International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

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

Remaz, M. Juma[1], Nagwa B. Elhag[2]

[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 out breaks. 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. Staphylocoocus 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 *Stapylococcus. aureus*, while the coagulase-negative *Staphylococci* (CoNS) include species that are part of the normal flora of the skin in humans such as *Staphlococcus 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.

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^{o}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

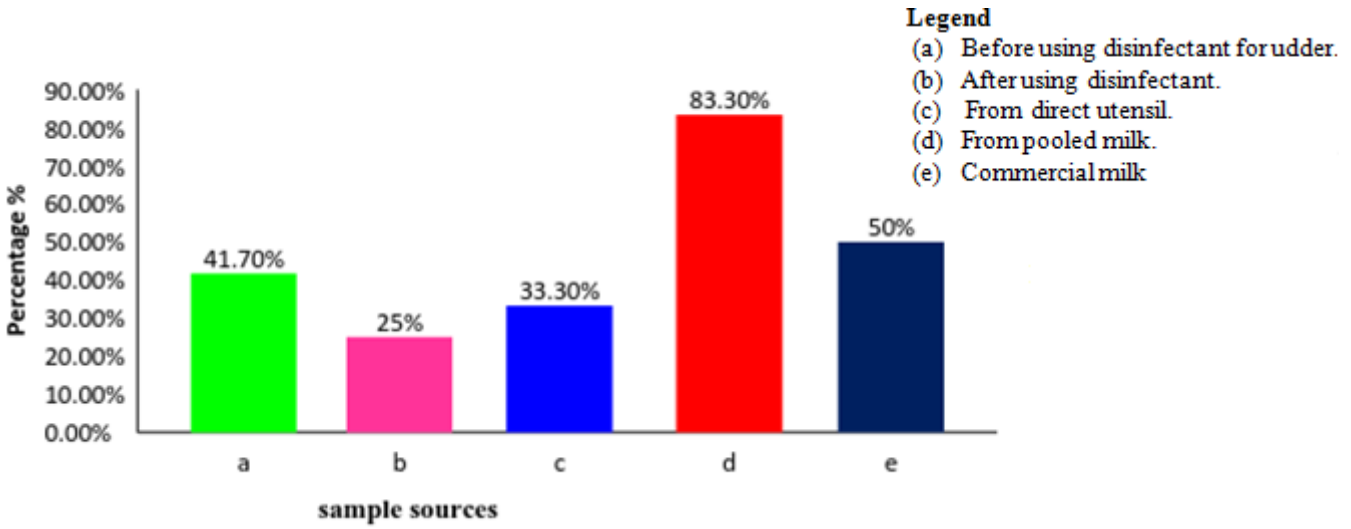

Figure 1: Percentage of *Staphylococci* isolates obtained from different sources

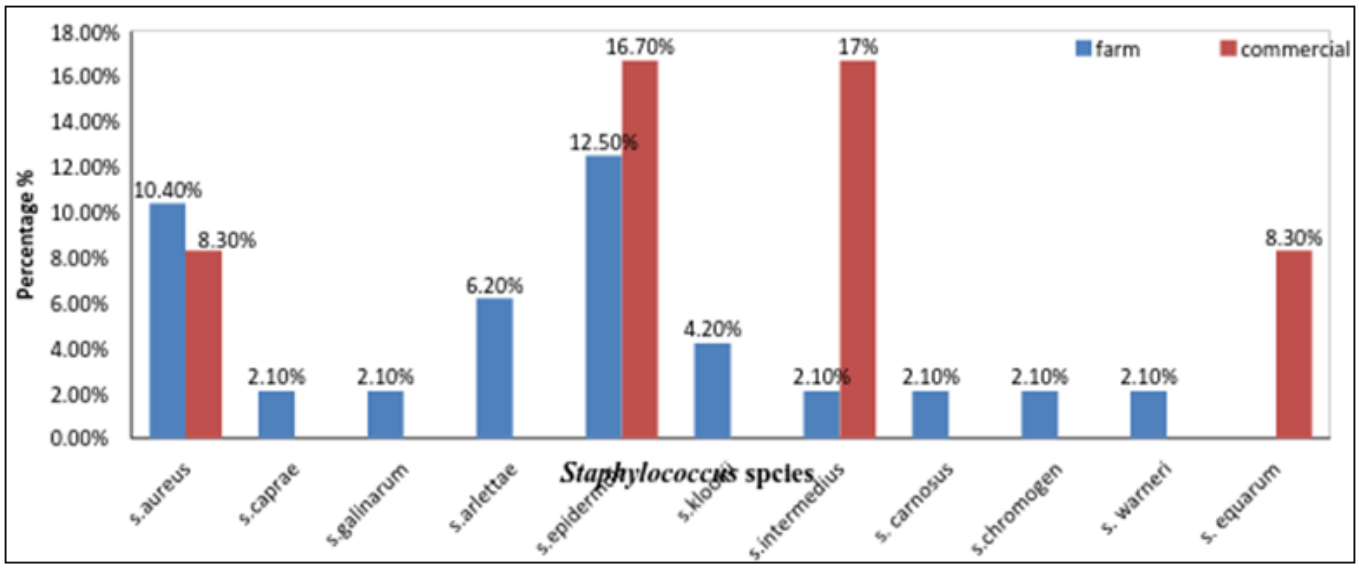

Figure 2: Occurrence percentage of *Staphlococci* species in farm and commercial camel milk samles

.

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 prevelance 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.

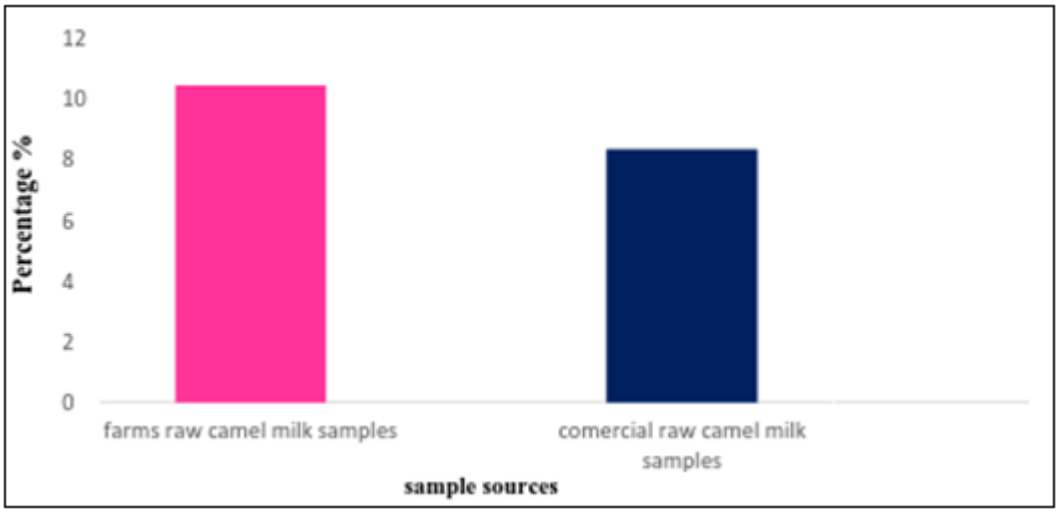

Figure 3: The prevalence of Methicillin Resistant *S.aureus* (MRSA) in farm and commercial camel milk

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.

References

[1] G.A.Alhadrami. Camel. InH. Roginski, J.W. Fuquay & P.F. Fox, eds. Encyclopedia of dairy sciences. London, Academic Press, , pp. 616–622.2003.
[2] FAO. Statistics year-book. – FAO, Rome.2003.
[3] R.P. Arrowal, R. Beniwal, D.K. Kochar, F.C. Tuteja, S.K. Ghorui, M.S. Sahaui, and S. Sharma. Camel milk as an adjunct to insulin therapy improves long-term glycaemia control and reduction in doses of insulin in patients with type-1 diabetes. A 1 year randomized controlled trial. – *Diabetes Res. Clin. Pract.* 68:17.2005.
[4] W.Heeschen.MilchalsLebensmittel,In:Wendt,K.,H.Miele&H.W.Fuchs(Eds):*EuterundGesaugekrankheitenGustavFischerVerlag,Jena,Stuttgart*, Germany,pp;138-180.1994.
[5] F. Kalsoom, N.H.S. Syed, and J. Farzana. Antibiotic resistance pattern against various isolates of *Staphylococcus aureus* from raw milk samples. *J. Res. Sci* , 15:145-151.*2004*.
[6] H.J. Sinell . Food Infections, from Animals. In: The Microbiological Safety of Foods. Hobbs, B. C. and J. H. B. Christian (Eds). Academic Press, London and New York.1973.
[7] D.A. Robinson, W.J. Edgar, G.L. Gibson, A.A. Matcheit and A.A. Robertson. Campylobacter enteritis associated with consumption of unpasteurized milk . *Brit. Medical J.*1:1171.1979.
[8] M.L. De Buyser, B. Dufour, M. Maire, and V. Lafarge. Implication of milk and milk products in food-.borne disease in France and different industrialized countries-*Int. J. Food Microbiol*., 67: 1-17. 2001.
[9] G.A. Somervilleand R.A. Proctor. The Biology of staphylococci. In: staphylococci in Human Disease, Crossley , K.B., K.K. Jefferson, G.L. Archer and V.G. Fowler (Eds.).2nd Edn., Wiley-Blackwell, Chichester, UK. 2009.
[10] A.L. Casey, P.A. Lambert and T.S.J. Elliott. *Staphylococci. Int. J. Antimicrob. Agents*, 29:S23-S32.2007.
[11] F. Irliger. Safety assessment of dairy microorganism: Coagulase-negative *staphylococci.Int. J. Food Microbiol*., 126:302-310.2008.
[12] R.V. Goering. Mims' Medical Microbiology. 4th Edn., Elesevier, Philadelphia, USA., ISBN-13: 978-0808923725.2008.
[13] G. Ippolito, S. Leone,F.N. Lauria, E. Nicastri and R.P. Wenzel. Methicillin-resistant *Staphylococcus aureus*: The superbug. *Int.J. Infect. Dis.*, 14: S7-S11.2010.
[14] M. Morgan. Methicillin-resistant *Staphylococcus aureus* and animals: Zoonosis or humanosis*?. J. Antimicrob. Chemther*., 62: 1181-1187.2008.
[15] J.A. Kluytmans, Methicillin-resistant *Staphylococcus aureus* in food products: Cause for concern or case for

complacency. *Clin. Microbiol. Infec*., 16:11-15.2010.
[16] A. Piette and G. Verschraegen. Role of coagulase – negative *staphylococci* in human disease. *Vet. Micrbiol*., 134: 45-54.2009.
[17] G. Normanno, G. La Salandra, A. Dambrosio, N.C. Quaglia and M. Corrente *et al*.,. Occurrence , characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *Int. J. Food Microbiol*., 115:290-296.2007.
[18] M. Lancellotti, M.P. de Oliveira and F.A. de Avile. Research on Staphylococcus sp. In biofilm formation in water pipes and sensibility to antibiotic. *Braz. J. Oral Sci*., 6:1283-1288.2007.
[19] M. Acco, F.S. Ferreira, J.A.P. Henriques and E.C. Tondo. Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handelers. *Food Microbiol*., 20:489-493.2003.
[20] S. Stefani and A . Goglio. Methicillin-resistant *Staphylococcus aureus*: Related infections and antibiotic resistance. *Int. J. Infect. Dis*., 14:S19-S22. 2010.
[21] M. Wulf and A. Voss. MRSA in livestock animals-an epidemic waiting to happen.*Clin. Microbiol. Infect*., 519-521.2008.
[22] E.Klein, DL.Smith, R.Laxminarayan. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. *Emerg. Infect. Dis*. 13:1840–1846.2007.
[23] T. Khanna, R.Friendship, C.Dewey, JS.Weese. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Vet. Microbiol*. 128:298–303.2008.
[24] P.Declercq, D. Petre, B. Gordts, A.Voss. Complicated community-acquired soft tissue infection by MRSA from porcine origin. Infection 36:590–592.2008.
[25] A. Josephine, Morello, A. Paul, Granato Helen Ecked Mizer. Laboratory Manual and Workbook in Microbiology.7th ed., The McGraw–Hill Companies, ISBN: 0-07-246354-6.2003.
[26] G.L.Barrow, and R.K.A. Feltham. Cown and Steel Manual for the identification of medical bacteria.3rd ed. Cambridge University Press, Cambridge, U.K.pp.50-55.2003.
[27] ES. Shuiep , T. Kanbar , N. Eissa , J. Alber , C. Lämmler , M. Zschöck , IE. El Zubeir and R.Weiss . Phenotypic and genotypic characterization of *Staphylococcus aureus* isolated from raw camel milk samples. *Res Vet. Sci*. 86(2):211-5. 2009.
[28] F. Peles, M. Wagner, L. Varga, I. Hei and P.Rieck et al. characterization of staphylococcus aureu strain isolated from bovine milk in Hungary. Int. J. Food Microbiol. 118:186-193.2007.
[29] R.H. Omer and A.H. Eltinay. Short Communication of Microbial quality of camel's raw milk in central & southern regions of United Arab Emirates. Emir. *J. Food Agric*. Available at www cfa.uaeu.ac.ae/research/ejfa.htm76.2008.
[30] A. Tsegalem, L. Yoseph, M. Behar and U. Befekadu. Bacteriological quality of raw camel milk along the market value chain in Fafen zone, Ethiopian Somali regional state. **DOI:** 10.1186/s13104-016-2088-1, 2016.
[31] A.E. Elhag, A.B. Freigoun, and T.T. Mohamed. Aerobic Bacteria And Fungi Associated With Raw Camel's Milk .*Online J. Anim. Feed Res*., 4 (1): 15-17. 2013.
[32] M.G. El-Ziney and A.I. Al-Turki. Microbiological quality and safety assessment of camel milk (*Camlus Dormedaries*) in Saudi Arabia (Qassim Region). Appl. Ecology and Environ. Res., 5: 115-122.2007.
[33] P.D. Fey and M.E.Olson. "Current concepts in biofilm formation of *Staphylococcus epidermidis*". *Future Microbiology* 5 (6): 917–933.2010.
[34] K.H. Schleifer, R. Kilpper-Bälz and L.A. Devriese . "*Staphylococcus arlettae* sp. nov., *S. equorum* sp. nov. and *S. k1oosii* sp. nov.: Three New Coagulase-Negative, Novobiocin-Resistant Species from Animals". *System. Appl. Microbiol*.5 (4): 501–509.1984.
[35] V. Hájek. "*Staphylococcus intermedius*, a New Species Isolated from Animals". *Int. J. System. Bacteriol.* 26 (4): 401–408.1976.
[36] E. Carretto, D. Barbarini, I. Couto, D. De Vitis , P. Marone, J. Verhoef , H. De Lencastre, and S. Brisse. "Identification of coagulase-negative *Staphylococci* other than *Staphylococcus epidermidis* by automated ribotyping.". *Clin Microbiol Infect.* 11 (3): 177–184.2005.
[37] L.A. Devriese, B. Poutrel, R. Kilpper, and K. H. Schleifer. "*Staphylococcus gallinarum* and *Staphylococcus caprae*, Two New Species from Animals". *Int. J. System. Bacteriol.* 33 (3): 480–486.1983.
[38] Y. Ohara-Nemoto, H. Haraga, S. Kimura and T.K. Nemoto. "Occurrence of staphylococci in the oral cavities of healthy adults and nasal oral trafficking of the bacteria". *J.Med. Microbiol* 57 (1): 95–99.2008.
[39] K.H. Schleifer, and U. Fischer. "Description of a New Species of the Genus Staphylococcus: *Staphylococcus carnosus*". *Int. J. System. Bacteriol.* 32 (2): 153–156.1982.
[40] S.C. Nickerson. "Control of heifer mastitis: antimicrobial treatment-an overview.". *Veterinary Microbiology* 134 (1-2): 128–35.2009.
[41] E. K. Barbour, N. H. Nabbut, W. M. Frechs, and H. M.AL-Nakhli. Inhibition of Pathogenic Bacteria by Camels Milk Relation to Whey Lysozyme and Stage of Lactation.*J. of Fd Prod*. 47:838-840.1984.
[42] E. I. El-Agamy, R. Ruppanner, A. Ismail, C. P. Champagne and R. Assf. Antibacterial and Antiviral Activity of Camel Protective Proteins. *J. Dairy Research*. (59):169–175.1992.
[43] M.H. Abdulkadhim . Prevalence of Methicillin Resistant *Staphylococcus aureus* in Cattle and She-camels Milk at Al-Qadisyia Province. *Al-Anbar .J. Vet. Sci.,5*. (2). 2012.

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.