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Faculty of Veterinary Medicine
Department of Microbiology

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Containment principals of *S. aureus* isolated from mastatic buffaloes

Thesis presented by

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(B. V. Sc., 2003)
(M.V.Sc., 2008)

For fulfilling the degree of PhD.
(Bacteriology-Immunology-Mycology)

Under the Supervision of

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(Bacteriology, Immunology and Mycology)

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Abstract

Prevalence of mastitis among the examined farms showed that 183 animals out of 352 buffaloes showing signs of mastitis with a percentage of 51.98% distributed on the farms included as follow 53.91%; 67.50%; 26.92%; 38.09 % and 53.57% respectively. Results showed that, the percentage of quarters showing clinical mastitis were 23.53%; 17.65%; 22.46% and 36.36 %. Results obtained were 31.3%; 29.1%; 18.2% and 21.4% at the right hind, left hind, right fore and the left fore respectively. Obtained results revealed that 84 isolates of *S. aureus* from overall 183 mastitic animals with an incidence of 45.90% meanwhile, the incidence of isolation according to the total number of quarters 374 was 22.46%. The distribution of isolation from mastic animals and mastitic quarters were 43.55%;21.60,59.26%; 24.43, 14.29%; 13.33, 12.5%; 11.11% and 48.89%; 23.40 from Farms 1, 2, 3, 4 and 5 respectively. Regarding the virulence factors among the examined isolates, out of 84 *S. aureus* isolates only 39 were toxigenic 46.43% including 12 isolates had enterotoxin (A) 30.77% and the remaining 27 isolates had enterotoxin (D) 69.23%; all isolates of *S. aureus* had each staph protein A with an incidence of 100%; coagulase activity and hemolytic activity showed that all isolates were hemolytic (100%), 69 isolates (82.14%) were β hemolytic, and 15 isolates were α hemolytic (17.86%). Results of Polymerase chain reaction indicated that 5 isolates of *S. aureus* representing the five farms proved by culturing and biochemically were used for the detection of virulence factors and the results obtained showed that all isolates used (5) were positive to *16sRNA; sed; spa* and *coa, m*eanwhile 2 isolates were carrying both genes for *hlb* & *icaA.* Biosafety measures were assessed on our study using WHO hecklist (biosafety Level 1 and 2) plus data supported from material safety data sheet. Assessment of biosecurity among the 5 selected farms indicating that one farm 3 warned potential for failure of a biosecurity element and some action required, one farm 4 ; safe- meeting basic biosecurity practices and no action required and three farms (1, 2 and 5) alert biosecurity gap identified, action required. Correlation was exixted between the level of biosecurity and prevalence of *S. aureus* isolation and the results obtained clear it indicating that good biosecurity measure showing the lowest percentage of isolation *S. aureus* (12.5%) in farm 4, followed by farm 3 (14.29%) , farm1 (43.55%), farm 5 (48.89%) and farm 2 (59.26%) .

Dedicated to;

My dear parents,

dear husband : Shenouda ,

dear brothers
Maged,Ehab,Micheal

and

lovely son :Fady

lovely daughter :Maria

for their encouragement, efforts, support and endless help through out the whole work.

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1. Introduction

Mastitis is a multietiologic disease of the mammary gland characterized mainly by reduction in milk production and milk quality due to intramammary infection by pathogenic bacteria **(Harjanti *et al*., 2018).** The economic losses from mastitis due to severe drop in milk production, potential health risks for other animals and human beings, increased cost of treatment and culling processes, are tremendous **(Dhakal and Thapa, 2002).** Moreover, bacterial agents that are involved in bovine mastitis may represent a health risk for the human population via the food chain **(Kadariya *et al*., 2014).**

Among the animal diseases which affect the profitability of rearing animals, mastitis is considered to be one of the expensive diseases in terms of production losses **(Bardhan, 2013).** The losses are the potential revenues not earned, while the control costs are actual expenditures related to treatments, preventive measures, and additional labour used by them **(McInerney *et al*,. 1992).** The economic calculations of production losses and knowledge of the cost component are very essential in farmer's decision to develop control mechanism. Many studies have been conducted on preventive and microbial aspects of this disease as well as simulative form and few studies are based on data of the field farms to estimate production related losses and treatment costs **(Hogeveen , 2005 and Selvaraju *et al*., 2013) .**

Staphylococci are very common human and animal pathogens. A variety of staphylococcal virulence determinates leads to vast range of infections. One of them is mastitis which is a common disease of the mammary glands. The incidence of this disease is widespread all over the world and depends on bacterial virulence and on prevention programs **(Lisowska-lysiak *et al*., 2018).**

Raw milk contaminated with *Staphylococcus aureus* at elevated temperatures can result in the production of staphylococcal enterotoxins, especially type A (*SEA*),

which is most frequently associated with food poisoning outbreaks **(Sabike *et al.*, 2014).**

Biofilm production by the microorganisms is considered an important virulence factor responsible for adhesion of these microorganisms with living or non-living surfaces. *Staphylococcus aureus* isolates which produce biofilm lead to chronic mastitis in dairy animals**.** The ability of *S. aureus* to grow and produce *SEs* under a wide range of conditions is evident by the variety of foods that have been implicated in *Staphylococcus aureus* Food Poisoning (SFP) **(Rosengren *et al*., 2010).**

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Pathogenesis of mastitis may be caused by extracellular toxins, enzymes and surface antigens **(O'Riordan and Lee, 2004).** Coagulase gene of *S. aureus* is considered an important virulence factor. Amplification of *S. aureus* coagulase gene *(coa)* has been recommended as an accurate method for identification of virulent strains of *S. aureus* **(Morandi *et al.*, 2010)** Sequencing of the coagulase gene shows great diversity in *S. aureus* population **(Costa *et al.*, 2012).** Information regarding the genetic diversity of *Staphylococcus aureus* isolated from mastitis in cow is available but such information regarding *S. aureus* from buffalo mastitis is limited **(Firyal *et al.*, 2009).** Various studies described that bovine mastitis is caused by a wide variety of *Staphylococcus aureus* genotypes **(Smith *et al.*, 2005)** and *Staphylococcus aureus* from mastitis represents a genetic heterogeneity **(Fournier *et al.*, 2008).**

Milk is a good substrate for *S. aureus* growth, and milk and milk products have been the source of many SFP outbreaks **(Loncarevic *et al*., 2005).** Contamination with *S. aureus* may be attributed to occurrence of *S. aureus* in raw milk or handling during the manufacturing process.

Staphylococcus aureus is responsible for variety of infections in human and animals **(Bartlett and Hulten, 2010; Gu *et al.*, 2013)** and treatments become more difficult due to its emerging strains. Biosafety is a discipline that focuses on the safe handling and containment of infectious microorganisms and hazardous biological materials. Recently, research on infectious pathogens has been on the rise due to the