**Faculty of Veterinary Medicine**
**Food Hygiene & Control Department**

**Cairo University**

# *Staphylococcus aureus* in raw and pasteurized milk: phenotypic & molecular studies

**Presented by**

**Aml Elsayed Ibrahim**

B.V.Sc. (2013), Cairo University

**For the degree of master in Veterinary Medical Science**

**(Hygiene and Control of Milk and its products)**

**Under the Supervision of**

**Dr. Nagah Moustafa Hafiz**

Professor of Milk Hygiene and Control

Faculty of Veterinary Medicine

Cairo University

**Dr. Mena Fouad Saad**

Lecturer of Milk Hygiene and Control

Faculty of Veterinary Medicine

Cairo University

**Dr. Adel Sayed Amin**

Chief Researcher and Head of Biotechnology Research Unit

Animal reproduction research institute

**(2017)**

Cairo University
Faculty of Veterinary Medicine
Food Hygiene & Control Department

# APPROVAL SHEET

**This is to approve that thesis presented by:**

**Aml Elsayed Ibrahim**

**Entitled: *Staphylococcus aureus* in raw and pasteurized milk: phenotypic & molecular studies.**

**For the degree of master**

**(Hygiene and Control of Milk and its products)**

## APPROVAL COMMITTEE

**Prof. Dr. Mohamed Ahmed Mansour**
Professor of Milk Hygiene& Control
Faculty of Veterinary Medicine
Zagazig University

**Prof. Dr. Said Sayed Elsayed Sallam**
Professor of Milk Hygiene& Control
Faculty of Veterinary Medicine
Cairo University

**Prof. Dr. Nagah Mostafa Hafiz (Supervisor)**
Professor of Milk Hygiene& Control
Faculty of Veterinary Medicine
Cairo University

**Faculty of Veterinary Medicine**
**Food Hygiene & Control Department**

**Cairo University**

# SUPERVISION SHEET

**Dr. Nagah Moustafa Hafiz**

Professor of Milk Hygiene and Control

Faculty of Veterinary Medicine

Cairo University

**Dr. Mena Fouad Saad Faheem**

Lecturer of Milk Hygiene and Control

Faculty of Veterinary Medicine

Cairo University

**Dr. Adel Sayed Amin**

Chief Researcher and Head of Biotechnology Research Unit

Animal reproduction research institute

**(2017)**

**Faculty of Veterinary Medicine**
**Food Hygiene & Control Department**

**Cairo University**

***Abstract***

**Name:** Aml Elsayed Ibrahim
**Date and place of birth:** 8/5/1991, Cairo.
**Nationality:** Egyptian.
**Degree**: master in Veterinary Medicine Science.
**Specialty:** Food hygiene and control (Milk and its products).
**Supervisors:**
**Dr. Nagah M. Hafiz**
Professor of Milk Hygiene and Control, Faculty of Veterinary Medicine, Cairo University.
**Dr. Mena F. Saad**
Lecturer of Milk Hygiene and Control, Faculty of Veterinary Medicine, Cairo University.
**Dr. Adel Sayed Amin**
Chief Researcher and Head of Biotechnology Research Unit Animal reproduction research institute

**Title of thesis**: *Staphylococcal aureus* in raw and pasteurized milk: phenotypic & molecular studies.

## Abstract

**" *Staphylococcal aureus* in Raw and Pasteurized Milk: Phenotypic & Molecular Studies. " Aml Elsayed Ibrahim, Cairo Univ. Fac. Vet. Med. master; Food Hygiene and Control, 2017.**

Fifty raw milk samples and sixty pasteurized milk samples (30 unflavoured and 30 flavoured) were obtained from shops and supermarkets in Cairo and Giza Governorates, Egypt. Staphylococci were present in all of the examined samples, with mean count of $7.0x10^6$, $5.5x10^3$ and $3.5x10^5$ CFU/ml of raw, unflavoured and flavoured pasteurized milk, respectively. Coagulase Positive Staphylococci was present with mean count of $2.7x10^6$, $1.4\ x10^3$ and $6.4\ x10^2$ CFU/ml of raw, unflavoured and flavoured pasteurized milk, respectively. The Coagulase Positive Staphylococci (CPS) and TNase isolated from the examined raw milk samples were in a percentage of 38.06 and 30.77, respectively. While CPS isolated from the examined unflavoured and flavoured pasteurized milk were in a percentage of 30.21 and 19.11 respectively. CPS beta hemolytic isolates of raw milk samples were found in a percentage of 74.47, while in unflavoured and flavoured pasteurized milk were found in a percentage of 44.83 and 16.67 respectively. The CPS isolates of examined milk samples gave 100.00% positive for staphytect plus test. Coagulase Positive *S. aureus* was found in raw, unflavoured and flavoured pasteurized milk samples in a percentage of 84.04, 68.97 and 36.67 respectively based on mannitol test. With applying of multiplex PCR on *S. aureus* isolates which were positive with mannitol test, all *S. aureus* isolates were positive encoding *16s rRNA* gene but that encoding *nuc* gene were found in raw, unflavoured and flavoured pasteurized milk samples in a percentage of 74.05, 50.00 and 45.45 respectively. Isolates positive β- lactamase inhibitors were found in raw, unflavoured and flavoured pasteurized milk samples in a percentage of 41.88, 90.00 and 100.00 respectively, while that positive MRSA were found in a percentage of 29.06, 20.00 and 0.00 respectively. The percentage of positive isolates for toxin production was (8.1) of SEE toxin and (2.7) of SEC toxin. By applying statistical study for sensitivity and specificity of the previous tests, Sensitivity and specificity of coagulase, β-hemolytic, mannitol and staphytect plus tests were 100% and 50% respectively, while TNase was 62.5% sensitivity and the highest 100% specificity. The percent of unacceptable samples were 68.00% and 10.00% in raw and pasteurized milk samples respectively based on Egyptian standards.

**Key words:** raw milk, pasteurized milk, phenotypic, PCR, *S. aureus*.

# *Dedication*

*To*

*My Mother and Father*

*My Husband*

*My Sister*

*&*

*My friends*

## *Acknowledgment*

*I am grateful to the Almighty God the most beneficent for this graciousness bestowed upon me and the opportunity given to study and complete this master.*

*No words adequately express my sincere gratitude and great appreciation to my supervisor* ***Prof. Dr. Nagah Moustafa Hafiz*** *who offered me a lot of her time and devoted her experience to provide me the best possible piece of advice and suggestion to complete this work.*

*My deepest thanks to my supervisor* ***Dr. Mena Fouad Saad*** *for his valuable help, criticism, unfailing efforts and kind advice.*

*And my deepest thanks to my supervisor* ***Prof. Dr. Adel Sayed Amin*** *for his help in biotechnology department.*

*With special thanks to* ***Prof. Dr. Sabry Darwish Morgan, Dr. Lamiaa Ibrahim Ahmed*** *and all members of Food Hygiene & Control Department for their encouragement and help.*

*Special thanks to Dr,* ***Ameen Ibrahem AlHababi*** *who provided me with Staphytect Plus Test kits.*

*Deep thanks to all member of Biotechnology Research unit, Animal Reproduction Research Institute, for their help and great advice.*

*Finally I would like to thank my family and my husband for their love and support*

# LIST OF CONTENTS

# LIST OF TABLES