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Isolation of *Staphylococcus aureus* from Milk and Human with Reference to its Survival on Surfaces

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Abstract: This study was conducted to isolate *Staphylococcus aureus* from milk and human and studying its survival on different surfaces in dairy environment. A total of 40 individual raw milk samples (20 cows and 20 buffaloes, 10 ml each), skin and throat swabs of 11 veterinarians, were collected from Academic Veterinary Hospital, Zagazig University. Of the total 62 examined raw milk and human swab samples 18(29.03%) were positive to *Staph. aureus*. *Staphylococcus aureus* was isolated from cow`s milk, buffaloes milk, throat swabs and skin swabs with the following percentages 30.0 (6/20), 25 (5/20), 27.3 (3/11) and 36.4 (4/11), respectively. The survival of *Staphylococcus aureus* on various surfaces at room temperature suspended in physiological saline and nutrient broth was recorded for at least 35 days. Staphylococcal viability was longest on polyethylene (49 days) and stainless steel and glass (42 days), while it was the shortest (35 days) on aluminum surface. Log $_{10}$, reductions in *Staph. aureus* cell number on aluminum, stainless steel, glass and polyethylene were 5.67, 5.0, 4.0 and 4.0 in saline suspensions and 5.34, 4.67, 3.67 and 3.34 in nutrient broth suspensions respectively. The obtained results are useful for designing strategic plans of prevention and control program against *Staph. aureus* in dairy ecosystem.

Key words: *Staph. aureus* · Milk · Human · Aluminum · Stainless Steel · Glass and Polyethylene

INTRODUCTION

Staphylococcus aureus is an important food borne pathogen and causes a mild skin infection to more severe diseases, such as pneumonia and septicemia [1]. *Staphylococcus aureus* is a major causative pathogen of clinical and subclinical mastitis [2]. Milk has been reported as a common food that may cause staphylococcal poisoning [3]. The update studies of epidemiology of *Staph. aureus* may help in prevention and control strategies. Isolation of *Staph. aureus* from milk was previously studied in Sharkia Governorate [4] and Alexandria [5]. Enteropathogenic *Staph. aureus* in milk should be regarded as a part of the risk analysis of milk and milk products [6]. The importance of contaminated surfaces in relation to potential transmission of pathogens to food is apparent in food processing, catering and domestic environments [7]. One factor for transmission of a microorganism from a person to the environment and then to another person is the ability of that microbe to survive on that environmental surface [8]. The most common materials in dairy farms and industry are made of aluminum foil, stainless steel, polyethylene and glass.

The aim of present study was to investigate the occurrence of coagulase positive *Staph. aureus* in bovine milk and skin and throat swabs of human at Zagazig district, Egypt. The survival of *Staph. aureus* on the most commonly used surfaces in dairy farms and industry was also studied.

MATERIALS AND METHODS

Isolation of *Staph. aureus*: A total of 40 individual raw milk samples (20 cows and 20 buffaloes, 10 ml each) were collected from clinical cases, Academic Veterinary Hospital, Zagazig University between June and August 2012, the udder of the sampled cows were apparently normal. Each milk sample was collected in a sterile screw cap bottle aseptically from each mammary gland after washing with water and cleaning the teats with cotton soaked in 70% ethanol and previous discard of the first 3

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streams of milk [2]. The samples were immediately taken to the laboratory for bacteriological analysis. The skin and throat swabs of 11 veterinarians, Academic Veterinary hospital, Zagazig University were collected and sent to the laboratory. Identification of suspected *Staph. aureus* colonies was carried out [9]. Further identification was done by API staph system (Bio Merieux, Marcy-L` Gtoile, France).

Preparation of coupon was carried out after Cervenka *et al*. [7] with some modification. Approximately 1.5x 1.5 cm coupons of stainless steel (SS), polyethylene (PE), aluminum foil (AF) and glass (GL) were thoroughly cleaned with 70% ethanol. The coupons of SS, AF and GL were sterilized for 2 hours at 160°C and the polyethylene coupons were autoclaved for 15 min at 121°C before inoculation with the bacterial suspension. Coupons were lined up in rows next to, but not touching, each other. A 20 µl aliquot of the suspension ($2x10^6$ CFU) was placed in the centre of each coupon to form one droplet and the coupons were carefully placed in the incubator at room temperature (37ºC).

Every week, triplicate coupons of each material was picked up and placed into a 10 ml tube of sterile saline and vortexed to liberate the attached cells. Subsequently, 100 µl of suspension was streaked onto nutrient agar plate and the bacterial count (log_{10} CFU per coupon) was determined after aerobic cultivation at 37°C for 24 h. Ten fold serial dilutions in saline were done if necessary. When no colony forming has been detected, freshly inoculated coupons were deposited on the surface of nutrient agar plate for 5 min. After being aseptically removed, the plates were incubated at 37°C for 24 h in aerobic condition and the viable cells were observed (Coupon-printing method) according to Cervenka *et al*. [7].

The data were transformed to the log_{10} scale for analysis purposes. The mean and standard deviation (SD) of the logarithmic CFU for all the coupons were calculated.

RESULTS

Of the total 62 examined raw bovine milk and human swab samples 18 (29.03%) were positive to *Staph aureus*. *Staphylococcus aureus* was isolated from cow`s milk, buffaloes milk, throat swabs and skin swabs with the following percentages 30.0 (6/20), 25 (5/20), 27.3 (3/11) and 36.4 (4/11), respectively as shown in Table 1.

In the present study *Staph. aureus* survived for at least 35 days on all tested surfaces (Table 2). Staphylococcal viability was longest on polyethylene (49 days) and stainless steel and glass (42 days). The shortest survival time for tested *Staph. aureus* was on aluminum surface (35 days) (Table 2).

Table 1: Occurrence of *Staphylococcus aureus* in examined raw milk and human samples

Source	No. examined	No. of positive	% of positive
Cows` milk	20	6	30.0
Buffalos` milk	20	5	25.0
Human throat swab	11	3	27.3
Human skin swab	11	4	36.4
Total	62	18	29.0

Table 2: Survival of *Staphylococcus aureus* suspended in physiological saline and nutrient broth on surfaces at room temperature

	Mean counts of *Staphylococcus aureus* as log CFU/coupon ±SD							
	Aluminum		Stainless steel		Glass		Polyethylene	
Contact time (d)	s	b	s	b	s	b	s	b
0	6.66±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
7	3.66±1.15	4.00±1.00	4.00±1.00	4.33±0.58	4.00±1.00	4.33±0.58	5.00±1.00	5.33±0.58
14	3.00±0.00	3.66±0.58	3.00±0.00	3.66±0.58	4.00±1.00	4.33±0.58	4.66±0.58	5.00±0.00
21	2.33±0.58	3.00±1.00	2.66±0.58	3.00±0.00	3.33±0.58	4.00±1.00	4.00±1.00	4.66±0.58
28	0.33±0.58	1.66±0.58	2.00±1.00	2.33±0.58	2.66±0.58	3.66±1.53	3.00±1.00	3.66±1.53
35	0.33±0.58	0.66±0.58	1.00±0.00	1.33±0.58	2.00±1.00	2.33±1.15	2.00±1.00	2.66±0.58
42	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.58	0.33±0.58	0.66±0.58	0.33±0.58	0.66±0.58
49	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.58
56	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

s = suspended in saline; b = suspended in nutrient broth

Table 3: Log 10 reduction in *Staph. aureus* cell, number during 35 days contact time with surfaces at room temperature

Suspension	Aluminum	Stainless steel	Glass	Polyethylene
Saline	5.67	5.00	4.00	4.00
Nutrient broth	5.34	4.67	3.67	3.34

Table 3 shows that log 10 reduction in *Staph. aureus* cells number during 35 days contact time on aluminum, stainless steel, glass and polyethylene surfaces was 5.67, 5.0, 4.0 and 4.00, respectively in saline suspensions and 5.34, 4.67, 3.67 and 3.34, respectively in nutrient broth suspensions.

DISCUSSION

The obtained results in Table 1 showed that *Staphylococcal aureus* frequently occurred in buffaloes' milk (25%) and cows' milk (30.0%). Three out of 11 human throat isolates (27.3%) were coagulase positive. Concerning the result of buffalos` milk, it is lower than that previously reported [10,11] whose results were 78 and 28%, respectively. Result of cows` milk is lower than that recorded by Mohamed and El Zubeir [12] who reported prevalence rate of *Staph. aureus* were 46.7%. Nevertheless, lower frequencies [13-15] whose results were 16.47%, 16.7 and 30%, respectively were recorded. The variation in frequencies of *Staph*. *aureus* in raw milk may be due to the improper hygiene and poor farm management [16]. Moreover, the current results reveal the public health significance of examined milk samples. The isolation rate of coagulase positive *Staph*. *aureus* from throat (27.3%) and skin swabs (36.4%) revealed that the examined humans are major reservoir for staphylococci. Human nasal swabs were Staphylococcus *aureus* positive with the frequencies of 20-55% [17-20]. El-Jakee *et al*. [21] coagulase positive *Staph*. *aureus* was previously isolated from nasal swabs of 10 apparently healthy humans. [21]. *Staph*. *aureus* is among the most important nasocomial pathogens because of both the diversity and the severity of the infections. It causes superficial and deep skin and soft tissue infections, endocarditis and bacteriemia and a variety of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded-skin syndrome and toxic shock syndrome [1].

The importance of contaminated surfaces in dairy industry is apparent in milk processing, catering and dairy environments. The obtained results in table 2 indicated that *Staph*. *aureus* can survive for a period up to 49 days on the examined polyethylene surfaces. Surfaces contamination can contribute to transmission [22]. The recorded survival period was near to what reported by Neely and Maley [8], whose results indicated survival of *Staph*. *aureus* on hospital fabrics and plastics (up to 51 days). A lower result was recorded (4-24 hours) on clothes [23] by lower inoculate density (10^2 CFU). Dose-response effect on the survival of *Staph*. *aureus* on aluminum foil was previously studied [24].

In the present study, the contaminated surfaces such aluminum, stainless steel, glass and polyethylene are potential reservoir for staphylococci in dairy environment. The potential public health hazard is considered. The variation in log $_{10}$ reduction of *Staph*. *aureus* cell counts during 35 days contact time with tested surfaces at room temperature between saline (4-5.67) and nutrient broth (3.34-5.34) suspensions might be due to lack of nutrient with physiological saline compared with nutritionally rich medium on hard surfaces as recorded before [7]. The diverse survival period of the tested strain on different surfaces may be due to the difference in hydrophobicity of used surface. The more hydrophobic surfaces may result in a smaller droplet diameter (a higher droplet height), a slower drying rate and hence a longer log time, since the organisms remain viable in hydrated environment for a longer period [25]. The previous study on the persistence of *A. buzleri* was determined on glass, plastic and stainless steel coupons [7] and interpreted. The longer survival period on polyethylene than stainless steel is due to the difference in surface free energy. Attachment of bacteria to surfaces occurs more readily to hydrophobic materials such as polyethylene than to hydrophilic substances such as glass [26]. The present results indicated that the ability of *Staph. aureus* to survive on environmental dairy surfaces is a critical factor for transmission of zoonotic *Staph. aureus* from milk to humans.

It can be concluded that used surfaces in dairy farms and industry could become vector for staphylococcal infections. During prevention and control programs, the obtained survival results should be considered.

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