

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى 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



MONA MAGHRABY





Antibacterial activity of nanoparticles against multi-drug resistant Staphylococci

Thesis submitted for The award of the degree of doctor philosophy in microbiology

By

Mahmoud Alfituri Alamari Abushiba

(B.Sc. in Medical Laboratory, Faculty of Medical Technology, Misurata University, 1997) (M.Sc. in Microbiology, Basic Sciences School, Libyan Academy, 2008)

Supervisors

Prof. Dr. Einas Hamed El-Shatoury

Professor of Microbiology Microbiology Department Faculty of Science Ain Shams University

Dr. Saad Atteya Mahmoud Moghannem

Associate Professor of Microbiology Botany and Microbiology Department Faculty of Science Al-Azhar University

Prof. Dr. Gamal Mohamed Elsaid El-Sherbiny

Professor of Medical Microbiology Botany and Microbiology Department Faculty of Science Al-Azhar University

Dr. Ali Mohamed Ali Saeed

Lecturer of Microbiology Microbiology Department Faculty of Science Ain Shams University

Microbiology Department Faculty of Science Ain Shams University 2020





Title of thesis: Antibacterial activity of nanoparticles against multi-drug

resistant Staphylococci.

Name of student: Mahmoud Alfituri Alamari Abushiba.

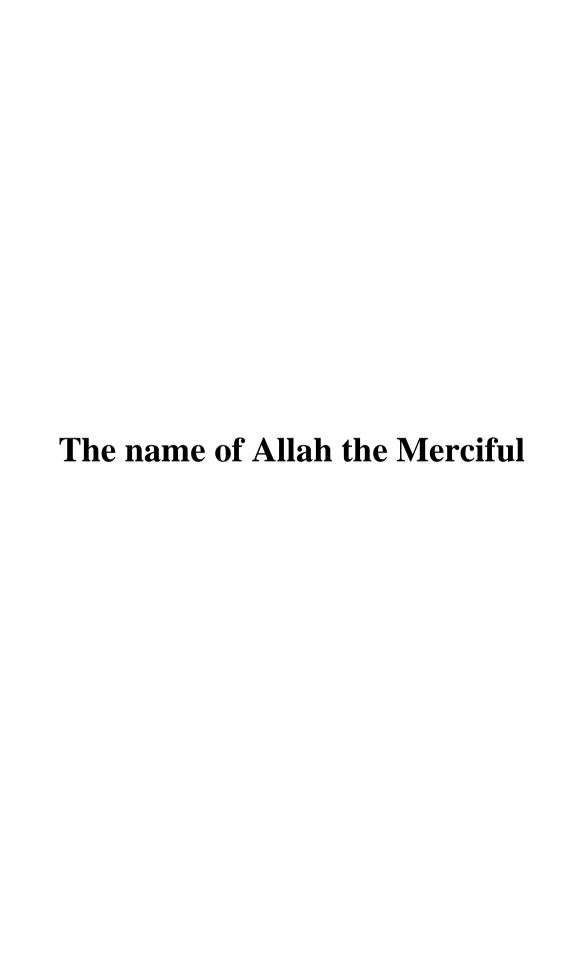
Degree: Doctor philosophy in Science in Microbiology.

Department: Microbiology.

Faculty: Science.

University: Ain Shams.

Graduation year: 2020.



Dedication

To

The soul of my Parents,

Brothers, Sisters,

Sons, Daughters,

Wives,

And,

My Lovely Family

I dedicate this to them, as my great happiness is theirs.

Mahmoud Alfituri Alamari Abushiba

Acknowledgements

In the Name of Allah, the Merciful, The Beneficent, Praise be to the Lord of all worlds. Prayers and peace be upon our Prophet, Muhammad, his family and all of his companions. Praise be to God for the countless grace and provision, praise be to God for success and charity, praise be to God introduced me to my wonderful supervisors who helped me to complete this work. It is a pleasure to express my sincere thanks and appreciation to: Prof. Einas H. El-Shatory, Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University and **Prof. Gamal M. El-Sherbiny**, Professor of Medical Microbiology, Botany and Microbiology Department, Faculty of Science Al-Azhar University, who have been a source of inspiration and guidance to me throughout my dissertation. Their careful corrections during regular meetings and discussions always ensured a more thoughtful approach towards problem-solving. Deep thanks are conveyed to Dr. Saad A. Moghannem, and Dr. Mohamed H. Kalaba, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, and Dr. Ali M. Saeed, Lecturer of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for suggesting the subject of this thesis and for their constant supervision and encouragement throughout the work which without this work would not have been completed. Sincere thanks are due to the head of Microbiology Department Prof. Youssria M. Shetaia and members of Microbiology Department, Faculty of Science, Ain Shams University, my thanks go particularly to Prof. Khaled Z. El-**Baghdady** and Prof. **Mohamed A. Abouzeid**, Prof. **Sahar T. Tolba**. I would also like to express my appreciation to Bacteriology Lab, Botany and Microbiology Department, Al-Azhar University, for providing necessary laboratory facilities to execute these studies. Also, I would like to thanks my family for their moral support. Special thanks to my brothers, sisters, and sons for their support, help and encouragement. I am grateful to everyone who has helped and encouraged me.

I thank you all Mahmoud F. Abushiba



My Thanks and Appreciation to the Professors, members of the Examination committee from right to left Prof. Einas H. El-Shatoury, Prof. Gamal M. El-Sherbiny, Prof. Maged S. Ahmed, Prof. Khalid Z. El-Baghdady, and Prof. Soad A. Abdallah,

Researcher. **Mahmud A. Abushiba** on the far left of picture.

Declaration

I declare the thesis entitled "Antibacterial activity of nanoparticles against multi-drug resistant Staphylococci" has been composed solely by myself, and it has not been submitted, in whole or in part, in any previous application for a degree.

Mahmoud Alfituri Alamari Abushiba

Table of Contents

No.	Subject	Page no
	List of Tables	i
	List of Figures	ii
	List of Abbreviations	vi
	Abstract	1
	Introduction	3
	Aim of the work	6
	Part I: Literature review	
1.1	Antimicrobial agents	7
1.1.1	Short history	7
1.1.2	Importance of antibiotics	8
1.1.3	Action mode of antibacterial drugs	8
1.1.4	Emergence of antibiotic resistance	10
1.1.5	Causes of antibiotic resistance	11
1.1.6	Bacterial resistance mechanisms	13
1.1.7	The responsibility of antibiotics for the emergence of resistant strains	17
1.1.7.1	Antibiotic-induced mutagenesis	17
1.1.7.2	Antibiotic-induced DNA damage and genomic instability	18
1.1.7.3	Antimicrobial-induced single nucleotide mutagenesis	19
1.1.7.4	Antibiotic-induced disable DNA repair	20
1.1.7.5	Antibiotic-induced gene transfer	21
1.1.7.6	Antibiotic-induced multiple-resistance	21
1.2	The multi-drug resistant problem and ways to solve this crisis	22
1.2.1	Discovery of novel antimicrobial agents	23
1.2.2	Finding new culturing techniques	24
1.2.3	New strategies for target sites	24
1.2.4	DNA recombination	25
1.2.5	Prevention of antibiotic resistance	25
1.2.6	Bacteriophage therapy	26
1.2.7	Bacteriocins and antimicrobial peptides	27

1.2.8	Synergistic combination between antimicrobials	27
1.3	Metal nanoparticles	27
1.3.1	Preparation of nanoparticles	29
1.3.2	Mechanisms of nanoparticles formation	33
1.3.3	Factors affecting synthesis of nanoparticles	36
1.3.4	Purification, separation and storage of nanoparticles	41
1.3.5	Characterization techniques of nanoparticles	43
1.3.6	Stability of nanoparticles	48
1.3.7	Preparation of nanofluids	50
1.3.8	Nanoparticles applications	51
1.3.9	Antimicrobial activity of metal nanoparticles	53
1.3.10	Mechanism of antimicrobial activity of metal nanoparticles	55
1.3.11	Antimicrobial effect of combination metal nanoparticles and	60
	antibiotics	
1.3.12	Antibacterial mechanism of nanoparticle-antibiotics conjugates	66
1.3.13	Human toxicity of metal nanoparticles	67
	Part II: Materials and methods	
2.1	Materials	70
2.1.1	Reagents	70
2.1.2	Media	71
2.1.2.1	Media for Staphylococci isolates	71
2.1.2.2	Media for Actinobacterial isolates	72
2.2	Methods	74
2.2.1	Collection of clinical samples	74
2.2.1.1	Isolation of Staphylococci isolates	74
2.2.1.2	Bacterial storage and revival	75
2.2.1.3	Inoculum preparation of tested isolates	75
2.2.1.4	Identification of Staphylococcal isolates	75
2.2.1.5	Selection of multi-drug resistant Staphylococci	76

2.2.2	Isolation, purification, and identification of Actinobacterial	79
	isolate	
2.2.2.1	Soil sample collection	79
2.2.2.2	Isolation and purification of Actinobacteria	80
2.2.2.3	Preservation and maintenance of Actinobacterial isolates	80
2.2.2.4	Screening of metal nanoparticles synthesizing Actinobacteria	80
2.2.2.5	Characterization of the selected Actinobacterial isolate	81
2.2.2.5.1	Morphological identification	81
2.2.2.5.2	Biochemical and Physiological identification	82
2.2.2.5.3	Antimicrobial activity of selected isolate	85
2.2.2.5.4	Molecular Identification of the selected isolate	85
2.2.3	Nanoparticles preparation	87
2.2.3.1	Biosynthesis and purification of metal nanoparticles	87
2.2.3.2	Characterization of metal nanoparticles	89
2.2.3.3	Antibacterial activity of metal nanoparticles	91
2.2.3.4	Assessment of synergistic activity between metal nanoparticles and	92
	antibiotics	
2.2.3.5	Determination of minimum inhibitory concentration	93
2.2.3.6	Checkerboard assay	95
2.2.3.7	Time-kill assay	98
2.2.3.8	Morphological test of treated bacterial cells	100
	Part III: Results	
3.1	Isolation and identification of Staphylococci isolates	106
3.1.1	Cultural, microscopical, and biochemical characteristics	106
3.1.2	Antimicrobial assay	108
3.2	Isolation, purification, and identification of Actinobacterial	114
	isolate	
3.2.1	Isolation and purification of the most potent isolate	114
3.2.2	Screening of potential Actinobacteria isolates for nanoparticles	116
	biosynthesis	
3.2.3	Characterization of selected Actinobacterial isolate	116
3.2.3.1	Morphological identifications	116

3.2.3.2	Biochemical characteristics	121
3.2.3.3	Physiological characteristics	122
3.2.3.4	Antagonistic activity	122
3.2.3.5	Molecular identification of Actinobacterial isolate	123
3.3	Silver nanoparticles	125
3.3.1	Biosynthesis of silver nanoparticles	125
3.3.2	Purification of biosynthesized silver nanoparticles	130
3.3.3	Preparation of silver nanofluid	130
3.3.4	Characterization of silver nanoparticles	131
3.3.4.1	Direct visual observation	131
3.3.4.2	UV-Vis spectrometry	132
3.3.4.3	X-ray diffraction	132
3.3.4.4	Particle size distribution	133
3.3.4.5	High-resolution transmission electron microscopy	134
3.3.4.6	Zeta potential measurement	134
3.3.4.7	Fourier transform infrared spectroscopy	135
3.3.5	Antibacterial activity of silver nanoparticles	136
3.3.6	Assessment of synergistic activity between Ag-NPs and antibiotics	137
3.3.7	Determination of minimum inhibitory concentration	141
3.3.8	Checkerboard assay	147
3.3.9	Time-kill assay	149
3.3.10	Morphological test of treated bacterial cells	152
3.3.11	Cytotoxicity assay of Penicillin, Ag-NPs, and their combination	154
	Part IV: Discussion and Conclusion	
	Discussion	159
	Conclusion	175
	Recommendations	177
	Summary	178
	References	180
	Arabic summary	

List of Tables

No.	Subject	Page no
Table 1.1	Representing the mechanism of drug resistance of common antibiotics.	16
Table 2.1	Frequency of S. aureus from different clinical specimens.	85
Table 2.2	Normal value of antibiotic resistant breakpoints for Staphylococci species.	78
Table 2.3	Characteristics of S. aureus ATCC 25923.	79
Table 2.4	The different concentrations of both the Ag-NPs and Penicillin combination used in a killing time assay.	99
Table 3.1	The biochemical tests for the Staphylococci isolates.	107
Table 3.2	Sensitivity pattern of S. aureus from clinical specimens.	110
Table 3.3	Zone of inhibition of different antibiotics against <i>S. aureus</i> SA-185, <i>S. aureus</i> SA-325, and <i>S. aureus</i> ATCC-25923 as control.	113
Table 3.4	The source and morphological characteristics of Actinobacteria isolates.	115
Table 3.5	Physiological and biochemical characteristics of Actinobacterial isolate E-37.	120
Table 3.6	Antibiosis activity of Ag-NPs and Ag-NF against tested isolates.	136
Table 3.7	Screening of synergistic effect between antibiotics and Ag-NPs.	138
Table 3.8	Determination of minimum inhibitory concentrations of Ag-NPs and Ag-NF against test isolates using agar well diffusion method.	142
Table 3.9	Determination of minimum inhibitory concentrations of Ag-NPs and Ag-NF against test isolates using resazurin assay.	144
Table 3.10	Determination of minimum inhibitory concentrations of Penicillin against test isolates.	145
Table 3.11	Checkerboard assay results of Penicillin and Ag-NPs combinations against <i>S. aureus</i> SA-325.	148
Table 3.12	Time kill assay of Penicillin and Ag-NPs against S . $aureus$ SA-325.	150
Table 3.13	Evaluation of different concentration of Penicillin, Ag-NPs, and their combination on WI-38cell viability determined by MTT assay.	155