

بسم الله الرحمن الرحيم

 $\infty\infty\infty$

تم رفع هذه الرسالة بواسطة / سلوي محمود عقل

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

AIN SHAMS UNIVERSITY

Since 1992



Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology Department

Effect of Selected Nanoparticles on Biofilm Formation by Enterococcal Urinary Pathogens

A Thesis

Submitted in Partial Fulfillment of the Requirements for

Master's Degree

In Pharmaceutical Sciences (Microbiology and Immunology)

By

Nada Swidan Hassan

Bachelor of Pharmaceutical Sciences, Faculty of Pharmacy, Ain Shams University, 2014



Ain Shams University Faculty of Pharmacy Microbiology & Immunology Department

Effect of Selected Nanoparticles on Biofilm Formation by Enterococcal Urinary Pathogens

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master's Degree

In Pharmaceutical Sciences (Microbiology and Immunology)

By

Nada Swidan Hassan

Bachelor of Pharmaceutical Sciences, Faculty of Pharmacy, Ain Shams University, 2014

Under Supervision of

Prof. Dr. Mahmoud Abdul-Magead Yassien, PhD

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

Prof. Dr. Walid Faisal Elkhatib, PhD

Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University and Vice Dean of Faculty of Pharmacy, Galala University

Dr. Yomna Abdelgawad Hashem, PhD

Lecturer of Microbiology
Faculty of Pharmacy, The British University in Egypt





طريقالس الغطين

Acknowledgments

First of all, all thanks to **Allah**, for his valuable rewards and blessings that covered me throughout this thesis till I have finished it.

I would like to express my sincere gratitude to **Prof. Dr. Mahmoud Abdul-Magead Yassien,** Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University for his invaluable advice, continuous guidance, and patience during my master's study. His immense knowledge and plentiful experience have encouraged me all the time in my academic research.

I am extremely grateful to **Prof. Dr. Walid Faisal ElKhatib,** Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, and Vice Dean of Faculty of Pharmacy, Galala University for his invaluable guidance and endless support throughout this project. He was always there when I need help.

I would like to thank my supervisor **Dr. Yomna Abdelgawad Hashem,** Lecturer of Microbiology, Faculty of Pharmacy, The British University in Egypt. Her guidance and emotional support helped me in all the time of research and writing of this thesis. She did not hesitate to give any help and advice in every step in my thesis.

I'm proud of, and grateful for, having **Prof. Dr. Abdelgawad Hashem,** Vice Dean of Faculty of Pharmacy, The British University in Egypt, in my life. He is like a godfather to me and my colleagues. Without his precious support, it would not be possible to conduct this research.

I am sincerely grateful to **Prof. Dr. Mohamed Mohey El-Din Elmazar**, Dean of Faculty of Pharmacy, The British University in Egypt, for his continues encouragement, support, and caring.

I would like to thank **Prof. Dr. Maha Salama**, Head of Microbiology and Pharmacognosy Department, Faculty of Pharmacy, The British University in Egypt, for her kindness and infinite support.

I am also grateful to my friends and my colleague in the Faculty of Pharmacy, BUE University, and Ain Shams University, who supported me and always motivated me whenever the situation arose. You make my work life easier even on those very dreaded days.

Finally, I must express my very profound gratitude to my father Mr. Swidan Hassan, my mother Dr. Ghada Abdel Tawab, and my brother Omar for providing me with unconditional love, unfailing support, continuous encouragement throughout my life, and infinity Duaa. I am at a loss for words when I try to express what they mean to me. I couldn't have wished for a better family. This accomplishment would not have been possible without them. Thank you.

و لله الحَمدُ و المِنّة كَما ينبَغِي لجَلال وجههِ وعظيم سُلطانِه

Nada Swidan Hassan

Table of Content

Item	S	P	age no
Table	of Cor	ntent	I
List of	Abbr	reviations	IV
List of	Figur	res	VI
List of	Table	es	VIII
Abstra	act		1
Introd	uction	n	3
Litera	ture R	Review	6
1.	Biofi	ilm	6
	1.1.	Reasons for biofilm formation	7
	1.2.	Steps of biofilm formation	8
2.	Enter	гососсі	10
	2.1.	Habitat	11
	2.2.	Species differentiation and laboratory identification	11
	2.3.	Factors influencing enterococcal biofilm formation	12
	2.4.	Biofilm resistance to antibiotics	15
	2.5.	Enterococcal biofilm associated infections	16
3.	Agen	nts to combat biofilm	17
	3.1.	Bacteriophages	17
	3.2.	Quorum sensing inhibitors	18
	3.3.	Enzyme Inhibitors	18
	3.4.	Plant derived antibiofilm products	19
	3.5.	Metabolites combined with antibiotics	19
4.	Nano	oparticles	20
5.	Silve	er nanoparticles	21
	5.1.	Antibacterial activity of silver nanoparticles	21
	5.2.	Antibiofilm activity of silver nanoparticles	22
	5.3.	Synthesis of silver nanoparticles	23

		5.4.	Catheters coated silver nanoparticles	25		
Mate	eri	als and	d Methods	26		
Mate	eri	als		26		
1	l.	Micro	organisms	26		
2	2.	Chem	icals	26		
3	3.	Plants	3	27		
4	1 .	Culture media				
5	5.	Equip	ment	29		
Metl	hoo	ds		31		
6	ó.	Purifi	cation and maintenance of the collected isolates	31		
7	7.	Identi	fication of the collected isolates	31		
		7.1.	Microscopical examination	31		
		7.2.	Biochemical tests	32		
		7.3.	Genetic identification of the collected isolates to species level using <i>ddl</i> specific primers	•		
8	3.	Asses	sment of biofilm formation	34		
		8.1.	Tube Method	34		
		8.2.	Crystal violet assay	34		
ç).	Silver	nanoparticle synthesis	35		
		9.1.	Green synthesis of silver nanoparticles	35		
1	0.	Chara	cterization of silver nanoparticles	37		
		10.1.	UV-visible spectra analysis	37		
		10.2.	Particle size and zeta potential analysis	37		
		10.3.	Fourier transform infrared, energy dispersive X-ray and X-ray Diffraction analysis	•		
		10.4.	Transmission electron microscope	38		
1	1.	Antib	acterial effect of silver nanoparticles	38		
		11.1.	Determination of minimum inhibitory concentration of the sil nanoparticles			
		11.2.	Determination of minimum bactericidal concentration of the silver nanoparticles	39		

	12.	Antib	iofilm effect of sub-MIC of silver nanoparticles	.39
		12.1.	Microtiter plate assay	.39
		12.2.	Effect on bacterial adherence onto urinary catheter surfaces	.40
		12.3.	Scanning electron microscope of the catheters	.40
	13.	Statis	tical analysis	.41
Re	sults	S		.42
	1.	Purifi	cation and identification of Enterococcus isolates	.42
	2.		cic identification of the collected isolates to species level by using pecific primers	_
	3.	Asses	sment of biofilm formation	.46
		3.1.	Tube assay	.46
		3.2.	Crystal violet assay	.47
	4.	Synth	esis of green silver nanoparticles	.51
	5.	Chara	cterization of silver nanoparticles	.51
		5.1.	UV-visible spectra analysis	.51
		5.2.	Particle size and zeta potential analysis	.52
		5.3.	Fourier Transform Infrared analysis	.53
		5.4.	Energy dispersive X-ray analysis	.57
		5.5.	X-ray diffraction analysis	.59
		5.6.	Transmission electron microscope	.60
	6.	Antib	acterial effect of silver nanoparticles	.61
	7.		iofilm activity of silver nanoparticles by using microtiter plate	.64
	8.		dherent effect of silver nanoparticles against tested <i>E. faecalis</i> es on urinary catheter surfaces	.66
	9.	Scann	ing electron microscope of treated catheters	.67
Di	scuss	sion		.69
Su	mma	ary		.80
Re	fere	nces		.83
ہے	ں العر	. الملخص		1

List of Abbreviations

Abbreviations Definitions

ABC ATP-binding cassette

AgNPs Silver nanoparticles

AgNO₃ Silver nitrate

ATR Attenuated total reflection

CAUTI Catheter associated urinary tract infection

ddl D-alanine D-alanine ligase

bee Biofilm enhancer in enterococci

DLS Dynamic light scattering

Ebp Endocarditis and biofilm associated pili

EDX Energy dispersive X-ray

Esp Enterococcal surface protein

EPS Extracellular polymeric substances

FCC Face centered cubic crystal

Fsr Enterococcus faecalis sensor regulator

FT-IR Fourier transform infrared

GelE Gelatinase

GBAP Gelatinase biosynthesis-activating pheromone

HSL Homoserine lactone

ICDD-PDF International center for diffraction data- Powder

diffraction file

MBC Minimum bactericidal concentration

List of Abbreviations

Abbreviations Definitions

MIC Minimum inhibitory concentrations

MW Molecular weight

OD Optical density

PBS Phosphate buffered saline

PCR Polymerase chain reaction

PGCs Pilin gene clusters

PdI Poly dispersive index

PVP Polyvinylpyrrolidone

QSI Quorum sensing inhibitor

ROS Reactive oxygen species

SEM Scanning electron microscope

SPR Surface plasmon resonance

SprE Serine protease

TEM Transmission electron microscope

tetS Tetracycline resistance gene

TSBG Trypticase soy broth with 1% glucose

UTI Urinary tract infection

UV Ultra violet

XRD X-ray Diffraction