



Impact of Gamma Radiation and Some Natural Compounds on Quorum Sensing System of Some Pathogenic Gram Negative Bacteria

**Thesis Submitted for the award of the degree of doctor
philosophy in microbiology**

By

Reham Rashad Mahmoud EL-Behery

(M.Sc . in Microbiology, Ain Shams University)

Supervisors

Prof. Fawqia M. EL-Beih

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

Prof. Hala Abdallah Farrag

*Professor of Microbiology
Drug Research Department,
National Center for Radiation Research
Technology*

Dr. Khaled Zakaria El-Baghdady

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

**Microbiology Department
Faculty of Science
Ain Shams University
2015**

APPROVAL SHEET

Name: Reham Rashad Mahmoud EL-Behery

Title:

Impact of Gamma Radiation and some Natural Compounds on
Quorum Sensing System of some Pathogenic Gram Negative
Bacteria

Supervisors:

- 1- Prof. Fawqia Mohamed EL Beih*
- 2- Prof. Hala Abdallah Farrag*
- 3- Dr. Khaled Zakaria EL Baghdady*

Examiners:

- 1- Prof. Hassan Moawad Abdelaal*
- 2-Prof. Heba AbdelMonem El Refay*
- 3- Prof. Fawqia Mohamed EL Beih*
- 4- Prof. Hala Abdallah Farrag*

DEDICATION

*I gratefully would like to dedicate this work to my dearest
parents, husband, son and daughter for their continuous,
unconditional and unlimited support through the years of this
study, wishing them a life overwhelmed with good health,
success and joy*

Many thanks to all of you

Reham

Declaration

This thesis has not been submitted previously for this or other degree in this or any other university.

Reham Rashad EL-Behery

ACKNOWLEDGEMENT

*I wish to express my deepest and most sincere gratitude to dear **God** for supporting me through this study and giving me the strength of patience and insistence to accomplish this work,*

*My sincere thanks to **Prof. Fawqia M. EL-Beih**, Professor of Microbiology, Microbiology Department, Faculty of science, Ain Shams University, for her kind supervision and support.*

I wish to express my sincere appreciation, deepest thanks and gratitude to Prof.Dr.Hala Abdallah Farrag Professor of Medical Microbiology and Head of Biotechnology sector, National Center for Radiation Research and Technology for her valuable time in the supervision during all stages of this study, continuous facilities offered to accomplish this work, Prof. H.Farrag suggested the idea, designed the study, wrote the protocol, managed the analysis of the study and revised the thesis.I am honored to be under her supervision.

*I deeply value and appreciate the effort of **Dr. Khaled Z. EL-Baghdady**, Associate Professor of Microbiology, Microbiology Department, Faculty of science, Ain Shams University, for devoting much of his valuable time and effort in the supervision of this study, in addition to his valuable remarks, suggestions, encouragement and unlimited help.*

*A special gratitude is in order to **Dr. Sahar Tolba**, Associate Professor of Microbiology, Microbiology Department, Faculty of science, Ain Shams University, for her cooperation and valuable help and support concerning the section of PCR and molecular identification of the isolates.*

I am also seizing this opportunity to thank all staff members of the Microbiology Laboratory at the Drug Radiation Research Department at NCRRT for their sincere help and support.

Reham EL-Behery

CONTENTS

Title	Page
ABSTRACT	1
INTRODUCTION	4
AIM OF THE WORK	8
REVIEW OF LITERATURE	9
1.1 Common pathogenic Gram negative bacteria and their pathogenicity	12
1.2 Bacterial virulence factors	23
1.3 Ionizing Radiation	25
1.3.1 Interaction of Ionizing radiation with biological materials	25
1.3.2 Effect of ionizing radiation on microorganisms	26
1.3.3 Radiolysis of water	30
1.4 Quorum Sensing	31
1.5 Detection of AHLs using bioreporters	36
1.6 Quorum sensing inhibition(QSI)	38
MATERIALS AND METHODS	
2.1 MATERIALS	42
2.1.1 Microorganisms	42
2.1.1.1 Clinical isolates	42
2.1.1.2 Bioreporter organism	42
2.1.2 Media used for isolation and cultivation of pathogenic bacteria	42
2.1.3 Reagents buffers and solutions	51
2.1.4 Chemicals	53

2.1.5 Chemicals and Reagents of GC/MS	54
2.1.6 Cell Line	55
2.1.7 Radiation source	55
2.2 Methods	56
2.2.1 Collection of clinical samples and specimens	56
2.2.1.1 Isolates recovery from urine samples	56
2.2.1.2 Sputum specimens	57
2.2.1.3 Blood samples	57
2.2.1.4 Isolates recovery from wounds and vaginal secretions	57
2.2.1.5 Isolates recovery from ascitic fluid	58
2.2.2 Maintenance of clinical isolates	58
2.2.3 Preliminary identification of clinical isolates	59
2.2.3.1 Growth on MacConkey's agar	59
2.2.3.2 Citrate utilization test	59
2.2.3.3 Hemolytic activity	59
2.2.3.4 Indole production test	60
2.2.3.5 MIU test	60
2.2.3.6 Nitrate reduction test	60
2.2.3.7 Oxidase test	61
2.2.3.8 Oxidation-fermentation test	61
2.2.4 Preliminary assay of some virulence factors	61
2.2.4.1 Preparation of bacterial inoculum	62
2.2.4.2 Growth conditions for production of virulence factors by the test	62
2.2.4.3 Assay of protease production	62

2.2.4.4 Assay of lipase production	63
2.2.4.5 Assay of elastase production (Elastin hydrolysis)	63
2.2.4.6 Detection of slime production	64
2.2.4.7 Assay of pyocyanin production	64
2.2.4.8 Twitching motility	64
2.2.4.9 Cytotoxicity assay	65
2.2.5 Culturing and preparation of reporter strain	66
2.2.6 Agar-Based plate bioassays for detection of Acyl HSL	67
2.2.6.1 Cross-feeding assay for AHL detection	67
2.2.6.2 Diffusion assay	67
2.2.7 Antibiotic sensitivity test	68
2.2.8. Effect of gamma radiation on QS producing samples	71
2.2.9. Preparation of natural products	72
2.2.9.1. Extraction of clove and lavender essential oils	72
2.2.9.2. Extraction of garlic	72
2.2.9.3 Extraction of rosemary	73
2.2.10. Biosensor bioassay for detecting quorum sensing inhibitory activity (QSI) of the different natural compounds	73
2.2.10.1 Screening of QSI activity by well diffusion assay	73
2.2.10.2. Screening of QSI activity by disc diffusion assay	74

2.2.11 Disk Diffusion Method for determination of Minimum Inhibitory Concentration MIC	75
2.2.12. Effect of anti-quorum sensing compounds and radiation on some virulence factors	76
2.2.12.1 Quantitative assay of protease activity	76
2.2.12.2 Quantitative assay of lipase activity	77
2.2.12.3 Quantitative assay of elastase activity	78
2.2.12.4 Quantitative assay for Biofilm formation	79
2.2.12.5 Cytotoxicity	80
2.2.13 Gas Chromatography-Mass Spectrometry (GC/MS) analysis of N-Acyl Homoserine Lactones before and after treatments	80
2.2.13.1 Ethyl acetate extraction	80
2.2.13.2 GC/MS Instrumentation and Conditions	81
2.2.14 Molecular analysis	82
2.2.14.1 Identification of the selected bacterial isolates by 16SrRNA gene sequencing	82
2.2.14.2 sequencing of 16SrRNA genes	83
2.2.15 Statistical analysis	83
RESULTS	84
3.1 Clinical isolates	84
3.2 Preliminary identification of clinical isolates	88
3.3 Preliminary assay of some virulence factors of the bacterial isolates	89
3.3.1 <i>Pseudomonas</i> spp.	89
3.3.2 <i>Escherichia coli</i>	95

3.3.3 <i>Klebsiella</i> spp.	95
3.3.4 <i>Acinetobacter</i> spp.	98
3.3.5 <i>Providencia</i> spp.	98
3.4 Detection of quorum sensing signals (AHLs) in clinical isolates	101
3.5 Antibiotic sensitivity test	105
3.6 Screening of antiquorum sensing activity of different plants extracts	107
3.7 Determination of Minimum Inhibitory Concentration (MIC) of selected plant extracts	110
3.8 Effect of antiquorum sensing compounds and radiation on bacterial virulence factors	112
3.9 Identification of selected clinical isolates	129
DISCUSSION	135
CONCLUSION&RECOMENDATIONS	161
SUMMARY	163
REFERENCE	166
ARABIC SUMMARY	

