Cairo University

Faculty of Veterinary Medicine

Microbiology Department

"Phenotypic and Genotypic characterization of Staphylococcus species"

A Thesis presented by

Noha Elsayed Aref Abd El-Megeed Abd El-Dayem

B.V.Sc. (1995) M.V.Sc. (2011)

For the degree of PhD thesis. In Veterinary Medical Science, (Bacteriology, Immunology and Mycology)

Under the Supervision of

Prof. Dr. Jakeen Kamal Abdel-Haleem El-Jakee

Prof. of Microbiology and Vice Dean for graduate studies and research affairs, Faculty of Veterinary Medicine, Cairo University, Egypt

Dr. Ahmed Samir

Ass. Prof. of Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt

Dr. Alaa Mohamed Gomaa Bayoumi

Chief Researcher and Head of Mastitis and Neonates disease Department, Animal Reproduction Research Institute

Dr. Samah Fekry Mohamed

Chief Researcher, Biotechnology Research unit, Animal Reproduction Research Institute

2017

Supervision committee

Prof. Dr. Jakeen kamal Abdel-Haleem El-Jakee

Prof. of Microbiology and Vice Dean for graduate studies and research affairs Faculty of Veterinary Medicine, Cairo University, Egypt.

Dr. Ahmed Samir

Ass. Prof. of Microbiology Department Faculty of Veterinary Medicine, Cairo University, Egypt.

Dr. Alaa Mohamed Gomaa Bayoumi

Chief Researcher and Head of Mastitis and Neonates disease Department Animal Reproduction Research Institute (ARRI).

Dr. Samah Fekry Mohamed

Chief Researcher, Biotechnology Research unit Animal Reproduction Research Institute (ARRI).

Cairo University Faculty of Veterinary Medicine Microbiology Department

Name: Noha El Sayed Aref Abd El Megeed Abd Eldayem.

Date of birth: 5 / 1 / 1972 **Place of birth:** Giza

Degree: PhD in Veterinary Medical Sciences.

Specialization: Microbiology "Bacteriology, Immunology and Mycology"

Supervisors:

Prof. Dr. Jakeen kamal Abdel-Haleem El-Jakee

Prof. of Microbiology and Vice Dean for graduate studies and research affairs Faculty of Veterinary Medicine, Cairo University, Egypt.

Dr. Ahmed Samir

Ass. Prof. of Microbiology Department Faculty of Veterinary Medicine, Cairo University, Egypt.

Dr. Alaa Mohamed Gomaa Bayoumi

Chief Researcher and Head of Mastitis and Neonates disease Department Animal Reproduction Research Institute (ARRI).

Dr. Samah Fekry Mohamed

Chief Researcher, Biotechnology Research unit Animal Reproduction Research Institute (ARRI).

Title of thesis: "Phenotypic and Genotypic characterization of Staphylococcus species"

Abstract

In the present investigation, The confirmation of 45 strains of coagulase negative Staphylococcus isolates was studied among mastitic cows, buffaloes. 23 from cow milk and 22 from buffalo milk. In addition to 18 S.aureus isolates, 11from cow milk and 7 from buffalo milk. S.aureus was isolated from the examined cow and buffaloes samples in pure culture (61.1 and 38.9 % respectively). CNS were isolated from the examined cow and buffaloes pure cultures was 51.1 and 48.9 % respectively. Identification of isolates was achieved by using convetional identification methods, the API staph ID test (BioMerieux) and PCR amplification. 14 S. xylosus. 12 S. hominis. 7 S. lugdunensis. 6 S. cohnii, 4 S. saprophyticus, 1 S. simulans, 1 S. lentus strains were confirmed. All B lactamase CNS producers (100%) detected from the examined buffaloe milk samples were resistant to penicillin. While 72.7% of B lactamase CNS producers detected from the examined cows were resistant to it. Most of CNS isolates were sensitive to neomycin, gentamicin, florfenicol and erythromycin. Mean while the examined isolates were resistant to cloxacillin, amoxycillin, ampicillin, penicillin and oxacillin. 52.2% and 18.2% CNS isolated from cows and buffaloes respectively were toxin (A) producers, while 4.35% and 4.5% respectively were toxin (A+D) producers, And 4.35% isolated from cows were toxin A+C+D and 4.5% isolated from buffaloes were toxin A+C producers. Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) showed the protein profile analysis of 41 CNS strains. It obvious that protein profile of CNS strains is separated into 1-12 fractions. The proportion of similarity in the samples that were separated ranged between 88% and 97%

Key word: Coagulase negative staphylococci (CNS)- Bovine Mastitis- Gel Electrophoresis -Toxin.

Acknowledgment

First of all, I greatly indebted in all my work and success to our gracious Allah.

No word can express my high appreciation, deep gratitude and sincere thanks to Prof. **Dr. Jakeen Kamal abd El-Haleem El-Jakee**, Prof. of Microbiology and Vice Dean for graduate studies and research affairs, Faculty of Veterinary Medicine, Cairo University, Egypt for her valuable and keen supervision, ideal guidance, great support, constructive criticism and continual encouragement throughout the course of this study.

I would like to thank **Dr. Ahmed Samir** Ass. Prof. of Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt, for his supervision, for his support, providing the facilities throughout the course of this work.

I am greatly indebted to **Dr. Alaa Mohamed Gomaa Bayoumi** Head of Mastitis and Neonates disease Department, Animal Reproduction Research Institute, for his supervision, encouragement and great help and advice throughout the work.

I also would like to present all thanks to **Dr. Samah Fekry** Mohamed Chief Researcher in Biotechnology Research Unit, Animal Reproduction Research Institute, for her supervision, cooperation, sincere guidance and help in performing the PCR assay.

I express my thankes to **Dr. Ibrahim Gad** head of Animal Reproduction Research Institute for his supportive attitude and sustained encouragement.

I am also grateful to **Dr. Inas M. Gamal** and **Dr. Noha abdelkhalek** in Animal Reproduction Research Institute for their kindness effective and great help during this work.

CONTENTS

Subject	Pages
INTRODUCTION	1
Aim of study	5
REVIEW OF LITERATURE	6
2.1. History and Classification of <i>Staphylococcus</i> spp	6
2.2. Importance of <i>Staphylococcus</i> spp	7
2.3. Identifecation of <i>Staphylococcus</i> spp	20
2.3.1 Phynotypic Identifecation of <i>Staphylococcus</i> spp	20
2.3.2 Genotypic Identifecation of <i>Staphylococcus</i> spp	27
2.4. Sensitivity of Coagulase negative staphylococci to chemo therapeutic agents	37
2.5. Protein profile analysis of <i>Staphylococcus</i> spp	46
MATERIALS & METHODES	49
3.1. Materials	49
3.1. 1. Isolates	49
3.1. 2. Standard strains	49
3.1.3. Media:	49
3.1.4. API-Staph Kit (bioMerieux)	51
3.1.5. Reagents and solutions used for biochemical identification	51
3.1.6. Stain	52
3.1.7. Plasma from rabbits for Coagulase test	52
R 1.8. Materials used for PCR	52

3.1.9. B-LACTA strip (Test-Line Ltd. Krizikova 68, 612 00 BRNO CZ.)	53
3.1.10. Antibiotic sensitivity discs(Oxoid)	54
3.1.11. Materials used for (SDS-PAGE)	54
3.1.12.Equipment	56
3.2. Methods	57
3.2.1. Identification of the Staphylococcus species	57
3.2.2. Biochemical identification of CNS	58
3.2.3. Detection of CNS by PCR	60
3.2.4. β lactamase activity test	61
3.2.5. Antibiotic sensitivity test among isolates	62
3.2.6. Sodium dodecyl sulfate - Poly Acrylamid Gel Electrophoresis (SDS-PAGE)	64
RESULT	65
DISCSSION & CONCLUSION	109
SUMMARY	122
REFERANCE	125
APARIC SUMMARY	

LIST OF ABBREVIATIONS

ACME Arginine catabolic mobile elements

α hemolysis Alpha hemolysis

AFLP Amplified fragment length polymorphism

API Analytical Profile Index

β hemolysisβ-lactamasebeta hemolysisbeta-lactamase

blaZ genes β-lactam-resistant gene

CNS Coagulase negative staphylococci

Coa Coagulase

CPS Coagulase positive staphylococci

EU European Union
IBM Individual bulk milk
IMI Intramammary infection

mecA gene Methicillin resistant gene A

MIC Minimum inhibitory concentration

MR-CNS Methicillin resistant Coagulase negative staphylococci

MRSA Methicillin resistant S. aureus

NBG No bacterial growth

NCCLS National Committees for clinical laboratory standards

PCR polymerase chain reaction

PFGE pulsed field gel electrophoresis rDNA Ribosomal Deoxyribonucleic acid

RFLP Restriction fragment length polymorphism

rRNA Ribosomal Ribonucleic acid

SCC Staphylococcal cassette chromosome

SCM Subclinical mastitis

SDS-PAGE Sodium Dodecyl Sulfate -PolyAcrylamide Gel Electrophoresis

SEs Staphylococcal enterotoxins SFP Staphylococcal food poisoning

LIST OF TABLES

NO.	Title	Pages
1	Oligonucleoide primers used for identification of staphylococci	52
2	Zone dimeter interpretation, standard of different antibacterial agents used	63
3	Staphylococcus isolates among the mastitic animals	66
4	CNS isolates among the mastitic animals	67
5	Pigmentation activity among the CNS isolated from milk samples	69
6	Pigmentation activity among the S. aureus isolated from milk samples	70
7	Hemolytic activity among the CNS isolated from milk samples	73
8	Hemolytic activity among the S. aureus isolated from milk samples	74
9	β- lactamase activity among the examined S. aureus isolates	80
10	β- lactamase activity among the examined CNS isolates	81
11	The antibacterial resistance among the <i>S. aureus</i> isolated from milk samples	84

12	Correlation between B lactamase S. aureus producers and resistant to antibacterial agents	85
13	The antibacterial resistance among the CNS isolated from milk samples	87
14	Correlation between β- lactamase CNS producers and resistant to antibacterial agents among cow isolates	88
15	Correlation between β- lactamase CNS producers and resistant to antibacterial agents among buffalo isolates	89
16	Detection of mecA and blaz among Staphylococcus strains	93
17	Detection of Toxins among S. aureus isolates	97
18	Detection of Toxins among CNS isolates	99
19	Gel Electrophoresis (SDS-PAGE) Profile analysis of CNS samples of Gel 1	105
20	Gel Electrophoresis (SDS-PAGE) Profile analysis of CNS samples of Gel 2	106
21	Gel Electrophoresis (SDS-PAGE) Profile analysis of CNS samples of Gel 3	107
22	Gel Electrophoresis (SDS-PAGE) Profile analysis of CNS samples of Gel 4	108

LIST OF FIGURES

NO.	Title	Pages
1	S. aureus and CNS on mannitol salt agar	71
2	CNS Alpha hemolysis on blood agar	75
3	CNS Beta hemolysis on blood agar	75
4	CNS and S. aureus Alpha and Beta hemolysis on blood agar	76
5a-b	Multiplex PCR assay to detect Staphylococcus isolates	78
6	B lactamase activity among CNS isolates	82
7	CNS antibiotic sensitivity test	90
8	Multiplex PCR assay to detect mecA and blaz among Staphylococcus aureus isolates	92
9a-b-c	Multiplex PCR assay to detect mecA and blaz among CNS isolates	94
10	Multiplex PCR assay to detect Staphylococcus aureus toxins	96
11a-b-c-d	Multiplex PCR assay to detect CNS toxins	98
12a-b	SDS-PAGE gel showing bands of Staphylococcus xylosus and Staphylococcus saprophyticus isolates and the Dendrogram analysis of expressed	101
13a-b	SDS-PAGE gel showing bands of <i>Staphylococcus cohnii</i> and <i>Staphylococcus saprophyticus</i> isolates and the Dendrogram analysis of expressed	102
14a-b	SDS-PAGE gel showing bands of Staphylococcus Lugdunensis and Staphylococcus lentus and Staphylococcus Simulans and Staphylococcus hominis isolates and the Dendrogram analysis of expressed	103
15a-b	SDS-PAGE gel showing bands of <i>Staphylococcus hominis</i> isolates and the Dendrogram analysis of expressed	104