



بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة / سامية زكى يوسف

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد





Prevalence of *OXA* carbapenemases genes in multidrug resistant *Acinetobacter* and *Pseudomonas* spp.

Thesis

Submitted for Partial Fulfilment of Master Degree in
Microbiology

By

Nermin Mahmoud Kamel Mohamed

(B.Sc. in Microbiology, Faculty of Science, Ain Shams University,
2011)

Supervisors

Prof. Khaled Zakaria El-Baghdady

*Professor of Microbiology
Microbiology Department,
Faculty of Science, Ain Shams University*

Prof. Iman Mohamed Amin El-Kholy

*Consultant of Microbiology
Ain Shams University Specialized Hospital,
Ain Shams University*

Prof. Gehan Mohamed Fahmy

*Consultant of Infection Control, Clinical Pathology
Ain Shams University Specialized Hospital,
Ain Shams University*

**Microbiology Department
Faculty of Science
Ain Shams University
2022**



Approval sheet

Prevalence of OXA carbapenemases genes in multidrug resistant *Acinetobacter* and *Pseudomonas* spp.

By

Nermin Mahmoud Kamel Mohamed

Supervisors Approved

Prof. Khaled Zakaria El-Baghdady

*Professor of Microbiology, Microbiology Department,
Faculty of Science, Ain Shams University*

Prof. Iman Mohamed Amin El-Kholy

*Consultant of Microbiology, Ain Shams Specialized Hospital,
Ain Shams University*

Prof. Gehan Mohamed Fahmy

*Consultant of Infection Control, Clinical Pathology
Ain Shams University Specialized Hospital, Ain Shams University*

Examination committee

Prof. Abeer Ahmed Rushdy Mohamed

*Professor of Microbiology
Faculty of Women for Arts, Science and Education,
Ain shams University.*

Prof. Marwa Saad Mohammed Fathi

*Professor of Medical Microbiology and Immunology
Faculty of Medicine, Ain shams University.*

Prof. Dr. Khaled Zakaria El-Baghdady

*Professor of Microbiology, Microbiology Department,
Faculty of Science, Ain Shams University*

Prof. Iman Mohamed Amin El-Kholy

*Consultant of Microbiology, Ain Shams Specialized Hospital,
Ain Shams University*

Date of Discussion 22 / 06 /2022

Approval date / / 2022

University Council Approved / /202



ANNOUNCEMENT

*This thesis has not been
previously submitted for any
degree.*

Nermin Mahmoud Kamel



بسم الله الرحمن الرحيم

"قالوا سبحانك لا علم لنا إلا
ما علمتنا إنك أنت العليم الحكيم"

صدق الله العظيم

سورة البقرة الآية
(٣٢)



DEDICATION

I would like to dedicate this work to my authors for their efforts and assistance during the research period also beloved parents and brothers, my dear friend for their encouragement. Specially thanks to my dear husband for putting up with me and supporting me all through this work.

Nermin Mahmoud



ACKNOWLEDGMENT

*First, I am deeply thankful to **ALLAH** for giving me everything I wish, strengthen and helping me to complete this work.*

*I would like to express my gratitude and appreciation to **Prof. Dr. Khaled Zakaria**, Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for his help and continuous support. He was best advisor, perfect in work and expert supervisor to bring this thesis to more than satisfactory finish.*

*My grateful and sincere thanks to **Prof. Dr. Eman El Kholy** consultant of Microbiology, Microbiology Department, specialized hospital, Ain Shams University, for her help, encouragement, continuous guidance, valuable comments, and constructive criticism which was a great asset for this work.*

*My deep thanks to my dear **Gehan Fahmy**, consultant of infection control, Microbiology Department, specialized hospital, Ain Shams University for her help, support, and friendly way in her supervision.*

I would like to express my thanks to all of my friends, and colleagues for their encouragement and support.

Nermin Mahmoud

LIST OF CONTENTS

Title	Page NO
1. Introduction	1
2. Literature Review	5
2.1. Site of infection	5
2.2 Antibiotics resistant	6
2.3. β-lactamase	10
2.4. OXA Extended-spectrum-lactamases	11
2.5. OXA groups in <i>P. aeruginosa</i>	12
2.6. OXA-type in <i>A. baumannii</i>	15
2.6.1. OXA-23-like β -lactamases	16
2.6.2. OXA-40/24-like β -lactamases	17
2.6.3 OXA-51-like β -lactamases	18
2.6.4 OXA-58-like β -lactamases	19
2.7. Formation of biofilm in <i>A. baumannii</i> and <i>P. aeruginosa</i>	21
2.7.1 Biofilm formation in <i>P. aeruginosa</i>	24
2.7.2 biofilm formation in <i>A. baumannii</i>	25
2.8. Colistin	26
2.8.1. Mechanism of action to colistin	28
2.8.2. Resistance Mechanisms to colistin	29

3. Materials	31
3.1.1 Isolates collection	31
3.1.2 Media	31
3.1.3 Antibiotics	37
3.1.4 Chemicals and reagents	37
3.1.4 Biofilm formation	39
3.1.5 Vitek 2 cards	40
3.1.6 Kits for PCR	40
3.2 Methods	41
3.2.1 Clinical isolates and patient's data	41
3.2.2 Preliminary identification of clinical isolates	41
3.2.2.1 Growth on MacConkey agar	41
3.2.2.2 Microscopic examination of Gram-stained smears	42
3.2.2.3 Oxidase test	42
3.2.2.4 Carbohydrate fermentation and hydrogen sulfide production by TSI Test	42
3.2.2.5 Urea utilization test	43
3.2.2.6 Citrate utilization test	43
3.2.2.7 MIO reaction test	43
3.2.2.8 Methyl red (MR) test	44
3.2.2.9 Voges Proskauer test	44

3.2.3 Maintenance and preservation of clinical isolate.	44
3.2.4 Determination of minimum inhibitory concentrations (MICs) of antibiotics	45
3.2.4.1 Suspension Preparation	46
3.2.4.2 Inoculation	46
3.2.4.3 Card Sealing and incubation	47
3.2.4.4 Optical System	47
3.2.4.5 Test Reactions	47
3.2.4.6 Analytical techniques	48
3.2.5 Antibiotic susceptibility test	49
3.2.6 Genotypic detection of OXA genes	52
3.2.6.1 Preparation of DNA template	52
3.2.6.2 PCR primers	53
3.2.7 Method for biofilm formation	54
3.2.8 Determination of minimum inhibitory	55
3.2.8.1 MIC of colistin by VITEK 2	55
3.2.8.1.2 MIC of colistin using microtiter plate	55
4. Results	56
4.1 Collection of isolates' data	56
4.1.1. Percentage of gender and age in both species	58
4.1.2 Source of collected isolates	60

4.1.3 Types of clinical infection in relation to isolates' gender	62
4.1.4 Types of clinical infection according to patients' age	63
4.2. Different antibiotics using disc diffusion method	65
4.3. MIC of different antibiotics using Vitek 2	68
4.4. PCR results in <i>P. aeruginosa</i> and <i>A. baumannii</i>	73
4.5. Formation of biofilm both <i>P. aeruginosa</i> and <i>A. baumannii</i>	74
4.6. Relation between forming biofilm and <i>OXA</i> genes in <i>P. aeruginosa</i>	75
4.7. Relation between forming biofilm and <i>OXA</i> genes in <i>A. baumannii</i>	77
4.8. MIC of colistin for both <i>P. aeruginosa</i> and <i>A. baumannii</i>	79
Discussion	82
General Conclusion and Recommendations	
Summary	
References	
Arabic summary	

LIST OF TABLES

Title	Page NO
(1) Antibiotics on AST-N 22 cards, their concentrations, and the range of MICs as provided by supplier (BioMerieux)	48
(2) Standards of antibiotics susceptibility test	51
(3) Primers used for detection of OXA genes by real time PCR	53
(4) Isolates data (age, gender, department and source of isolates)	56
(5) Biochemical tests for identification <i>P. aeruginosa</i> and <i>A. baumannii</i>	58
(6) Percentage of gender in <i>P. aeruginosa</i> and <i>A. baumannii</i>	59
(7) Percentage of clinical isolates distribution among different isolation sites	61
(8) Types of clinical infection according to gender	62
(9) Types of clinical infection according to age	64
(10) Antibiotic susceptibility of <i>P. aeruginosa</i> from (1-25) and <i>A. baumannii</i> (25-50) clinical	66

isolates	
(11) MIC of different antibiotics which used to detect resistant in <i>P. aeruginosa</i> and <i>A. baumannii</i>	69
(12) Prevalence of <i>OXA</i> both <i>P. aeruginosa</i> and <i>A. baumannii</i>	73
(13) Prevalence of biofilm formation both <i>P. aeruginosa</i> and <i>A. baumannii</i>	74
(14) Relation between forming biofilm and <i>OXA</i> genes in <i>P. aeruginosa</i>	76
(15) Relation between forming biofilm and <i>OXA</i> genes in <i>A. baumannii</i>	78
(16) Susceptibility of isolates to colistin	80

LIST OF Figures

Title	Figures NO
(1) Mode of action for different groups of antibiotics.	10
(2) A, AST-N 22 cards and B, Vitek 2 system compact 15 used in the determination of MIC National Cancer Institute	45
(3) Frequency of <i>P. aeruginosa</i> and <i>A. baumannii</i> isolates according to patients' gender	59
(4) Frequency of <i>P. aeruginosa</i> and <i>A. baumannii</i> isolates according to category age	60
(5) Percentage of clinical isolates distribution among different isolation sites	61
(6) Types of clinical infection according to gender	63
(7) Types of clinical infection according to age	64
(8) MIC of different antibiotics which used to detect resistant in <i>P. aeruginosa</i> and <i>A. baumannii</i>	72
(9) Prevalence of OXA both <i>P. aeruginosa</i> and <i>A. baumannii</i>	73

(10) Prevalence of biofilm formation both <i>P. aeruginosa</i> and <i>A. baumannii</i>	75
Figure (11): the melt curve shows a TM about 84°C in a single peak the absence of a non-specific amplified product	
(11) Relation between forming biofilm and <i>OXA</i> genes in <i>P. aeruginosa</i>	77
(12) Relation between forming biofilm and <i>OXA</i> genes in <i>A. baumannii</i>	79
(13) Susceptibility of isolates to colistin	80