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Isolation of Coagulase Positive *Staphylococci* from She-Camel Milk at Eastern Libya and Their Drug Susceptibility Patterns

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Abstract

Aim: This study was carried out with aim to isolate *Staphylococcus aureus* (*S. aureus*) from she camel milk and determine antibiogram pattern of *S. aureus* isolates. A total of 220 she camel milk samples were collected from seven different locations in Al Jabal Al Akhdar, Eastern of Libya. The samples were collected under aseptic precautions and were enriched in blood agar medium, followed by direct plating on selective media, Baird-Parker Agar, and mannitol salt agar. The presumptive *S. aureus* isolates were identified by biochemical test, AVIPATH-STAPH test. Antibiogram pattern of *S. aureus* to antimicrobial agents were evaluated by disk diffusion method. Analysis of the results revealed that coagulase positive *Staphylococci* were identified as 2.7% out of the total examined milk samples (220). In the present study *S. aureus* isolates were found variably resistant to the antibiotics tested. The *S. aureus* isolates showed highest sensitivity towards both gentamycin, 10 mg and cephalexin, 30 mg (100%), followed by chloramphenicol, 10 mg and amoxicillin as 66.7% and clavulanic acid, 30 as 33.3%. The pattern clearly indicated that the overall high percent of S. aureus isolates were resistant to ampicillin 10 mg (100%), followed by cephalexin, 30 mg (83.3 %), streptomycin, 10 mg (66.7 %), and neomycin, 30mg (50 %). Conclusions: Results clearly suggested a possibility of potential public health threat of S. aureus resulting from contamination of camel milk with pathogenic bacteria mainly due to unhygienic processing, handling and unhygienic environment.

Keywords: Antibiogram pattern, milk products, coagulase positive *S. aureus*, eastern Libya, liver.

INTRODUCTION

Milk is an excellent source of nutrients for humans and the same nutrients provide a most suitable medium for microbial growth and metabolism, their rate of multiplication in milk depends mainly on storage temperature and handling conditions (Richard, 2002). In Libya camel milk is traditionally consumed as raw by the pastoralists. For a long time, a very limited amount was being sold. Due to the changing in the life style, the demand for camel milk has increased. The pastoralists now sell camel milk as alternative for income generation (Farah, 1996). The bulk of marketed milk reaches consumers through informal marketing. The increase in marketing of camel milk for herders' household income generation has raised concern over the hygienic management and preservation of the milk. Camel milk is supposed to have medicinal properties, as it contains insulin - like protein, so it has hypoglycemic effect (Richard, 2002).

Milk contaminated with harmful bacteria has been linked to several serious diseases including typhoid fever, diphtheria, septic sore throat, scarlet fever, dysentery, Q-fever, and other kinds of food borne illness. Other diseases, including tuberculosis and undulant fever (brucellosis), can be transmitted to people via raw milk from diseased animals. *S. aureus* is one of the most common agents in bacterial food poisoning outbreaks (Loncarevic *et al*., 2005; Pelisser *et al*., 2009). It is a versatile pathogen of humans and animals and causes a wide variety of diseases, ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicaemia (Rahimi & Alian, 2013). *S. aureus* produces different extra-cellular protein toxins and virulence factors, which enhance its pathogenicity due to their enterotoxins (Akineden *et al*., 2001). *S. aureus* was isolated from milk and dairy products and are often associated with food-borne disease outbreaks due to the ability of some strains to produce a thermostable enterotoxin. Diseases are usually associated with coagulase and thermonuclease positive *Staphylococcus spp* (Gabriela *et al*., 2009). One of the major problems concern *S. aureus* strains is their ability to develop resistance to antibiotics (methicillin, gentamicin and others), also the ability to coagulate citrated plasma and to produce a number of enzymes and toxins

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which are the main factors of its pathogenecity. The problem with *S. aureus* became more complicated when it was found that it quickly developed resistance and was capable of producing many antibiotic resistant strains (Kitara *et al*., 2011).

MATERIALS AND METHODS

Collection of Milk Samples

A total of 220 milk samples from 55 teats of apparently healthy lactating she-camels were collected for investigation from seven different locations in Al Jabal Al Akhdar, Eastern of Libya (Table 1). The udders of she-camels were thoroughly cleaned with tap water and mopped to dry with clean cloth. The milkers' hands were thoroughly washed with soap and water prior to collection of samples, the first flow of the milk stream was discarded, sample from each quarter were collected separately in sterile test tubes, then tubes were marked. Collection was first done from the near side and then from the offside to avoid contamination of the cleaned teat apices. History and relevant information such as animal number, age, stage of lactation, milk yield and physical condition of the udders were recorded. Samples were kept in an ice box and then transported to bacteriological laboratory, Department of Microbiology, Faculty of Veterinary Medicine, University of Omar–Al Muktar Libya.

Table (1): Locations and numbers of teat milk samples

Location	No of milk samples		
	No of animals		No of samples
	No	%	
Gardas	5	9.1%	20
Tanemlo	7	12.7%	28
Om – Alsafsaf	7	12.7%	28
Labrag	5	9.1%	20
Tubrak	14	25.5%	56
Shahat	11	20%	44
Al- Makailie	6	10.9%	24
Total	**55**	**100%**	**220**

Isolation and identification procedures

Primary isolation procedures were performed in two stages. Firstly, a loop full from each milk sample was inoculated into "nutrient broth tube". Secondly, test milk samples were directly incubated, considering milk a growth medium. Both inoculated broth tubes and milk samples were incubated at 37 ℃ for 24-48 hours. Subcultures from the broth tube and milk bottles were made onto "nutrient agar and blood agar media", with incubation at 37 ℃ for 24-48 hours; and pure cultures were obtained by subculturing on the same agar media. Selective and special media such as "mannitol salt agar", "Baird Parker agar" and "deoxyribonucleolease agar", were cultured for identification of colonies suspected as genus *Staphylococci* according to the methods described by Langlois *et al*. (1990) and Barrow & Feltham (1993). Identification of isolates was based on colonial characteristics, microscopic features and biochemical reactions. All isolation, identification procedures were done according to (Barrow & Feltham, 1993). Media and reagents used in this study were prepared according to the manufacturer's recommendations.

AVIPATH-STAPH

The AVIPATH-STAPH test was performed for detection of coagulase and Protein A, where both factors are associated with *S. aureus*. When examining the test slides under a strong light source, a positive result is indicated by the obvious agglutination pattern of the latex in a clear solution. A negative result is indicated by no change in the latex suspension on the test slide. Agglutination indicates the presence of either coagulase or protein A. Positive AVIPATH STAPH test was confirmed as *S. aureus* by biochemical tests.

Antimicrobial susceptibility testing

All *S. aureus* strains were tested for susceptibility to a panel of nine antimicrobial disk by the disc diffusion agar method on Mueller agar (oxoid), following the National Committee on Clinical Laboratory Standard. Each *S. aureus* was cultured on nutrient broth and incubated at 37^{o}C for 12 hours. 0.05 MacFedan adjusted samples were then spread over the surface of Muller & Hinton agar. Antibiotic discs were disposed on the surface of inoculated agar media aseptically and incubated at 37^{o}C for 18-24 hours. The inhibition zones of each disk were measured, and the results were interpreted based on instruction of the antibiotic disk manufactory as susceptible, intermediate, and resistant. Antibiotics discs used were: ampicillin (10 mg), amoxicillin/clavulanic acid (30 mg), cloxacillin (30 mg), gentamicin (10 mg), streptomycin (10 mg). chloraamphenicol (10 mg), oxytetracycline (30 mg), neomycin (30 mg) and cephalexin (30 mg). Antibacterial agents, their abbreviations, potency and source are given in (Table2).

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Table 2: Antibacterial agents, their abbreviations, potency and source

Antimicrobial agent	Abbreviation	Disc potency	Source
Ampicillin	AM	10 mg	Bioanalyse
Gentamicin	GM	10 mg	Difco
Coxacillin	C	30mg	Difco
Cephalexin	CF	30 mg	Difco
Oxytetracycline	OT	30 mg	Bioanalyse
Amoxycillin/ Clavulanicacid	AMC	30 mg	Difco
Neomycin	N	30	Bioanalyse
Streptomycin	S	10	Difco
Chloraamphenicol	CL	10	Difco

RESULTS AND DISCUSSION

Coagulase positive *Staphylococci* were identified as 2.7% out of the total examined milk samples (220). Tanemlo region showed the highest percentage of the isolation of coagulase positive *Staphylococci* as 7.1% followed by Al- Makailie, Om–Alsafsaf and Tubrak as 4.2, 3.6, 3.6 respectively (Table 3). Contamination of raw milk with *S. aureus* is mainly due to handling and unhygienic environment; hence the occurrence of these bacteria in milk can cause severe health hazards to people. These findings confirm the finding of Vasavada, (1988), Bonfoh *et al* (2003) and Soomro *et al.* (2003).

It is well known that about 50 % strain of *S. aureus* are able to produce enterotoxins associated with food poisoning (Payne and Wood, 1974). Little information is available at present about the toxinogenic potential of this bacterial species in camel milk and about the role of the raw camel milk in intoxication of the consumer by staphylococcal enterotoxins. Milk with pathogenic bacteria is mainly dangerous and harmful for the human health. *S. aureus* is recognized as one of the most important bacterial pathogen that seriously contributes to the problem of nosocomial and community acquired infections (Jan *et al*., 2002). The isolation of the *S. aureus* from camel raw milk in many countries was previously reported by some authors; in Sudan by Shuiep *et al*. (2009), in Brazil by Fagundes *et al. (*2010), and in India by Thaker *et al*. (2013).

Based on cultural properties as shown in table (4), all the isolates showed β-hemolysis on blood agar media (Fig. 1), fermentation of mannitol with production of small yellow colonies at the mannitol salt agar (Fig. 2), production of black, shiny, convex colonies with entire margins and clear zones on Baird-Parker agar medium (Fig. 3), development of Dnase positive colonies (Fig. 4). All isolates were Gram-positive clusters cocci, positive for both catalase and coagulase tests. The AVIPATH-STAPH test showed obvious agglutination pattern of the latex in a clear solution.

Figure 1: Development of β-hemolysis on 5% sheep blood agar by isolated *S. aureus*.

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Figure 2: Fermentation of mannitol salt agar by *S. aureus*..

Figure 3: At Baird-Parker Agar medium *S. aureus* produce black, shiny, convex colonies with entire margins and clear zones

Figure 4: *S. aureus* on DNase Agar, appears as a clear zone around the growth

The susceptibility of the isolated *S. aureus* is presented in Table (5), the bacteria showed the highest resistant (100%) toward ampicillin (10 mg) followed by cephalexin (30 mg, 83.3%) and streptomycin (10 mg, 66.7%). 50% of the isolates showed resistant to neomycin (30 mg). On the other hand, all isolated bacteria showed clear susceptibility toward both gentamycin (10 mg) and oxytetracycline (30 mg) as (100%). The isolated bacteria showed susceptibility of 66.7%, 66.7% and 33.3% for chloramphenicol (10 mg), amoxicillin/ clavulanic acid (30 mg) and streptomycin (10 mg), respectively. Intermediate susceptibility was shown with cloxacillin (30 mg) as 66.7%.

Knowledge of the pattern of antibiotic resistance among isolates is very important both clinically and epidemiologically. The results of antimicrobial resistance patterns are of great concern due to these predominant bacterial isolates which are highly resistant to commonly available antimicrobial agents (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3220124/ - R14). These were stated previously by Ojulong *et al*. (2009). Sensitivity of the isolated bacteria to gentamycin could explain the possibility of sensitivity to all aminoglycosides. However, in this study isolated *S. aureus* was found resistant to streptomycin as 66.7%, and to neomycin as 50%. These might be attributed to the fact that certain aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation and impermeability of membranes (AL Masaud *et al*., 1991). The antimicrobial pattern of *S. aureus* was studied by Beatriz *et al.* (1999), Marais *et al*. (2009) and Zerfie *et al*. (2014) who reported susceptibility to ciprofloxacin and ceftriaxone as 95.4% and 80% respectively. They added that *S. aureus* was highly resistant to penicillin G, amoxicillin and nalidixic acid.

Table 4: Prevalence of *S. aureus* isolated from she- camels milk in Libya

Location	No. of samples	Positive isolation for *Staph. aureus*		Negative isolation for *Staph. aureus*	
		No	%	No	%
Gardas	20	0	0%	20	100%
Tanemlo	28	2	7.1%	26	92.9%
Om – Alsafsaf	28	1	3.6%	27	96.4%
Labrag	20	0	0%	20	100%
Tubrak	56	2	3.6%	54	96.4%
Shahat	44	0	0%	44	100%
Al- Makailie	24	1	4.2%	23	95.8%
Total	**220**	**6**	**2.7%**	**214**	**97.3%**

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The present study confirms the results of Zerfie *et al*. (2014), where no one of the isolates was susceptible to all of the tested antibiotics, and also none of the *S. aureus* isolates were pan-resistant (resistant to all the tested antibiotics).

Table 5: Colonial characteristics of *S. aureus* isolated from She- Camels Milk

Colony characteristic					
Nutrient agar	**Blood agar**	**MacConkey agar**	**MSA**	**Baird parker Agar**	**DNase**
creamy buff or golden yellow colonies, about 1-3 mm in diameter	creamy, buff, color and surrounded by narrow zones of clear haemolysis	No growth	bright yellow zones around the colonies	black colonies and clear zones around respective colonies	clear zone around the growth, surrounding medium

CONCLUSION

In conclusion, the results of this study highlight the potential risk of consuming raw she-camel milk, especially in the absence of strict hygienic and preventative measures to avoid the presence of *S. aureus* isolates in milk. It is apparent from the present study that public health and food hygiene practices during milking, transportation, and storage should be implemented to reduce the risk of *S. aureus* related camel milk poisoning.

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