

**MOLECULAR GENETIC IDENTIFICATION OF  
*STAPHYLOCOCCUS SP.* AND THEIR  
GENOTOXIC ACTIVITY**

By

**MARWA HASSAN MOHAMMED KHALEL**  
B.Sc. Agric. Sc. (Biochemistry), Ain Shamus University (2007)

**A Thesis Submitted in Partial Fulfillment  
Of  
The Requirement for the Degree of**

**MASTER OF SCIENCE**

**in**

**Agricultural Sciences  
(Genetics)**

**Department of Genetics  
Faculty of Agriculture  
Ain Shams University**

**2019**

**Approval Sheet**

**MOLECULAR GENETIC IDENTIFICATION OF  
*STAPHYLOCOCCUS SP.* AND THEIR  
GENOTOXIC ACTIVITY**

By

**MARWA HASSAN MOHAMMED KHALEL**

B.Sc. Agric. Sc. (Biochemistry), Ain Shamus University (2007)

**This thesis for M. Sc. degree has been approved by:**

**Dr. Samir Khalaf Abd El-Aal** .....  
Researcher Prof. Emeritus of Genetics, The National Research Center

**Dr. Khaled Abd El-Aziz Soliman** .....  
Prof. of Genetics, Faculty of Agriculture, Ain Shams University

**Dr. Ashraf Bakry Abd El-Razik** .....  
Prof. of Genetics, Faculty of Agriculture, Ain Shams University

**Dr. Samir Abd El-Aziz Ibrahim** .....  
Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams  
University

**Date of Examination:** / / 2019

**MOLECULAR GENETIC IDENTIFICATION OF  
*STAPHYLOCOCCUS SP.* AND THEIR  
GENOTOXIC ACTIVITY**

By

**MARWA HASSAN MOHAMMED KHALEL**

B.Sc. Agric. Sc. (Biochemistry), Ain Shamus Univ. (2007)

**Under the supervision of:**

**Dr. Samir Abd El-Aziz Ibrahim**

Prof. Emeritus of Genetic, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

**Dr. Ashraf Bakry Abd El- Razik**

Prof. of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

**Dr. Sawsan Youssef Youness Mohamed Elateek**

Lecturer of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

## ABSTRACT

**Marwa Hassan Mohammed: Molecular Genetic Identification of *Staphylococcus Spieces* and Their Genotoxic Activity. Unpublished Master thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2019.**

Methicillin resistant *Staphylococcus aureus* strains (MRSA) are considerably responsible for most hospital infection all over the world. Due to the presence of *mec A* gene resistance acquired for  $\beta$  - lactam group. Fifty clinical samples were collected from Ain Shams University Hospitals from March 2016 to July 2016, identified to the species level as *S. aureus* and detected as MRSA, MSSA isolates based on *mec A* gene in PCR. MRSA isolates were represented in 68% of the isolates, whereas MSSA isolates were represented in 32% of the latter. To study genotoxic activity of these isolates, forty five males' albino mice were injected interproteanally with  $1 \times 10^6$  C.F.U of different MRSA isolates and performed in National Research Center (NRC). Damage of liver tissues were shown by comet assay while micronuclei formation (as indicator on DNA damage) measured were by micronucleus test. This study revealed that our MRSA isolates which have been isolated from our hospitals were capable of inducing genotoxicity and furthermore mutation in mice. Our finding shed some light on how hygiene in the hospital should play important rule to decrease the spread of *Staphylococcus aureus* bacteria and MRSA specifically. Should be further investigation to be able to track these bacteria for better control management

**Keywords:** *Staphylococcus aureus*, *mecA* gene, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Polymerase Chain Reaction (PCR), Comet assay, Micronucleus assay.

## **ACKNOWLEDGEMENT**

I would like to thank all my professors in the Department of Genetics and I would like to thank all the professors at the Faculty of Agriculture Ain Shams University.

I would like to give my gratitude to the professors supervising this study, Prof. Dr. Samir A. Ibrahim, Prof. Dr. Ashraf Bakry, for their support until the completion of this study. Furthermore, I would like to thank Dr. Sawsan Elateek for her support and assistance in every step of this study.

Moreover, I am very grateful for Prof. Dr. Ekram Salah Eldin, Prof. Dr. Mahrossa M. Hassanane and Prof. Dr. Wagdy Khalil at the National Research Center who contributed to the completion of this study by conducting genotoxicity experiments in their unit and special thanks for the microbiology department at Ain Shams University hospital, which provided us with bacterial isolates.

I would like to give my gratitude to Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB) – Faculty of Agriculture – Ain Shams University.

# CONTENTS

Title	Page
<b>LIST OF TABLES</b> .....	V
<b>LIST OF FIGURES</b> .....	III
<b>INTRODUCTION</b> .....	1
<b>REVIEW OF LITERATURE</b> .....	3
1. Genus of <i>Staphylococcus</i> .....	3
2. <i>Staphylococcus aureus</i> transmission .....	3
3. <i>Staphylococcus aureus</i> Characteristics.....	4
4. <i>Staphylococcus aureus</i> structure and Virulence factors.....	5
5. <i>Staphylococcus aureus</i> genome .....	8
6. Clinical manifestation for <i>Staphylococcus aureus</i> .....	9
7. Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)...	10
7.1. Staphylococcal Chromosome Cassette <i>mec A</i> (SCC <i>mec</i> )	11
7.1.1. <i>Mec A</i> gene.....	11
7.1.2. <i>MecB</i> gene .....	11
7.2. <i>MecC</i> gene .....	12
7.3. <i>MecD</i> gene .....	12
8. Mechanism of resistance to methicillin in MRSA strains...	12
9. Types of MRSA .....	13
9.1. Community Acquired MRSA (CA-MRSA).....	13
9.1.1. Community acquired MRSA transmission.....	14
9.1.2. CA-MRSA transmission for athletes.....	14
9.2. Hospital-Acquired MRSA (HA-MRSA).....	14
9.2.1. Hospital - Acquired MRSA transmission.....	15
9.2.2. MRSA infection in children .....	15
9.3. Livestock-associated MRSA(LA-MRSA) .....	15
10. Biochemical test for <i>Staphylococcus aureus</i> detection .....	16
10.1. Catalase test.....	16
10.2. Coagulase test .....	16
11. Method for MRSA detection in clinical Sample.....	16

11.1.Traditional method .....	16
11.1.1. Detected media.....	16
11.1.1.1. Chromogenic Agar medium.....	17
11.1.1.2. Baired Parker medium.....	17
11.1.2. Susceptibility test.....	17
11.2. Molecular assay method.....	17
12.Difference in detection <i>Mec A</i> and <i>Mec C</i> in MRSA isolates	18
13.Genotoxicity of MSSA and MRSA strains.....	19
14. <i>Staphylococcus aureus</i> leads to genotoxicity .....	20
15. <i>Staphylococcus aureus</i> and the innate immunity system.....	20
16.Role of <i>S. aureus</i> extracellular protein.....	21
17.Infection Mice with MRSA strains.....	22
18.Genetic toxicity test.....	22
18.1.Micronucleus test.....	22
18.2.Comet assay.....	23
<b>MATERIALS AND METHODS</b> .....	25
<b>Materials</b> .....	25
1.Sample collection.....	25
2. Media.....	25
2.1. A selective and diagnostic medium for the isolation and enumeration of <i>Staphylococcus aureus</i> .....	26
2.2. Media for antimicrobial susceptibility testing for Methicillin Resistance <i>Staphylococcus aureus</i> (MRSA) Identification.....	26
2.3. Medium for <i>Staphylococcus aureus</i> preservation.....	26
3. Reagents used for bacterial identification.....	27
3.1. Gram stain.....	27
3.2. Catalase test.....	27
3.3.1.Oxicillin Disc.....	27
3.3.2. Cefoxitin Disc.....	27
4. Reagents used for DNA Extraction.....	28

### III

5. Material for measurement of DNA Concentration.....	28
6. Reagents used in PCR.....	28
7. Reagents for Electrophoresis .....	29
8. Genotoxicity assay experiment on mice.....	29
9. Reagent used for the neutral comet assay to genotoxicity screen .....	31
10. Micronucleus test .....	31
<b>Methods.....</b>	<b>32</b>
1. Samples activation.....	32
2 Identification of <i>Staphylococcus aureus</i> isolates.....	32
2.1. Preparation of Baird parker medium .....	32
2.2. Microscopic examination .....	33
2.3. Catalase test .....	33
2.4. Disc diffusion test.....	33
3. Sample preservation .....	33
4. Samples preparation for DNA Extraction.....	33
5. DNA Isolation .....	33
6. DNA measurements .....	34
7. Polymerase Chain Reaction (PCR) for detection of <i>mec A</i> gene.....	34
8. DNA gel electrophoresis .....	35
9. Genotoxicity test.....	35
9.1. Preparation of bacterial inoculum.....	36
9.2. Comet Assay .....	37
9.3. Micronucleus test.....	37
10. Statistical analysis.....	37
<b>RESULTES AND DISCUSSIONS.....</b>	<b>39</b>
1. Samples collection.....	39
1.1. Clinical samples from different (gender – age) groups of patients.....	39
1.2. <i>Staphylococcus aureus</i> percentages in different clinical samples .....	39

1.3. <i>Staphylococcus aureus</i> percentages in different age group.....	42
1.4. Division of each type of samples depended on gender.....	44
2. Identification of <i>Staphylococcus aureus</i> and MRSA isolates by microbiological and biochemical tests.....	45
2.1. <i>Staphylococcus aureus</i> growth on Baird parker media .....	46
2.2. <i>Staphylococcus. aureus</i> under light microscope.....	47
2.3. Catalase test .....	47
3. Oxycillin-Cefoxitin sensitivity test.....	48
3.1. Percentage of MRSA and MSSA isolates by sensitivity test.	50
4. DNA concentration assay of <i>Staphylococcus aureus</i> isolates.....	51
5. Polymerase Chain Reaction.....	52
6. Measurement of genetic toxicity using albino mice.....	57
7. Comet assay.....	59
8. Micronucleus test.....	64
<b>SUMMARY AND CONCLUSSION.....</b>	<b>70</b>
<b>REFERENCES .....</b>	<b>74</b>
<b>ARABIC SUMMARY .....</b>	

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
1. Bacterial isolates isolated from different clinical samples	30
2. PCR reaction preparation.....	34
3. PCR program for detecting <i>mecA</i> gene in MRSA isolates..	35
4. Experiment of MRSA treatment effect on mice in the liver and bone marrow (ACGEB) at NRC.....	36
5. Different types of samples from different gender and age groups of patients from isolate number (1) to isolate number (49).....	40
6. Samples divided into different age groups.....	44
8. Different clinical samples based on gender.....	45
9. MRSA detection of <i>Staphylococcus aureus</i> isolates using Oxicillin Cefoxitin sensitivity disc test from isolate number (1) to isolate number (50).....	49
10. Percentage of MRSA and MSSA isolates by Cefoxitin disc.....	50
11. Percentage of MRSA and MSSA by Oxicillin disc.....	51
12. DNA Concentration assay of <i>S. aureus</i> isolates by Fluorometer instrument from isolate number (1) to isolate number (50) test.....	52
13. MRSA isolates after PCR.....	54
14. Percentage of MRSA and MSSA isolates using PCR product results .....	55
15. Comet assay using infected liver mice tissue with MRSA suspension.....	60
16. The comet parameters of DNA damage.....	62
17. Statistical Analysis of the formation of Micronucleated polychromatic erythrocytes (MnPCEs) in mice bone marrow cells treated with MRSA isolates and its effect on	65

	2000 PCE .....	
<b>18.</b>	Micronucleus parameters of DNA damage.....	68

## LIST OF FIGURE

No.		Page
1.	Percentage of <i>Staphylococcus aureus</i> isolate in different clinical sample .....	39
2.	Distribution of <i>Staphylococcus aureus</i> in different age groups.....	42
3.	<i>Staphylococcus. aureus</i> isolates in different gender (%)..	45
4.	Distribution of <i>Staphylococcus aureus</i> growth on Baird Parker medium.....	46
5.	<i>Staphylococcus aureus</i> after stained with gram stain under light microscope (Gram positive).....	47
6.	<i>Staphylococcus aureus</i> Catalase (+) test.....	47
7.	Oxacillin-Cefoxitin sensitivity disc test for MRSA detection in <i>S. aureus</i> isolates.....	48
8.	Comparison between Oxacillin and Cefoxitin disc in sensitivity test .....	50
9.	Detection of MRSA isolates between <i>Staphylococcus aureus</i> isolates using PCR.....	52
10.	Comparison between MRSA strain by PCR, MRSA isolates by sensitivity test (Cefoxitin disc) and (Oxacilin disc).....	53
11.	Injected mouse intraperitoneally with suspension of MRSA isolate.....	58
12.	Dissecting mouse after sacrifice.....	58
13.	Visual of Mouse liver after injection with MRSA isolates .....	59
14.	Normal Change in color of mouse liver after injection with MRSA isolates (pale color).....	59
15.	Visual score of normal DNA (class 0, 1, 2, 3) using Comet assay in liver samples exposed to MRSA isolates from different clinical sample.....	61

VIII

<b>16.</b>	Microscope visual of (MnPCEs) of male mice exposed to selected MRSA isolates.....	67
<b>17.</b>	Microscope visual of mouse bone marrow cells showing PCE and NCE.....	68

## LIST OF ABBREVIATION

<b>Abbreviation</b>	<b>Mean</b>
W	wound
B	Blood
P	Pus
Sp	Sputum
U	Urine
Ab	Abscess
NR	Not Reported
Ox	Oxacillin Disc
Fox	Cefoxitin Disc
C1	Positive Control
C2	Negative Control
M	Marker
PCE	PolyChromatic Erythrocytes
NCE	NormoChromatic Erythrocytes
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin sensitivity <i>Staphylococcus aureus</i>
VRSA	Vancomycin Resistant <i>Staphylococcus aureus</i>
CA-MRSA	Community – Acquired MRSA
HA-MRSA	Healthcare – Associated MRSA
LA-MRSA	Live – associated MRSA
MnPCEs	Micronucleated PolyChromatic Erythrocytes
PCR	Polymerase Chain Reaction
PVL	Panton-Valentine leukocidin
Efb	Extracellular fibrinogen-binding protein

SpA	Surface protein A
CHIPS	Chemotaxis Inhibiting Proteins of <i>S. aureus</i>
SCIN	Staphylococcal Complement Inhibitor
Hlg	gamma-Hemolysin
Ecb	extracellular complement-binding protein
PSM $\alpha$	alpha-type Phenol Soluble Modulins