

Characterization of *Staphylococcus* species and Detection of Methicillin Resistant *Staphylococcus aureus* in Camel Milk at Khartoum North, Sudan

Remaz, M. Juma¹, Nagwa B. Elhag²

^{1,2} Department of Food Hygiene and Safety, College of Public & Environmental Health, University of Bahri, Khartoum North, Sudan

Abstract: Camel milk is well known for its health benefits and it has been consumed in many countries worldwide. It is usually consumed fresh and unpasteurized. It is contaminated with different microorganisms particularly the pathogenic one during milking, handling, transportation and marketing. Daily consumption of fresh cow milk may contribute to the foodborne diseases outbreaks. Thus the aim of this study is to isolate and identify *Staphylococcus* species and detect methicillin resistant *Staphylococcus aureus* associated with fresh udder and commercial camel milk samples. The streak method on Blood agar medium and disc diffusion method were used for investigation and detection of *Staphylococci* and methicillin resistant *Staphylococcus aureus* respectively. A total of 60 samples were collected from camel farms at different stages of milking and commercial fresh camel milk samples purchased from supermarkets outlets at Bahri locality. Results revealed that the percentage occurrence of *Staphylococci* species was 41.7%, 25%, 33.3%, 83.3% and 50% for the samples obtained before udder sterilization, after udder sterilization, directly from utensils of milking, pooled milk and commercial raw camel milk respectively. *Staphylococcus* species farm isolates were identified as *S. aureus* (10.4%), *S. epidermidis* (12.5%), and *S. arlettae* (6.2%), *S. kloosii* (4.2%), *S. caprae*, *S. gallinarum*, *S. intermedius*, *S. carnosus*, *S. chromogenes* and *S. warneri* (2.1%) for each. The *Staphylococcus* species isolated from the commercial raw camel milk were *S. epidermidis* and *S. intermedius* (16.7%) for each, *S. aureus* and *S. equorum* was (8.3%) for each. The percentages of methicillin resistant *Staphylococcus aureus* in all samples from farms and commercial milk were 10.4% and 8.3% respectively. The results indicated that raw camel milk can be contaminated with *Staphylococcus* species and methicillin resistant *Staphylococcus aureus* due to improper hygiene i.e. conditions of milking. Fresh camel obtained from farms and offered for sale are highly contaminated with positive and negative coagulase *Staphylococci*. The presence of methicillin resistant is potential health risk. Therefore they are considered as a health risk for human consumption. Proper procedure, good manufacturing practices (GMP) and good hygienic practices are recommended to improve milk quality.

Keywords: raw camel milk, coagulase positive, coagulase negative, methicillin resistant *Staphylococcus aureus*, Sudan.

1. Introduction

Camel milk is the key food in arid and semi-arid areas of the African and Asian countries [1]. Food Agriculture Organization has reported that more than 18 million camels around the world support the survival of millions of people [2]. Camel milk contains more nutrients compared to the cow milk [3] and it influences microorganisms growth. The growth of bacteria in milk depends mainly on temperature and presence of other bacteria [4]. Bacterial contamination of milk usually occurs during the milking process which depends on the sanitary conditions of the environment, utensils used for milking, the milking personnel hygiene and microorganisms that introduced from udder through the teat canal [5] and also during handling, distribution, transportation and displaying at the selling points.

Raw camel milk may contain pathogenic microorganisms, and their source may lie either within or outside the udder. Pathogenic bacteria may present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis are *Staphylococcus aureus*, and *Escherichia coli* [6]. Contamination of raw milk by pathogenic bacteria from source external to the udder may be caused by *Salmonellae* strains, which produce many outbreaks of enteritis [7]. Many studies revealed that non-heat treated milk and raw-milk products are considered as the main factors responsible for illnesses caused by food borne pathogens [8].

Staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. The genus *Staphylococcus* has at least 40 species which are separated in to two major groups on the basis of their ability to clot (coagulate) blood plasma by the action of *Staphylocoagulase* [9]. The coagulase-positive *Staphylococci* (CoPS) include pathogenic species such as *Staphylococcus aureus*, while the coagulase-negative *Staphylococci* (CoNS) include species that are part of the normal flora of the skin in humans such as *Staphylococcus epidermidis* [10]. *Staphylococci* are ubiquitous in the environment and found as part of the normal flora in soil, water, skin and mucous membranes of humans and warm-blooded animals and have been frequently isolated from a wide range of foodstuffs such as dairy products and meat [11].

The coagulase-positive *Staphylococcus aureus* is a major cause of various community and hospital acquired infections [12]. It causes skin and soft tissues infections, surgical site infections and bone and joint infections [10, 13]. Generally, *Staphylococcus aureus* is a common cause of hospital-acquired bacteraemia and it is associated with hospital-acquired respiratory tract infections [10, 13]. It is an important food-borne pathogen that usually associated with raw unpasteurized milk of dairy cattle suffering *Staphylococcal*-associated mastitis [11, 14]. [15] recorded that 30% of the *Staphylococcus aureus* found in the nasal carriage of the healthy adult animals.

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The coagulase-negative *Staphylococci*, are common components of the human skin microflora and play an important role in flavor and aroma formation through the production of fermented foods, such as cheese and sausage. Many researchers reported there was an increase in cases of nosocomial infections in which coagulase-negative *Staphylococci* are implicated [16].

Antibiotic resistant *Staphylococci* are major public health concern since this bacteria can be easily circulated in the environment. Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have increased world-wide during the past twenty years [13]. Multiple drug-resistant *Staphylococcus aureus* have been frequently isolated from foodstuffs [17], biofilm formation [18], nasal mucosa of humans [19], clinical cases [20] and livestock [21].

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a major cause of health care-associated (HA) and community-associated (CA) infections [22]. In addition, livestock-associated (LA) MRSA genotypically classified under clonal complex 398 (CC398) has been detected among pigs and swine farmers in the Netherlands and other countries [23] and it is known to cause infections in humans and animals [24].

Therefore the objectives of this research is to isolate and identify *Staphylococcus spp.* from fresh udder milk and commercial raw camel milk and to determine the presence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in fresh raw and commercial camel milk.

2. Materials and Methods

2.1. Sample collection

A total of 60 samples of raw and commercial camel milk samples were obtained from al-Zakyab, Shambat, Kafouri farms and different supermarkets at Bahri locality. Sixteen samples from each farm and 12 samples of commercial camel milk were collected. From each farm 4 samples were collected before using disinfectant for udder from different camels, 4 samples collected after using disinfectant of udder, 4 samples obtained from utensil during milking and 4 samples collected from pooled milk. All samples were kept in sterile containers and transported into water iced box to the laboratory for immediate investigation. This study was conducted in tropical medicine research institute laboratory, Sudan.

2.2. Microbiological investigation

2.2.1. Primary isolation

A large drop of camel milk sample was placed onto blood agar plate near the edge, spread it with a loop and then streaked for the isolation [25]. The streaked plates were incubated at 37°C for 48 hours.

2.2.2. Purification and Identification of *Staphylococcus* isolates

Predominant *Staphylococci* isolates were selected and sub-cultured onto nutrient agar medium. The purified isolates were then kept in refrigerator for further studies.

Identification of *Staphylococci* isolates was carried out by the conventional methods which based on the cultural morphological and biochemical tests [26].

3. Results and Discussion

Results revealed that *Staphylococci* were recovered in 28 samples of all camel milk samples investigated comprises 46.7%. The coagulase positive *Staphylococcus aureus* were identified in 9 samples out of 28 samples making 32.1%, while coagulase negative *Staphylococcus* were detected in 19 samples with percentage of 67.9%. [27] investigated the presence of *S. aureus* in raw camel milk samples collected from healthy camels of three different location in Sudan. They identified twenty-five *S. aureus* phenotypically and by PCR. The prevalence of coagulase positive bacteria in the studied samples may be referred to the lactating animals that suffer from *Staphylococcus*-associated mastitis [28].

With respect to the presence of *Staphylococcus* in farm milk samples, results revealed that the percentage of *Staphylococcus* isolated from pooled milk was 83.3% which was higher than that recorded for the samples collected before using disinfectant for udder (41.7%), samples collected directly from utensil of milking (33.3%) and samples collected after using disinfectant for udder (25%) while the percentage of *Staphylococcus* isolated from commercial raw milk samples was 50% (Fig. 1). The prevalence of *Staphylococcus* in commercial camel milk samples was higher (50%) than that of the farm samples (45.8%). These findings (except for the samples collected after udder sterilization) were higher than those obtained by [29] who reported that 32% of *Staphylococcus sp.* were isolated from camel's milk and lower than those obtained by [30] who isolated 89.8 % of *Staphylococcus spp.* from raw camel milk in Ethiopian Somali regional state. Another study conducted by [31] who found that the existence percentage of *Staphylococcus spp.* was 28.69% for camel milk samples that collected from farms at Bhari (Khartoum North). The high percentage of *Staphylococcus* in pooled milk and commercial raw camel milk samples may be attributed to the mixing of milk after milking process and contamination from air, camel's udder, hide, milking utensil, during transportation, storage, hands and clothing of the workers during milking. [32] reported that, approximately 50% of the examined raw camel's milk samples were produced and handled under poor hygienic conditions with high health risk to the consumers. Results showed that *Staphylococcus* species isolated from farm camel milk samples were identified as *Staphylococcus aureus* (10.4%), *S. arlettae* (6.2%), *S. epidermidis* (12.5%), *S. kloosii* (4.2%), *S. caprae*, *S. gallinarum*, *S. intermedius*, *S. carnosus*, *S. chromogen* and *S. warneri* (2.1%) for each, while *Staphylococcus* isolates of commercial camel milk samples were identified as *S. aureus* and *S. equarum* (8.3%), *S. epidermidis* and *S. intermedius*, (16.7%) as presented in Fig. 2. *Staphylococci* are ubiquitous in the environment and found as part of the normal flora in soil, water, skin and mucous membranes of humans and warm-blooded animals and have been frequently isolated from a wide range of foodstuffs such as dairy products and meat. The identified *Staphylococcus epidermidis* is part of the normal human flora, typically the skin flora, and less commonly the

mucosal flora. This bacterium is not usually pathogenic but people with compromised immune systems are at risk of developing infection [33]. *Staphylococcus arlettae* has been isolated from the skin of mammals and birds. *Staphylococcus kloosii* Strains of this species were originally isolated from the skin of various wild animals [34].

The negative coagulase *Staphylococcus intermedius* were originally isolated from the anterior nares of pigeons, dogs, mink, and horses [35]. *Staphylococcus caprae* occurs as a commensal on human skin and occasionally cause infection

of bloodstream, urinary tract, bones and joints. It was originally isolated from goats ("*caprae*" means of a goat"). The members of this species have also been isolated from human samples [36]. However, *Staphylococcus gallinarum* were firstly isolated from chickens and a pheasant. Its cell wall is similar to that of *Staphylococcus epidermis*[37]. Also it has been found in the saliva of healthy human adults[38]. This bacterium is not generally pathogenic. The identified *S. carnosus* was originally isolated from dry sausage and is

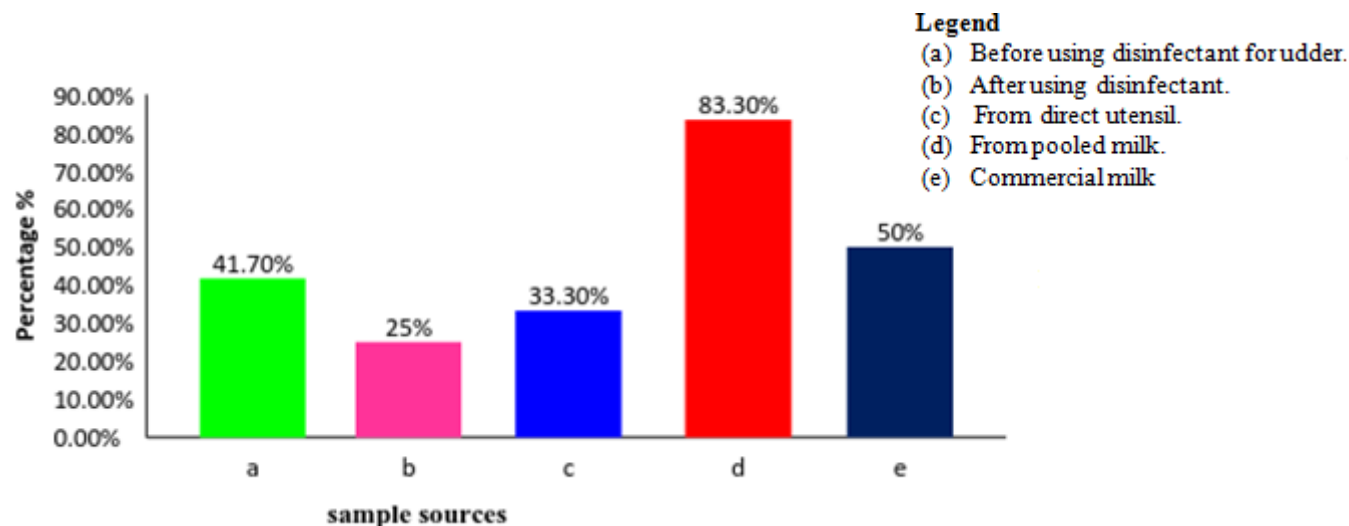


Figure 1: Percentage of *Staphylococci* isolates obtained from different sources

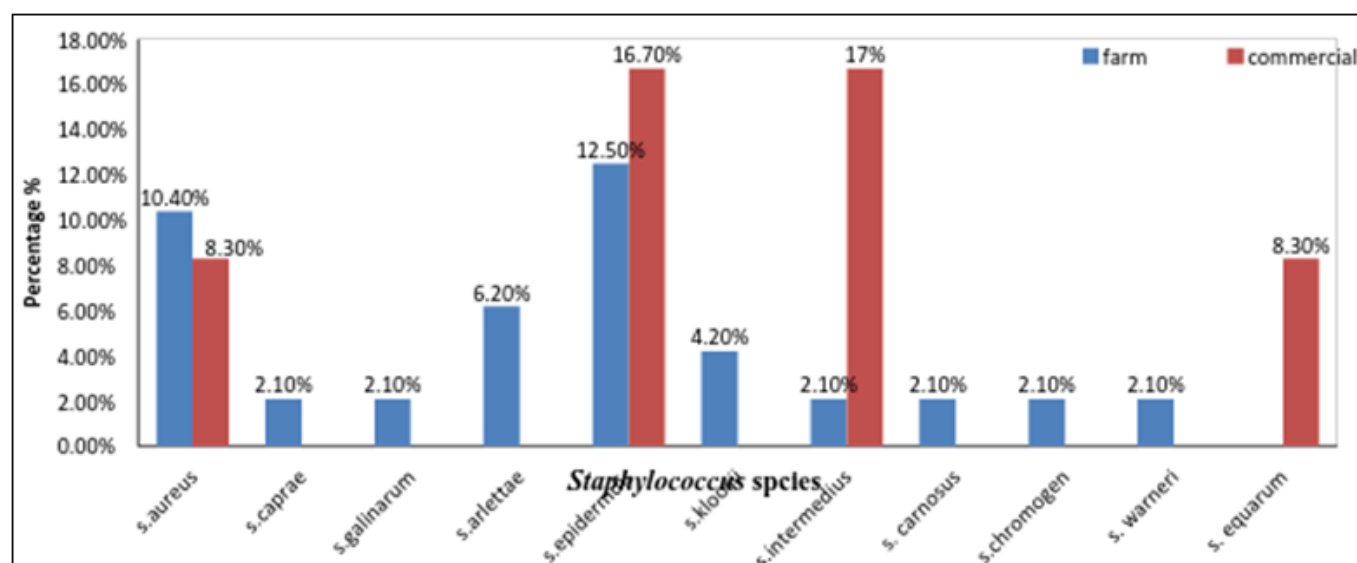


Figure 2: Occurrence percentage of *Staphylococci* species in farm and commercial camel milk samples

involved in the production of meat products [39]. *Staphylococcus chromogenes* species is associated with mastitis in dairy animals [40]. *Staphylococcus equorum* is originally isolated from the skin of healthy horses [34]. *Staphylococcus warneri* is a common commensal organism found as part of the skin flora on humans and animals. Like other coagulase-negative *Staphylococci*, *S. warneri* rarely causes disease, but may occasionally cause infection in patients whose immune system is compromised.

Generally, the presence of *S. aureus* (10.4%) in studied raw camel milk samples might be due to the weak effect of the

protective proteins (lysozyme, lactoferrin, lactoperoxidase, immunoglobulin G and A) of camel's milk on this bacterium. [41] and [42] found that camel milk lysozyme was effective against *Salmonella*, but not effective against *S. aureus*. Also they revealed that camel lactoperoxidase is bacteriostatic against gram positive bacteria and bacteriocidal against gram negative bacteria.

The high prevalence rate of positive and negative coagulase *staphylococci* in the raw camel milk samples in this study reveals the poor hygienic conditions during milk production in Sudan and it reflects the poor personal hygiene of the

milk dealers. Lack of standards hygiene measures among workers in the farms and at the markets may lead to the serious health hazards concerning consumers. In this study all of these species were isolated from camel milk. And there is no previous information about their isolation from camel milk. Therefore, the presence of these bacteria in raw camel milk make alarming signs for health authorities towards their prevalence in raw camel milk produced under poor hygienic conditions.

The prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in all camel milk

samples was 10%. Farm samples exhibited higher percent (10.4%) than the commercial camel milk samples (8.3%) (Fig.3). These findings were higher than those obtained by [[43]. He found *S. aureus* (13.4%) in all samples of camel milk samples tested. Also he claimed that no MRSA in his study. The high percent of this bacterium in this research may attributed to the improper hygiene and poor farm management, variation in geographical regions and collection of the samples at different stages during milking may account for success in detecting MRSA.

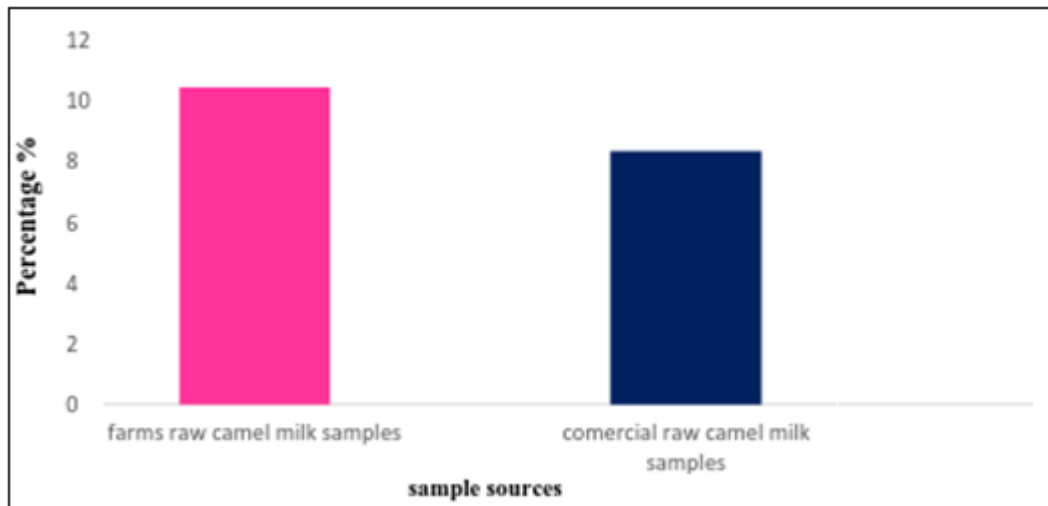


Figure 3: The prevalence of Methicillin Resistant *S.aureus* (MRSA) in farm and commercial camel milk

The presence of methicillin-resistant *Staphylococcus aureus* in raw camel milk play a significant role in the spreading of multi-drug resistant staphylococci and represent a great public health concern in Sudan. Proper procedure, good manufacturing practices (GMP) and good hygienic practices are recommended to improve milk quality.

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Author Profile

Remaz Mohammed Juma Gabash received the B. Sc. degree in Microbiology from - Juba University- Sudan in 2010, College of Applied and Industrial Sciences, Department of Microbiology, M.Sc. degree in Science of Microbiology from University of Khartoum Faculty of Sciences - Sudan in 2016. Now I join University of Bahri - Food Hygiene and Safety Department, College of Public & Environmental Health

Dr. Nagwa Babiker Elhag Khalifa received the B. Sc. degree in Food Science and Technology from Alexandria University- Egypt in 1993, Faculty of Agriculture, M.Sc. degree in Food Microbiology from University of Khartoum Faculty of Agriculture - Sudan in 2003 and PhD degree in Food Microbiology and Biotechnology from University of Khartoum-Sudan Faculty of Agriculture in 2013. During this period she stayed teaching in different Universities. Now she joined University of Bahri - Food Hygiene and Safety Department, College of Public & Environmental Health, teach and doing different researches.