Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Isolation, Identification and Antibiotics Resistance Pattern of *Staphylococcus aureus* Isolated from Raw Sheep Milk Samples in Ghebaish City West Kordofan State- Sudan

Alnazeer G. Alseed (1), Mohamed Hassan Fadlalla(1), Rawda Y. Ahmed(1), K. Rodwan (2) Muna O. Elhaj (3)
Faculty of Veterinary Medicine, West Kordofan University, Ghebaish.
College of Veterinary Medicine, Sudan University of Science and Technology
Central Veterinary Research Laboratory, Soba

ABSTRACT:
The diseases associated with Staphylococcal outbreaks are wide spread throughout the Sudan affecting both animals and humans. The most endemic types which affect the population health are those related to *Staphylococcus aureus*. *Staphylococcus aureus* is an important pathogen that can cause Staphylococcal Food Poisoning (SFP). Milk and dairy products are frequently contaminated with exterotoxigenic *Staphylococcus aureus*, which is often involved in Staphylococcal food poisoning. The objective of the present study was to evaluate the occurrence and antibiotic resistance of this bacterium. For this purpose sixty raw sheep milk samples were collected from different flocks throughout Ghebaish city. The samples were investigated for the presence of Staphylococci. The *Staphylococcus aureus* was isolated in Blood agar and Mannitol salt agar where typical and atypical colonies were selected and submitted to coagulase and other confirmatory tests.Out of 60 samples, (22) 36% isolates of *Staphylococcus aureus* were obtained and identified by biochemical tests.The results of sensitivity test reflected high antibiotic resistance rates of bacteria, Vancomycin (54%), Tetracycline (0%), Erythromycin (18%), Clindamycin (27%), Chloramphenicol (9%), Ceftrioxone (63%) and Methicillin (27%).It was concluded that *S. aureus* survives around animal environment causing infectious illnesses and begin to acquire resistance against different antibacterial agents and the presence of this pathogenic microorganism indicates a potential health hazard to those who consume milk from this region.
Key words: Milk, *Staphylococcus aureus*, Antibiotic resistance

Introduction:

Worldwide, millions of people suffer from communicable and non-communicable diseases caused by contaminated food. These diseases take a heavy toll in human life and suffering, particularly among infants, children, elderly and other susceptible persons. They also create an enormous social, cultural and economic burden on communities and their health system (van der vanter, 1999).

Harmful bacteria are the most common causes of food borne illnesses, milk is an excellent source of nutrients for human, and yet in a different context it provides a most suitable medium for microbial growth and metabolism (Richard, 2002). Bacteria in raw milk can affect the quality, safety and consumer acceptance of dairy products. Non pathogenic bacteria may affect milk and milk products quality (Sørhaug and Stepaniak, 1997; Barbano *et al*., 2006) and many countries have milk quality regulations, including limits on the total number of bacteria in raw milk, to ensure the quality and safety of the final product. The number and types of microorganisms in milk immediately after milking are affected by factors such as animal health, equipment cleanliness, season and feed. It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Bramley and McKinnon, 1990).

More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and Entero-pathogenic *Escherichia coli*. These bacteria are commonly found on many raw foods. Normally a large number of food-poisoning bacteria must be present to cause illness (Jack and Marianne, 1999).

Staphylococcus aureus is the most prevalent and economically significant pathogen causing inflammatory infections in dairy ruminants (Akineden *et al*., 2001), Approximately 30%-40% of all mastitis cases are associated with the bacterium (Asperger *et al*., 2003).

The infections vary greatly in severity. There may be a mild skin infection to severe pneumonia and septicemia The bacterium is frequently found associated with subclinical mastitis in dairy cattle (Adesiyun *et al*., 1998) and may be present in milk and other dairy products (Capurro *et al*., 2010).

Milk is a completely balanced diet with the right amount of carbohydrate, protein, fats, vitamins and minerals. Bacteria of sorts thrive in milk and as a result reduce its quality. The presence of pathogenic bacteria in milk is of immense public health significance. Milk can be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the contamination, as this bacteria colonizes the nasal pathways in human beings ,the hands of unhygienic milk handlers, the housing environments and instruments, and the cow itself are possible sources of milk contamination by pathogenic bacteria (Abera *et al*, 2010).

Staphylococcus aureus which is one of the causes of mastitis in animals could have its source from milk handlers since most humans carry the organisms in their nostrils. This is the most common type of mastitis and has great economic importance to dairy farmers (Abebe *et al*, 2013). Mastitis in cattle caused by S. *aureus* can either be subclinical or clinical. According Bachaya *et al* (2011), sub-clinical mastitis is of global importance in the dairy industry. It shows no noticeable alterations in the appearance of the milk or the udder, but there is decrease in milk production. The symptoms of clinical mastitis include swelling, hardness, redness, heat, and pain.

Antimicrobial resistance has been detected in all parts of the world; it is one of the greatest challenges to global public health today, and the problem is increasing. It is now accepted that increased antimicrobial resistance (AMR) in bacteria affecting humans and animals in recent decades is primarily influenced by misuse and over use of antimicrobials for a variety of purposes , AMR can occur naturally through adaption of microorganisms to their surrounding environment, it is exacerbated by inappropriate and excessive use of antimicrobials(O'Neill, 2014).

The widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multidrug resistant bacteria. *Staphylococcus aureus* is one of the bacteria that have a dramatic increase in resistance to antibiotics in the last decade. *Staphylococcus aureus* is a normal flora both human and animals. It often colonizes the skin and nose in healthy individuals; however, it can also cause severe diseases. In animals, it causes diseases such as mastitis in most species of domestic animals, pyaemia dermatitis in dogs, botryomycosis in horses, septicaemia and arthritis in poultry (Soltys, 1979; Tortora et al, 1989).

Bacteria can acquire resistance to antibiotics as a result of chromosomal mutation, expression of a latent chromosomal gene, by exchange of genetic material through transformation, transduction, or conjugation by plasmids (Neu, 1992).

There are few studies on the prevalence of potentially pathogenic staphylococci in raw milk in the Sudan. This study was carried out to isolate and characterize the bacterium *Staphylococcus aureus* from raw sheep milk at Ghebaish City western Kordofan State.

Materials and Methods:

Sample collection: A total of 60 raw sheep milk samples (approximately 10 mL from each) of apparently healthy sheep were collected in sterile clean universal bottles. This was done by applying gentle pressure with fingers on the udders for the presence of swelling, hardness, redness, heat, and pain. Moreover, the physical characteristic of the milk from each quarter was checked for any alterations. Before milk samples were collected, each quarter was washed with tap water and dried. The teats were swabbed one after the other with cotton soaked in 70% ethanol. 10 ml of milk was then collected aseptically from the udders into sterile universal bottles after discarding the first three milking streams. The samples were immediately transported on ice to Microbiology Research Laboratory of the College of Veterinary Medicine, West Kordofan University, for culturing and biochemical analysis and bacteriological investigation.

The samples were collected from February 2018 to April 2018 and investigation was carried out following collection.

Isolation and identification of S. *aureus*:

The collected milk samples (0.01 mL) were streaked onto 5% sheep blood agar (HiMedia®, India) incubated at 37°C for overnight. The presumptive colonies of *S. aureus* were further cultured onto mannitol salt agar (MSA) and repeatedly sub-cultured to get pure culture. These isolates were preserved for further bacterial identification. The isolates were identified as *S. aureus* on the basis of Gram staining, colony morphology on mannitol salt agar (MSA) (HiMedia®, India), beta-hemolytic patterns on blood agar enriched with 5% (v/v) sheep blood, catalase and coagulase tests (Ekici *et al* , 2004).

Biochemical identification of isolates was by standard microbiological methods as described by Cheesbrough (2000) and Cowan and Steel (2003).

Antibiotic Susceptibility Assay:

All of the *S. aureus* isolates were subjected to antibiotic sensitivity testing by standard disc diffusion method on Muller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Diluted solution of pure colony culture was prepared by suspending 2 to 3 colonies in 0.1ml sterilized normal saline. Normal saline was then added until the solution had the same turbidity with 0.5 McFarland standard solutions. This indicates that the suspension that was made contain approximately 108 cells/ml. Then a swab was dipped in the standardized solution and spread onto the Mueller Hinton agar (CLSI, 2007). Then antibiotic disc Vancomycin, Tetracycline, Erythromycin, Clindamycin, Chloramphenicol, Ceftrioxone and Methicillin Were placed on the agar .The plate was incubated overnight at 37°C. Upon completion, the diameter of the inhibition was measured and compared to the standard antibiotic susceptibility table (NCCLS, 2002). The isolates were assigned as resistance, intermediate and sensitive towards antibiotic tested.

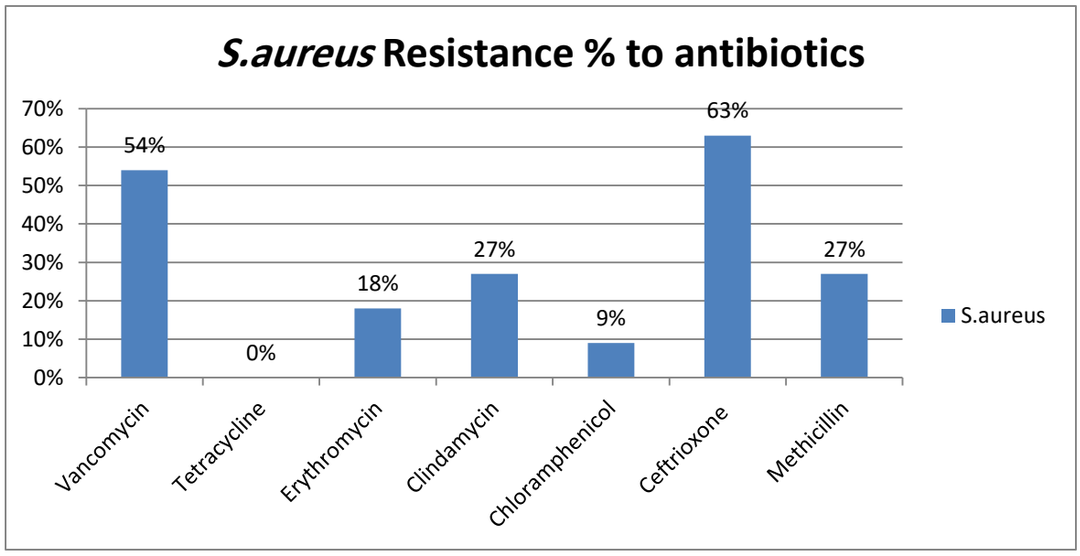
Results:

A total of 60 raw sheep milk samples were tested and *S. aureus* was isolated from 22 (36%) samples based on cultural and biochemical properties. All the 22 isolates showed β-hemolysis on blood agar media enriched with 5% sheep blood. Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci. The isolates also fermented mannitol with the color change of MSA (Mannitol Salt Agar) and production of small yellow colonies. These isolates were positive for catalase and coagulase test. In catalase test; Hydrogen peroxide was broken-down into water and oxygen. Production of oxygen was indicated by bubble formation, whereas the negative control did not produce any bubble . The isolates were identified as *S. aureus* by coagulase test. The positive result of coagulase test was confirmed by the formation of curd like clotting compared to negative control.

Based on antibiotic sensitivity test, all *S. aureus* isolates were found multidrug resistant (MDR). The isolates were found 54% resistant to Vancomycin , 18% resistant to Erythromycin , 27% resistant to Clindamycin and Methicillin, 9% resistant to Chloramphenicol and Ceftrioxone 63%. All the isolates found 100% sensitive to Tetracycline (0% resistance).

Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Figure (1): Resistance of *Staphylococcus aureus* to each antibiotic



Discussion:

The prevalence of *S. aureus* in raw milk and dairy product was found to be 56% in Turkey by Gundogan and Avci (2014), and 75% in Bangladesh by Begum *et al*. (2007) and, which were significantly higher than the present study (36%). This prevalence was found to be lower than 25.3% obtained by Alla Eldein *et al* (2018).

Several studies reported that *Staphylococcus aureus* is the main cause of mastitis (Kerro and Tareke, 2003; Hundera *et al*., 2005; Mekonnen *et al*., 2005). A study carried by Abera *et al*., (2013) in Ethiopia, showed the percentage of *Staphylococcus aureus* isolated from mastitis was 42.1 % which renders it as one of the problem causing in dairy farms,

The variability in prevalence of mastitis results may be affected by different factors such as the season, farm management practices sampling procedures, method of isolation and hygienic practice of milking and selling (Abera *et al*., 2013; Lili *et al*., 2016; Patel *et al*., 2018). An important feature of *Staphylococcus aurues* is it that it usually survives in the udder resulting in clinical or sub clinical mastitis, but it also can shed into the milk and it becomes a source for infection to other healthy animals during milking process and become a main source of contaminants (Patel *et al*., 2018).

The high incidence of *S. aureus* is indicative of poor hygienic measures during production, handling and distribution, stated in the findings of Zakary *et al*. (2011). Results of the sensitivity tests showed variety levels of Staphyllococci response to different antibiotics and this may be due to antibiotic misuse. The antibiotics to which Staphyllococci showed high resistance percentage were Ceftrioxone (63%) of isolates, and (54%) to Vancomycin. All isolates included in this study were found to be sensitive to tetracycline and this result may be attributed to limited use of tetracycline to treat Staphylococcal infections in veterinary field.

There are several factors that can make *S. aureus* to be resistance to antibiotics. Over prescribing of antibiotics by clinician is one way that can lead to resistance of *S. aureus*. When there is over usage and incomplete course of antibiotics by patients, example when appropriate antibiotics are given to the animals, the owner may only give part of the course of antibiotic and not finish the remainder, possibly leaving bacteria partially treated. This will result in an increase resistance towards the antibiotics. The availability of antibiotics which is this is a big concern inters nationally where many antibiotics are available without prescriptions. Many pharmacists in these countries act as the caregiver and give out antibiotics based on patients' complaints without adequate diagnosis or testing and lead to the resistance of the bacteria towards antibiotic. High cost and lack of adequate medications also can lead to the resistance to antibiotic. In several

lesser developed countries, many antibiotics are very expensive. This may contribute to only partial use of an antibiotic (Horwitch, 2000).

To minimize conditions which may favor the development of antibiotic resistance and to reduce the chances of treatment failure, it is important that antibiotics are taken correctly, following the instructions for dosage and duration of treatment. Some antibiotics need to reach a particular concentration in the body (concentration-dependent) or are provided for a certain length of time (time-dependent) in order to be fully effective. Importantly, sub-optimal dosage or duration of antibiotic treatment is important contributors to the development of resistance. This is why it is essential that treatment doses are calculated correctly and that the treatment duration instructions are followed exactly as directed by the vet who prescribed the antibiotic. Drug-resistance surveillance and epidemiological analysis of patient data is needed periodically and can be informative for appropriate management of antimicrobial resistance (O'Neill, 2014).

Conclusions:

This study concluded that. *aureus* could be isolated from the raw sheep milk at Ghebaish city as the etiological agent of mastitis and Some animals may be asymptomatic carriers of the bacterium . The isolates shows high resistance rates when subjected to antimicrobial agent with MDR (Multi-drug resistant).

References:

1. Abebe M, Daniel A, Yimtubezinash W, Genene T (2013). Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. Afr. J. Microbiol. Res. 7(27): 3501-35105.

2. Abera, A, Demie,B, K. Aragaw,, F. Regassa,, and A. Regassa.2013. Isolation and identification of Staphylococcus aureus from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. African Journal of Dairy Farming and Milk Production Vol. 1 (2), pp. 019-023.

3. Adesiyun AA, Webb LA, Romain HT (1998). Prevalence and characteristics of Staphylococcus aureus strains isolated from bulk and composite milk and cattle handlers. Journal of Food Protection, 61: 629-632.

4. Akineden O, Annemuller C, Lamler C, Zschock M (2001). Toxin Genes and Other Characteristics of Staphylococcus aureus Isolates from Milk of Cows with Mastitis. Clinical and Diagnostic Laboratory Immunology, 8: 959-964.

5. Alla Eldein Ibrahim, Hind A. Elnasri, Mona A.M. Khaeir and Adil M.A. Salman (2018). Isolation, Characterization and Sensitivity Pattern of Staphylococcus aureus Isolated from Milk Samples in Khartoum State, Sudan nternational Journal of Current Microbiology and Applied Sciences .ISSN: 2319-7706 Volume 7 Number 07 (2018) .Journal homepage: http://www.ijcmas.com.

6. Asperger M, Zangeri I (2003). Isolation and Identification of Staphylococcus aureus from Bovine mastitis. Journal of Clinical Microbiology, 23: 456-477.

7. Bachaya H, Raza AMA, Murtaz S, Akbar IUR (2011). Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). J. Anim. Plant Sci. 21:16-19.

8. Barbano, D. M.; Ma, Y. and Santos, M. V. (2006). Influence of raw milk quality on fluid milk shelf life.J. Dairy Sci. 89:15-19.

9. Barrow, G. I. and Feltham, R. K. A (2003).Cowan and steel's Manual for the identification of Medical Bacteria. London: Cambridge university press

10. Begum HA, Uddin MS, Islam MJ, Nazir KHMNH, Islam MA, Islam MT (2007). Detection of biofilm producing coagulase positive Staphylococcus aureusfrom bovine mastitis, their pigment production, hemolytic activity and antibiotic sensitivity pattern. Journal of Bangladesh Society for Agricultural Science and Technology, 4: 97-100.

11. Bramley, A. J. (1982). Sources of Streptococcus uberisin the dairy herd I. Isolation from bovine feces and from straw bedding of cattle. J. Dairy Res. 49. 369.

12. Capurro A, Aspán A, Unnerstad HE, Waller KP, Artursson K (2010). Identification of potential sources of Staphylococcus aureus in herds with mastitis problems. Journal of Dairy Science, 93: 180-191.

13. Cheesbrough, M. (2000). District oratory practice in tropical countries, part 2.Cambridge university press. Comparison of microbial contaminationon sheep/goa

14. CLSI (2007). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute. M100-S17, Vol. 27 No. 1.
15. Ekici K, Bozkurt H, Isleyici O (2004). Isolation of some pathogens from raw milk of different milch animals. Pakistan Journal of Nutrition, 3: 161-162
16. Gundogan N, Avci E (2014). Occurrence and antibiotic resistance of Escherichia coli, Staphylococcus aureusand Bacillus cereus in raw milk and dairy products in Turkey. International Journal of Dairy Technology, DOI: 10.1111/1471-0307.12149.
17. Horwitch(2000): www.depts.washington.edu/eminf/2000/ resistance/ resist2. Html (accessed on 8 June 2007).
18. Hundera S, Ademe Z, Sintayehu A (2005). Dairy cattle mastitis in and aroundSebeta, Ethiopia. Intern. J. Appl. Vet. Med. 3(4): 1525-1530.
19. Jack, R. S. and Marianne, P.(1999). Food and Drug Administration Center for Safety and Applied Nutrition. September 1999.
20. Jim O'Neill.(2014) Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. The Review on Antimicrobial Resistance.
21. Kerro OD, Tareke F. 2003. Bovine Mastitis in selected areas of Southern Ethiopia. Trop. Anim. Health Prod. 35: 197-205.
22. Lili Zhang, Yuchen Li, HongduoBao, Ruicheng Wei, Yan Zhou, Hui Zhang,Ran Wang. 2016. Population structure and antimicrobial profile of Staphylococcus aureus strains associated with bovine mastitis in China. Microbial Pathogenesis 97: 103-109.
23. Mekonnen H, Workineh S, Bayleyegne M, Moges A, Tadele K. 2005.Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. Med. Vet. 176(7): 391-394.
24. NCCLS (2002): Performance standards for antimicrobial disk susceptibility test, approved standards 7th edition, vol 20.
25. Neu, H.C. (1992). The crisis in antibiotic resistance. Science, 257: 1064- 1073.
26. Patel, R.K., Kumar, R., Savalia, C.V and. Patel, N.G. 2018 Isolation of Staphylococcus aureus from Raw Cattle Milk and their Drug Resistance Pattern. Int.J.Curr.Microbiol.App.Sci. 7(2): 836-840.
27. Richard, K. R. (2002).Dairy Microbiology Hand Book.Third Edition. John Wiley and Sons Inc. xi.
28. Soltys, M.A. (1979): Introduction to Veterinary Microbiology. P 91-95. Penerbit Univerisiti Putra Malaysia, Serdang, Selangor.
29. Sørhaug, T. and Stepaniak.L. (1997). Psychrotrophs and their enzymes in milk and dairy products quality aspects.Trends Food Sci. Technol.8:35–40.
30. Tortora, G.J.,Funke, B.R. and Case, C.L. (1989) Microbiology: An introduction. 3rd ed p 478-479. Benjamin Cummings Publishing Company, Inc California USA
31. Van der Vanter, T. (1999); Prospects for the future: Emerging problem of chemical /biological Conference of International Food Trade Beyond 2000:Science based Decision, equivalence and mutual Recognition Melbourne, Australia, Pp.11-15.
32. Zakary E, Marionette M, Nassif Z, Mohammed GMO (2011). Detection of Staphylococcus aureus in Bovine milk and Its Product by Real Time PCR Assay Global Journal of Biotechnology & Biochemistry, 6: 171-177