

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



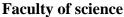
يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY





#### Microbiology department



Management of some opportunistic microbial pathogens isolated from cancer patients using fungal nanoparticles.

#### **Thesis**

**Submitted for Degree of Doctor Philosophy of Science (Microbiology)** 

By

#### Mohammed Kasem Mohammed Ali Lila

M.Sc. in Microbiology (2016)

#### **Supervisors**

Dr. Yousseria Mohamed Hassen Shetaia	Dr. Gamal Mohammed El-Saied El- Sherbiny
Professor of Mycology	Professor of Medical Microbiology
Head of Microbiology department	Botany and Microbiology department
Faculty of Science- Ain-Shams University	Faculty of science- Al-Azhar University

#### **Dr. Samar Samir Mohamed Elsayed**

Assistant professor of Microbiology

Department of Microbiology

Faculty of Science- Ain-Shams University

### **Dr.** Marwa Mohammed Fathy Elswify

Lecturer of clinical pathology

National cancer Institute

Cairo University

Microbiology Department
Faculty of science
Ain shams University

2021

#### **Approval sheet**

## Management of some opportunistic microbial pathogens isolated from cancer patients using fungal nanoparticles.

<u>By</u>

#### Mohamed Kasem Mohamed Ali Lila

M.Sc. in Microbiology (2016)

<u>Supervisors</u>	<b>Approved</b>
Prof. Dr. Yousseria Mohamed Hassen Shetaia.	
Professor of Microbiology (Mycology) and head of Microbiology	lepartment -Faculty of Science,
Ain-Shams University	
Prof. Dr. Gamal Mohammed El-Saied El-Sherbiny.	
Professor of medical Microbiology, Botany and Microbiology dep	artment, Faculty of science.
Al-Azhar University	
Dr. Samar Samir Mohamed Elsayed	
Assistant professor of Microbiology, Department of Microbiology,	Faculty of Science,
Ain-Shams University	
Dr. Marwa Mohammed Fathy Elswify	
Lecturer of clinical pathology. National cancer Institute, Cairo University	versity
Examiner committee	<b>Approved</b>
Prof. Dr. Efreen Mohamed Khalifa el-tonsiy	
Professor of pediatrics, faculty of medicine El-Azhar University (B	oys)
Prof. Dr. Eman Fathy Abdelhamid Sharaf	
Professor of Microbiology, faculty of science, Cairo University, Ca	airo
Prof. Dr. Yousseria Mohamed Hassen Shetaia	
Professor of Microbiology (Mycology) and head of Microbiology	lepartment, Faculty of Science,
Ain-Shams University,	
Prof. Dr. Gamal Mohammed El-Saied El-Sherbiny	
Professor of medical Microbiology, Botany and Microbiology depart	artment, Faculty of science.
Al-Azhar University.	

### بِسْمِ اللَّهِ الرَّحْمَانِ الرَّحِيمِ

وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلْنَا عَلَى كَثِيرٍ مِّنْ عَلَى عَبَادِهِ الْمُؤْمِنِينَ وَوَرِثَ سُلَيْمَانُ دَاوُودَ

[النمل: 15-16].

عن أبى الدرداء قال: سمعت رسول الله صلى الله عليه وسلم يقول:

(من سلك طريقا يطلب فيه علما؛ سلك الله به طريقاً من طرق الجنة، والملائكة تضع أجنحتها رضا لطالب العلم، وإن العالم يستغفر له من في السموات، ومن في الأرض، والحيتان في الماء، وفضل العالم على العابد كفضل القمر ليلة البدر على سائر الكواكب، إن العلماء ورثة الأنبياء، إن الأنبياء لم يورثوا ديناراً ولا در هما، وأورثوا العلم، فمن أخذه؛ أخذ بحظ وافر.

#### 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, Mohammad, his family and all of his companion. Praise be to God for the countless grace and provision, praise be to God for success and charity, praise be to God that provided 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. Dr. Yousseria Mohamed Hassen Shetaia, Professor of Microbiology and head of Microbiology department Faculty of Science, Ain-Shams University and Prof. Dr. Gamal Mohammed El-Saied 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. Samar Samir Mohamed Elsayed Assistant professor of Microbiology, Department of Microbiology, Faculty of Science, Ain-Shams University and Dr. Marwa Mohammed Fathy Elswify Lecturer of clinical pathology, national cancer Institute, Cairo University for suggesting the subject of this thesis and for their constant supervision and encouragement throughout the work. Sincere thanks to the head and members of microbiology department, faculty of science, Ain shams university and Botany and microbiology department, al-Azhar university, Cairo, Egypt, for providing necessary laboratory facilities to execute this studies, I would like to thank my family for their moral support, special thanks to my wife for her support, help and encouragement. I am grateful to everyone who has helped and encouraged me.

I thank you all

Mohamed Kassem Mohamed Ali

#### **Declaration**

I declare the thesis entitled "Management of some opportunistic microbial pathogens isolated from cancer patients using fungal nanoparticles" has been composed solely by myself, and it has not been submitted, in whole or in part, in any previous application for a degree.

#### **Tables of contents**

No.	subject	page no.
	List of tables	I
	List of figures	v
	Abbreviations	viii
	Abstract	1
	Introduction	3
	Aim of the work	8
	Part I: literature review	
1.1	Immunocompromised patients and bacterial	12
	infection	
1.1.1	Escherichia coli	12
1.1.2	Klebsiella pneumoniae	14
1.1.3	Acinetobacter sp.	15
1.1.4	Pseudomonas sp.	16
1.1.5	Enterobacter sp.	17
1.1.6	Pantoea sp	18
1.1.7	Serratia	18
1.1.8	Burkholderia cepacia	19
1.1.9	Cedecea davisae	20
1.1.10	Citrobacter sp.	21
1.1.11	Proteus mirabilis	21
1.1.12	Staphylococcus aureus	22
1.2	Immunocompromised patients and fungal infection	23
1.3	Antibiotics	27
1.3.1	Antibacterial classification	27
1.3.2	Antibacterial that inhibit cell wall synthesis	27
1.3.3	Antibacterial agents that breakdown cell membrane	28
	structure	
1.3.4	Antibacterial agents that inhibit the structure and	29

No.	subject	page no.
	function of nucleic acid.	
1.3.5	Antibacterial agents that inhibit the protein synthesis	30
1.3.6	Antibiotics Block key metabolic pathway	31
1.4	Antimicrobial resistance	31
1.4.1	Immunocompromised patients and resistant bacterial	32
	isolates.	
1.4.2	Methods to solve the crisis of antibiotic resistance	33
1.5	Preparation of nanoparticles	34
1.5.1	Biogenic synthesis of nanoparticles	36
1.5.2	Biosynthesis of silver nanoparticles by fungi	38
1.5.3	Application of nanoparticles	40
1.5.4	Antimicrobial activity of nanoparticles	42
1.5.5	Factors affecting on antimicrobial activity of	44
	nanoparticles	
1.5.6	Mechanism of antimicrobial activity of nanoparticles	46
1.5.7	Antimicrobial activity of silver nanoparticles	48
1.6	Amino acids enhance the activity of antibiotics.	51
	Part II: materials and methods	
2	Materials	53
2.1.1	Blood Agar Base Medium	53
2.1.2	MacConkey Agar Medium.	53
2.1.3	Sabouraud Dextrose agar	54
2.1.4	Chrom agar for Candida (Odds and Bernaerts 1994)	55
2.1.5	Muller-Hinton Agar (Mueller and Hinton, 1941)	56
2.1.6	Materials used for antibiotic susceptibility test.	56
2.1.7	Antibiotic discs used for antimicrobial susceptibility	56
	testing	
2.2	Methods	57
2.2.1	Samples collection	57

No.	subject	page no.
2.2.2	isolation and purification of microbial isolates.	57
2.2.3	Identification of microbial	58
	isolates.	
2.2.3.1	Morphological Studies	58
2.2.4	Identification of candida isolates	58
2.2.4.1	Chromogenic media	58
2.2.5	Automated Identification by Using the Biomerieux	59
	Vitek 2 System	
2.3	Suspension Preparation	60
2.3.1	Inoculation.	61
2.3.2	Card Sealing and Incubation	61
2.3.3	Optical System	62
2.3.4	Test Reactions	62
2.3.5	Database Development	62
2.3.6	Analytical Techniques	63
2.3.7	Identification Levels	63
2.4	Antibiotic susceptibility of microbial isolates	64
2.4.1	Antibiotic susceptibility of bacterial isolates	64
2.4.2	Antibiotic susceptibility of candida isolates	67
2.4.3	Detection of extended-spectrum $\beta$ -lactamase (ES $\beta$ L)	68
	producing Gram- negative bacteria	
2.5	Biosynthesis of Silver Nanoparticles	69
2.5.1	Characterization of the Biosynthesized Silver	70
	Nanoparticles	
2.5.1.1	UV-visible spectroscopy	70
2.5.1.2	X-Ray Diffraction (XRD)	70
2.5.1.3	Transmission Electron Microscopy (TEM)	70
2.5.2	Antimicrobial activity of Biosynthesized Silver	70
	Nanoparticles	

No.	subject	page no.
2.5.3	Cytotoxicity of Silver Nanoparticles	71
2.6	D-Amino acids enhance antimicrobials activity	72
	Part III: Results	
3.1	Sample's collection and microbial isolation	73
3.2	Identification of bacterial isolates	79
3.2.1	Characterization and identification of E.coli	79
3.2.2	Characterization and identification of Klebsiella	80
	pneumoniae	
3.2.3	Characterization and identification of Acinetobacter	81
	baummannii	
3.2.4	Characterization and identification of Pseudomonas	83
	aeruginosa Isolates	
3.2. 5	Characterization and identification of Enterobacter sp.	84
	Isolates	
3.2.6	Characterization and identification of Proteus mirabilis	85
	isolates	
3.2.7	Characterization and identification of Citrobacter	86
	freundii isolates	
3.2.8	Characterization and identification of Pantoea	87
	agglomerans isolates	
3.2.9	Characterization and identification of Serratia	88
	marcescens isolates	
3.2.10	Characterization and identification of Cedecea advisae	89
	isolate	
3.2.11	Characterization and identification of Burkholderia	90
	cepacia isolate	
3.2.12	Characterization and identification of Staphylococcus	91
	aureus isolates	
3.2.13	Characterization and identification of Staphylococcus	92

No.	subject	page no.
	haemolyticus isolates	
3.2.14	Characterization and identification of Staphylococcus	93
	epidermidis isolates	
3.3	Identification of Candida isolates using chromogenic	94
	agar	
3.4	In vitro susceptibility of bacterial isolates to antibiotic	97
3.4.1	Susceptibility of E. coli	97
3.4.2	Susceptibility of Klebsiella pneumonia	99
3.4.3	Susceptibility of Acinetobacter baumannii	100
3.4.4	Susceptibility of Pseudomonas aeruginosa.	102
3.4.5	Susceptibility of Enterobacter sp.	103
3.4.6	Susceptibility of Proteus mirabilis	105
3.4.7	Susceptibility of Citrobacter freundii	106
3.4.8	Susceptibility of Serratia marcescens	108
3.4.9	Susceptibility of Cedecea davisae	109
3.4.10	Susceptibility of Burkholderia cepacian	111
3.4.11	Susceptibility of Pantoea agglomerans	112
3.4.12	Susceptibility of total Gram -negative bacteria	114
3.4.13	Susceptibility of Staphylococcus sp.	115
3.5	Detection of extended-spectrum β-lactamase (ESβL)	117
	producing Gram-negative bacteria	
3.6	In-vitro susceptibility of Candida sp. to different	118
	antifungal	
3.6.1	Susceptibility of Candida albicans	118
3.6.2	Susceptibility of Candida tropicalis	119
3.6.3	Susceptibility of Candida glabrata	120
3.6.4	Susceptibility of Candida krusei	121
3.6.5	Susceptibility of all Candida sp.	123
3.7	Biosynthesis of silver nanoparticles	124

No.	subject	page no.	
3.7.1	Assessment of Antimicrobial activity of the	127	
	biosynthesized silver Nanoparticles		
3.8	Assessment of Cytotoxic activity of biosynthesized	128	
	AgNPs		
3.9	Assessment the role of amino acids in enhancement of	129	
	antimicrobial activity against resistant microbes isolated		
	from clinical samples of cancer patient		
Part IV: Discussion and conclusion			
	Discussion	132	
	Conclusion	152	
	Recommendations	153	
	Summary	154	
	References	157	
	Arabic summary (الملخص العربى )		

#### List of tables

No.	Subject	page no
Table (1):	Suspension turbidities used for card inoculation.	60
Table (2):	Identification levels of Vitek 2.	63
Table (3):	Standard inhibition zone dimeter in the protocol chart of bacterial isolates, sensitive, intermediate or resistance according to CLSI, 2018.	66
Table (4):	Standard inhibition zone dimeter in the protocol chart of candida isolates, sensitive, intermediate or resistance according to NCCLS 2008, Rosco Diagnostic Company (Neosensitabs, Denmark). and CLSI, 2018.	68
Table (5)	Percentage of occurrence of the collected samples.	74
Table (6):	Bacterial species isolated from different clinical samples collected from cancer patients.	75
Table (7):	Candida species isolated from different clinical samples in cancer patients.	76
Table (8):	Characterization of <i>Escherichia coli</i> isolates using the VITEK2 system.	80
Table (9):	Characterization of <i>Klebsiella pneumoniae</i> isolates using VITEK2 system.	81
Table (10):	Characterization of <i>Acinetobacter baummannii</i> isolates using VITEK2 system.	82
Table (11):	Characterization of <i>Pseudomonas aeruginosa</i> isolates using VITEK2 system.	83
Table (12):	Characterization of <i>Enterobacter</i> sp. isolate using VITEK2 System.	84
Table (13):	Characterization of <i>Proteus mirabilis</i> isolate using VITEK2 system.	85
Table (14):	Characterization of <i>Citrobacter freundii</i> isolates using VITEK2 system.	86
Table (15):	Characterization of <i>Pantoea agglomerans</i> isolate using VITEK2 system.	87
Table (16):	Characterization of <i>Serratia marcescens</i> isolate using VITEK2 system.	88