# ROLE OF FATTY ACIDS AS ENVIRONMENTAL REGULATOR ON PROTEUS MIRABILIS SWARMING AND BIOFILM FORMATION IN CATHETER-ASSOCIATED URINARY TRACT INFECTION

#### **THESIS**

Submitted for Partial Fulfillment of the Medical Doctorate Degree in Medical Microbiology and Immunology

> By Amal Aly Aly El-Shimy M.B.,B.Ch (M.Sc.)

#### SUPERVISED BY

#### PROF. DR. MOHAMED AMR EL-KHOLY

Professor of Medical Microbiology & Immunology
Faculty of Medicine
Cairo University

#### DR. ZEINAB ABD EL- KHALEK IBRAHIM DR. NADIA HAFEZ SALAH EL-DIN AUDA

Assistant Professor of Medical Microbiology & Immunology Faculty of Medicine Cairo University Assistant Professor of Medical Microbiology & Immunology Faculty of Medicine Cairo University

Faculty of Medicine Cairo University 2009

# بسم الله الرحمن الرحيم

#### **ABSTRACT**

*P. mirabilis* is a common cause of C-UTI. This study evaluates the effect of stearic, plamitic and myristic acids to LB culture medium, on swarming, biofilm formation and EPS production of 30 *P. mirabilis* strains isolated from 300 urine samples collected from 300 catheterized patients. Myristic and palmitic acids could inhibit swarming and EPS of *P. mirabilis*. Only myristic acid could inhibit biofilm formation, while stearic acid enhanced swarming and EPS production of *P. mirabilis*.

#### Key Words:

. C-UTI. Catheter - associated urinary tract infection

. EPS: exopolysaccharides

. hrs: hours

. LB: Lauri Bertani

P. mirabilis: Proteus mirabilis

#### **ACKNOWLEDGEMENT**

First and foremost, thanks to ALLAH, the most beneficent, the most merciful.

The greatest credit goes to *Prof. Dr. Mohamed Amr El-Kholy*, Professor of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, for his valuable assistance continued support, and precious guidance, throughout the development of this thesis.

I would like to express my sincere thanks and greatest gratitude to *Dr. Zeinab Abd El-Khalek Ibrahim*, Assistant Professor of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University.

My heartful gratitude is given to *Dr. Nadia Hafez Salah El-Din*, Assistant Professor of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, for her splendid cooperation, valuable advise and expert guidance. My deepest thanks for her kind help and true assistance in fulfilling this work.

To whom I belong, I'm deeply grateful, to all my respectable professors, staff members and colleagues at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University.

I'm honored to thank the staff members of the Urology Department of Kasr El-Aini University Hospital for their generous cooperation during samples collection.

Finally, I owe a great appreciation to my husband and daughters for their kind support althrough this thesis.

# **CONTENTS**

	Page
LIST OF ABBREVIATIONS	I
LIST OF TABLES	VI
LIST OF FIGURES	VIII
INTRODUCTION AND AIM OF THE WORK	1
REVIEW OF LITERATURE	
PROTEUS MIRABILIS	
Morphology	5
Culture	7
Colonial appearance	8
Swarming	9
Anti-swarming agents	17
Metabolic activities and extracellular products	19
Susceptibility to physical and chemical agents	24
Virulence factors and pathogenesis	25
A) Cystitis	27
1) Flagellae	27
2) Fimbriae and adhesins	34
B) Pyelonephritis and stone formation	40
1) Urease	40
2) Hemolysin	46

C) Iron sequestration	47
D) Immune system avoidance	49
E) Antimicrobial resistance	51
EPIDEMIOLOGY OF P. MIRABILIS UTI	54
1) Risk factors of <i>P. mirabilis</i> UTI	55
2) Rheumatoid arthritis and <i>P. mirabilis</i> UTI	56
3) Incidence of <i>P. mirabilis</i> UTI	58
4) Susceptibility to antimicrobial agents	59
5) Vaccines for <i>P. mirabilis</i> in UTI	63
BIOFILM FORMATION AND CATHETER ENCRUSTATION	67
1) Mechanism of biofilm formation	67
2) Antimicrobial susceptibility of biofilms	71
3) Biofilm in C-UTIs	72
4) Strategies for the control of catheter encrustation	74
5) Management of biofilm infection	83
EFFECTS OF FATTY ACIDS ON BACTERIA	86
SUBJECTS AND METHODS	91
RESULTS	111
DISCUSSION	124
CONCLUSION AND RECOMMENDATIONS	135
SUMMARY	138
REFERENCES	139
APPENDIX	180
ARABIC SUMMARY	

# LIST OF TABLES

	Table	Page
1)	Distinguishing reactions of species of Proteus, Morganella and	
	Providencia	4
2)	Rate of <i>P. mirabilis</i> isolation in relation to sex	112
3)	Relationship between rate of <i>P. mirabilis</i> isolation and age	
	of the patients	
4)	Relationship between P. mirabilis isolation and duration of	113
	urinary catheter insertion	
5)	Results of the effect of stearic, palmitic and myristic acids	114
	on swarming of <i>P. mirabilis</i>	
6)	Results of swarming migration of P. mirabilis at different	116
	concentrations of palmitic and myristic acids	
7)	Results of the effect of stearic, palmitic and myristic acids	115
	on biofilm formation by <i>P. mirabilis</i>	
8)	Results of biofilm formation by P. mirabilis at different	119
	palmitic and myristic acids concentrations	

9)	Effects of addition of fatty acids on EPS production by	120
	P. mirabilis	
10)	Results of EPS production by <i>P. mirabilis</i> at different	122
10)		122
	palmitic and myristic acids concentrations	

# LIST OF FIGURES

Figure	Page
Figure (1) Simplified scheme of some of the factors and genes involved in the cycle of swarming growth in <i>Proteus</i>	12
Figure (2) Virulence factors produced by <i>P. mirabilis</i> and possible sites of action during UTI.	26
Figure (3) Proportion of UTI caused by <i>P. mirabilis</i>	55
Figure (4) Formation of biofilm	68
Figure (5) Composition of biofilm	69
Figure (6) Scanning electron microscope of a developing biofilm	70
Figure (7) Encrustation of catheters in relation to the catheter material	79
Figure (8) Relationship between time of catheter blockage and the catheter material	80
Figure (9) The effect of heparin coating on biofilm formation	82

Figure (10) Effect of antibiotics on biofilm progression	85
Figure (11) Methods for obtaining a catheter specimen of urine	92
Figure (12) Swarming appearance on nutrient agar	97
Figure (13) Biochemical reactions of <i>P. mirabilis</i>	100
Figure (14) Effects of fatty acids on swarming of 3 different strains of <i>P. mirabilis</i> (1, 2, 3)	102
Figure (15) Microtitre plate after addition of crystal violet	104
Figure (16) Crystal violet stained biofilms of <i>P. mirabilis</i> in the microtitre plate wells.	105
Figure (17) Extraction of the biofilms with ethanol	106
Figure (18) Resuspension of the cell pellets with high salt buffer	108
Figure (19) Effects of fatty acids on EPS production by <i>P. mirabilis</i>	109
Figure (20) Rate of <i>P. mirabilis</i> isolation in relation to sex of the patients	112

Figure (21) Relationship between rate of <i>P. mirabilis</i> isolation and	113
age of the patients	
Figure (22) Relationship between rate of <i>P. mirabilis</i> isolation and	114
duration of urinary catheters insertion	
Figure (23) Effects of different fatty acids on swarming of <i>P</i> .	115
mirabilis	
Figure (24) Effects of fatty acids on <i>P. mirabilis</i> biofilm formation	118
Figure (25) Effects of fatty acids on EPS production by <i>P. mirabilis</i>	121
rigate (20) Effects of fact, acids off Eff b production by r. mirabilis	121

#### LIST OF ABBREVIATIONS

α: Alpha.

aad: Amino acid deaminase.

Adon: Adonitol.

AI-2: Autoinducer 2.

AmpC: Ambler's molecular class C.

ATF: Ambient temperature fimbriae.

bp: Base pair.

 $\beta$ : Beta.

C: Complement.

Ca<sup>2+</sup>: Calcium.

CFU/ml: Colony forming unit per milliliter.

CFU: Colony forming unit.

CLED: Cystine lactose electrolyte deficiency.

*Ccm:* Cell-cell migration.

*cmfA*: Colony migration factor.

CPG: Casamino- peptone-glucose.

C-terminus: Carboxy terminus.

CTX: Cefotaxime.

C-UTI: Catheter-associated urinary tract infection.

C $\beta$ L: AmpC  $\beta$ -lactamases.

DKPs: Diketopiperazines.

DNA Deoxyribonucleic acid.

DPPC: Diplamitoylphosphatidylcholine.

E. coli: Escherichia coli.

EDTA: Ethylene diamine tetra-acetic acid.

EPS: Exo/Extracellular polysaccharides.

ESBLs: Extended spectrum β-lactamases.

FAS: Fatty acid synthetase.

Fig.: Figure.

fla: Flagellin gene.

flh: Flagellar hyperexpression gene.

Fur: Ferroxamine upregulator.

γ: Gamma.

gidA: Growth and intiation of differentiation.

GIT: Gastrointestinal tract.

h $\beta$ D1: Human  $\beta$ -defensin 1.

HAUTI: Hospital-acquired urinary tract infection.

HLA: Human leucocytic antigen.

Hly: Hemolysin.

HPF: High power field.

hrs.: Hours.

 $ID_{50}$ : Infective dose 50.

Ig: Immunoglobulin.

K. aerogenes: Klebsiella aerogenes.

K. penumoniae: Klebsiella pneumoniae.

KCN: Potassium cyanide.

kD<sub>a</sub>: Kilo dalton.

L. Lactis: Lactococcus lactis.

LB: Luria – Bertani.

 $LD_{50}$ : Lethal dose 50.

LD<sub>50</sub>S: Lethal dose 50 strain.

Log: Logarithmic.

LPS: Lipopolysaccharides.

*Lrp*: Leucine-responsive regulator protein.

M. morganii: Morganella morganii.

mg/l: Milligram per liter.

mg/ml: Milligram per milliliter.

Mg<sup>2+</sup>: Magnesium.

MIC: Minimal inhibitory concentration.

min.: Minute.

mM: Millimole.

mm: Millimeter.

mol: Mole.

MPC: Methacryloyloxyethyl phosphoryl choline.

MR/K: Mannose - resistant/*Klebsiella*-like.

MR/P: Mannose - resistant/*Proteus*-like.

mRNA: Messenger ribonucleic acid.

*mrp*: Mannose resistant pilin.

μg/ml: Microgram per milliliter.

μL: Microliter.

μm: Micrometer.