Cairo University

Faculty of Veterinary medicine
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Staphylococcus aureus as a causative agent of subclinical mastitis in dairy farms with special reference to MRSA at El- Gharbia Governorate

Thesis Presented by

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(Hygiene and Control of Milk and its products)

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ABSTRACT

***"Staphylococcus aureus* as a causative agent of subclinical mastitis in dairy farms with special reference to MRSA at El- Gharbia Governorate." Marwa Nabil Sayed, Cairo Univ. Fac. Vet. Med. Master; Food Hygiene and Control, 2018.**

Three hundred and fifty quarter milk samples were collected from apparently healthy cows at Al-Gharbia governorate, Egypt. Samples were tested using California Mastitis Test (CMT) and somatic cell count, of which 235 samples were detected as positive. Overall, 168 quarter milk samples (71.48%) were found to be contaminated with coagulase positive *Staphylococcus aureus* with a mean value of $64x10^3 \pm 19x10^3$cfu/ml. Isolated *Staphylococcus aureus* strains were tested for methicillin to identify Methicillin resistant strains (MRSA). Antibiotic sensitivity test was carried out by using two antibiotic disks against fourty six identified *Staphylococcus aureus* isolates. The obtained results indicated that resistance against cefoxitin was 34.78% and sensitivity was 65.2٢%, while the resistance against vancomycin was 26.08% and sensitivity was 73.9٢%. PCR technique was used to detect presence of mecA gene that coded for penicillin-binding protein 2a. The results on sixteen positive isolates which suspected to have mecA gene by antibiotic sensitivity test were 93.75%. The total of subclinical mastitis cases infected with MRSA was 31.11%. The results provided evidence that the presence of *Staphylococcus aureus*, as well as Methicillin–resistant strains (MRSA) have become remarkably widespread in subclinical mastitic quarter milk samples. This calls for better control of the sources of milk contamination as well as spread of antimicrobial resistance organisms.

Key words: Subclinical mastitis, California mastitis test, *Staphylococcus aureus* and MRSA.

DEDICATION

To my Mother and Father, the reason of what I became today. thanks for your great support and continuous care and all you have done for me.

MY husband Amir for your patience, encouragement and support all the time.

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INTRODUCTION

Milk has long been referred to as the most perfect food for human from birth to senility, as it contains all the nutrients required for rapid growth and healthy development of the body. When the milk is drawn from the udder of a healthy animal, it contains large number of microorganisms that have entered the teat canal through its opening. They are mechanically flushed out during milking. The number of microorganisms is ranged between several hundred to several thousand per milliliter **(Farzana et al., 2004 and Oliver et al., 2005)**.

Bovine mastitis is an inflammation of the udder of dairy cows that can occur in one or multiple quarters. It costs the dairy industry great economic losses due to reduced milk production and alteration of its quality. Moreover, causative agents of mastitis with zoonotic potential may represent a health risk for human populations via the food chain **(Le Marechal et al., 2011 and Awale et al., 2012).**

Subclinical mastitis is more prevalent than clinical one. The subclinical form is difficult to detect because there are no apparent symptoms. It is mainly characterized by an increase in milk somatic cell count (MSCC) and decrease in milk production **(Hogeveen et al., 2011 and Yang et al., 2017)**.

Animals are considered to have a subclinical mammary gland infection if milk contains a threshold of milk somatic cell count more than 2×10^5 cells/mL **(Idriss et al.,2013b and Yang et al., 2017)**.

Subclinical mastitis causes a great economic loss in dairy industry**.** Milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing **(Zdunczyk et al., 2003 and Abdel- Rady&Sayed 2009).**

Cows with subclinical mastitis considered as a source of infections within herds with particular pathogens that can be spread via automatic milking systems **(Barkema et al., 2009 and Hovinen and Pyorala 2011**).

Because subclinical mastitis is one of the most costly and troublesome diseases in dairy cows in Egypt, it is considered of vital importance in its association with many pathogens causing public health hazard **(Seleim et al., 2002)**.

California Mastitis Test (CMT) has a useful role in the dairy herd monitoring programs as a simple, inexpensive and rapid screening test to detect dairy cows with subclinical mastitis **(Sargeant et al., 2001).** When CMT being regularly performed as a control measure, significantly lower risks of subclinical mastitis being observed **(Busato et al.,2000).**

Milk Somatic cell counts (MSCC) are expressed as the number of somatic cells in a millilitre (ml) of milk. The somatic cells found in milk are primarily leukocytes, or white blood cells which include phagocytes, lymphocytes and body cells that enter from animal´s blood stream. During mastitis the major increase in MSCC is due to the influx of neutrophils to the milk to fight infection. It is widely accepted criterion as a better tool for monitoring subclinical mastitis **(Rysanek et al., 2007).**

Bacteria are by far the most common cause of mastitis. Although mastitis can be caused by 137 different microorganisms, the epidemiology of bovine mastitis has been characterized worldwide by an increase in the prevalence of Staphylococci. *Staphylococcus aureus* is the most frequently isolated, coagulase-positive microorganism among the forty-four-species belonging to the Staphylococci family and is considered the main cause of chronic and subclinical mastitis **(Hussain et al., 2012; Raza et al., 2013 and Jaradat et al., 2014)**.

Staphylococcus aureus is known as the most important pathogens in human and animal medicine due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. This bacterium is a significant cause of nosocomial infections, as well as community-acquired-diseases It is a common member of the natural microflora of human skin and nasal passage. In addition, as a potential pathogen, it may adversely affect human and animal health by causing sever necrotic lesions, abscesses and bacteremia (**Hermans et al., 2004 and Hanson et al., 2011).**

Staphylococcus aureus intoxication ranked third of food poisoning cases all over the world and had been implicated with different categories of food including raw milk **(Asao et al., 2003** and **Jorgensen et al., 2005a).** Staphylococcal food poisoning, rely on one single type of virulence factor, the SEs. Contamination of food with enterotoxin toxigenic Staphylococcus can occur, directly from infected food producing animal or at any stage of food production, processing, transportation, storage or retail as a result of poor personal hygiene that leads colonized individuals (30-50%) to provide the main source for the dissemination of Staphylococci. *Staphylococcus aureus* possesses several proteins which are virulent in nature and can pose an elevated health hazard **(Maksymiec &Mikolajczyk, 2012 and Guimarães et al., 2017).**

Coagulase is considered the most important virulence factors that clot plasma and coats the bacterial cell, so prevent the phagocytosis **(Panizzi et al.,2004; Kalorey et al., 2007 and Cengiz et al., 2015)**. Currently, the main therapy for subclinical mastitis is the administration of antibiotics; but, this approach is associated with a risk of the development of antimicrobial resistant bacteria. There are many factors associated with an increase in drug resistance such as in correct treatment of the pathogens, overuse or misuse of antibiotics that do not quickly kill microorganisms. Therfore, it is important to regularly test sensitivity to these antibiotics and determine their continued efficacy in treating bacterial diseases (**Quinn et al., 2002 and Ventola, 2015**).