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PUBLIC HEALTH HAZARD OF STAPHYLOCOCCUS AUREUS ISOLATED FROM RAW MILK AND ICE CREAM IN QENA GOVERNORATE

(With 3 Tables and One Figure)

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المخاطر الصحية على الإنسان من المكور العنقودي الذهبي المعزول من الألبان الخام والآيس كريم بمحافظة قنا

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نظرا لأهمية ميكروب المكور العنقودي الذهبي وخطورتة الصحية علي الإنسان لما يسببة من حالات التسمم الغذائى نتيجة استهلاك اللبن الخام وبعض منتجاتة، بالاضافة الى هذا فهو يعتبر المسبب الاساسى لالتهاب الضرع فى الابقار، الأغنام، الماعز والجمال. لذا فقد تم تجميع عدد 125عينة من اللبن الخام والآيس كريم بمحافظة قنا بواقع 50 عينة من لبن الأبقار ، 30 عينة من لبن الأغنام ، 15 عينة من لبن الجمال و 30 عينة من الآيس كريم . وثم فحصها بكتريولوجيا لعزل المكور العنقودي الذهبي. وقد أشارت النَتائِج المكتسبة بأن ميكروب المكور العنقودي الذهبي تم عزلة بنسبة (24 %) منْ عينات لبن الأبقار، (13.3 %) من لبن الأغنام ، 20 % من لبن الجمال و(40 %) مِنْ عيناتِ الآيس كريم. بالاضافة لما سبق فقد تم اختبار المكور العنقودي الذهبي لمقاومة 12نوع من مختلف مضادات ال ميكروبلت باستخدام طريقة إنتشار القرص. وقد اشارت النتائج الى وجود نسبة عالية من العترات المقاومةِ للبنسلينِ بنسبة (96.77%)، داى كلوكساسيلين بنسبة (80.64%)، الامبيسيلين والايرسروميسين بنسبة (74.19%). وعلى العكس فقد لوحظ ان الميكروب كان حساس للجينتاميسين بنسبة (90.32%). وتمت مناقشة الأهمية الصحية والوبائية لهيكروب المكور العنقودي الذهبي والإجراءات الصحية الواجب إتباعها لمنع تلوث الالبان المختلفة ومنتجاتها بهذا الميكروب.

SUMMARY

In this study, a total of 125 samples of raw cow, sheep, camel milk, and ice cream were collected from different sources in Qena Governorate and were analysed for the presence of *Staphylococcus aureus*. There were 31 *S. aureus* isolates could be detected from these samples, which were investigated for antibiotic resistance. The obtained results indicated that *S. aureus* was isolated from (24%) of cows' milk samples, (13.3%)

of sheep milk samples, 20% from the camel milk samples and (40%) of ice cream samples. Resistance of *S. aureus* to different antibiotics was determined by the disc diffusion method. There was a high rate of resistance to penicillin (96.77%), dicloxacillin (80.64%), ampicillin and erythromycin (74.19%) was frequent for *S. aureus* strains. A low prevalence of gentamycin resistance (9.67%) was detected in (28) *S. aureus* strains. The public health hazard of Staphylococcus species and the sanitary measures for improving milk quality were recommended.

Key words: *Milk, S. aureus, Antibiotics, Resistance, Public health hazard*

INTRODUCTION

Milk and milk products have the potential to transmit pathogenic organisms to humans, because milk is an ideal medium for the growth of many microorganisms, including food pathogens. Ice cream is considered one of the popular frozen milk products consumed widely all over the world. Although it is a wide spread dairy food consumed by people of all ages, yet it may be subjected to contamination by various microorganisms at different stages of manufacturing, handling and packaging Moreover, the processing of ice cream requires adding substances as sugar, fruits, eggs or other products of animal origin (Bryan, 1983) These additives may contribute to food poisoning outbreaks or gastroenteritis in human or other bacterial problems (Kumari *et al.*, 1996).

Among the predominant bacteria involved in food-borne diseases, *Staphylococcus aureus* (*S. aureus*) is a leading cause of gastroenteritis resulting from the consumption of contaminated food (Loir, *et al.*, 2003). Also *S. aureus* is the most predominant contagious pathogen responsible for clinical and subclinical infections in lactating cows (Kerro-Dego, *et al*., 2002) and in small ruminant (Las Heras, *et al.*, 1999). Moreover, the isolation of this bacterial pathogen from raw camel milk has been described from different parts of the world namely Saudi Arabia (Zahran and Al-Saleh, 1997), Ethiopia (Semereab & Molla, 2001), and Morocco (Benkerroum, *et al*., 2003). *S. aureus*, was also isolated from mastitic camel milk in Sudan (Abdurahman, *et al*., 1995) India (Tuteja, *et al*., 2003) and Egypt, (Saad and Thabet 1993, Aly and Abo-Al- Yazeed, 2003 and Korashy and Moustafa 2006)

It is well established that foodborne diseases cause significant economic and social losses. As consumption of raw milk remains a well-identified risk factor for foodborne disease. It was reported that milk, ice

cream, and cheese have been identified as the vehicle for less than 1.5% of all foodborne disease outbreaks investigated by the Centers for Disease Control (Bean, *et al*., 1996).

S. aureus strains produce heat-resistant enterotoxins, which cause nausea, vomiting and abdominal cramps when ingested by human and are responsible for staphylococcal food poisoning outbreaks (Kluytmans, *et al.*, 1997).

Antimicrobial therapy has been a valuable tool for controlling mastitis and as a consequence an increase in the frequency of staphylococci resistance has been recorded (Ochoa-Zarzosa, *et al*., 2008), because, some *S. aureus* strains have antibiotic resistance resulting from genetic mutations (Lowy, 1998).

The potential threats to human health related to milk and dairy products include errors in pasteurization, consumption of raw milk products, contamination of milk products by heat-resistant pathogens and emergence of antimicrobial resistance. Therefore the objectives of this study was to allow qualitative checking of hygienic conditions of examined raw cow, sheep, camel milk and ice cream for the prevalence of *S. aureus* in Qena Governorate as well as to estimate their antimicrobiol sensitivity pattern.

MATERIALS and METHODS

a) Samples collection:

A total of 125 random milk samples were aseptically collected from dairy shops, street vendors and farmers' houses in Qena Governorate. These samples include raw marketable cow milk (50), raw sheep milk (30), raw camel milk (15) and street vendors' ice cream (30), were transferred to the laboratory with a minimum of delay to be examined for the concerned microorganism.

b) Samples preparation:

Milk samples were examined by storch test according to Lampert, (1975), to detect heat treated samples. Ice cream samples were left to melt at room temperature.

c) Enrichment procedure:

One ml of each milk sample or melted ice cream was transferred to 10 ml of selective enrichment broth [brain heart infusion broth (BHI)]. The inoculated broth was incubated at 37°C for 48 hours.

d) Isolation and identification of *S. aureus*:

Loopful of the incubated broth was streaked into plates of selective media Baird- Parker agar. (Finegold & Martin, 1982) Inoculated plates were incubated at 37°C for 2 days (Oxoid). The suspected colonies were inoculated into slope of nutrient agar for morphological and biochemical tests. The identification was carried out using the following tests: Gram staining, production of coagulase, catalase and fermentation of mannitol (Bennett & Lancette, 1995).

e) Antimicrobial susceptibility of *S. aureus* isolates

S. aureus isolates were evaluated for antibiotic susceptibility with the disc diffusion method on Baird Parker agar. The following Gram-positive multi discs (Oxoid) were used: ampicillin (AMP), 10 µg; cephalotin (CEP), 30µg; cefotaxime (CTX), 30µg; ceftazidime (CAZ), 30µg; cefuroxime (CXM), 30µg; dicloxacillin (DX), 1µg; erythromycin (ER), 15µg; gentamicin (GE), 10µg; pefloxacin (PEF), 5µg; penicillin (PEN), 10U; tetracycline (TET), 30µg and trimethoprim (TRM), 25µg. In parenthesis are indicated the diameters (mm) of inhibition zones used to consider an isolate as resistant, according to the manufacturer's instructions. A Baird Parker agar plate without antimicrobials was used as control for each isolate. Plates were incubated at 37°C for 24 h.

RESULTS

The obtained results were illustrated in Tables 1-3 and Figure 1

Table 1: Incidence of *S. aureus* and *CNS in the examined samples of raw milk and ice cream

Examined samples	No. of examined samples	Positive samples		Isolated strains			
				S. *aureus*		*CNS	
		No.	%	No.	%	No.	%
Raw cow milk	50	14	28%	12	24%	2	4%
Raw sheep milk	30	6	20%	4	13.3%	2	6.6%
Raw camel milk	15	3	20%	3	20%	0	0%
Ice cream	30	15	50%	12	40%	3	10%
Total	125	38	30.4%	31	24.8%	7	5.6%

* CNS= Coagulase negative staphylococci

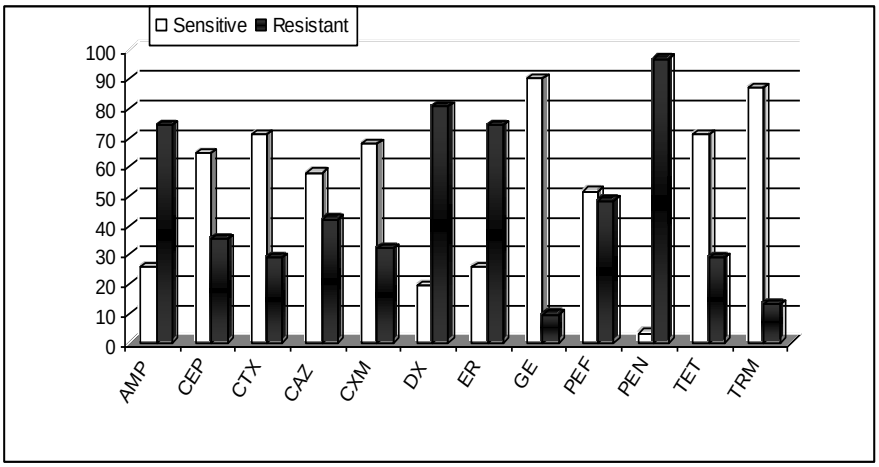
Table 2: Frequency distribution of *S. aureus* and CNS in the examined samples raw milk and ice cream

Examined samples	Isolated strains		S. *aureus*		CNS	
	No./38	%	No./38	%	No./38	%
Raw cow milk	14	36.8 %	12	31.5 %	2	5.2%
Raw sheep milk	6	15.7 %	4	10.5 %	2	5.2%
Raw camel milk	3	7.8 %	3	7.8%	0	0.0%
Ice cream	15	39.4 %	12	31.5	3	7.8%

Table 3: Antibiotic susceptibility of *S. aureus* (31 strains) isolates by disc diffusion method

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Ampicillin, 10 µg	8	25.80	23	74.19
Cephalotin, 30 µg	20	64.51	11	35.48
Cefotaxime, 30 µg	22	70.96	9	29.03
Ceftazidime, 30 µg	18	58.06	13	41.93
Cefuroxime, 30 µg;	21	67.74	10	32.25
Dicloxacillin, 1 µg	6	19.35	25	80.64
Erythromycin, 15 µg	8	25.80	23	74.19
Gentamicin, 10 µg	28	90.32	3	9.67
Pefloxacin, 5µg;	16	51.61	15	48.38
Penicillin, 10 U	1	3.22	30	96.77
Tetracycline, 30µg	22	70.96	9	29.03
Trimethoprim, 25µg	27	87.09	4	12.90

Fig. 1: Antibiotic sensitivity of *S. aureus* isolated from raw milk and ice cream



AMPp =Ampicillin, CEP =Cephalotin, CTX =Cefotaxime, CAZ =Ceftazidime, CXM =Cefuroxime, DX= Dicloxacillin, ER= Erythromycin, GE= Gentamicin, PEF= Pefloxacin, PEN= Penicillin, TET= Tetracycline, TRM= Trimethoprim,

DISCUSSION

S. aureus is found in a wide variety of habitats, including human skin, where many strains are commensals that may be clinically significant or contaminants of food. In the present study, 31 *S. aureus* strains were isolated and identified from 125 samples obtained from raw cow, sheep, camel milk and ice cream samples (Table 1). According to our results, *S. aureus* was isolated from all types of examined samples.

As it was isolated from (24%) of cows' milk samples, higher estimates were detected by Chye, *et al*. (2004) and Ekici, *et al*. (2004), they showed that *S. aureus* was isolated from more than (60%) and (75%) of the raw cow milk samples. However lower result was detected by Abdel hameed, *et al*. (2004) and Abdel hameed, (2006) who isolate *S. aureus* from cows milk samples in percentages of 14.38% and 9.28% respectively.

Regarding raw sheep milk samples *S. aureus* was detected in (13.3%). Higher result of (16.6%) was reported by Ekici, *et al*. (2004). On the contrary lower record (4.04%) was obtained by El-Bassiony, *et al.* (2008).

Although *S. aureus* is a well known bacterial pathogen in human and animal infections little information is available at present about the occurrence of this bacterial species in camel milk. In the present study 15 milk samples were investigated for the presence of this pathogen leading to the isolation of *S. aureus* in 20% from the camel milk samples examined. Lower results were recorded by Saad & Thabet (1993) and Shuiep, *et al*. (2009) who found that (6.88 and 8.8%), of camel milk samples were positive for *S. aureus.*

A high percentages of *S. aureus* were isolated from ice cream samples (40%). Kanbakan, *et al*. (2004) and Yaman, *et al.* (2006), in Turkey, reported similar results of *S. aureus* in the fresh ice cream. On the contrary lower result (10%) was detected by Leloglu, *et al.* (1998) in the open ice cream samples in Turkey.

Concerning CNS, it was recorded in (4%) of cows' milk samples. A higher result (5.4%) was obtained by Abdel hameed, (2006). It was found that CNS was isolated from (6.6%) of sheeps' milk samples. This was in accordance to the results detected by El-Bassiony, *et al.* (2008). Additionally, (10%) of ice cream samples was contaminated by CNS, but it was not detected in camels' milk samples.

Table 2 indicated that the lowest frequency distribution for *S. aureus* was reported for camels' milk samples. Whereas the highest

frequency was calculated for cows' milk samples and ice cream samples which show similar frequency (31.5%). Additionally, similar frequency for CNS was estimated for cows' and sheeps' milk samples (5.2%). While the highest frequency distribution for CNS was recorded for ice cream samples (7.8%).

The presence of *S. aureus* may be resulted from either insufficient pasteurization of milk, or human exposure. In humans, the main reservoir of *S. aureus* is the nasal cavity. The organism finds their way to the skin and into wounds either directly or indirectly. The most common skin sources are arms, hands, and face. In addition to skin and nasal cavities, *S. aureus* may be found in the eyes, throat and intestinal tract. From these sources, the organism finds its way into air and dust, onto clothing, and in other places from which it may contaminate foods (Jay, 1996). It is obvious from the previous and the present data that ice cream samples are frequently subjected to *Staphylococci* spp. contamination which may indicate inadequate personel hygiene of workers or sales people.

According to the results of the present study *S. aureus* isolated from camel milk display the typical properties of this bacterial species. However, because of the rare isolation of *S. aureus* from raw camel milk, camel milk at this stage seems to be of minor importance as vector of staphylococci.

Antimicrobial resistant *S. aureus* can be transmitted by different foods, including contaminated milk (da Silva, *et al*., 2005). Such transfer can occur by means of antibiotic residues in food, through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms (Pesavento, *et al*. 2007). The overall antibiotic resistance of *S. aureus* strains isolated from raw milk, and ice cream samples is shown in Table 3. and Fig. 1. According to these results, more strains were resistant to penicillin (96.77%), dicloxacillin (80.64%), ampicillin and erythromycin (96.77%). A low prevalence of gentamycin resistance was detected in *S. aureus* strains (9.67%). *S. aureus* is frequently resistant to antibiotic therapy due to its capacity to produce an exopolysaccharide barrier and because of its location within microabscesses, which limit the action of drugs (Gündoğan, *et al*., 2006). Even though all strains were susceptible to gentamycin (90.32%) attention should be given to the fact that the *S. aureus* strains have demonstrated resistance to one or more antibiotics.

In conclusion, the large amount of *S. aureus* found in raw milk and ice cream samples represents a health hazard to the consumers, and emphasises the need for improved hygiene practise at levels in the dairy. Our results indicate that antibiotic-resistant strains might be transmitted to humans by the consumption of milks containing such multi resistant bacteria, and that use of antibiotics common both in human and animal care should be avoided.

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