

PREVALENCE OF STAPHYLOCOCCUS AUREUS AND IT'S TOXINS IN STREET MARKETING MILK AND SOME MILK PRODUCTS

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ABSTRACT

In this study, the bacteriological examination of 130 street marketing milk samples and 251 milk product samples revealed that 47 isolates of *Staphylococcus aureus* were recovered from 130 milk samples with a percentage of 36% and 31 isolates of *S.aureus* were recovered from 251 milk product samples with a percentage of 12.4%.

The pathogenic activity of the isolated *S.aureus* from milk and milk products were studied including Hemolytic activity, DNase activity and coagulase activity. The results proved that 70 isolates out of 78 tested isolates were Hemolytic and 72 isolates have DNase activity and 60 isolates have coagulase activity.

By using latex slide agglutination Test was used for detection of Protein A in isolated *S.aureus* from milk samples. The results proved that 46 out of 47 isolates contained protein A. Concerning the ice cream samples 11 out of 13 tested isolates contained protein A. and 16 out of 18 tested isolates from Kariesh cheese contained protein A.

The results showed that, out of 78 tested isolates 20 isolates were proved to be enterotoxin A

producer, 2 isolates were enterotoxin B producer and 5 isolates were enterotoxin C producer by using ELISA.

INTRODUCTION

S.aureus is reorganized world wide as a major pathogen causing subclinical intramammary infections in dairy cows (*Salasia et al., 2004*).

Identification of bacterial pathogens in milk from cows with mastitis is the definitive diagnosis of mastitis infections. It also provides information important for prevention and control of the disease. In most clinical laboratories, identification methods are based on microbiological culture of milk and biochemical identification of bacterial isolates recovered. Subclinically infected cows are intermittent shedders of organisms through low and high shedding patterns during lactation that leading to negative cultures. (*Phuektes et al., 2001*).

Defective pasteurization of raw milk is a potential source of *S.aureus* which act as a source of infection of milk products like Kariesh cheese and ice cream (*Bahout and Moustfa 2006, Aly et al., 2007*)

The pathogenic *Staphylococci* produce a battery of toxins (Quinn et al., 1994) which make a contribution of the ability of this organism to cause disease on the mammalian hosts. (Salasia et al., 2004).

These exotoxins include haemolysins, various enzymes and a family related pyrogenic toxins, namely *Staphylococci* enterotoxin, toxic shock syndrome toxins and exfoliative toxins (Dinges et al., 2000). Recently a novel gene cluster encoding Staphylococcal exotoxins like protein have been described (William et al., 2000).

Production of enterotoxins by *S.aureus* isolated from milk and it's products, has received attention and extensive work has been conducted by different investigators in various

countries (Tollersrud et al. 2006,Zecconi et al 2006).

The work of this study was directed mainly to revealed the successful ability for detection of pathogenic *S.aureus* directly in milk and milk products also detection of the enterotoxigenic property of the isolated *S.aureus* by ELISA

MATERIAL AND METHODS

Samples:

A total of 381 samples were collected from milk and some milk products (ice cream and Kareish cheese).130 of them were cow's milk samples, 116 were ice cream samples and 135 were Kariesh cheese samples. All these samples were collected from street marketing (Table ,1).

Table (1) Type and Number of the collected milk and milk product samples:-

Source of samples	Type of samples	No. of examined samples
Bovine milk	Street marketing	130
<u>Milk products :</u>		
Ice cream	Street marketing	116
Kariesh cheese	Street marketing	135
Total		381

Collection and Preparation of Samples:

1-Milk:

Samples of raw milk (250 ml) were randomly collected from street peddlers and dairy shops in clean, dry and sterilized sampling bottles. Then incubated aerobically at 37°C for 24 hr then centrifuged at 3000 r.p.m. for 20 min. The cream and supernatant fluids were discarded. A loopfull from the sediment was streaked onto the surface of different media (**Breed et al, 1957**).

2-Ice cream:

Random ice cream samples were collected from street vendors and shops as it is sold in cups, biscuits, sticks or bars or taken by the retailer's server, or spatula into a sterile airtight jar of 100g capacity containers. The samples were kept in thermostatically controlled water bath at 44± 0.2°C for not more than 15 min. with periodical shaking till melting before being examined

3-Kariesh cheeses:

Samples of Kariesh cheeses (each about 250 g.) Were randomly collected in sterile airtight sampling jars from street peddlers, groceries and small dairies. 11 grams of sample were added to 99 ml. of sterile 2% sod.citrate in 18x30 cm polyethylene bags. The bags were locked into jaws of stomacher (Stomacher 400

laboratory blend a Seward Medical, London) and blended for 2 min.

The collected samples were transferred to lab. In an insulated ice box without delay to be subjected for bacteriological examination.

Isolation of *Staphylococci*:

The collected samples were cultivated onto Nutrient agar, sheep blood agar (**Oxoid CM3**), Baired Parker medium and Manitol Salt agar (**Mackie and McCartney, 1996**). The inoculated plates were incubated for 24-48 hours at 37°C. The suspected isolates were identified primarily as Gram- positive and Catalase positive cocci. The suspected colonies were picked up and propagated onto nutrient agar slop for further examination.

Identification and Characterization of *Staphylococcus aureus*:

The isolates were identified according to **Cruick Shank et al.,(1975)** , **APHA,(1992) Quinn et al.,(1994) and Mackie and McCartney, (1996)**, by using Catalase test, Coagulase test , Mannitol fermentation activity, Pigment production on to nutrient agar, DNase activity, Gelatine Liquefaction, growth on Baired -Parker Medium and detection of SpA by SpA agglutination Kits according to **Essers and Radobold (1980)**.

Detection of *Staphylococcus aureus* Enterotoxins A,B and C by ELISA
According to **Burdova et al.,(1994)**.

Table (2) Prevalence of *Staphylococcus aureus* in examined samples

Source of the samples	Type of the samples	No. of examined samples	<i>Staphylococcus aureus</i> isolates	
			No.	%
Bovine milk	Street marketing	130	47	36
Milk products:				
Ice cream	Street marketing	116	13	11
Kariesh cheese	Street marketing	135	18	13.3
Total		381	78	20.5

Table (3) Collective table showed the characteristic features of the examined *S.aureus*.

Source of the samples	Type of the samples	No. of examined samples	<i>Staphylococcus aureus</i> isolates		Heamolytic activity		DNase activity		Coagulase activity	
			No.	%	No.	%	No.	%	No.	%
Bovine milk	Street marketing	130	47	36	43	91.5	45	96	36	76.6
Milk products:										
Ice cream	Street marketing	116	13	11	11	84.6	11	85	10	67.9
Kariesh cheese	Street marketing	135	18	13.3	16	88.9	16	89	14	77.8
Total		381	78	20.5	70	89.7	72	92.3	60	77

Table (4):Correlation between *S.aureus* protein A and *S.aureus* enterotoxins

Staphylococcal protein A		Staphylococcus Enterotoxins by ELISA					
No.	%	A		B		C	
		No.	%	No.	%	No.	%
46	97.9	17	36.2	0	0	10	21.2
11	84.6	3	23	2	15	0	0
16	88.8	0	0	0	0	5	28
73	93.6	20	25.6	2	2.6	5	6.4

Staphylococcus aureus is one of the leading causes of bacteremia with high levels of accompanying morbidity and mortality (Qinfany et al 2007).In this study, a total of 381 milk samples and milk products samples were examined bacteriologically to reveal the prevalence of pathogenic ***S.aureus***.

Fourty seven isolates of ***S.aureus*** out of 130 (36%) examined milk samples were identified as ***S.aureus***.

The same finding were recorded by Haltia et al., (2006), Abd el Aal, (2008);, and Tamminga et al., (2008) who isolated ***S.aureus*** from row milk samples and from milk product samples with a percentage of isolation reached (15-18%) , while Devi et al.,(1997) reported higher incidence of ***S.aureus*** mastitis reached (75.3%) in India.Wide variation in the prevalence of ***S.aureus*** has also been reported elsewhere Radostitis et al., (2000).

Concerning the type of examined milk samples, the high incidence of ***S.aureus*** in street milk might be due to

the interference of man hazard during handling and preparing of milk before marketing.

Thirteen isolates out of 116 ice cream samples and 18 out of 135 Kareish cheese samples identified as ***S.aureus*** with a percent of isolation 11% and 13.3% respectively. The results of isolation of ***S.aureus*** from ice cream were supported by Feng et al., (2007) and Tamminga et al., (2008) who proved that the incidence of ***S.aureus*** from ice cream ranged from (5 to 20%) due to man hazard effect like hand, skin , sneeze and cough which produce droplet infection during transportation, storage and retailing.

As regards to Kareish cheese, our results supported by Haltia et al., (2006) and Abd El Aal (2008); who isolated and identified ***S.aureus*** from kariesh cheese samples with nearest percentage of isolation.

Boerlin et al.,(2003) recorded that, only half of ***S.aureus*** isolates were

positive coagulase tests after 4 hr incubation and overnight incubation were necessary to obtain reliable results. This long incubation time represents an important drawback for diagnostic applications and quicker method would be preferable.

Staphylococcus aureus are usually identified as B hemolysis producing, catalase positive and Gram positive cocci. Additional tests are needed to identify B hemolysis negative **S.aureus**, other tests such as heat resistant DNase test can replace or can be used together with the coagulase tube test or the clumping factor for the identification of **S.aureus** (Laevens et al., 1996).

In this study, the results presented in table (3) proved that, the isolates recovered from street marketing milk 43 isolates were hemolytic and 45 isolates has DNase activity, while 36 isolates has the ability to coagulate citrated rabbit plasma.

These findings supported by Boerlin et al., (2003); and El-Jakee et al., (2008) who recorded that, only half of the **S.aureus** isolates were positive in coagulase test after 4 hrs incubation, and an overnight incubation is necessary to obtain reliable results and concluded that testing for the presence of hemolysis on blood agar plates represents a first simple and rapid methods. Same finding which recorded by Laevens et al., (1996); and Annemüller and Zschock (1999) who reported that the most **S.aureus** produced typically B hemolysis.

As regards to the examined street marketing ice cream 11 out of 13 isolates of **S.aureus** showed

hemolytic activity and DNase activity while 10 of the isolates were coagulase positive.

Examination of street marketing Kariesh cheese 16 out of 18 isolates of **S.aureus** showed hemolytic and DNase activity while 14 isolates were coagulase positive strains. These observations were supported by Anuranjini et al., (2008) and Tamminga et al., (2008); who detected the pathogenic characters of **S.aureus** recovered from milk products either from company marketing or street marketing.

Forty six isolates recovered from 47 **S.aureus** isolates from street milk samples harboured protein A. while 11 isolates recovered from street ice cream samples showed agglutination (contain protein A) and 16 isolates out of 18 isolates of **S.aureus** showed agglutination. The same finding were recorded by Rodgers et al., (1999) who proved that protein A was represented an all strains recovered from different origins. Also El-Jakee (2008) and Sindhu et al., (2008); who recovered that both human and animal **S.aureus** isolates were positive for the presence of protein A by using agglutination test. On the other hand, the tested street milk samples proved that, 17 samples out of 47 milk harboured **S.aureus** enterotoxins type A. and 10 milk samples harbouring enterotoxin type C.

Regarding the examined milk products samples the obtained results showed that 3 isolates of **S.aureus** out of 13 tested isolated from street ice cream produced enterotoxin A (23%) and 2 isolates produced type B and all 13

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tested isolates could not produce enterotoxin type C. On the other hand, only 5 isolates out of 18 tested isolates from Kariesh cheese samples 28% exhibited enterotoxin C.

These findings were in agreement with **Moon et al., (2007)** ;**Sakai et al.,(2008)** ; and **Labib et al., (2009)** who detect 57 isolates produced enterotoxins out of 297 tested isolates (31.8%) from raw milk and the high proportion of the isolates produced enterotoxins (A and B) by using antibody sandwich ELISA and concluded that , it can used as reference method for detection of **S.aureus** enterotoxins in different samples causing food poisoning.

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