



## Bacteriological and molecular studies on toxigenic *Staphylococcus aureus* in milk and some milk products

Ashraf A. Abd El Tawab<sup>1</sup>, Ahmed M. Ammar<sup>2</sup>, Fatma I. El-Hofy<sup>1</sup>, Hoda A. Aideia<sup>3</sup>, Eman A. Hammad<sup>4</sup>

<sup>1</sup> Bacteriology, Immunology and Mycology Dept., Fac. Vet. Med. Benha Univ. <sup>2</sup> Microbiology Dept., Fac. Vet. Med. Zagazig Univ. <sup>3</sup> Animal Health Research Institute Dokki, Giza. <sup>4</sup> General Authority for Vet. Services.

### ABSTRACT

A total of 200 random samples of milk and milk products represented by kareish cheese, yoghurt and ice-cream (50 for each) were examined microbiologically for the presence of *Staphylococcus aureus*, its enterotoxigenicity and its antibiotic sensitivity. *Staphylococcus aureus* was isolated from 8 (16%) milk samples, 15 (30%) kareish cheese, 4 (8%) yoghurt and 11 (22%) ice-cream samples. All *S. aureus* isolates exhibited clumping factor using kits for reliable latex agglutination test. The susceptibility of the isolates was determined for 12 antimicrobial drugs using disc diffusion assay. The majority of strains were susceptible to ofloxacin and ampicillin + sulbactam (100%), vancomycin and tetracycline (94.7%), norfloxacin and sulphamethoxazole + trimethoprim (89.5%), chloramphenicol (73.3%) but they were resistant to oxacillin and metronidazole (100%). Amplification of coagulase gene (*coa*) using uniplex PCR, staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) and methicillin-resistant *S. aureus* (*mecA*) gene using multiplex PCR revealed that, 11/11(100%) of the examined samples were positive for both *coa* and *mecA* genes. While, *sea* produced by 5 (45.45%) strains, *sec* and *sed* produced by 4 (36.36%) strains and *seb* and *see* were not produced by any strains.

**Key words:** milk, *S. aureus*, enterotoxigenicity, antibiotic sensitivity, PCR.

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-31(2): 202-209, 2016)

### 1. INTRODUCTION

Milk serves as an optimum medium for propagation of various pathogenic and spoilage microorganisms (Gatti et al., 2013). Staphylococcal intoxication is considered the second or the third most common food intoxication of microbiological origin (Atanassova et al., 2001). Coagulase positive *S. aureus* is the causative agent of two thirds of food-borne disease outbreaks (Busani et al., 2005). Staphylococcal food poisoning (SFP) is one of the most prevalent causes of gastroenteritis worldwide. Symptoms of SFP have a rapid onset (2 to 6 hours) of abdominal cramps, nausea, and vomiting, sometimes followed by diarrhoea. Patients become symptomatic within 2-4 hours after ingestion of thermostable staphylococcal enterotoxins (SEs) of an approximate dose of 0.1 to 1.0 mg/kg of body weight (Stewart et al., 2005). There are serologically several distinct enterotoxins that *S. aureus* produced including staphylococcal

enterotoxins (SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity (Argudín et al., 2010). Antimicrobial resistance is also of an important public health concern worldwide. Methicillin resistant *S. aureus* (MRSA) strains resistant to quinolones or multi-resistant to other antibiotics have been emerging, leaving a limited choice for their control (Mee-Marquet et al., 2004; Nejma et al., 2006). PCR assay for detection of genes for *S. aureus* was developed and proved to be specific, sensitive, and rapid method (Omoe et al., 2002; Zschock et al., 2005). As the level of contamination of both milk and its products with different food-borne pathogens constitutes serious problems for consumers, so, the purpose of this study was the evaluation of bacteriological patterns of *Staphylococcus aureus* as one of the food poisoning micro-organisms in milk and milk products.

## 2. MATERIAL AND METHODS

### 2.1. Samples collections:

A total of 200 random samples of milk and milk products including yoghurt, kareish cheese and ice-cream (50 of each) were collected from different large and small dairy plants, street vendors and dairy house in El-Sharkia and Giza Governorates.

### 2.2. Bacteriological examination:

#### 2.2.1. Preparation of serial dilutions (APHA, 1992):

#### 2.2.2. Isolation and identification of *staphylococcus aureus*:

Isolation on Baird-Parker medium, Blood agar medium and Milk Salt agar medium was performed according to (APHA, "American Public Health Association" 1992), morphological identification by Gram stain (Cruickshank et al., 1975), biochemical tests (Arora, 2003; Quinn et al., 2002) and serologically by latex agglutination test Dry Spot kit (Staphylect plus) (Oxoid, 1990).

#### 2.2.3. In-Vitro anti-microbial sensitivity method (Finegold and Martin, 1982):

Using agar diffusion method.

### 2.3. Molecular biology technique (PCR):

#### 2.3.1. Uniplex PCR:

For detection of staphylococcal Coagulase (*coa*) gene using specific oligonucleotide primers sequences for these genes with the length of amplified products at 850 bp according to Goh et al. (1992).

#### 2.3.2. Multiplex PCR:

For detection of *S. aureus* enterotoxins genes (*sea seb, sec, sed* and *see*) and the methicillin-resistant (*mecA*) genes of *S. aureus* using specific oligonucleotide primers sequences with the length of amplified products for enterotoxins at (102 bp for *sea*), (164 bp for *seb*), (451 bp for *sec*), (278 bp for *sed*) and (209 bp for *see*). While, the length of amplified products for methicillin-resistant (*mecA*) genes at different base pairs (613, 398, 280, 776, 493, 200, 881 and 325 bps) according to Mehrotra et al. (2000).

## 3. RESULTS

The bacteriological examination of milk and milk products (kareish cheese, yoghurt and ice-cream) samples was shown in (Table, 1). The results revealed that *S. aureus* was isolated from 38 (19%) of the examined samples represented as, 8

(16%) milk samples (1 from large scale dairy plants, 1 from small scale dairy plants, 3 from farmer houses and 3 from street vendors), 15/50 (30%) kareish cheese samples (1 from large scale dairy plants, 2 from small scale dairy plants, 7 from farmers houses and 5 from street vendors), 4 (8%) yoghurt samples (0 from large scale dairy plants, 0 from small scale dairy plants, 2 from farmers houses and 2 from street vendors) and 11 (22%) ice-cream samples (0 from large scale dairy plants, 2 from small scale dairy plants, 3 from farmers houses and 6 from street vendors). The results of in-vitro sensitivity test for the isolated *S. aureus* indicated the isolated strains were susceptible to ofloxacin and ampicillin + sulbactam (100%), vancomycin and tetracycline (94.7%), norfloxacin and sulphamethoxazole + trimethoprim (89.5%), chloramphenicol (73.3%) (Table, 2). On the other hand, moderate sensitivity was observed to cephalothin and clindamycin with percentages of 57.9% and 42.1%, respectively. Moreover, the majority were resistant to oxacillin and metronidazole (100%). Confirmation of 11 randomly selected *S. aureus* isolates from milk and milk products using molecular PCR (Table, 3) showed that, *coa* genes produced by all of the examined 11 (100%) samples (Photo, 1) using uniplex PCR, *mecA* genes produced by all of the examined 11(100%) samples (Photo, 2) by multiplex PCR. Multiplex PCR also used for detection of staphylococcal enterotoxin genes (*sea, seb, sec, sed* and *see*) and showed that, *sea* genes produced by 5/11 isolates (45.45%), *sec* and *sed* produced by 4/11 isolates (36.36%). While, *seb* and *see* genes did not produced by any isolate (Photo, 3).

## 4. DISCUSSION

The examined samples of milk and milk products (kareish cheese, yoghurt and ice-cream) have been showed *S. aureus* in 38 (19%) of milk and milk products samples Table (1). *Staphylococcus aureus* was isolated from 8 (16%) milk samples. Nearly similar findings were postulated by Alnakip (2009); Aman and Ahmed (1996); El-Bessary (2006); El-Jakee et al. (2008); Jakeen et al. (2010); Nassar (2013) who isolated *S. aureus* from 15%, 16.66%, 17.5%, 16% from buffalo's milk and 22.7% from cow milk, 16.7%, 19.13% and 16%, respectively. On the other hand, higher incidence was reported by El-Gendy (2015); Hammad (2004); Mohammed (2002); Ralls et al. (2008); Tondo et al. (2000); Wafy (2006) who isolated *S. aureus* from 90.4%, 64%, 80%, 60%,

Table 1. Prevalence of *S. aureus* in milk and milk products (n=50):

Type of samples	No. of samples	Dairy plants				Farmers houses		Street vendors		Total	
		Large scale		Small scale		No./15	%*	No./15	%*	No./50	%**
		No./10	%*	No./10	%*						
Milk	50	1	10	1	10	3	20	3	20	8	16
Kareish cheese	50	1	10	2	20	7	46.7	5	33.33	15	30
Yoghurt	50	-	-	-	-	2	13.33	2	13.33	4	8
Ice-cream	50	-	-	2	20	3	20	6	40	11	22
Total	200	2	5	5	12.5	15	25	16	26.7	38	19

\*percentage in relation to No. of each examined samples. \*\* percentage in relation to total No. of each 50 examined samples.

Table 2. In-Vitro antimicrobial sensitivity test for isolated *S. aureus* (CLSI, 2014):

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No. of <i>S. aureus</i> isolates	%*	No. of <i>S. aureus</i> isolates	%*	No. of <i>S. aureus</i> isolates	%*
Ofloxacin	38	100	-	-	-	-
Ampicillin+ Sulbactam	38	100	-	-	-	-
Vancomycin	36	94.7	-	-	2	5.3
Tetracycline	36	94.7	2	5.3	-	-
Norfloxacin	34	89.5	-	-	4	10.5
Sulphamethoxazole- Trimethoprim	34	89.5	4	10.5	-	-
Chloramphenicol	28	73.7	6	15.8	4	10.5
Clindamycin	18	47.4	16	42.1	4	10.5
Amoxicillin+ Clavulinic acid	14	36.8	8	21.1	16	42.1
Cephalothin	6	15.8	22	57.9	10	26.3
Metronidazole	-	-	-	-	38	100
Oxacillin	-	-	-	-	38	100

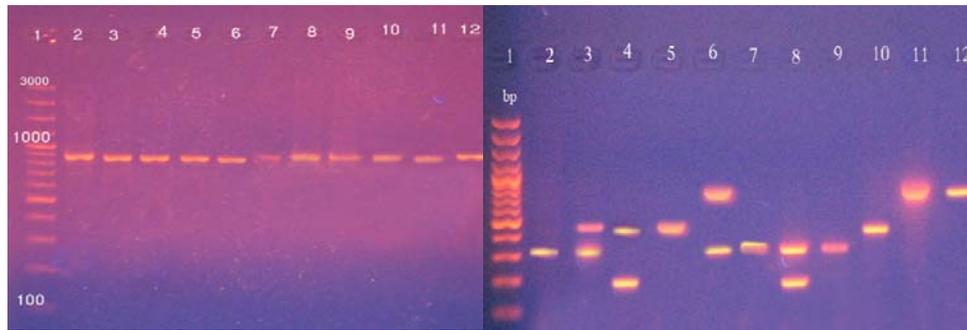
\*Percentage in relation to total number of isolated *S. aureus*

Table 3. Incidence of coagulase positive, methicillin resistant *S. aureus* and enterotoxins in randomly selected 11 examined samples of milk and milk products by PCR:

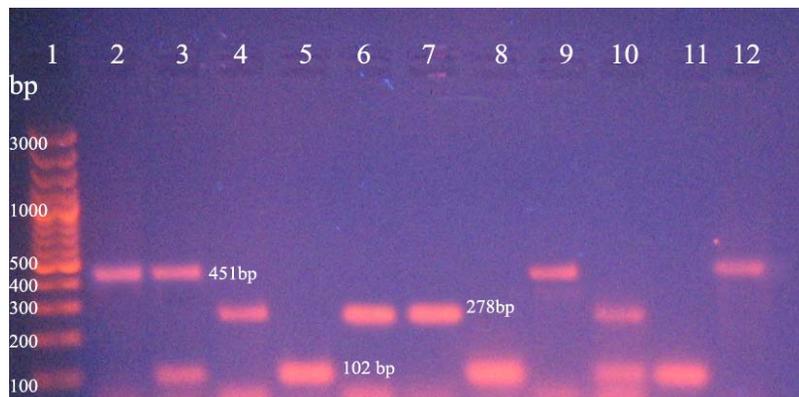
Examined <i>S. aureus</i> for	No. of + ve samples	%*
Coagulase positive	11	100%
Methicillin resistant	11	100%
Enterotoxins A	5	45.45%
Enterotoxins B	0	0
Enterotoxins C	4	36.36%
Enterotoxins D	4	36.36%
Enterotoxins E	0	0

\*Percentage in relation to total number of selected *S. aureus* isolates

Photos 1, 2 and 3. Agarose gel electrophoresis patterns showing: 1. Uniplex PCR for the *S. aureus*. 2. Multiplex PCR for methicillin. coagulase (*coa*) gene, resistant *S. aureus* (*mecA*) gene



Lanes 1: DNA molecular size marker (100-bp ladder). Lane 2-12: positive sample for coagulase gene and methicillin resistant gene



Multiplex PCR amplification products for the *S. aureus* enterotoxin genes. Lane 1: DNA molecular size marker (100-bp ladder). Lanes 3, 5, 8, 10 and 11: positive samples *sea* gene at 102 bp. Lanes 2, 3, 9 and 12: positive samples *sec* gene at 451 bp. Lanes 4, 6, 7 and 10: positive samples *sed* gene at 278 bp

70.4% and 80%, respectively, but relatively lower incidence was achieved by Amer et al. (2007); Demo et al. (1999); Kivaria et al. (2006); Sudershan and Ashwani (1996) who isolated *S. aureus* from 12.96%, 14.5%, 6.3% and 13%, respectively. Moreover, *S. aureus* was isolated from 15/50 (30%) kareish cheese samples. Nearly similar finding to that postulated by Abo-Donia et al. (1975); Ahmed et al. (1988); El-Shater (2010); Hassan (2003) who isolated *S. aureus* from 33.3%, 27.5%, 30% and 28%, respectively. On the other hand, higher incidence was reported by Awida (2009); (1999); Hassan (2008); Kolta (2011); Said and Fahmy (1991) who isolated *S. aureus* from 78%, 42.5%, 72%, 50% and 70%, respectively, but lower incidence was reported by Ahmed Ahmed et al. (2004); Awad Allah (2004); El-Bessary (2006) who isolated *S. aureus* from 10 % and 11.25 % in Kafr El-Shaikh and Gharbia Governorates, 12% and 5%, respectively. *S. aureus* was isolated from 4/50 (8%) of the examined yoghurt. Nearly similar

finding to that postulated by El-Biaa (2011); El-Shinawy (1987) who isolated *S. aureus* from 10% and 12%, respectively. On the other hand, higher incidence was reported by Abdel-Fatah (2007) who isolated *S. aureus* in 64.44% of samples. *S. aureus* could not be detected in industrial processing yoghurt samples; this is in agreement with Al-Tahriri (2005). *S. aureus* was isolated from 11/50 (22%) ice-cream samples. Nearly similar finding to that postulated by Abdel-Fatah (2010); Kamal (2009); Masud (1989) who isolated *S. aureus* from 26%, 22.9% and 20%, respectively. On the other hand, higher incidence was reported by Abdel-Haleem (1995); Abo-Risha (1998); Allam (1999); Hammad (2004); Hassan (2003); Patr et al. (2007) who isolated *S. aureus* from 84.72%, 50%, 76%, 56.67%, 55% and 44% , respectively, but lower incidence was reported by Amuramjimi et al. (2008); Kock et al. (1998); Kruy et al. (2001); Little and Louvois (1999); Manzanera-Pelegrin et al. (1995) who isolated *S. aureus* from 2.7 % , 4.3%

,0.5% , 12.2 and 4.4%, respectively. Moreover, Caglayanlar et al. (2009); Korel et al. (2002); Maiereni et al. (1993); Sagdic et al. (2002) could not detect *S. aureus* in any one of the examined ice-cream samples. Moreover, the results of antibiotic sensitivity tests for the isolated *S. aureus* Table (2) showed that, *S. aureus* isolates were highly sensitive to ofloxacin and ampicillin + sulbactam (100%), vancomycin and tetracycline (94.7%), norfloxacin and sulphamethoxazole + trimethoprim (89.5%), chloramphenicol (73.3%) and they were the most proper antibiotics with the highest efficiency against isolated *S.aureus*. Meanwhile, they were resistant to oxacillin and meropenidazole (100%). Nearly similar results of norfloxacin were recorded by Ahmed (2015); Khalil (2014). Nearly similar results of vancomycin were recorded by Nassar (2013) at which the sensitivity to vancomycin was 100%. The sensitivity to ofloxacin (65%) is also recorded by Ikeagwn et al. (2008). The sensitivity to sulphamethoxazole + trimethoprim (95%) is also recorded by Nassar (2013). The sensitivity to chloramphenicol is also recorded by Centorbi et al. (1992); Rossetti (1993) at which it was detected in 94% and 100%, respectively, also Bobu et al. (2007) mentioned that chloramphenicol was the most effective antibiotics. There is no resistance was detected for ampicillin + sulbactam as recorded by Gentilini et al. (2000). The sensitivity to tetracycline is also recorded by Ahmed (2015); Dutta and Rangnekar (2008); Edward et al. (2009) at which it was detected in 100%, 70% and 85.7%, respectively. Compound oxacillin used to detect MRSA strains and it is also named ORSA (Oxacillin resistant *S. aureus*). This study explained that all examined *S. aureus* strains were resistant to oxacillin, this result come in agreement to that reported by Khalil (2014) and dis-agree to those reported by Gentilini et al. (2000); Rossetti (1993) who showed that 100% of the examined strains are susceptible to oxacillin. Our PCR results Table (3) revealed that, uniplex PCR results for *coa* gene showed that 11/11 (100%) of the examined isolates were positive for *coa* gene. Detection of coagulase gene by PCR was discussed by several authors as by Malahat et al. (2009) at which 21% of the samples were positive for coagulase gene and Shalaby (2012) reported that, out of the 75% of 12 *S. aureus* isolates were positive for coagulase gene. Our multiplex PCR results for enterotoxin genes were so interesting as, *sea* produced by 5/11 (45.45%) samples, *sec* and *sed* produced by 4/11(36.36%) samples, *seb* and *see* not produced by any strains, some of the isolates may carried more than one toxin gene. Detection of staphylococcal enterotoxins by multiplex PCR was

discussed by several authors as by Adwan et al. (2005) who reported that, out of the 100 *S. aureus* isolates (milk sheep origin= 52, milk cows origin= 48) tested for SE-genes by PCR method, 37% were positive. None of these isolates carried more than one toxin gene. The majority of these positive toxin gene isolates 20 (54.1%) were *seb* positive and (Shalaby, 2012) at which the multiplex PCR test revealed that enterotoxin A found in 58.3% of *S. aureus* isolates, both enterotoxins B and C was found in 66.7% of the isolates and enterotoxin D was the predominant one found in 75% of the isolates. The results of multiplex PCR for methicillin resistant *S. aureus* showed that 11/11(100%) of the examined isolates were carrying *mecA* gene (MRSA). Methicillin resistant *S. aureus* by multiplex PCR was also detected by several authors as by Bakeet and Darwish (2014); Mulders et al. (2010); Nematı et al. (2008); Oke and Adewale (2013).

The obtained results allow concluding that milk and its products were highly contaminated with high number of *S. aureus* which may lead to undesirable changes that render them unfit for human consumption and indicate unpersonal hygiene and unsanitary conditions during processing and handling. Also, Antimicrobial resistance are widely spread among isolated strains and has been reported to negatively affect the treatment of its associated infections in human and animals.

## 1. REFERENCES

- Abdel-Fatah, E.N. 2007. Sanitary studies on fermented milk marketed at Zagazig Markets.M.V.Sc. , Zag. Univ. Egypt.
- Abdel-Fatah, E.N. 2010. Role of small and large scale producers in transmission of food poisoning organisms through consumption of ice- cream.Ph.D. Thesis, Zag. Univ., Egypt.
- Abdel-Haleem, A.A. 1995. Microbiological evaluation and sanitary improvement of ice-cream.Ph. D. Thesis, Assiut Univ. Egypt.
- Abo-Donia, S.H., Sirry, I., Abdel-Rahman, S.M., 1975. Chemical and microbiological studies on kareish cheese. . Alex. J. Agric. Res. 23, 243-247.
- Abo-Risha, N.E. 1998. Occurrence of some food poisoning microorganisms in some dairy products. M.V.Sc. Thesis, Kafr El-Sheikh Univ., Egypt.
- Adwan, G., Bassam, A.S., Kamal, A., 2005. Enterotoxigenic Staphylococcus aureus in

- raw milk in the North of Palestine. . Turk. J. Biol. 29, 229-232.
- Ahmed, A.H., Moustafa, M.k., Saad, N.M., Ahmed, S.H., 1988. Occurrence of staphylococci in milk and some dairy products. . Assiut Vet. Med. J. 19, 85-89.
- Ahmed, H.F., Aman, I.M., Mohamed, A.M., 2004. Bacteriological quality of kareish cheese and a trial to control *Staphylococcus aureus* in cheese. . Alex. J. Vet. 21, 514-523.
- Ahmed, W.A. 2015. Studies on antimicrobial activity of some plant extract against *Staphylococcus aureus* and *Candida albicans* with mastitic relevance. Ph.D. Thesis Benha Univ., Egypt.
- Al-Tahriri, R., 2005. A comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies. . Pakist. J. of nutr. 4, 345-348.
- Allam, H.A. 1999. Microbiological studies on milk and some milk products. Ph.D. Thesis., Benha Univ., Egypt.
- Alnakip, M.E. 2009. Prevalence of Gram positive bacteria in milk and some dairy products. M.V.Sc. Thesis., Zagazig Univ., Egypt.
- Aman, I.M., Ahmed, H.F., 1996. Incidence and survival of some food-borne pathogens in milk and cheese. . Egyptian. Vet. Med. Association 57, 151.
- Amer, I.H., Abdel-Aal, S.F., Awad, E.I., 2007. Prevalence of bacterial content and food borne organisms in raw cow's milk. . Alex. J. Vet. Science 26, 153-164.
- Amuramjimi, C., Geethu, S., Dhanashree, B., 2008. Bacteriological analysis of ice-cream from Mangalore. South India. Indian J. Med. Res. 127, 91-92.
- APHA, "American Public Health Association" 1992. Compendium of methods for the microbiological examination of food, 3rd ed, Washington, D.C., USA.
- Argudín, M.A., Mendoza, M.C., Rodicio, M.R., 2010. Food poisoning and *Staphylococcus aureus* enterotoxins Toxins 2, 1751-1773
- Arora, D.R., 2003. Text Book of Microbiology. 2nd Edition (Cultural characteristics of *Staphylococcus* spp (202-2013), *Aeromonas*, *Plesiomonas* (381- 388) Satish Kumar Jain for CBS publishers.
- Atanassova, V., Meindi, A., Ring, C., 2001. Prevalence of *Staph. aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham, a comparison of classical culturing detection and RPLA-PCR Int. J. Food Microbiol. 68, 105-113.
- Awad Allah, M.A. 2004. Studies on proteolytic bacteria in milk and cheese in Zagazig markets M. V. Sc. Thesis, Zag. Univ., Egypt.
- Awida, R.M. 2009. Some studies on *S. aureus* in milk and some milk products sold in Assiut city with special reference to antibiotic resistant *Staphylococcus aureus* Ph. D. Thesis, Assiut Univ., Egypt.
- Bakeet, A.A., Darwish, S.F., 2014. Phenotypic and genotypic detection of methicillin resistant *Staphylococcus aureus* (MRSA) in broiler chickens. Assiut Vet. Med. J. 60, 142-150.
- Bobu, Y.H., Sudhaker, B., Srinivas, C.S., Khan, M.A., 2007. Studies on incidence and vitro sensitivity of isolates to antimicrobial from subclinical mastitis in Jersey cows. Animal health Centre. . Kurnool. Andhrapradesh, India. Livestock. Adviser 8, 47-49.
- Busani, L., Cigliano, A., Tailoli, E., 2005. Prevalence of *Salmonella enterica* and *Listeria monocytogens* contamination in food of animal origin in Italy. . J. food prot. 68, 1729-1733.
- Caglayanlar, G.E., Buket Kunduhogul, B., Coksoyler, N., 2009. Comparison of microbiological quality of packed and unpacked ice-cream sold in Bursa, Turkey Journal of Arts and Sciences Sayi 12, 93-102.
- Centorbi, O.N., Cuadrado, A.M.A., Alcaraz, L.E., Laciari, A.L., Milan, M., 1992. Prevalence of *S. aureus* isolated from subclinical bovine mastitis in the dairies of San Luis city. Revista Argentina de Microbiol. 24, 73-80.
- Cruickshank, R., Duguid, J.P., Marmoin, B.P., Swain, R.H., 1975. Medical microbiology. The practice of medical microbiology page, 12th ed, Churchill, Edinburgh.
- Demo, M., Quinones, J., Martin, V., 1999. Dichotomous keys for identifying *Staphylococcus* isolated from bovine milk. Latinoa. Microbiol. 41, 53-57.
- Dutta, S., Rangnekar, A., 2008. Subclinical mastitis in Jersey herd Indian Vet. J. 78, 161-162.
- Edward, M., Henryka, L., Anna, K., Sebastian, S., Michal, K., 2009. Atypical *Staphylococcus aureus* as aetiological agent of mastitis in cows Bull Vet Inst Pulawy 53, 383-387.
- El-Bessary, M.M. 2006. Sanitary status of milk and some milk products marketed in suburbs of Assiut Governorate Ph.D., Assiut Univ., Egypt.
- El-Biaa, N.I. 2011. Evaluation of hygienic quality of large scale manufactured yoghurt M.V.Sc. Thesis, Zag. Univ., Egypt.

- El-Gendy, A.M. 2015. Bacteriological and molecular studies on staphylococcus species isolated from raw milk M.V.Sc. Thesis, Benha Univ., Egypt.
- El-Jakee, J., Ata-Nagwa, S., Bakry, M., Zouelfakar-Sahar, A., Elgabry, E., Gad El-Said, W.A., 2008. Characteristics of Staphylococcus aureus strains isolated from human and animal sources. American Eurasian J. Agri. Environ. Sci. 4, 221-229.
- El-Shater, N.S. 2010. Evaluation of immunological and bacteriological patterns of some food poisoning micro-organisms (Staphylococcus aureus and Clostridium perfringens) Ph.D. Thesis, Zagazig University.
- El-Shinawy, S.H. 1987. Microbiological studies on fermented milks Ph.D. Thesis, Zag. Univ., Egypt.
- Finegold, S., Martin, V., 1982. Bailey's and Scott's. Diagnostic Microbiology 6th ed. Mosby Company, St. Louis, Toronto, London.
- Gatti, M., Bottari, B., Lazzi, C., Neviani, E., Mucchetti, G., 2013. Invited review: Microbial evaluation in raw milk, long ripened cheese produced using undefined natural whey starters J. Dairy Sci. 97, 573-591.
- Gentilini, E., Denamiel, G., Liorente, P., Godaly, S., Rebuelto, M., DeGregorio, O., 2000. Antimicrobial susceptibility S. aureus isolated from bovine mastitis in Argentina J. Dairy Sci. 83, 1224-1227.
- Goh, S., Byrne, S.K., Zhang, J.L., Chow, A.W., 1992. Molecular typing of Staphylococcus aureus on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. 30, 1642-1645.
- Halawa, M.A., Moawad, A.A., 1999. Bacteriological quality of street-vended white soft cheese Alex. J. Vet. Sci. 15, 855-864.
- Hammad, A.M. 2004. Microbiological studies on raw milk and some dairy products M.V.Sc. Thesis, Minufiya Univ., Egypt.
- Hassan, G.M. 2003. Quality assessment of some dairy products at consumer level M.V.Sc., Cairo Univ., Egypt.
- Hassan, W.S. 2008. Microbiological evaluation of some white soft cheese locally manufactured and sold in Assiut city M.V.Sc. Thesis, Assiut Univ., Egypt.
- Ikeagwu, I.J., Amadi, E.S., Iroha, I.R., 2008. Antibiotic sensitivity pattern of Staphylococcus aureus in Abakaliki, Nigeria Pak. J. Med. Sci. 24, 231-235.
- Jakeen, k.A., Emad, R.Z., Randa, S.F., 2010. Properties of enterotoxigenic S. aureus isolated from mastitic cattle and buffaloes in Egypt. J. Am. Sci., 6, 170-178.
- Kamal, R.M. 2009. Sanitary status of some locally and imported dairy products Ph.D. Thesis, Zag. Univ., Egypt.
- Khalil, A.O. 2014. Studies on Staphylococcus aureus and Aeromonas hydrophila associated with a severe outbreak infection in farms rabbits Ph. D. Thesis, Benha Univ., Egypt.
- Kivaria, F.M., Noordhuizen, J.P., Kapaga, A.M., 2006. Evaluation of hygienic quality and associated public health hazards of raw milk marketed by small holder dairy producers in the Dar es Salam region, Tanzania. Trop. Anim. Health Protec. 38, 185-194.
- Kock, C., Akan, M., Yardimci, H., 1998. Bacteriological quality of ice-cream marketed in Ankara. Veterinary-Fakultesi-Dergisi-Ankara Univ. 45, 113-134.
- Kolta, M.N. 2011. Characterization of Staphylococcus aureus superantigens in milk and some dairy products Ph.D. Thesis, Assiut Univ., Egypt.
- Korel, F., Omeroglu, S., Tan, G., Odabaso, A.Z., 2002. The evaluation of chemical and microbiological quality of ice-cream sold in retail markets in Manisa. Turkey. Annual Meeting and food Expo-Anaheim, California.
- Kruy, S.L., Soares, J.L., Ping, S., Sainte-Marie, F.F., 2001. Microbiological quality of "ice cream. Sorbet" sold on the streets of Phnom Penh; April 1996- April 1997. Bulletin de la societede pathogenic exotique 94 411-421.
- Little, C.L., Louvois, J., 1999. Microbiological Quality of soft ice-cream from fixed premises and mobile vendors. Int. J. Environ. Health Res. 9, 223-232.
- Maiereni, M., Civilini, M., Domenis, C., Manzano, M., Di-Prima, R., Cami, G., 1993. Microbiological quality of Artisanal ice-cream. Zentralblatt fur Hygiene und Umwelt Medizin Biological. Abst. 194, 553-570.
- Manzanera-Pelegrin, C., Marin-Mesegure, D., Paredes-Garcia, P., Pelegrin, C.M., Mesegure, D.M., Gracia, P.P., 1995. Quality control of ice-cream. Health area III of Murcia region Alimentaria 33, 119-121.
- Masud, T., 1989. Microbiological quality and public health significance of ice-cream J. Pak. Med. Assoc. 39, 102-104
- Mee-Marquet, N.V., Blanchard, M., Domelier, A.S., Quentin, R., 2004. Virulence and antibiotic susceptibility of Staphylococcus aureus strains isolated from various origins.

- Survey Study Group of the Relais. . Hygie'ne du Centre. Pathol. Biol. 52 579-583.
- Mehrotra, M., Wang, G., Johnson, W.M., 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1 and methicillin resistance. *J. of Clin. Microbiol.* 38, 1032-1035.
- Mohammed, A.M. 2002. Quality investigation into beef frankfurter produced in Egypt. M.v.sc. Thesis Cairo Univ.
- Mulders, M.N., Haenen, A.P., Geenen, P.L., Vesseur, P.C., Poldervaart, E.S., Bosch, T., Huijsdens, X.W., Hengeveld, P.D., Dam-Deisz, W.D., Graat, E.A., 2010. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughter house personnel in the Netherlands *Epidemiol. Infect.* 138, 743-755.
- Nassar, M.K. 2013. Phenotypic and genotypic characterization of enterotoxigenic *S. aureus* isolated from different sources with reference to antimicrobial resistance M.V.Sc. Thesis, Zagazig Univ., Egypt.
- Nejma, M.B., Mastouri, M., Frih, S., Sakly, N., Salem, Y.B., Nour, M., 2006. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated in Tunisia. *Diagn. Microbiol. Infect. Dis.* 55 21-26.
- Nemati, M., Hermans, K., Lippinska, U., Denis, O., Deplano, A., Struelens, M., Devriese, L.A., Pasmans, F., Haesebrouck, F., 2008. Antimicrobial resistance of old and recent *Staphylococcus aureus* isolated from poultry: first detection of livestock-associated methicillin resistant strain ST398 *Antimicrob. Agents Chemother* 52, 3817-3819.
- Oke, A.J., Adewale, A.O., 2013. Incidence of methicillin resistant *Staphylococcus aureus* (MRSA) in a small poultry in south west, Nigeria. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 5, 53-55.
- Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D.L., Ueda, S., Shinagawa, K., 2002. Detection of seg, seh, and sei genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates Harboring seg, seh, or sei genes. *J. Clin Microbiol.* 40.
- Patr, B., Ztepe, G., Ihak, I., Bozkurt, P., 2007. Microbiological quality of ice-cream marketed in Elzag Veteriner Bilimleri Dergisi.
- Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., Maguire, D., 2002. *Veterinary microbiology and microbial disease* 2nd ed. Blackwell Science.
- Ralls, V.L., Vieira, F.P., Ralls, R., Vieitis, R.L., Fernandes Jr., A., Candies, J.M., Cardoso, K.F., Araujo Jr., J.P., 2008. PCR detection of staphylococcal enterotoxigenes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk *Vet. Microbiol.* 132, 408-413.
- Rossetti, C.A., 1993. Prevalence of subclinical mastitis caused by *S. aureus* in the Buenos Aires dairy area and its susceptibility to antibiotics *Vet. Argentina.* 10, 323-326.
- Sagdic, O., Tuluoglu, D.D., Ozcelik, S., Simsek, B., 2002. The chemical and microbiological quality of ice-cream consumed in Isparta market *Ziraat. Fakultesi-Dergisi, Ataturk Uni.* 33, 441-446.
- Said, M.R., Fahmy, M.A., 1991. A survey of incidence of some *S. aureus*, *E. coli* and *B. cereus* in some types of Egyptian cheeses in Assiut city *J. Assiut Agric. Sci.* 22, 239-246.
- Shalaby, M.I. 2012. Detection of *Staphylococcus aureus* enterotoxin genes in strains isolated from milk in cattle with reference to its antibiogram M.V.Sc. Thesis, Zag. Univ., Egypt.
- Stewart, C.M., Cole, M.B., Schaffner, D.W., 2005. Managing the Risk of Staphylococcal Food Poisoning from Cream-Filled Baked Goods to Meet a Food Safety Objective. *Journal of Food Protection* 66.
- Sudershan, K., Ashwani, K., 1996. Occurrence of some bacterial pathogens of public health significance in bovine raw milk. *Haryana veterinarian. Dairy Sci. Abst.* 6.
- Tondo, E.C., Guimaraes, M.C., Henriques, J.A., Ayub, M.H., 2000. Assessing and analyzing contamination of dairy products processing plant by *S. aureus* using antibiotic resistance PFGE *Candian J. Microbiol.* 46, 1108-1114.
- Wafy, Y.M. 2006. Sanitary improvement of serving milk and dairy products in Assiut University Hospitals Ph.D. Thesis, Assiut Univ., Egypt.
- Zschock, M., Kloppert, B., Wolter, W., Hamann, H.P., Lammler, C.H., 2005. Pattern of enterotoxin genes seg, seh, sei and sej positive *S. aureus* isolated from bovine mastitis. *Vet. Microbiol.* 108.