt which might play a role in *Staph. aureus* inhibition and hampering its growth.

The tabulated results in Table 3 showed that, *Staph. aureus* exhibited 52.78% resistance towards Oxacillin (Methicillin). Relatively similar result of 55.9% was found by Hoque *et al.* (2018). On the other hand, lower result (9%) was detected by Wang *et al.* (2017), while Omoshaba *et al.* (2018) revealed higher result of 88.2%.

It is worth mentioning that; Methicillin resistant *Staph. aureus* (MRSA) is a potential health hazard especially in immune-compromised individuals, the treatment of MRSA infections is difficult and costly. The ability of these bacteria to spread multiple antimicrobial resistance genes, turning it into a major public health problem. Therefore, monitoring MRSA from food is important and improving hygiene standards in food practices in order to reduce the microbiological risk to minimum (Kluytmans *et al.*, 1995; Pinchuk *et al.*, 2010 and Basanisi *et al.*, 2017).

Out of 36 *Staph. aureus* isolates, 4 isolates (11.11%) were resist to Vancomycin (Table 3). Lower result of 2.8% was revealed by Breves *et al.* (2015); while, higher result (20.13%) was reported by El-Banna *et al.* (2015).

All of the four Vancomycin resistant isolates obtained in this study were from raw milk samples and all of them were resisting simultaneously to Oxacillin (Methicillin), Amoxicillin and Erythromycin. While, 3 strains were resisting to Ceftriaxone and 2 strains were resist to Tetracycline.

From the public health point of view, Vancomycin has been the antibiotic of the last resort to treat the hospitalized patients critically infected with Methicillin resistant *Staph. aureus* MRSA (Moellering, 2008). Therefore, the presence of Vancomycin resistant *Staph. aureus* in raw milk at this study is of special public health concern and good hygienic measures must be applied during production of milk and milk products to safeguard consumers from being infected with such organism.

Concerning Amoxicillin, 77.78% of *Staph. aureus* isolates were resistant (Table 3). Lower result (42%) was revealed by Hoque *et al.* (2018). On the other hand 81.8%

resistant was revealed by Papadopoulos *et al.* (2018). For Ceftriaxone, 61.11% of the isolates were resistant (Table 3). Lower result of 34.8% was recorded by Sadek *et al.* (2014b). In regard to Gentamicin, 11.11% of the tested isolates were resistant. Relatively, similar result (13.2%) was found by Wang *et al.* (2017).

The tested isolates exhibited resistance to Tetracycline, Erythromycin and Trimethoprim-Sulfamethoxazole in percentages of 36.11, 63.89 and 16.67%, respectively (Table 3). Resistance of 38.2, 52.1 and 6.1% were reported respectively for the previous antibiotics by Wang *et al.* (2017). None of tested isolates was resistant to Ciprofloxacin in this study and this result coincided with Jung *et al.* (2005).

The illustrated results in Tables 4 and 5 revealed that, 55.56% of the tested isolates were Multi-antimicrobial resistant and with average resistance index (RI) of 0.37 and only 11.11% of the isolates showed resistance index of 0.1. This result indicated that, the isolates recovered from samples originated from high-risk sources and milk is a possible transmitter of these microorganisms to humans with difficulty to eradicate the infection (Krumperman, 1983; Pinchuk *et al.*, 2010 and Hammad *et al.*, 2012).

Concerning enterotoxins, out of the 36 *Staph. aureus* isolates 34 isolates (23 isolates from raw milk and 11 isolates from kareish cheese) (94.44%) were positive for *sea* gene (Table 6). Nearly similar result found by Wang *et al.* (2018) as the authors reported that, 94.8% *Staph. aureus* harbored at least one virulence gene from which *sea* (60.4%) genes was detected. Also, from Table (6 and Fig. 1) only one isolate (2.77%) was positive for *sed* gene which is co-positive with *sea* gene and this result simulated what obtained by Jung *et al.* (2005). Furthermore, no *seb* and *sec* genes were detected from the isolated

*Staph. aureus* in the present study and this result coincided with Jung *et al.* (2005).

From the obtained results in this study it is observed that, *sea* gene was the most prominent gene detected in *Staph. aureus* isolated from the examined samples and this observation go parallel with Argudin *et al.* (2010) as they concluded that, *sea* gene is the most common enterotoxin found in food and is frequently associated with staphylococcal food-poisoning outbreaks worldwide. Moreover, Enterotoxin does not affect foods' taste, odor, and appearance; they are heat resist and retain their biological activity even after pasteurization. Also, they resist the majority of proteolytic enzymes and thus remain their action in the gastrointestinal tract (Le Loir *et al.*, 2003; Orwin *et al.*, 2003 and Rall *et al.*, 2008).

It is worth mentioning that; all the methicillin resistant *Staph. aureus* (MRSA) detected in this study, harbored *sea* gene from which one isolate of them harbored both *sea* and *sed* genes simultaneously and this observation indicated that, the isolated *Staph. aureus* strains from this study were of potential public health hazards as they are Methicillin resistant and enterotoxins producers.

The correlation between *Staph. aureus* enterotoxin genes and its ability to resist different types of antibiotics revealed that, most of the enterotoxigenic strains were multi-antibiotics resistance and resist simultaneously to Amoxicillin, Ceftriaxone and Erythromycin. All the methicillin resistant *Staph. aureus* (MRSA) isolates harbored *sea* gene. Moreover, the enterotoxigenic *sea* and *sed* positive strain was Oxicillin (Methicillin) resistance. Furthermore, the 2 negative enterotoxigenic *Staph. aureus* strains were multi-antibiotic resistance; one of them was sensitive to Methicillin and Vancomycin while the other was resist to them, in addition all of the 2 strains were sensitive to gentamycin.

## CONCLUSION

The present study revealed that, *Staph. aureus* organisms isolated from raw milk and kareish cheese samples were resist to different varieties of antibiotics and with high multi-drug resistant (MDR) percent and with high resistance index (RI). Methicillin resistant *Staph. aureus* (MRSA) and Vancomycin resistant *Staph. aureus* (VRSA) were detected in the isolated strains which represent a potential hazard to consumers. Counts of the most isolated *Staph. aureus* were sufficient to produce enterotoxins responsible for occurrence of food poisoning to consumers. *Staph. aureus* enterotoxin A gene was the most prominent gene in the isolated strains. All the methicillin resistant *Staph. aureus* (MRSA) strains harbored *sea* gene representing potential risks of infection and intoxication by consumption of such food. Thorough food inspection and frequent bacteriological surveillance by food control agencies is highly recommended to control the incidence of *Staph. aureus* in raw milk and dairy products to safeguard the consumers from risks of food poisoning.

## REFERENCES

- A.P.H.A. (2001): Compendium of Methods for the Microbiological Examination of Foods. 4<sup>th</sup> Ed. American Public Health Association, Washington, DC, USA.
- Abdel-Baky, R.M.; Ahmed, H.R. and Gad, G.F.M. (2014): Prevalence and conjugal transfer of vancomycin resistance among clinical isolates of *Staphylococcus aureus*. *AIR.*, 2(1): 12-23.
- Argudin, M.A.; Mendoza, M.C. and Rodicio, M.R. (2010): Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins*, 2: 1751-1773.
- Bailey, W.R. and Scott, E.G. (1994): Diagnostic Microbiology. A text book for the isolation and identification of pathogenic microorganisms. 9<sup>th</sup> Ed., C.V. Mosby Co., St. Louis, Toronto, London.
- Basanisi, M.; La Bella, G.; Nobili, G.; Franconieri, I. and La Salandra, G. (2017): Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. *Food Microbiol.*, 62:141-146.
- Breves, A.; Miranda, C.; Flores, C.; De Filippis, I. and Clementino, M. (2015): Methicillin- and vancomycin-resistant *Staphylococcus aureus* in health care workers and medical devices. *J. Bras. Patol. Med. Lab.*, 51(3): 143-152.
- BSAC (British Society for Antimicrobial Chemotherapy) (2002): BSAC Standardized Disc Susceptibility Testing Method (version 14) January 2015 available at <http://bsac.org.uk/wp-content/uploads/2012/02/BSAC-disc-susceptibility-testing-method-Jan-2015.pdf>
- CLSI (Clinical and Laboratory Standards Institute) (2011): Performance standards for antimicrobial susceptibility testing: Twenty First Informational Supplement M100-S21. Wayne, PA.
- Cremonesi, P.; Luzzana, M.; Brasca, M.; Morandi, S.; Lodi, R.; Vimercati, C.; Agnellini, D.; Caramenti, G.; Moroni, P. and Castiglioni, B. (2005): Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. *Mol. Cell Probes*, 19: 299-305.
- Dinges, M.M.; Orwin, P.M. and Schlievert, P.M. (2000): Enterotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev.*, 13: 16-34.
- El-Banna, T.; Sonbol, F.; Abdel-Aziz, A. and El-Ekhnawy, E. (2015):

- Characterization of Vancomycin Resistant *Staphylococcus aureus* in Tanta University Hospital. *Int. J. Curr. Microbiol. App. Sci.*, 4(10): 1-11.
- Elmaghraby, Manal, S.; Abden, Eman, E.; Abou-Elros, Nahla, A. and Elbagory, A. (2018): Molecular identification of virulence genes of *Staphylococcus aureus* isolated from milk products. *Alexandria J. Vet. Sci.*, 54 (1): 57-64.
- FDA (2001): Food and Drug Administration (Bacteriological Analytical Manual). *Staphylococcus aureus*. Chapter: 12, January 2001. <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM071429.htm>
- Finegold, S.M. and Martin, W.J. (1982): Bailey and Scott Diagnostic Microbiology. 6<sup>th</sup> Ed., C.V. Mosby Co., St. Louis, Toronto, London.
- Hammad, A.M.; Watanabe, W.; Fujii, T. and Shimamoto, T. (2012): Occurrence and characteristics of methicillin-resistant and – susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. *J. Food Microbiol.*, 156: 286-289.
- Hoque, M.N.; Das, Z.C.; Rahman, A.N.; Haider, M.G. and Islam, M.A. (2018): Molecular characterization of *Staphylococcus aureus* strains in bovine mastitis milk in Bangladesh. *Int. J. Vet. Sci. Med.*, 6:53-60.
- Jablonski, L.M. and Bohach, G. (2001): *Staphylococcus aureus*. In: Doyle M.P.; Beuchat L.R. and Montville T.J. (eds): *Food Microbiology: Fundamentals and Frontiers*. ASM Press, Washington: p. 411-434.
- Janštová, B.; Necidová, L. and Janštová, B. (2012): Comparing the growth of *Staphylococcus aureus* and production of staphylococcal enterotoxin c in sheep's and goat's milk. *J. Microbiol. Biotechnol. Food Sci.*, 1: 758-768.
- Jay, M.J.; Loessner, J.M. and Golden, A.D. (2005): Staphylococcal gastroenteritis. In: *Modern Food Microbiology*. 7<sup>th</sup> ed. Springer Science, New York, pp 545-560.
- Johnson, W.M.; Tyler, S.D.; Ewan, E.P.; Ashton, F.E.; Pollard, D.R. and Rozee, K.R. (1991): Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J. Clin. Microbiol.*, 29: 426-430.
- Jung, H.; Cho, J.; Park, S.; Ha, S. (2005): Genotypic and phenotypic characteristics of *Staphylococcus aureus* isolates from lettuces and raw milk. *Korean J. Food. Sci. Technol.*, 37 (1): 134-141.
- Kamal, Rania, M.; Bayomi, M.A.; Abdel-Aal, S.F. (2013): MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. *Food Control*, 33: 49-53.
- Kluytmans, J.; Van Leeuwen, W.; Goessens, W. (1995): Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno and genotyping. *J. Clin. Microbiol.*, 33: 1121-1128.
- Krumperman, P.H. (1983): Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol.*, 46: 165-170.
- Lampert, L.M. (1975): *Modern Dairy Products*. 3<sup>rd</sup> Ed., Chemical Pub. Co., Inc., New York.
- Le Loir, Y.; Baron, F. and Gautier, M. (2003): *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.*, 2(1): 63-76.
- Lee, J. (2003): Methicillin (Oxacillin) resistant *Staph. aureus* strains isolated from major food animals and their potential transmission to

- humans. *App. Environ. Microbiol.*, 69 (11): 6489-6494.
- Mansour, A.S.; Wagih, G.E.; Morgan, S.D.; Elhariri, M.; El-Shabrawy, M.A.; Abuelnaga, A.S.M. and Elgabry, E.A. (2017): Detection of *Staphylococcus aureus* enterotoxigenic strains in bovine raw milk by reversed passive latex agglutination and multiplex polymerase chain response. *Vet. World*, 10(8): 843-847.
- Meshref, A.; Hassan, G.; Riad, E. and Ashour, W. (2019): Studies on enterotoxigenic *Staphylococcus aureus* in milk and some dairy products. *Assiut Vet. Med. J.*, 65 (163): 87-97.
- Moellering, R.C. (2008): Current treatment options for community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis.*, 46:1032-1037.
- Normanno, G; La Salandra, G.; Dambrosio, A.; Quaglia, N.C.; Corrente, M.; Parisi, A.; Santagada, G.; Firinu, A.; Crisetti, E. and Celano, G.V. (2007): Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *Int. J. Food Microbiol.*, 115(3):290-296.
- Omoshaba, E.O.; Ojo, O.E.; Sofela, O. and Onifade, O.I. (2018): Prevalence and antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* in raw milk and soft cheese (wara) sold in Abeokuta, Nigeria. *Sokoto J. Vet. Sci.*, 16 (1): 1-8.
- Orwin, P.M.; Fitzgerald, J.R.; Leung, D.Y.; Gutierrez, J.A.; Bohach, G.A. and Schlievert, P.M. (2003): Characterization of *Staphylococcus aureus* enterotoxin L. *Inf. Immunit.*, 71: 2916-2919.
- Papadopoulos, P.; Papadopoulos, T.; Angelidis, A.; Boukouvala, E.; Zdragas, A.; Papa, A.; Hadjichristodoulou, C. and Sergelidis, D. (2018): Prevalence of *Staphylococcus aureus* and of methicillin-resistant *Staph. aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiol.*, 69: 43-50.
- Pinchuk, I.V.; Beswick, E.J. and Reyes, V.E. (2010): Staphylococcal enterotoxins. *Toxins*, 2:2177-2197.
- Rall, V.L.M.; Vieira, F.P.; Rall, R.; Vieitis, R.L.; Fernandes, A.Jr.; Candeias, J.M.G.; Cardoso, K.F.G. and Araujo, J.P.Jr. (2008): PCR detection of staphylococcal enterotoxin genes in *Staph. aureus* strains isolated from raw and pasteurized milk. *Vet. Microbiol.*, 132: 408-413.
- Reischl, M.; Ehret, W. and Wolf, H. (1994): PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. *Bio Techniques*, vol. 17, no. 5, pp. 844-845.
- Sadek, O.A.; Amin, M.M. and Hussein, M.F. (2014a): Comparison between the microbiological status of raw and pasteurized milk yoghurt. *Assiut Vet. Med. J.*, 60 (142): 132-138.
- Sadek, O.A.; Sayed, S.M.; El Berbawy, S.M.; Mansy, Mariam, F. and Hussien, M.F. (2014b): Some antibiotic resistant bacteria of public health hazard isolated from raw milk sold in some Assiut City markets. *Assiut Univ. Bull. Environ. Res.*, 17(1): 97-107.
- Sidhu, M.S.; Oppegaard, H. Devor, T.P. and Sorum, H. (2007): Persistence of multidrug-resistant *Staphylococcus haemolyticus* in an animal veterinary teaching hospital clinic. *Microb. Drug. Resist.*, 13(4):271-280.
- Wang, W.; Baloch, Z.; Jiang, T.; Zhang, C.; Peng, Z.; Li, F.; Fanning, S.; Ma, A. and Xu, J. (2017):

- Enterotoxigenicity and Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Retail Food in China. *Front. Microbiol.*, 8:2256. doi: 10.3389/fmicb.2017.02256
- Wang, W.; Lin, X.; Jiang, T.; Peng, Z.; Xu, J.; Yi, L.; Li, F.; Fanning, S. and Baloch, Z. (2018): Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, China. *Front. Microbiol.*, 9:1123. doi: 10.3389/fmicb.2018.01123
- Wang, X., Meng, J., Zhang, J., Zhou, T., Zhang, Y., Yang, B. (2012): Characterization of *Staphylococcus aureus* isolated from powdered infant formula milk and infant rice cereal in China. *Int. J. Food Microbiol.* 153, 142–147. doi: 10.1016/j.ijfoodmicro.2011.10.030
- WHO (2007): The world health report – A safer future: global public health security in the 21<sup>st</sup> century.
- Yildirim, T.; Sadati, F.; Kocaman, B. and Siriken, B. (2019): *Staphylococcus aureus* and Staphylococcal enterotoxin detection in raw milk and cheese origin coagulase positive isolates. *Int. J. Sci. Letters*, 1(1): 30-41.
- Zakary, E.; Nassif, M. and Mohammed, G. (2011): Detection of *Staphylococcus aureus* in bovine milk and its product by real time PCR Assay. *Global J. Biotechnol. Biochemist.*, 6 (4): 171-177.

## الكشف الجزيئي لجينات السموم المعوية لميكروب المكور العنقودي الذهبي المعزول من اللبن الخام وبعض منتجات الالبان

أنسي أديب صادق ، أيمن منير إبراهيم كُرَيْم

E-mail: onsi\_2000@yahoo.com Assiut University web-site: www.aun.edu.eg

تهدف هذه الدراسة إلى تحديد مدى تواجد ميكروب المكور العنقودي الذهبي في اللبن الخام وبعض منتجات الالبان وكذلك ايجاد العلاقة بين وجود جينات السموم المعوية ومقاومة الميكروب للمضادات الحيوية. حيث تم تجميع عدد ١٢٠ عينة من اللبن الخام والجبن القريش والزيادي البلدي (٤٠ عينة لكل منهم) من محلات الالبان والباعة الجائلين في مدينة أسيوط ، مصر وتم فحص العينات بكتريولوجيًا للتأكد من وجود ميكروب المكور العنقودي الذهبي. فكانت نسب وجود المكور العنقودي الذهبي في اللبن الخام والجبن القريش والزيادي البلدي هي ٦٢,٥ ، ٢٧,٥ و صفر.٪ ، على الترتيب بمتوسط عد على التوالي هي ٣,٢٥ ، ٤,١٣ و  $1 >$  لو غار يتم مستعمرة/ملي. وقد تم اختبار جميع العترات المعزولة عن طريق فحص PCR لوجود جينات السموم المعوية A, B, C, D فكانت ٩٤,٤٤ ٪ من العترات المختبرة تحتوي على جين السموم المعوية A و ٢,٧٧ ٪ من العترات كانت إيجابية بالنسبة لجين السموم المعوية D ، بينما كانت جميع العترات سلبية لجينات السموم المعوية B و C. تم اجراء اختبار حساسية العترات لبعض المضادات الحيوية وكانت النتائج على الترتيب ٥٢,٧٨ ، ١١,١١ ، ٧٧,٧٨ ، ٦١,١١ ، ١١,١١ ، ٣٦,١١ ، ٦٣,٨٩ و ١٦,٦٧ ٪ لكل من أوكساسيلين (ميثيسيلين) ، فانكوميسين ، أموكسيسيلين ، سيفترياكسون ، جنتاميسين ، تيتراسيكلين ، إريثروميسين ، وتراي ثوبريم سلفا ميثازول. بينما كانت جميع العترات حساسة بنسبة ١٠٠٪ تجاه سيبروفلوكساسين. أظهرت العترات مقاومة متعددة للمضادات الحيوية بنسبة ٥٥,٥٦ ٪ وبمؤشر مقاومة متوسطة ٠,٣٧. وقد كانت هناك علاقة بين المكور العنقودي الذهبي المحتوية على جينات السموم المعوية وقدرة الميكروب على مقاومة العديد من المضادات الحيوية ومقاومة في نفس الوقت للمضادات الحيوية أموكسيسيلين وسيفترياكسون وإريثروميسين. وكانت كل العترات المقاومة للمضاد الحيوي أوكساسيلين (ميثيسيلين) تحتوي على جين السموم المعوية (A). وقد تمت مناقشة المخاطر الصحية لوجود الميكروب في اللبن وبعض منتجاته وكذلك الطرق الصحية الواجب اتباعها لتقليل خطورة الميكروب محل الدراسة.