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

**Ashraf A. Abd El Tawab1, Ahmed M. Ammar2, Fatma I. El-Hofy1, Hoda A. Aideia3 ,Eman A. Hammad4**

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

**ABSTRACT**

A total of 200 random samples of milk and milk products which included kareish cheese, yoghurt and ice-cream (50 for each) were examined microbiologically for the presence of *S. aureus* , their enterotoxigencity and their antibiotic sensitivity. *S. aureus* was isolated from 8/50 (16%) milk samples, 15/50 (30%) kareish cheese, 4/50 (8%) yoghurt and 11/50 (22%) ice-cream samples. All *S. aureus* isolates were showing clumping factor using kits for reliable latex agglutination test. The susceptibility of the isolates were determined for 12 antimicrobial drugs using disc diffusion assay. The majority 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* (*mec*A) gene using multiplex PCR revealed that, 11/11(100%) of the examined samples were positive for both *coa* and *mec*A genes. *Sea* produced by 5 (45.45%) strains , *sec* and *sed* produced by 4 (36.36%) strains and *seb* and *see* not produced by any strains.

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

**1. Introduction**

Milk serves as an optimum medium for propagation of various pathogenic and spoilage microorganisms (Gatti et al., 2013). Staphylococcal intoxication considered the second or the third most common food intoxication of microbiological origin (Atanassova et al., 2001). Coagulase positive *S. aureus* are the causative agents 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* produces including staphylococcal enterotoxins (SEs: SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity (Argudín et al., 2010). Antimicrobial resistance is also an important public health concern worldwide. Methicillin resistant *S. aureus* (MRSA) strains resistant to quinolones or multiresistant to other antibiotics have been emerging, leaving a limited choice for their control *(*Mee-Marquetet al.,2004 andNejmaet 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 and Zschock et al., 2005). As the level of contamination of both milk and milk 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 some milk products.

**2. MATERIAL AND METHODS**

*2.1. Samples collections*

A total of 200 random milk and milk products samples including yoghurt, kareish cheese and ice-cream (50 of each) were collected from different supermarkets, street vendors and dairy houses.

*2.2.Bacteriological examination*

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

*2.2.2. Isolation and identification of staphylococcus aureus*:

Isolation on specific media (APHA, 1992), morphological identification by Gram stain (Cruickshank et al., 1975), biochemical tests(Quinn et al., 2002 and Arora, 2003) and serologically by latex agglutination test Dry Spot kit (Staphytect 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 Staph 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 (A, B, C, D and E) and the methicillin-resistant *(mec*A*)* 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 *(mec*A*)* 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 studied milk and milk products (kareish cheese, yoghurt and ice-cream) samples (Table, 1)revealed that, *S. aureus* were isolated from 38/200 (19%) of the examined samples represented as, 8/50 (16%) milk samples (1 from large scale dairy plants, 1 from small scale dairy plants, 3 from farmers 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), 4/50 (8%) yoghurt samples (0 from large scale dairy plants, 0 from small scale dairy plants, 2 from farmers houses and 2 from street vendors) and11/50 (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* (Table, 2) showed that, the majority were susceptible to ofloxacin and ampicillin+sulbactam (100%), vancomycin and tetracycline (94.7%), norfloxacin and sulphamethoxazole+trimethoprim (89.5%), chloramphenicol (73.3%).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 11/38 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, *mec*A 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 (A, B, C, D and E) 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 not produced by any isolate (Photo, 3).

**4. DISCUSSION**

A total of 200 examined samples of milk and milk products (kareish cheese, yoghurt and ice-cream) having *S. aureus* in 38/200 (19%) of milk and milk products samples Table (1). *S. aureus* were isolated from 8/50 (16%) milk samples. Nearly similar finding to that postulated by Boddi et al., (1987), Aman and Ahmed (1996), El-Bessary (2006), El-jakee et al., (2008), Alnakip (2009), Jakeen et al., (2010) and Nassar (2013) at which *S. aureus* could be isolated from 15%, 16.66% , 17.5%, 16% from buffaloes milk and 22.7% from cow milk, 16.7%, 19.13% and 16%, respectively. On other hand, higher incidence was reported by Tondo et al., (2000), Mohammed et al., (2002), Hammad (2004), Wafy (2006), Ralls et al., (2008) and El-Gendy (2015) at which *S. aureus* could be isolated from 90.4%, 64%, 80%, 60%, 70.4% and 80%, respectively, but relatively lower incidence was achieved by Sudershan and Ashwani (1996), Demo et al., (1999), Kivaria et al., (2006) and Amer et al., (2007)at which *S. aureus* could be isolated from 12.96%, 14.5%, 6.3% and 13%, respectively. *S. aureus* were isolated from 15/50 (30%) kareish cheese samples . Nearly similar finding to that postulated by Abo-Donia et al.,(1985), Ahmed et al., (1988), Hassan (2003) and El-Shater (2010) at which *S. aureus* could be isolated from 33.3%, 27.5%, 30% and 28%, respectively. On other hand, higher incidence was reported by Said and Fahmy (1991), Halawa and Moawad (1999), Hassan (2008), Awida (2009) and Kolta (2011) at which *S. aureus* could be isolated from 78%, 42.5%, 72%, 50% and 70%, respectively, but lower incidence was reported by Ahmed et al., (2004), Awad Allah (2004) and El-Bessary (2006) at which *S. aureus* could be isolated from 10 % and 11.25 % in Kafr E l-Shaikh and Gharbia Governorates, 12% and 5%, respectively. *S. aureus* were isolated from 4/50 (8%) of the examined yoghurt. Nearly similar finding to that postulated by El-Shinawy (1987) and El- Biaa (2011) at which *S. aureus* could be isolated from 10% and 12%, respectively . On 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* were isolated from 11/50 (22%) ice-cream samples. Nearly similar finding to that postulated by Masud (1989), Kamal (2009) and Abdel-Fatah (2010) at which *S. aureus* was detected in 26%, 22.9% and 20%, respectively . On other hand, higher incidence was reported by Abdel-Haleem (1995), Abo-Risha (1998), Allam (1999), Hassan (2003), Hammad (2004) and Patr et al., (2007)at which *S. aureus* was isolated from 84.72%, 50%, 76%, 56.67%,55% and 44% , respectively, but lower incidence was reported by Manzanera-Pelegrin et al., (1995), Kock et al., (1998), Little and Louvois (1999), Kruy et al., (2001) and Amuramjimi et al., (2008) at which *S. aureus* was isolated from 2.7 % , 4.3% ,0.5% , 12.2 and 4.4%, respectively. Moreover, Maiereni et al., (1993), Korel et al., (2002), Sagdic et al.,(2002) and Caglayanlar et al., (2009) could not detect *S. aureus* in any one of the examined ice-cream samples. In this study all isolates of *S. aureus* were subjected for detection of clumping factor using dry spot kit (staphytect plus) (Oxoid). It is a latex slide agglutination test for differentiation of *S.aureus* than other staphylococci. Coagulase and clumping factor are the fibrinogen-binding determinant on the *S. aureus* cell surface which distinct entities. Specific mutants lacking coagulase retain clumping factor activity, while clumping factor mutants express coagulase normally, these results also described by Chiang et al., (2006). 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 metronidazole (100%). Nearly similar results of norfloxacin were recorded by Khalil (2014) and Ahmed (2015). Nearly similar results of vancomycin were recorded byNassar (2013) at which the sensitivity to vancomycin was 100%. The sensitivity to ofloxacin (65%) is also recorded byIkeagwn et al., (2008). The sensitivity to sulphamethoxazole + trimethoprim (95%) is also recorded by Nassar (2013). The sensitivity to chloramphenicol is also recorded by Centrobie et al., (1992) and 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 Dutta and Rangnekar (2008), Edward et al., (2009) and Ahmed (2015)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 strains are resistant to oxacillin, this result agree to that reported by Khalil (2014) and dis-agree to those reported by Rossetti (1993) and Gentilini et al., (2000) at which their results 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 andShalaby (2012) reported that, out of the75% 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) 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. Non of these isolates carried more than one toxin gene. The majority of these positive toxin gene isolates 20 (54.1%) were *seb* positiveandShalaby (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%ofthe 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 *mec*A gene (MRSA). Detection of methicillin resistant *S. aureus* by multiplex PCR was discussed by several authors as by Nemati et al.,(2008), Mulders et al., (2010) ,Oke and Adewale (2013) and Bakeet and Darwish (2014).

**5. REFERENCES**

Abdel-Fatah, E.N. 2007. Sanitary studies on fermented milk marketed at Zagazig Markets. M. V. Sc. Thesis, Fac. Vet. Med. 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.

Abdel-Haleem, A.A. 1995. Microbiological evaluation and sanitary improvement of ice-cream. Ph. D. Thesis, Fac. Vet. Med. Assiut Univ., Egypt.

Abo-Donia, S.H., Sirry, I. and Abdel-Rahman, S.M. 1975. Chemical and microbiologial 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, Fac. Vet. Med. Kafr El-Sheikh Univ., Egypt.

Adwan, G., Bassam, A.S. and 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. and Ahmed, S.H. 1988. Occurance of staphylococci in milk and some dairy products. Assiut Vet. Med. J., 19(38): 85-89.

Ahmed, H.F., Aman, I.M. and Mohamed, A.M. 2004. Bacteriological quality of kareish cheese and atrial to control *Staphylococcus aureus* in cheese. Alex. J. Vet., 21(2): 514:523.

Ahmed, W.A. 2015. Studies on antimicrobial activity of some plat extract against *Staphylococcus aureus* and *Candida albicans* with mastitic relevance. Ph. D. Thesis, Fac. Vet. Med. Moshtohor Benha Univ., Egypt.

Allam, H.A. 1999. Microbiological studies on milk and some milk products. Ph. D. Thesis, Fac. Vet. Med. Moshtohour, Benha Univ., Egypt.

Alnakip, M.E. 2009. Prevalence of Gram positive bacteria in milk and some dairy products. M. V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.

AI-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 (5): 345-348.

Aman, I.M. and Ahmed, H.F. 1996. Incidence and survival of some food-borne pathogens in milk and cheese. Egyptian. Vet. Med. Association, vol. 57(1):151.

Amer, I. H., Abdel-Aal, S.F. and Awad, E.I. 2007. Prevalence of bacterial content and food borne organisms in raw cow's milk. Alex. J. Vet. Science, 26(1): 153-164.

Amuramjimi, C., Geethu, S. and 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. and Rodicio, M.R. 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins, 2 (7): 1751-1773.

Arora, D.R. 2003. Text Book of Microbiology. 2nd Edition (Cultural characteristics of *Staphylococcus spp* (202-2013), *Aeromonas, Plesiomonas* (381- 388).Publishing by Satish Kumar Jain for CBS publishers.

Atanassova, V., Meindi, A. and 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 (1-2): 105-113.

Awad Allah, M.A. 2004. Studies on proteolytic bacteria in milk and cheese in Zagazig markets. M. V. Sc. Thesis, Fac. Vet. Med. 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, Fac. Vet. Med. Assiut Univ., Egypt.

Bakeet, A.A. and Darwish, S.F. 2014. Phenotypic and genotypic detection of methicillin resistant *Staphylococcus aureus*  (MRSA) in broiler chickens. Assiut Vet. Med. 60(143):142-150.

Bobu,Y.H., Sudhaker, B., Srinivas, C.S. and Khan, M.A. 2007. Studies on incidence and vitro sensitivity of isolates to antimicrobial from subclinical mastitis in Jersey cows. Animal heath Centre. Kurnool. Andhrapradesh, India. Livestock. Adviser. 8(4):47-49.

Busani, L., Cigliano, A. and Tailoli, E. 2005. Prevalence of *Salmonella enterrica* and *Listeria monocytogens* contamination in food of animal origin in Italy. J. food prot., 68(8): 1729-1733.

Caglayanlar, G. E., Buket Kunduhogul, B. and 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, AMAde, Alcaraz, L.E., Laciar, A.L. and Milan, M. 1992. Prevalence of *S. aureus* isolated from subclinical bovine mastitis in the dairies of San Luis city. Revista Argentina de Microbiol., 24(2): 73-80.

CLSI ''Clinical and Laboratory Standards Institute'' 2014. Performance standards for antimicrobial susceptibility testing, Twenty Fourth Informational Supplement. M100-S24, 34 (1).

Cruickshank, R., Duguid, J.P.; Marmoin, B.P. and Swain, R.H. 1975. Medical microbiology.The practice of medical microbiology page 434. 12th Editions, Vol.II. Churchill, Edinburgh.

Demo, M., Quinones, J. and Martin, V. 1999. Dichotomous keys for identifying *Staphylococcus* isolated from bovine milk. Rev. Latinoa. Microbiol., 41(2): 53-7.

Dutta, S. and Rangnekar, A. 2008. Subclinical mastitis in Jersey herd. Indian Vet. J., 78(2): 161-162.

Edward, M., Henryka, L., Anna, K., Sebastian, S. and 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. Thesis, Fac. Vet. Med., Assiut Univ., Egypt.

El-Biaa, N.I. 2011. Evaluation of hygienic quality of large scale manufactured yoghurt. M. V. Sc. Thesis, Fac. Vet. Med. Zag. Univ., Egypt.

El-Gendy, A.M. 2015. Bacteriological and molecular studies on *staphylococcus species* isolated from raw milk. M. V. Sc. Thesis, Fac. Vet. Med., Benha Univ., Egypt.

El-Jakee, J., Ata-Nagwa, S., Bakry, M., Zouelfakar-Sahar, A., Elgabry, E. and Gad El-Said, W.A. 2008. Characteristics of *Staphylococcus aureus* strains isolated from human and animal sources. American Eurasian J. Agri. Environ. Sci, 4 (2):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 ,Fact. of Vet. Med. Zagazig University.

El-Shinawy, S.H. 1987. Microbiological studies on fermented milks. Ph. D. Thesis, Fac. Vet. Med. Zag. Univ., Egypt.

Finegold and Martin 1982. Bailey's and Scott's. Diagnostic Microbiology. 6th Ed. The C. V. Mosby Company. St. Louis, Toronto, London.

Gatti, M., Bottari, B., Lazzi, C., Neviani, E. and Mucchetti, G. 2013. Invited review: Microbial evaluation in raw milk, long ripened cheese produced using undefined natural whey starters. J. Dairy Sci., 97(2): 573-591.

Gentilini, E., Denamiel, G., Liorente, P., Godaly, S., Rebuelto, M. and DeGregorio, O. 2000. Antimicrobial susceptibility *S. aureus* isolated from bovine mastitis in Argentina. J. Dairy Sci., 83(6):1224-1227.

Goh, S., Byrne, S.K., Zhang, J.L. and 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. and Moawad, A.A. 1999. Bacteriiological quality of street-vended white soft cheese. Alex. J. Vet. Sci., 15(4):855-864.

Hammad, A.M. 2004. Microbiological studies on raw milk and some dairy products. M. V. Sc. Thesis, Fac. Vet. Med. Sadat City, Minufiya Univ., Egypt.

Hassan, G.M. 2003. Quality assessment of some dairy products at cosumer level. M. V. Sc. ,Fac. Vet. Med., Bani-Suef, 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, Fac. Vet. Med. Assiut Univ., Egypt.

Ikeagwn, I.J., Amadi, E.S. and Iroha, I.R. 2008. Antibiotic sensitivity pattern of *Staphylococcus aureus* in Abakaliki, Nigeria. Pak. J. Med. Sci., 24(2): 231-235.

Jakeen, k.A., Emad, R.Z. and Randa, S.F. 2010. Properties of enterotoxigenic *S. aureus* isolated from mastitic cattle and buffaloes in Egypt. J. Am. Sci., 6(11): 170-178.

Kamal, R.M. 2009. Sanitary status of some locally and imported dairy products, Ph. D. Thesis Fac. Vet. Med. Zag. Univ., Egypt.

Khalil, A.O. 2014. Studies on *Staphylococcus aureus* and *Aeromonas hdrophila* associated with a sever outbreak infection in farms rabbits. Ph. D. Thesis, Fac. Vet. Med., Benha Univ., Egypt.

Kivaria, F.M., Noordhuizen, J.P. and 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. and Yardimci, H. 1998. Bacteriological quality of ice-cream marketed in Ankara. Veterinary-Fakultesi-Dergisi-Ankara Univ., 45(1):113-134.

Kolta, M.N. 2011. Characterization of *Staphylococcus aureus* superantigens in milk and some dairy products. Ph. D. Thesis. Fac. Vet. Med., Assiut Univ., Egypt.

Korel, F., Omeroglu, S., Tan, G. and 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 Exo-Anaheim, California.

Kruy, S.L., Soares, J.L., Ping, S. and 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 (5): 411-421.

Little, C.L. and Louvois, J. 1999. Microbiological Quality of soft ice-cream from fixed premises and mobile vendors. Int. J. Environ. Health Res., 9(3):223-232. Dairy Sci. Abst., 62(1):216(2000).

Maiereni, M., Civilini, M., Domenis, C., Manzano, M., Di-Prima, R. and cami, G. 1993. Microbiological quality of Artisanl ice-cream. Zentralblatt fur Hygiene und Umvelt Medizin, 194(5-6): 553-570.Biological. Abst.,, 97(4).

Manzanera-Pelegrin, C., Marin-Mesegure, D., Paredes-Garcia, P., Pelegrin, C.M., Mesegure, D.M. and Gracia, P.P. 1995. Quality control of ice-cream. Health area III of Mucria region. Alimentaria, 33(263):119-121.

Masud, T. 1989. Microbiological quality and public health significance of ice-cream. J. Pak. Med. Assoc., 39(4):102-104.

Mee-Marquet, N.V., Blanchard, M., Domelier, A.S. and 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. And 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(3):1032-1035.

Mohammed, A.M. 2002. Quality investigation into beef frankfurter produced in Egypt. M. v. sc. Thesis Fac. Vet. Med. 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. and 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.

Najera-Sanchez, G., Maldonado-Rodriguez, R., Olvera, P.R. and de la Garzal, M. 2003. Development of two multiplex polymerase chain reaction for the detection of enterotoxigenic strains of *Staphylococcus aureus* isolated from foods. J. Food Prot.; 66:1055-1062.

Nassar, M.K. 2013. Phenotypic and genotypic characterization of enterotoxic *S. aureus* isolated from different sources with reference to antimicrobial resistance. M. V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.

Nejma, M.B., Mastouri, M., Frih, S., Sakly, N., Salem, Y.B. and 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. and Haesebbrouck, 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(10):3817-3819.

Oke, A.J. and Adewale, A.O. 2013. Incidence of methicillin resistant *Staphylococcus aureus*  (MRSA) in a small poultry in south west, Nigeria. IOSR Journall of Agriculture and Veterinary Science (IOSR-JAVS), 5(3):53-55.

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D.L., Ueda, S. and 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 (3):857.

Oxoid Manual 1990. Culture media, ingredients and other laboratory services. 6th Ed. Unipath limited. Wade Road.

Patr, B., Ztepe, G., Ihak, I. and 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. and Maguire, D. 2002. Veterinary microbiology and microbial disease. 2nd Ed., Blackwell Science, 84.

Ralls, V.L., Vieira, F.P., Ralls, R., Vieitis, R.L., Fernandes Jr., A., Candies, J.M., Cardoso, K.F. and Araujo Jr., J.P. 2008. PCR detection of staphylococcal enterotoxigenes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. Vet. Microbiol.,132(3-4): 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(95): 323-326.

Sagdic, O., Tuluoglu, D.D. Ozcelik, S. and Simsek, B. 2002. The chemical and microbiological quality of ice-cream consumed in Isparata market Ziraat.Fakultesi-Dergisi,Ataturk Uni., 33(4):441-446.

Said, M.R. and 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.

Sawai, T., Tomono, K., Yanagihara, K., Yamamato, Y., Kaku, M., Hirakata, Y., koga, H., Tashiro, T. and Kohno, S. 1997. Role of coagulase in amurine model of hematogenous pulmonary infection induced by intravenous injection of *Staphylococcus aureus* enmeshed in agar beads. Infect. Immun. 65:466-471.

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, Fac. Vet. Med. Zag. Univ., Egypt.

Stewart, C.M., Cole, M.B. and 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) 7: 1310.

Sudershan, K. and Ashwani, K. 1996. Occurrenceof some bacterial pathogens of public health significance in bovine raw milk. Haryana veterinarian. Dairy Sci. Abst., 6(4): 281.

Tondo, E.C., Guimaraes, M.C., Henriques, J.A. and Ayub, M.H. 2000. Assessing and analyzing contamination of dairy products processing plant by *S. aureus* using antibiotic resistance PFGE. Candian J. Microbiol., 46(12):1108-1114.

Wafy, Y.M. 2006. Sanitary improvement of serving milk and dairy products in Assiut University Hospitals. Ph. D. Thesis, Fac. Vet. Med. Assiut Univ., Egypt.

Zschock, M., Kloppert, B., Wolter, W., Hamann, H.P. and Lammler, C.H. 2005. Pattern of enterotoxin genes seg ,seh,sei and sej positive *S. aureus* isolated from bovine mastitis. Vet. Microbiol., 108(3-4): 243.

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Antimicrobial agent | Sensitive | | Intermediate | | Resistant | |
| No. of  *S. aureus* isolates | %\* | No. of  *S. aureus* isolates | %\* | No. of  *S. aureus* 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:

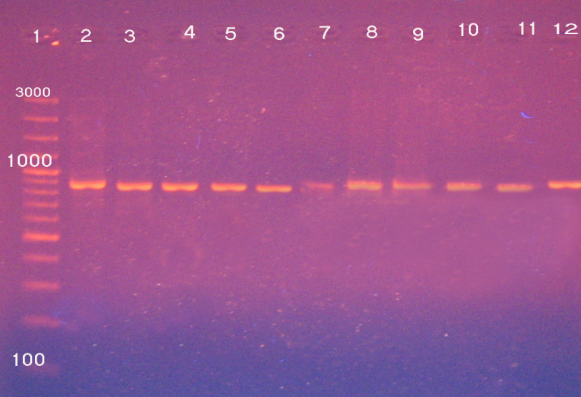
|  |  |  |  |
| --- | --- | --- | --- |
| Examined  *S. aureus* for | No. of  + ve samples | %\* | |
| Coagulase positive | 11 | | 100% |
| Methicillin resistant | 11 | | 100% |
| Enterotoxins A | 5 | | 45.45% |
| Enterotoxins B | 0 | | 0 |
| Enterotoxins C | 4 | | 36.36% |
| Enterotoxins D | 4 | | 36.36%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

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

Photoes 1,2 and 3. Agarose gel electrophoresis patterns showing :

1. Uniplex PCR for the *S. aureus* 2. Multiplex PCR for methicillin

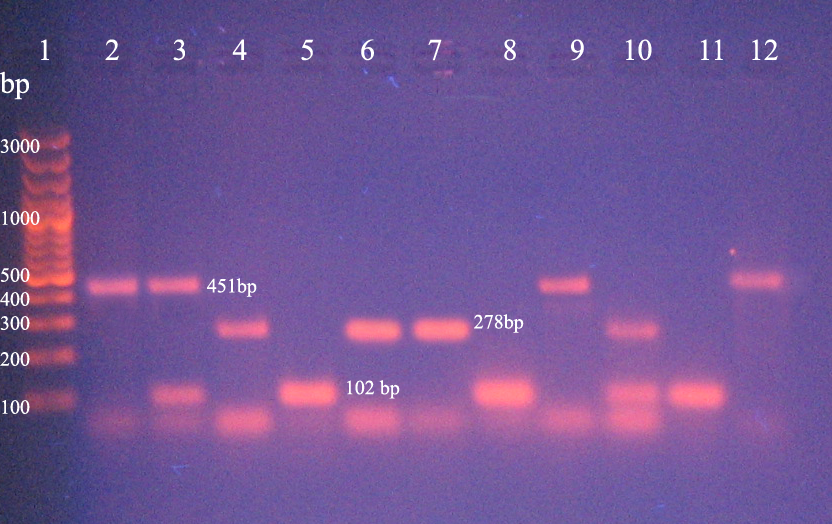
co-agulase (*coa*) gene resistant *S. aureus* (*mec*A) gene

Lanes 1: DNA molecular size marker (100-bp ladder)

Lane 2-12: positive sample for co-agulase gene and methicillin resistant gene

3. 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