ISOLATION AND IDENTIFICATION OF *Staphylococcus aureus* FROM BUFFALOES MILK INFECTED WITH SUBCLINICAL MASTITIS AND MILK WORKERS

Douaa A. Khaleel Rasha M. Othman Bassam Y. Khudaier

Department of microbiology, College of Veterinary Medicine,

University of Basrah, Basrah, Iraq.

(Received 10 November 2015 ,Accepted 9 December 2015)

Keywords: Mannitol salt agar, Buffaloes, *S. aureus*.

ABSTRACT

Atotal of 100/270 (37%) fermented Mannitol salt agar (MSA) isolates were obtained: 40/180 (22.2%) were from buffaloes milk with subclinical mastitis and 60/90 (66.7%) were from hands of milk workers. All suspected *Staphylococcus aureus* isolates which tested microscopically and biochemically were 15/100 (15%) of suspected isolates, 5/40 (12.5) from milk and 10/60 (16.7) from hands swabs of milk workers were diagnosed as *S. aureus*.

INTRODUCTION

Staphylococcus aureus is one of the most important pathogens in humans and animals [1]. It is an important food-borne pathogen involved in a variety of invasive diseases [2].There are many diseases affecting the milk yield of buffaloes. Of these, mastitis is at the top[3].Mastitis (inflammation of mammary gland) is one of the most devastating disease conditions leading to significant economic losses globally [4,5].Mastitis is the most common infectious disease affecting the dairy buffaloes and remains the most economically important disease of dairy industries around the world. A wide variety of bacteria can be involved, but the most common mastitis pathogens are *S. aureus* [6].Mastitis, which may be clinical (severe) or subclinical (moderate), is an important mammary gland disease that is usually caused by bacterial infection[7].*S. aureus* is frequently associated with subclinical mastitis and may contaminate milk and other dairy products [8].It is usually colonizes in the teat canal initially. After colonization, the bacteria adhere to the epithelium of ducts and alveoli in the gland and starts toxin production. The adherence of bacteria then stimulate

macrophage and migration of neutrophils from blood into the milk which will lead to high somatic cell number (SCC), swelling of the mammary gland, damage in the host defense system and epithelial cells [9].

S. aureus is able to produce a host of structural changes in udder and keeps on developing resistance against the most commonly used antibiotics .These resistant bacteria become part of the environment and are transmitted from animals to humans[10].*S. aureus* evolves resistance to many classes of antibiotics [11].The emergence of antibiotic-resistance *S. aureus* strains resulted in significant treatment difficulties which imposed burden on health care systems and simultaneously intensifying the need for new antibiotics [12].

The present study was conducted to isolation and identification *S. aureus* from buffaloes with subclinical mastitis and milk workers.

MATERIAL AND METHOD

Collection of Samples

One hundred and eighty milk samples were collected from buffaloes from different regions in Al-Basrah province .Prior to sampling, the California Mastitis Test (CMT) was carried out. Milk samples were collected from buffaloes milk after cleaning the udder from the grimes, bole and dirt by water and drying by a piece of clean cloth then used cotton moistened by alcohol 70% and removing the first flowage of milk and collecting 10 ml in sterile tube, transported by ice box immediately to the laboratory. Ninety samples were collected from humans hands swabs (milk workers),then direct transported to the laboratory.

Identification of *S.aureus*

Identification of *S.aureus* was carried out according to [13], each sample from milk and hand swab samples were directly inoculated onto mannitol salt agar (MSA) and incubated at 37 ºC for 24 hrs .Mannitol fermented colony from primary cultures were purified by subculture onto MSA medium and incubated at 37 ºC for 24-48 h.Gram stain slides were investigated according to [14] and biochemical tests that included catalase and coagulase tube were performed according to [15].

Biochemical Tests

Tube Coagulase Test

A 0.1 ml of 18-24 h bacterial cultured in brain heart infusion broth was added to 0.3 ml of rabbit plasma without dilution and incubated at 37ºC for 4 h. The clotting hourly noticed, then tubes was leaved at room temperature for 18-24 hrs. The appearance of the clotting indicates as a positive result comparable to control [15].

Catalase Test

The catalase test was carried out according to [15] as following: A small amount of pure growth was transferred with a wooden stick from MSA into clean slide, then a drop of catalase reagent was added. The evolution of gas bubbles indicates a positive result.

RESULTS

Table (1) shows the results of bacterial culturing on MSA 100 (37.03%) isolates out of 270 tested samples were suspected *S. aureus*. The percentage of frequency of *S. aureus* isolates were 40/180 (22.2%)and60/90 (66.6%) for buffaloes milk samples and humans hand swab samples, respectively. On the other hand the biochemical tests of*S. aureus* isolates revealed that the number and percentage of *S. aureus* isolates were 5/40(12.5%)for buffaloes milk and 10/60 (16.6%) in humans hands swabs ,Table (2).

Table (1): Prevalence of *S. aureus* Isolated from Buffaloes Milk and Hands Swabs From Milk Workers.

Type of sample	No. of samples	No of *S. aureus* fermented MSA	Percentage%
Milk from buffaloes	180	40	22.2
Hands swabs	90	60	66.6
Total	270	100	37.03

Table (2):Number and Percentage of *S aureus* with Biochemical Test.

Type of sample	*S. aureus* fermented MSA	Coagulase test (%)	Catalase test (%)
Buffaloes milk	40	5 (12'.5 %)	40 (100 %)
Hands swabs	60	10 (16.6 %)	60 (100 %)
Total	100	15 (15 %)	100 (100 %)

DISCUSSION

S. aureus is important milk borne pathogen and causes a wide variety of humans and animals diseases and it is frequently associated with subclinical mastitis in dairy animals and may contaminate milk and other dairy products which act as vehicles for *S. aureus* infection in humans[16].

S. aureus can be transmitted to humans through contaminated and untreated milk and milk products[17]. *S. aureus* presents on the skin and mucosa of food producing animal reservoirs that include ruminants and it is frequently associated with subclinical or clinical mastitis leading to the contamination of dairy products[18].

In present study (12.5 %) *S.aureus* strains were isolates from subclinical buffaloes mastitis that diagnosed by culturing, microscopically examination and biochemical tests, this percentage of *S.aureus*infection was similar to result that recorded by,[19 ,20,21] which were 10.23%, 10.9% and 15.62%, respectively. While the highest incidence of buffaloes subclinical mastitis were 78.12%; 58.33%; 48%;34.6%and 25.53%as reported (22, 23,24,25,26) respectively. The frequency of *S. aureus* isolated in the present study was 10 (16.66%) out of 60 hand swabs of dealers, these result is nearly to the result was reported by,[27].

In the other hand,[24] and,[28] were found more highest rates of *S. aureus* isolates from hand swabs of milk workers 70% and 56.52% ,respectively, in comparison with the rate of the present study, while [29] were found that 40% of *S.*

*aureus*in animal workers. Therefore, a comparison of the results of the present study and those reported by other authors is difficult because the occurrence of *S. aureus* as a causative agent of mastitis varies according to the area, handling practices of the animals and hygienic conditions during milking [30].To reduce the risk of the presence of *S. aureus* and other microorganisms in raw milk, it is necessary to gadget measures to reduce the prevalence of intramammary infections as well as increase the development of guidelines and support for dairy producers to improve production techniques that enhance the quality of milk in terms of microbiological, physical-chemical, sensual and nutritional aspects [31].

عزل وتشخيص المكورات العنقودية الذهبية من حليب الجاموس المصاب بالتهاب الضرع تحت السريري وعمال الحليب

دعاء عبد الرزاق رشا منذر عثمان بسام ياسين خضير

فرع الاحياء المجهرية ،كلية الطب البيطري ، جامعة البصرة ، البصرة ، العراق

الخلاصة

تم جمع 270 عينة، 180 عينة من حليب الجاموس بالتهاب الضرع تحت السريري و90مسحة من أيدي عمال الحليب من مختلف المناطق في مدينة البصرة. تم فحص جميع العينات لوجود بكتريا المكورات العنقودية الذهبية بوساطةزرعها على الوسط الملحي المانيتول (MSA). تم الحصول على 270/100 (37٪) من العزلات المخمرة لوسط المانيتول : 180/40 (22.2٪) من عينات الحليب و 90/60 (66.7٪) من ايدي عمال الحليب. تم فحص جميع عزلات المكورات العنقودية الذهبية المشتبه بها مجهريا وكيميائيا، حيث كانت 100/15 (15٪) من العزلات المشتبه بها كانت 40/5 (12.5) من الحليب و 60/10 (16.7) من مسحات من ايدي عمال الحليب تم تشخيصها كمكورات عنقودية ذهبية.

REFERENCES

1-Haghkhah, M. (2003). Study of Virulence Factors of *Staphylococcus aureus.* PhD. Thesis, Faculty of Biomedical and Life Sciences University Glasgow,Scotland, UK.

2-Morandi, S.; Brasca, M.; Andrighetto, C.; Lombardi, A. and Lodi R.(2009). Phenotypic and genotypic characterization of*Staphylococcusaureus* strains from Italian dairy

products. *International Journal of Microbiology*.:1–7. [PMC free article][PubMed].

3-Radostits, O. M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W.(2000). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9thEdn., W.B. Saunders Co., Philadelphia, USA., pp: 603-610.

4-Kumar, A.; Rahal, A.; Dwivedi, S. K. and Gupta, M.K.(2010). Bacterial Prevalence and Antibiotic Resistance Profile from Bovine Mastitis in Mathura, India. Egypt. *Journal of Dairy Science*. 38: 31-34.

5-AbdEllah, M. R. (2013). Role of Free Radicals and Antioxidants in Mastitis. Journal of *Advanced Veterinary* Research. 3: 1-7.

6-Chiang, Y. C.; Liao, W.W.; Fan, C. M. ; Pai, W. Y. ; Chiou, C.S. and Tsen, H. Y. (2008). PCR Detection ofStaphylococcal Enterotoxins (SEs), N, O, P, Q, Rand U and Survey of SE Types in *S. aureus*Isolatesfrom Food Poisoning Cases in Taiwan. *International Journal of Food Microbiology*.121(1): 66-73.

7-Seegers, H., C. Fourichon, and F. Beaudeau. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary* Research. 34:475–491.

8-Jones, F. T.; Creech, B. C.; Erwin, P.; Baird, G. S.; Woron, A. M. and Schaffner, W. (2006). Family Outbreaks of Invasive Community Associated Methicillin Resistant *Staphylococcus aureus* Infection. Clinical Infectious Diseases.42: 76-78.

9-Cucarella, C.; Tormo, M. A.; Peris, C.; Ubeda C.; Amorena, B.; Trotonda, M. P.; Lasa, I.; Monzon, M. and Penades, J. R. (2004). Role of Biofilm Associated Protein Bap in The Pathogenesis of Bovine *Staphylococcus aureus*, J. Infec. Immunol., 72: 2177-2185 http://dx.doi.org/10.1128/IAI.72.4.2177-2185.2004 PMCid:375157.

10-Booth, M.C.; Pence, L.M.; Mahasreshti, P.;Callegan, M. and Gilmore, M.(2001). Clonal Associations Among *Staphylococcus aureus* Isolates from Various Sites of Infections. Infect. Immun. 69(1): 345–352.

11-Aires de Sousa, M.; Conceicao, T.; Simas, C.; de Lencastre, H.(2005). Comparison of Genetic Backgrounds of Methicillin-Resistant and Susceptible *Staphylococcus aureus* Isolates from PortugueseHospitals and The Community. Journal of Clinical Microbiology.43(10): 5150–7.

12-Pravin R. D.; Anjana D. G. and Rajesh K. P.(2011). Phenotypic characterization and antibiotics combination approach to control the methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from the hospital derived fomites. *Asian Journal of* Medical Sciences.:72–8.

13-Talan, D. A. ; Staatz, D. ; Staatz, A. ; Goldstein, E. J. C. ; Singer, K. and Ocrturf, G. D. (1989). *Staphylococcus intermidius* in canine gingival and canine-infected human wound infections: Laboratory characterization of newly recognized zoonotic pathogen. J. Clin. Microbiol. 27:78-81.

14-Barrow and Feltham .(2003). Cowan and Steel's. Manual for the Identification of Medical Bacteria. 2nd Ed. Cambridge University press Cambridge, London, New York.USA.

15-Macfaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd Ed. Lippincott Williams and Wilkins USA.

16-Bharathy, S.; Gunaseelan, L.; Porteen, K. and Bojiraj, M. (2015). Prevalence of *Staphylcoccus aureus* in Raw Milk: Can it be a Potential Public Health Threat? International Journal of Advanced Research. Volume 3. Issue 2: 801-806

17-Seifu, E. ; Buys, E. M.; Donkin, E. F. and Petzer, I. M.(2004). Antibacterial activity of the lactoperoxidase system against food-borne pathogens in Saanen and South African indigenous goat milk. Food Control. 15: 447-452.

18-Arbeit, D.(1988). Laboratory Procedures for The Epidemiological Analysis of Staphylococci. In: Archer G, Crossley T. (eds.) Staphylococci and Staphylococci Diseases. New York: Churchill Livingstone; p. 203-286.

19-Khudaier, B. Y.; Anad, I. T. and Abbas, B. A.(2014). Isolation of *Staphylococcus aureus* from Buffalo Milk in Basra Governorate and Detection of Their

Antibiotic Susceptibility.Basrah Journal of Veterinary Research Vol.1,No.1:235-245.

20-Abd El-Hamid, M. I. and Bendary, M. M. (2013). Association between agr Alleles and Toxin Gene Profiles of S. aureus Isolates from Human and Animal Sources in Egypt. International Journal of Advanced Research,Volume 1, Issue 8, 133-144.

21-Abd El-Razik, K. A.; Abdelrahman, K. A.; Ahmed, Y. F.; Gomaa, A. M. and Eldebaky, H. A.(2010). Direct Identification of Major Pathogens of the Bubaline Subclinical Mastitis in Egypt using PCR.Journal of American Science 6(10) :652-660.

22-Hamed, M. I. and Zaitoun, A. M. A.(2014). Prevalence of *Staphylococcus aureus* Subclinical Mastitis in Dairy Buffaloes Farms. International Journal of Livestock ResearchVol. 4(3):21-28.

23-Prabhu, K. N.; Ruban, W. S.; Kumar, G. S. N.; Sharada, R.andPadalkar, R. D.(2015). Sub-clinical Mastitis in Buffaloes: Prevalance, Isolation and Antimicrobial Resistance of *Staphylococcus aureus*.Buffalo Bulletin Vol.34 No.2:215-222.

24-Sarkar, P.; Mohanta, D.; De, S. and Debnath, C.(2014).*Staphylococcus aureus* in Dairy Animals and Farm Workers in a Closed Herd in Karnal, North India: Assessment of Prevalence Rate and Coa Variations. International Journal of Innovative Research in Science, Engineering and TechnologyVol. 3, Issue 4:10962-10972.

25-Torky, H. A. and Kotb, S. E.A.(2013).The impact comparison of *Staphylococcus aureus* mastitis on the level of milk nitric oxide, immunoglobulin A and complement 3 between cows and buffaloes. New York Science Journal 6(11):90-95.

26-Jahan, M.; Rahman, M.; Parvej, M. S.; Chowdhury, S. M. Z. H.; Haque, M. E.; Talukder, M. A. K. and Ahmed, S.(2015). Isolation and Characterization of *Staphylococcus aureus* from Raw Cow Milk in Bangladesh. Journal of Advanced Veterinary and Animal Research 2(1): 49-55.

27-El-Gedawy, A. A., Ahmed, H. A. and Awadallah, M. A. I.(2014). Occurrence and molecular characterization of some zoonotic bacteria in bovine milk, milking equipments and humans in dairy farms, Sharkia, Egypt.International Food Research Journal 21(5): 1813-1823.

28-Abdel All, A. A. A.; Bashandy, M. M.;Yasin, M. H. and Ibrahim, A. K.(2010). Assessment of Conventional and Molecular Features of Staphylococcus aureus Isolated from Bovine Milk Samples and Contact Dairy Workers.GlobalVeterinaria 4 (2): 168-175.

29-Elhaig, M. M. and Selim, A.(2015). Molecular and bacteriological investigation of subclinical mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae* in domestic bovids from Ismailia, Egypt. Trop Anim Health Prod 47:271–276.

30-Jayarao, B. M.; Pillai, S. R.; Sawant, A. A.; Wolfgang, D.R. and Hegde, N.V. (2004): Guidelines for Monitoring Bulk Tank Milk Somatic Cell and Bacterial Counts. *Journal of Dairy Science*. 87(10): 3561–3573.

31-Zecconi, A., Cesaris, L., Liandris, E., Daprà, V. and Piccinini, R. (2006). Role of Several *Staphylococcus aureus* Virulence Factors on the Inflammatory Response in Bovine Mammary Gland. Microbial Pathogenesis,40,177-183. http://dx.doi.org/10.1016/j.micpath.2006.01.001