



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم

# بسم الله الرحمن الرحيم



**MONA MAGHRABY**



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم



# شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



**MONA MAGHRABY**



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



## يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



**MONA MAGHRABY**



# **Molecular and Physiological Characterization of Bacterial Macrolide Resistance Mechanisms as an Approach of Infection Control**

**A Thesis**

Submitted in Partial Fulfillment of the Requirements for the

**PhD degree**

In Pharmaceutical Sciences  
**(Microbiology and Immunology)**

By

**Amr Shaker Meselhy**

Master of Pharmaceutical Sciences, 2014  
Department of Microbiology and Immunology,  
Faculty of Pharmacy, Ain Shams University

**2020**





**Molecular and physiological characterization of bacterial macrolide resistance mechanisms as an approach of infection control**

**A Thesis**

Submitted in Partial Fulfillment of the Requirements for the

**PhD degree**  
In Pharmaceutical Sciences  
(**Microbiology and Immunology**)

By

**Amr Shaker Meselhy**

Master (MSc) degree of Pharmaceutical Sciences, 2014  
Faculty of pharmacy, Ain shams university

Under Supervision of

**Prof. Dr. Nadia Abd El-Haleem Hassouna, PhD**

Professor of Microbiology and Immunology,  
Faculty of Pharmacy, Ain Shams University

**Prof. Dr. Mahmoud Abd El-Mageed Yassien, PhD**

Professor of Microbiology and Immunology,  
Faculty of Pharmacy, Ain Shams University  
Chairman of the National Organization for Research and Control of Biologicals

**Prof. Dr. Khaled M. Anwar Aboshanab, PhD**

Professor of Microbiology and Immunology,  
Vice dean for postgraduate studies and researches,  
Faculty of Pharmacy, Ain Shams University

**2020**



# Acknowledgment

First, I thank "**Allah**" for granting me the power to accomplish this work.

I would like to express my deepest thanks to **Prof. Dr. Nadia A. El-Haleem Hassouna**, Professor of Microbiology and Immunology, and founder of the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University, for her valuable scientific supervision, constructive advice and continuous guidance throughout the work.

My deepest gratitude and appreciation are expressed to **Prof. Dr. Mahmoud Abd El-Mageed Yassien**, Chairman of National organization for research and control of biologicals, Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, for providing continuous scientific supervision, supplying facilities whenever needed and for his constructive criticism throughout this study.

I am also greatly indebted to **Prof. Dr. Khaled M. Aboshanab**, Vice dean for postgraduate studies and Research, Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, for his divine support, following up and giving his valuable time and effort. I also thank him for suggesting the point, planning the work and providing assistance throughout this study.

Grateful thanks are extended to my dear **colleagues** and to all the **workers** at the Faculty of Pharmacy, Ain Shams University for their help and support.

My everlasting thanks and appreciation are also directed to my beloved **family** for their continuous support, encouragement and sincere help throughout my whole life.

Last but not least, I would like to express my deepest and most sincere gratitude to my **wife** for her patience, understanding, motivation and everlasting support.

والحمد لله رب العالمين.....

*Amr Shaker Meselhy*





**Table of Contents**

**Acknowledgment ..... I**

**Table of Contents.....I**

**List of Abbreviations ..... V**

**List of Figures ..... VII**

**List of Tables..... X**

**Abstract ..... 1**

**Introduction ..... 4**

**Literature Review ..... 6**

1. Antibiotic resistance..... 6

    1.1. Current Situation of Antibiotic Use and Resistance worldwide ..... 6

    1.2. Reasons of irrational use of antimicrobials among healthcare providers..... 8

2. Macrolide antibiotics..... 9

    2.1. History ..... 9

    2. 2. Chemical structure..... 10

    2. 3. Mechanism of action ..... 12

    2. 4. Antimicrobial activity and clinical indications..... 15

    2. 5. Adverse effects ..... 19

3. Microbial resistance to macrolide antibiotics..... 20

    3. 1. Target site modification..... 21

    3. 2. Decreased macrolide accumulation ..... 31

    3. 3. Enzymatic inactivation ..... 32

## Table of Contents

---

<b>Materials and Methods .....</b>	<b>37</b>
Materials.....	37
1. Bacterial isolates, standard strains and vectors .....	37
1. 1. Bacterial isolates.....	37
1.2. Standard strains.....	38
1.3. Vectors.....	38
2. Chemicals, Enzymes and Kits.....	41
2. 1. Chemicals .....	41
2. 2. Enzymes and Kits .....	43
3. Instruments.....	44
4. Antibiotic discs .....	44
5. Media .....	45
5. 1. Ready-made culture media .....	45
5. 2. In house formulated media .....	46
6. Buffers and Solutions.....	48
6. 1. For antimicrobial susceptibility testing .....	48
6. 2. For preparation of competent <i>E. coli</i> DH5 $\alpha$ cells.....	48
6. 3. For agarose gel electrophoresis of DNA fragments.....	48
Ethidium bromide should be stored in a cool, dark place away from strong oxidizing agents. .....	49
7. Oligonucleotides .....	49
8. Computer programs.....	50
Methods.....	51
1. Antimicrobial susceptibility testing .....	51
1. 1. Kirby-Bauer disc diffusion method .....	51

## Table of Contents

---

1. 2. Determination of minimum inhibitory concentration .....	52
2. Growth and maintenance of bacterial strains .....	52
2. 1. Growth and maintenance of the collected clinical isolates .....	52
2. 2. Growth and maintenance of <i>E. coli</i> DH5 $\alpha$ .....	53
2. 3. Growth and maintenance of <i>E. coli</i> BL21 (DE3) .....	53
3. Detection of macrolides resistance-coding genes .....	53
3. 1. Chromosomal DNA extraction of the tested isolates .....	54
3. 2. PCR amplification of MACs resistance-coding genes .....	54
4. Agarose gel electrophoresis .....	54
5. Sequencing of PCR products .....	55
5. 1. PCR clean up .....	55
5. 2. Sequencing.....	55
5. 3. Assembling of sequence data and alignment against protein homologues in database	56
6. Homology modelling .....	56
7. Construction of the recombinant plasmid pUCPU21- <i>ermC</i> and pUCPU21- <i>msrA</i> and selection of the correct clone.....	57
7. 1. Restriction of PCR product and pUC .....	57
7. 2. Ligation.....	57
7. 3. Preparation of competent cells .....	57
7. 4. Transformation into <i>E. coli</i> DH5 $\alpha$ .....	58
7. 5. Plasmid extraction .....	58
7. 6. Restriction and gel electrophoresis.....	59
8. Construction of the recombinant plasmids pET22b- <i>ermC</i> and pET22b - <i>msrA</i> and selection of the correct clone.....	59
8.1 Extraction of passenger DNA using DNA gel extraction kit .....	59

## Table of Contents

---

8.2. Restriction of pET22b and ligation of <i>ermC</i> and <i>msrA</i> into pET22b .....	60
8.3. Preparation of competent cells, transformation and selection of the right clone .....	60
9. Checkerboard titration method for studying the effect of combinations of MACs and other antibiotics .....	60
<b>Results.....</b>	<b>62</b>
1. Antimicrobial susceptibility testing .....	62
1.1. Kirby-Bauer disc diffusion method .....	62
1.2. Distribution of MAC resistance phenotypes among the resistant isolates.....	63
1.3. Distribution of methicillin-resistance among the MAC resistant isolates .....	65
1.4. Minimum inhibitory concentration results by agar dilution method .....	66
2. Detection of MAC-resistance genes.....	71
3. Sequencing of PCR products .....	78
4. Homology Modelling.....	80
5. Cloning and expression of macrolide resistance proteins .....	82
6. Studying the combination of MACs and different antimicrobial agents.....	85
<b>Discussion .....</b>	<b>88</b>
Conclusion .....	99
Future perspectives.....	100
<b>Summary .....</b>	<b>101</b>
<b>References .....</b>	<b>104</b>
المخلص العربي.....	١

## List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>
<b>23S rRNA</b>	23S ribosomal ribonucleic acid
<b>ABC</b>	ATP-binding cassette
<b>AZM</b>	Azithromycin
<b>BLAST</b>	Basic Local Alignment Search tool
<b>CLI</b>	Clindamycin
<b>CLSI</b>	Clinical and Laboratory Standards Institute
<b>CoNS</b>	Coagulase-negative <i>Staphylococcus aureus</i>
<b>EDTA</b>	Ethylene diaminetetraacetic acid
<b>ERE</b>	Erythromycin esterase
<i>ere</i>	Gene encodes erythromycin esterase
<b>ERM</b>	Erythromycin ribosomal methylase
<b>ERY</b>	Erythromycin
<i>erm</i>	Gene encodes erythromycin ribosomal methylase
<b>MCS</b>	Multiple cloning site
<b>MAC(s)</b>	Macrolide(s)
<i>Mef</i>	The gene encoding macrolide efflux protein
<b>c/i MLS</b>	Constitutive/Inducible macrolide, lincosamide and streptogramin type-B
<b>MPH</b>	Macrolide 2'-phosphotransferase
<i>mph</i>	The gene encoding macrolide 2'-phosphotransferase enzyme
<b>MRSA</b>	Methicillin-resistant <i>Staphylococcus aureus</i>
<i>msr</i>	Gene encodes macrolide streptogramin resistance protein
<b>ORF</b>	Open reading frame
<b>SDS</b>	Sodium dodecyl sulphate

## List of Abbreviations

---

<b>SOB</b>	Super optimal broth
<b>SOC</b>	Super optimal broth with catabolite repression
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<b>TAE</b>	Tris acetic acid EDTA
<b>TE</b>	Tris EDTA
<b>Tris</b>	Trishydroxymethylaminomethane