Dispersion of biofilm formed by some Gram negative bacteria using microbial by-products

Thesis submitted for Partial Fulfillment of Master Degree in Microbiology

By

Nessma Mostafa Muhammed Gad

B.Sc. Microbiology, 2008

Supervisors

Dr. Einas H. Elshatoury

Associate Professor of Microbiology,

Faculty of Science,

Ain Shams University.

Dr. Sahar T.M. Tolba

Associate Professor of Microbiology,

Faculty of Science,

Ain Shams University.

Microbiology Department
Faculty of Science
Ain Shams University

2019

Approval Sheet

Dispersion of biofilm formed by some Gram negative bacteria using microbial by-products

By

Nessma Mostafa Muhammed Gad

B.Sc. Microbiology, Faculty of Science,

Ain Shams University, 2008

Supervisors: **Approval**

Dr. Einas H. Elshatoury
Associate Professor of Microbiology,
Faculty of Science, Ain Shams University.

Dr. Sahar T.M. Tolba
Associate Professor of Microbiology,
Faculty of Science, Ain Shams University.

Examination committee:

Dr. Mehrshan Taha El-Mokadem
Professor of Microbiology
Faculty of Arts and Science (girls)-Ain Shams University

Dr. Azza Abd El-Fattah Mohamed
Professor of Microbiology
Egyptian Atomic Energy Authority

Dr. Einas H. Elshatoury
Associate Professor of Microbiology,
Faculty of Science, Ain Shams University.

Dr. Sahar T.M. Tolba
Associate Professor of Microbiology,
Faculty of Science, Ain Shams University.

Examination date: **Approval date:**
University Council approval:

Acknowledgements

I would like to thank my supervisors, Dr. Sahar Tolba & Dr. Einas El-Shatoury for the patient guidance, encouragement and advice they have provided throughout my time as their student.

I sincerely place on record my thanks to the Microbiology Department, Ain shams university for facilitating my research and for all the support rendered.

I would like also to thank the Clinical laboratory department at International Medical Center (IMC) & Professors at the Regional Center for Mycology & Biotechnology at Al-Azhar University for their cooperation & support.

Completing this work would not have been possible without the support and friendship provided by my best friend Nermine Mohammad. I'm very grateful for your continuous support, encouragement & advice throughout this journey. No words of thanks can sum up the gratitude I owe her.

I would like to express my sincere gratitude to my family for their spiritual support and continuous encouragement throughout my life. I would not have been here without you.

Last but not least, I dedicate this dissertation to my late father who has been a constant source of support and encouragement. No

words can express how proud I am to be his daughter and I dedicate every success to him.

Contents

List of Tables

List of figures

Abbreviations

AST	Antibiotic Susceptibility Testing
BAIs	Biofilm Associated Infections
BAL	Broncoalveolar Lavage
BS	Biosurfactants
BSI	Blood Stream Infection
CCU	Coronary Care Unit
CDC	Centers for Disease Control and Prevention
CF	Cystic Fibrosis
CFS	Cell Free Supernatant
CLSI	The Clinical and Laboratory Standards Institute
CMC	Critical Micelle Concentration
CoNS	Coagulase Negative *Staphylococcus*
CSF	Cerebrospinal Fluid
CVCs	Central Venous Catheters
CVL	Central Venous Line
DA-HAIs	Device-Associated Health Care–Associated Infections
DANIs	Device-Associated Nosocomial Infections
DNA	Deoxyribonucleic Acid
eDNA	Extracellular Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EMCC	Egyptian Microbiological Culture Collection
EPS	Extracellular Polymeric Substances
ETT	Endotracheal Tube
Global PPL	Global Priority Pathogens List

GRAS	Generally Regarded As Safe
HAIs	Health care–associated infections
ICUs	Intensive Care Units
INICC	International Nosocomial Infection Control Consortium
IPC	Infection Prevention and Control Interventions
IUD	Intrauterine Devices
MDR	Multi-Drug Resistant
MIC	Minimum Inhibitory Concentration
NCCLS	National Committee for Clinical Laboratory Standards
NHSN	National Healthcare Safety Network
NICU	Neonate Intensive Care Unit
NIH	National Institutes of Health
NVE	Natural Valve Endocarditis
OD	Optical Density
OM	Otitis Media
PICU	Pediatric Intensive Care Unit
PVE	Prosthetic Valve Endocarditis
QS	Quorum Sensing
RNA	Ribonucleic Acid
RTI	Respiratory Tract Infection
SD	Standard Deviation
ST	Surface Tension
TCP	Tissue Culture Plate
TM	Tube Method

TSB	Tryptone Soy Broth
UTI	Urinary Tract Infection
WHO	World Health Organization

Abstract

Antimicrobial agents have been used for the last 70 years to treat patients who have infectious diseases. However, these drugs have been used for long time that the infectious organisms have adapted to them through different mechanisms. Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics and disinfectants as well as body's defense system.

Bacteria in biofilms embed themselves in extracellular matrix that acts as shield from antibacterials and help them to overcome harsh nutritional and environmental conditions. One promising way of disabling biofilm resistance is through breaking up this matrix using biosurfactants. *A. baumannii* & *P. aeruginosa* isolated from Egyptian hospitals were tested for their antibiotic resistance and ability to form biofilm. Out of 22 Multi-drug resistant (MDR) *P. aeruginosa* isolates, 100 % were biofilm formers. While in *A. baumannii* out of 36 MDR, 77% showed biofilm formation ability.

The anti-biofilm activity of a bio-surfactant extracted from *Bacillus subtilis* was tested against biofilms of *P. aeruginosa* and *A. baumannii*. The dispersion of biofilm occurred in 44-46 % of *P. aeruginosa* and 64% - 66 % of *A. baumannii*. using 0.25 and 0.5 mg/ml of the *Bacillus subtilis* extract.

Keywords: Biofilm, Multi-drug resistant, Anti-biofilm, Bio-surfactant, *Bacillus subtilis.*