



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



HANAA ALY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



HANAA ALY



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 تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



Molecular characterization of resistance mechanisms to fluoroquinolones of some *Acinetobacter baumannii* clinical isolates in Upper Egypt

A thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in pharmaceutical sciences (Microbiology & Immunology)

By

Mostafa Ahmed Mohammed Ahmed

Assistant lecturer of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut

Department of Microbiology & Immunology

Faculty of Pharmacy, Ain Shams University

Cairo, Egypt

2021



Molecular characterization of resistance mechanisms to fluoroquinolones of some *Acinetobacter baumannii* clinical isolates in Upper Egypt

A thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in pharmaceutical sciences (Microbiology & Immunology)

By

Mostafa Ahmed Mohammed Ahmed

Assistant lecturer of Microbiology and Immunology, Faculty of Pharmacy,
Al-Azhar University, Assiut

Under supervision of

Prof. Dr. Mohammad M. Aboulwafa

Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain Shams University,
Acting Dean of Faculty of Pharmacy,
King Salman International University

Prof. Dr. Khaled M. Anwar Aboshanab

Professor of Microbiology and immunology,
Vice Dean for Postgraduate Studies and Research,
Acting Dean of Faculty of Pharmacy,
Ain Shams University

Assist. Prof. Mohammed Taha Ahmed

Assist. Professor of Microbiology and
Immunology, Faculty of Pharmacy,
Al-Azhar University, Assiut

Dr. Bahaa El-Din Anwer Abdel-Raady

Lecturer of Microbiology and immunology,
Faculty of Pharmacy,
Al-Azhar University, Assiut

Department of Microbiology & Immunology

Faculty of Pharmacy, Ain Shams University

Cairo, Egypt

2021

APPROVAL SHEET

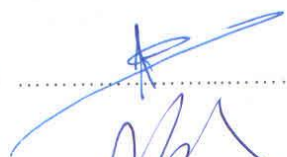
Molecular characterization of resistance mechanisms to fluoroquinolones of some *Acinetobacter baumannii* clinical isolates in Upper Egypt

By

Mostafa Ahmed Mohammed Ahmed

Assistant lecturer of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University,
Assiut (2016)

This Ph.D thesis with the title and applicant name shown above has been approved by:

- Prof. Dr. Mohammad M. Aboulwafa 
- Prof. Dr. Khaled M. Anwar Aboshanab 
- Prof. Dr. Abd Alnaser Abas Almogazy 
- Prof. Dr. Amal Emad-ELDin Mohammed 

(Committee in Charge)

Date: / /

Acknowledgments

Alhamdulillah, I praise and thank Allah SWT, the most merciful and compassionate, for all his blessings and generosity. The accomplishment of this thesis is one of ALLAH's continuous gifts to me. We will never be able to count ALLAH blessings upon us, since they are innumerable.

I would like to thank my supervisor: **Prof. Mohammad M. Aboulwafa** for accepting me as a Ph.D. student at the Microbiology and Immunology Department. My deep and sincere thanks for attentive supervision and the continuous support, the valuable advice, careful revision, encouragement, and the flexibility he has given to me to complete this work.

My deep and sincere thanks to **Prof. Khaled M. Anwar Aboshanab** for accepting me as a Ph.D. student at the Microbiology and Immunology Department, and for allowing me to perform my Ph.D. I am sincerely grateful for his attentive supervision, excellent guidance, careful revision, flexibility, and encouragement throughout this work.

All my appreciation to all staff members of the Department of Microbiology, Faculty of pharmacy, Al-Azhar University, Assiut branch especially **Assist. Prof. Mohammed Taha Ahmed, Dr. Baha Al-din Anwer**, and **Dr. Mamdouh Yones Ali** sincere thanks for attentive supervision and the continuous support, for all the friendship and support they gave me, and for really great times spent in and outside the laboratory.

I would like to thanks **Prof. Dr. Nahla Al Sherbiny** and **Prof. Dr. Enas Daef**, Assiut Hospitals University for their support, providing facilities and specimens that were collected at the Medical Research Center, Faculty of Medicine, Assiut University, and the valuable advice. I wish to give my warmest thanks to all those who helped throughout my work in the infection control unit and bacteriology department at Assiut University Hospital and AL-Azhar University hospital, Assiut Governorate.

Also, more gratitude is extended to **Dr. Mohammed Eissa**, INRS, University of Quebec, Canada, and **Dr. Samira M. Hamed**, Department of Microbiology and Immunology, Faculty of Pharmacy, October University for Modern Sciences and Arts, and **Dr. Mohamed A. Abdel-Lateef**; Department of Pharmaceutical Analytical Chemistry; Faculty of Pharmacy, Al-Azhar University for their support and help during this study.

All my love and appreciation for my brother **Prof. Dr.Abd Elnaser A. Mohammed**,
Faculty of Agriculture, Assiut University, for their continuous encouragement and love.

Last but not least, I dedicate this thesis to the soul of my mother, my father, my wife, son,
and daughter for their love and continuous support.

والحمد لله رب العالمين.....

Mostafa Ahmed Mohammed

Table of contents

Acknowledgments	
Table of contents	i
List of tables	v
List of figures	vii
List of Abbreviations	ix
Abstract	x
I. INTRODUCTION & AIM OF THE WORK	1
I.1 Introduction.....	1
I.2 Aim of the work.....	4
II. LITERATURE REVIEW	5
II.1 Genus <i>Acinetobacter</i>	5
II.1.1 Historical background.....	5
II.1.2 Nomenclature and <i>Acinetobacter</i> taxonomy.....	5
II.1.3 Current taxonomy.....	6
II.2 Laboratory identification.....	7
II.2.1 Sample collection.....	7
II.2.2 Identification at the genus level.....	7
II.2.2.1 Cultural characteristics.....	7
II.2.2.2 Microscopic characteristics.....	8
II.2.3 Identification at the species level.....	8
II.2.3.1 Phenotypic method.....	8
II.2.3.2 Biochemical methods.....	8
II.2.3.3 Molecular identification methods.....	8
II.3 Infections with <i>A. baumannii</i>	9
II.4 Clinical significance of <i>A. baumannii</i>	9
II.3.1 Community-acquired <i>Acinetobacter</i> pneumonia.....	10
II.3.2 Bloodstream infections, bacteremia and sepsis.....	10
II.3.3 Skin, soft tissue and wound infections.....	10
II.3.4 Neonatal Nosocomial Infections.....	10
II.3.5 Urinary tract infections.....	11
II.3.6 Meningitis infections.....	11
II.5 Epidemiology of <i>A. baumannii</i> infection.....	11
II.5.1 Global epidemiology of <i>A. baumannii</i>	12
II.5.2 Middle East epidemiology of <i>A. baumannii</i>	13
II.5.3 Epidemiology of an outbreak.....	13

II.6 Molecular typing methods of <i>Acinetobacter</i> strains	14
II.7 Resistance mechanisms of antimicrobial agents	14
II.7.1 Non-enzymatic mechanisms of resistance.....	15
II.7.2 Enzymatic mechanisms of resistance	16
II.7.2.1 Aminoglycoside resistance.....	16
II.7.2.2 β -lactam resistance	16
II.7.2.3 Carbapenem resistance	19
II.7.2.4 Polymyxins resistance	19
II.7.2.5 Tetracyclines and Glycylcyclines resistance	20
II.7.2.6 Resistance to other Antibiotics.....	20
II.8 Transfer of resistance genes among <i>Acinetobacter</i> spp.	22
II.9 Fluoroquinolones.....	23
II.9.1 Historical background	23
II.9.2 Classification	24
II.9.3 Mechanism of Action	24
II.9.4 Molecular Characterization of fluoroquinolones resistance Mechanisms.....	27
II.9.4.1 Target-site gene mutation.....	27
II.9.4.2 Plasmid-Mediated resistance	32
II.9.4.3 Chromosome-Mediated resistance	35
II.9.4.4 Association between Fluoroquinolone Resistance and ESBLs Production.....	37
II.10 Diminishing of <i>A. baumannii</i> resistance.....	38
II.10.1 Antimicrobial therapy.....	38
II.10.1.1 Monotherapy.....	38
II.10.1.2 Combination therapies.....	40
II.10.2 Association of antimicrobial agents with Non-antimicrobial agents.....	41
II.10.3 Prevention and control.....	43
III. PATIENTS, MATERIALS, AND METHODS.....	45
III.1 PATIENTS	45
III.1.1 Patients data	45
III.1.2 Samples collection	45
III.2 MATERIALS	45
III.2.1 Microorganisms reference strains	45
III.2.2 Chemicals, Reagents, and kits.....	45
III.2.3 Antimicrobial agents and their sources or suppliers	46
III.2.4 Antimicrobial discs and their suppliers.....	47
III.2.5 Equipment	47

III.2.6 Primers and suppliers	47
III.2.7 Readymade medium	48
III.3 METHODS	48
III.3.1 Culture media and Agarose gel preparation	48
III.3.1.1 Blood agar	48
III.3.1.2 Indol test medium	48
III.3.1.3 Brain Heart Infusion broth	49
III.3.2 Preparation of Buffers, solutions, reagents, and agarose gel	49
III.3.2.1 Tris –EDTA buffer (10X)	49
III.3.2.2 TAE buffer (10X)	49
III.3.2.3 TBE buffer (10X)	49
III.3.2.4 EDTA (20 mM)	50
III.3.2.5 EDTA (0.5M)	50
III.3.2.6 Bromothymol blue solution	50
III.3.2.7 Ethidium bromide solution	50
III.3.2.8 Normal saline	51
III.3.2.9 McFarland turbidity standard	51
III.3.2.10 Preparation of Agarose gel	51
III.3.3 Maintenance of microorganisms	51
III.3.4 Culture techniques	51
III.3.5 Identification of collected isolates	52
III.3.5.1 Isolation and identification of <i>Acinetbacter baumannii</i>	52
III.3.6 Antimicrobial susceptibility of <i>A. baumannii</i>	54
III.3.6.1 Disc diffusion method	54
III.3.6.2 The minimum inhibitory concentrations	54
III.3.7 Genotypic method (ERIC-PCR)	55
III.3.8 Molecular characterization of FQ-resistant mechanisms	56
III.3.8.1 Detection of target site mutation	56
III.3.8.2 Detection of chromosomal mediated of ESBLs encoding genes	57
III.3.8.3 Plasmid-associated fluoroquinolone resistance (PAFQR) genes.	57
III.3.9 Evaluation of drug combination to combat drug resistance of tested <i>A. baumannii</i>	59
III.3.9.1 Evaluation of FQs combination with other antimicrobial agents	60
III.3.9.2 Evaluation of FQs combination with non-antimicrobial agents	61
III.3.10 DNA Sequencing of PCR amplified products	61
III.2.11 Assembly, alignment, and analysis of target gene sequences	61
III.3.12 Statistical analysis	61

IV. RESULTS	62
IV.1 Identification of Bacterial isolates	62
IV.2 Identification of <i>A. baumannii</i>	63
IV.2.1 Phenotypic identification	63
IV.2.2 Genotypic identification	64
IV.3 Prevalence of <i>A. baumannii</i> isolated from different wards	65
IV.4 Prevalence of <i>A. baumannii</i> infection according to the gender of the patients	66
IV.5 Prevalence of <i>A. baumannii</i> isolation according to the risk factors of the patients	66
IV.5.1 Prevalence of <i>A. baumannii</i> infection according to the patient age	66
IV.5.2 Prevalence of <i>A. baumannii</i> infection among patients treated with fluoroquinolone	67
IV.5.3 Prevalence of <i>A. baumannii</i> infection according to different health illnesses	67
IV.5.4 Prevalence of <i>A. baumannii</i> infection among patients according to the stay time	68
IV.6 Antimicrobial susceptibility tests	69
IV.6.1 Disc diffusion method	69
IV.6.2 Susceptibility of <i>A. baumannii</i> as determined by minimum inhibitory concentrations	75
IV.7 Genotypic of <i>A. baumannii</i> isolates as determined by ERIC-PCR	79
IV.8 Molecular characterization of FQs resistance mechanisms	81
IV.8.1 Detection of target site mutation	81
IV.8.2 Detection of chromosomal ESBLs encoding genes among <i>A. baumannii</i> isolates	86
IV.8.3 Plasmid-associated fluoroquinolone resistance (PAFQR) genes	87
IV.9 Studying the correlations between resistance to CIP and resistance to other tested	96
IV.9.1 Studying the correlations between resistance to CIP and resistance to other tested antimicrobial agents among <i>A. baumannii</i> test isolates	96
IV.9.2 Correlations between FQ resistance genes and resistance to CIP resistance	97
IV.9.3 Correlations between FQ resistance genes and MIC values of CIP	98
IV.9.4 Correlations between FQ resistance genes and their resistance profiles	99
IV.9.5 Correlations between FQ resistance genes acquired by the tested <i>A. baumannii</i> isolates and types of specimens from which the tested isolates were recovered	100
IV.9.6 Distribution of <i>A. baumannii</i> isolates according to plasmid profiles	101
IV.10 Evaluation of drug combinations for combating resistance of <i>A. baumannii</i> isolates	101
IV.10.1 Evaluation of FQs plus other antibiotics combinations	105
IV.10.2 Evaluation of FQs plus non-antibiotics combinations	110
V. DISCUSSION	116
VI. SUMMARY	133
VII. REFERENCES	137
VIII. ARABIC SUMMARY	178