# BIOFILM PRODUCTION AND ANTIBIOTIC SUSCEPTIBILITY OF UROPATHOGENS ISOLATED FROM AHMADU BELLO UNIVERSITY TEACHING HOSPITAL ZARIA, NIGERIA

**BY**

# Michael Nosano YAKUBU

**B. Sc. MICROBIOLOGY (UNIMAID) 2009 M. Sc./PHARM-SCI/16491/2010-2011**

# A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACEUTICAL MICROBIOLOGY.**

# DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY

**FACULTY OF PHARMACEUTICAL SCIENCES AHMADU BELLO UNIVERSITY, ZARIA NIGERIA**

# SEPTEMBER, 2014

**DECLARATION**

, GHFODUH WKDW WKH ZRUN SUHVHQWHG LQ WKLV

Antibiotic Susceptibility of Uropathogens Isolated from Ahmadu Bello University

Teaching Hospital Zaria, Nigeria´ KDV EHHQ FDUULHG RXW E\ PH Pharmaceutics and Pharmaceutical Microbiology, under the supervision of Dr. B. O.

Olayinka and Dr (Mrs) G. O. Adeshina. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Name of student Signature Date

# CERTIFICATION

7KLV WKHVLV HQWLWOHG ³%N,TI2BI)O,TIC/S0U SC3E5PT2IB'IL8IT&Y 7,21 $1

OF UROPATHOGENS ISOLATED FROM AHMADU BELLO UNIVERSITY

TEACHING HOSPITAL ZARIA, NIGERIA´ E\ 0LFKDHO 1RVDQR <DNX

regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its scientific contribution to knowledge and literary

|  |  |
| --- | --- |
| presentation. |  |
| **Dr B. O. Olayinka** |  |  |
| **Chairman, Supervisory Committee** Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. | Signature | Date |
| **Dr. (Mrs) G. O. Adeshina** |  |  |
| **Member, Supervisory Committee** Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. | Signature | Date |
| **Prof. A. B. Isah** |  |  |
| **Head of Department** Department of Pharmaceutics and Pharmaceutical Microbiology,Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. | Signature | Date |
| **Prof. Hassan Zoaka** |  |  |
| **Dean, Post Graduate School**Ahmadu Bello University, Zaria. | Signature | Date |

# ACKNOWLEDGEMENT

I must recognize First of all the Almighty God whose presence is always with me, a present help in times of trouble and whose mercy I see morning by morning.

I will ever live to remember the unprecedented input and effort of my humble and able supervisors, Dr. B. O. Olayinka and Dr. (Mrs) G. O. Adeshina who have been as a father and a mother to me in all ramifications. It is their dedication of time and energy for thorough scrutiny and supervisions that has put this work in the best shape. They will ever live to be remembered and reckoned in high esteem in my heart.

I will also want to appreciate and acknowledge my late father, Mr. Yakubu Danwazam and my beloved mother, Kyakyawa Beauty Yakubu whose motherly care, concern and encouragement has brought me this far. My spiritual fathers, Pastor W. F. Kumuyi, Pastor

L. Innocent, Pastor I. Zamdayo, Pastor E. Meshubi, Pastor L. Ejeh, Pastor A. Elisha and Pastor G. Solomon will ever be appreciated for their prayers, cares, concern and encouragement.

I most appreciate the impact and effort of my elder brothers, Mr. Danlami Jatau and family, Mr. Jeremiah D. Yakubu and family, Mr. Shibayino Yakubu, Mr Gabriel Jatau and Family, Mr. Luka Jatau and family, Mr. Moses Jatau and family for all their assistance, my sisters, Mrs. Mercy H., Mrs Mary M., Mrs. Zainab N., Mrs. Paulina S., Mrs. Justina T. and Mrs. Rebecca D. for all their helps and contributions.

I also wish to express my sincere appreciation to my professors and able lecturers, Prof.

J.A. Onaolapo, Prof. J.O. Ehinmidu, Prof. Y.K.E. Ibrahim, Prof. A. B. Isah, Prof.

Adikwu, Dr. Olowosulu, Prof. A. Agunu, Dr. Hassan, Dr. B.A. Tytler, Prof. C.N. Kwanashie, Prof. J.A. Anoka, Dr. E. Ella of Microbiology Department, the chief laboratory technician (Mr Daniel), our always available laboratory technician (Pastor Ezekiel), Mr. Y. Y. Pala of Veterinary Medicine, Dr K. Mshelbwala, Dr. Abdulsamad, Dr. Awasum, Mrs Olayemi, Mr Abas, Mrs. Vicky, Mrs. Okeke, Mrs. Regina, Mr. Apeji (my daddy), Mr. Innocent, Mr. Yonni, Mr. Falaki, Mr. Godwin, Mr. Mike, Mal. Rufai, Mal. Usman, Mrs. Obajuluwa, Mal. Umor Mohammed, Mr. Mba and all the staff of ABUTH Medical Microbiology unit and many others whose names are too numerous to mention within and outside Faculty of Pharmaceutical Sciences who all contributed wonderfully to the success of this work.

This write up cannot be brought to conclusion without acknowledging the presence of my friends: Mr. Bello Omiogbemi, Mr. Victor Babatunde, Mr. Austine Ejegwa, Mr. Elisha Ikpe, Mr. Mari Mshelia, Pharm. Josiah, Mr. Danjuma N., Mr. Sekan S., Mr Abdulwahid B., Mr. James I, Mr. Polycarp F. Mrs Ruth O, Miss Uzoma L., Mr. Aremu O, Mr. Abdullahi M, Mr. Abdulmalik, Mr. Yerima, Mrs Nkechi O, Mrs Mercy A, Mrs Sophie N, Mr. Dagogot, Mr. Yahaya, Mr. Chidi E, Mr. Awal, Miss Aisha, Miss Rahila, Mrs Kemi etc whose assistance and contributions have resulted to this great success.

What shall I say more, for the time will fail me to mention categorically individuals both home and abroad who have in one way or the other contributed to the success of this work, may God bless you all. Amen

# DEDICATION

I dedicated this work to God Almighty who picked me from nothingness and set me to seat among the princes, my beloved mother, Mrs. Yakubu Kyakyawa, my brothers, sisters and dear beloved wife to be.

# TABLE OF CONTENTS

|  |  |
| --- | --- |
| Title Page - | - - - - - - - - i |
| Declaration Page | - - - - - - - - ii |
| Certification Page | - - - - - - - - iii |
| Acknowledgements | - - - - - - - - iv |
| Dedication - | - - - - - - - - vi |
| Table of Contents | - - - - - - - - vii |
| List of Tables - | - - - - - - - - xiii |
| List of Figures | - - - - - - - - xiv |
| List of Plates - | - - - - - - - - xv |
| List of Appendices | - - - - - - - - xvi |
| Abbreviations - | - - - - - - - - xvii |

[Abstract - - - - - - - - - xx](#_TOC_250019)

[Chapter One](#_TOC_250018)

* 1. Introduction - - - - - - - - 1
	2. The Background of the Study - - - - - 1
	3. [Statement of Research Problem - - - - - 4](#_TOC_250017)
	4. [Justification - - - - - - - - 5](#_TOC_250016)
	5. [Research Aim - - - - - - - - 6](#_TOC_250015)
	6. [Specific Objectives - - - - - - - 6](#_TOC_250014)
	7. [Null Hypothesis - - - - - - - 6](#_TOC_250013)
	8. [Alternate Hypothesis - - - - - - - 6](#_TOC_250012)

Chapter Two

* 1. Literature Review - - - - - - - 7
	2. Urinary System - - - - - - - 7
	3. Urinary Tract Infection - - - - - - 10
		1. [: Catheter-Associated Urinary Tract Infection - - - - 11](#_TOC_250011)
		2. [: Risk Factors for Urinary Tract Infections **- - - - 11**](#_TOC_250010)
		3. [: The Symptoms of a Urinary Tract Infection - - - - 12](#_TOC_250009)
		4. [: Epidemiology of Urinary Tract Infection in Nigeria - - - 12](#_TOC_250008)
		5. [: Diagnosis of Urinary tract infection - - - - - 13](#_TOC_250007)
		6. [: Treatment of Urinary tract infection - - - - - 14](#_TOC_250006)
		7. [: Prevention of Urinary Tract Infection - - - - - 15](#_TOC_250005)
		8. [: Uropathogens - - - - - - - - 16](#_TOC_250004)
		9. : Escherichia coli - - - - - - - 16
		10. : Klebsiella species - - - - - - - 17
		11. [: Proteus species - - - - - - - 17](#_TOC_250003)
		12. [Pseudomonas aeruginosa - - - - - - 18](#_TOC_250002)
		13. [: Staphylococcus aureus - - - - - - - 19](#_TOC_250001)
		14. : Methicillin-Resistant *S. aureus* (MRSA) - - - - 20
	4. [:0 Microbial Biofilms - - - - - - - 21](#_TOC_250000)
		1. : The Composition of Microbial Biofilm - - - - 24
		2. : Dynamics of Biofilm Colonization and Dispersion - - - 24
		3. : Biofilm Formation on Inanimate Objects - - - - 25
		4. : The Biofilm Complications - - - - - - 25
		5. : Biofilms and Host Defenses Resistance - - - - 27
		6. : Effect of Biofilm Production on Chemotherapy of Urinary Tract

Infection - - - - - - - - 28

* + 1. : Work on Biofilm Formation of Public Health Importance in Nigeria 29

2.5: Antibiotics - - - - - - - - 30

* + 1. : Antibiotics used for the Treatment of Urinary Tract Infections - 31
		2. : Ciprofloxacin - - - - - - - - 32
		3. : Chloramphenicol - - - - - - - 34

2.5.4: Gentamicin - - - - - - - - 37

2.5.5: Amoxicillin - - - - - - - - 39

2.5.6: Categorization of some commonly used antibiotics against

Uropathogens into their respective Classes - - - - 40

* + 1. : Antibiotic Resistance - - - - - - - 44
		2. : Antimicrobial Resistance in Uropathogens - - - - 44
		3. : Biofilm Formation and Antimicrobial Resistance - - - 44
		4. : Mechanisms of Biofilm Resistance to Antimicrobial Agents - - 47
		5. : Quorum Sensing (QS) and Microbial Biofilms - - - 52
		6. : Peptide Auto*-*inducers - - - - - - 52
		7. : Acyl Homoserine Lactones (AHL) - - - - - 53

2.7.3: Auto-inducer - - - - - - - - 54

2.7.4: Fungal QS Systems - - - - - - - 55

2.8.0: Quinolone and Biofilms - - - - - - 55

* + 1. : Clindamycin Inducible Resistance - - - - - 56
		2. : D-test showing inducible clindamycin resistance - - - 56

Chapter Three

3.0: Materials and Methods

3.1: Materials - - - - - - - - - 59

3.1.1: Equipment - - - - - - - - 59

3.1.2: Glasswares - - - - - - - - 59

3.1.3: Reagents - - - - - - - - 59

* + 1. : Culture Media - - - - - - - - 60
		2. Sentivity Dics (Oxiod LTD, England) - - - - 60

3.2:0: Methods - - - - - - - - 61

* + 1. : Retrospective Study - - - - - - - 61
		2. : Collection of Isolates - - - - - 61
		3. : Procedure for Culture Media Preparation - - - - 61
		4. : Purification of the Test Organisms - - - - - 61
		5. : Identification of the Isolates - - - - - - 61
		6. : Biofilm Production Assay - - - - - - 64
		7. : Quantitative Assay of Biofilm - - - - - - 65
		8. : Antibiotic Susceptibility Testing - - - - - 65
		9. : Determination of Inducible Clindamycin Resistance -- - - 66
		10. : Minimum Inhibitory Concentration - - - - - 66
		11. : Molecular Characterization of Quinolone Resistant Isolates of Klebsiella spp, E coli, Proteus spp and Pseudomonas aeruginosa - - - 67

Chapter Four

4.0: Results - - - - - - - - - 72

* 1. : Retrospective Studies - - - - - - - 72
	2. : Prospective Study - - - - - - - 74
		1. : Isolates Collection and Identification - - - - - 74
	3. : Biofilm Production assay of the Uropathogens - - - - 76
	4. : Antimicrobial Susceptibility Testing of the Uropathogens - - 80
		1. : The Antibiotic Susceptibility Testing of Klebsiella spp, E. coli and

Proteus spp - - - - - - - - 81

* + 1. : Antibiotic Susceptibility Testing of P. aeruginosa isolates and S. aureus

Isolates - - - - - - - - 84

* 1. : Relationship between MDR and Biofilm formation among the Uropathogens-- - - - - - - - 92
	2. : Inducible Clindamycin Resistance - - - - - 94
	3. : Methicillin Resistance Staphylococcus aureus (MRSA) - - 96
	4. : The Minimum Inhibitory Concentration of Ciprofloxacin to Resistant Isolates of Klebsiella spp, E. coli, Proteus spp and Pseudomonas

Aeruginosa - - - - - - - - 97

* 1. : Molecular Characterization of Resistant Isolates - - - 99
		1. : Inclusion criteria - - - - - - - 99
		2. : Extraction of Genomic DNA - - - - - - 99
		3. : Polymerase Chain Reation Amplification of Quinolones Resistant

Isolates Using Primers For Qnr Genes - - - - 102

Chapter Five

5.0: Discussion - - - - - - - - 107

Chapter Six

6.0 Summary, Conclusion and Recommendation - - - 117

6.1 Summary - - - - - - - - 118

6.2 Conclusion - - - - - - - - 119

6.3 Recommendation - - - - - - - 120

References - - - - - - - - - 121

Appendices - - - - - - - - - 141

|  |  |  |
| --- | --- | --- |
|  | **LIST OF TABLES** |  |
| Table 2.1: | Categorization of some commonly used Antibiotics against |
|  | Uropathogens into their respective Classes - - - | 43 |
| Table 3. 1a: | PCR Conditions for Primers: qnrA and qnrC - - - | 69 |
| Table 3. 1b: | PCR Conditions for Primers: qnrB and qnrS - - - | 69 |
| Table 3.2: | The Primers for the qnr genes and their references - - | 71 |
| Table 4.1: | The Summary of Biofilm Production by Uropathogens - | 79 |
| Table 4.2: | Interpretative chart according to EUCAST (2011) - - | 82 |
| Table 4.3: | Percentage Susceptibility of isolated *Klebsiella spp, E. coli* |  |
|  | and *Proteus spp* (Enteric bacteria) - - - - | 82 |
| Table 4.4: | The isolated Uropathogens with the most prevalent resistance |  |
|  | Phenotypes - - - - - - - | 88 |
| Table 4.5: | Multiple Antibiotic Resistance (MAR) Indices of bacteria in |  |
|  | ABUTH Zaria, Nigeria - - - - - | 89 |
| Table 4.6: | The summary of MAR Indices of the Uropathogens - - | 89 |
| Table 4.7: | The Relationship between Multidrug Resistance and |  |
|  | Biofilm Formation among the Uropathogens - - - | 93 |
| Table 4.8: | Inducible Clindamycin Resistance (D ±Test) Result among the |  |
|  | Erythromycin resistant isolates of *S*. *aureus* (N=5) - - | 95 |
| Table 4.9: | Determination of MRSA using Cefoxitin Disc (N=23) - | 96 |
| Table 4.10: | The Minimum Inhibitory Concentration of Ciprofloxacin |  |
|  | to Resistant Isolates of *Klebsiella spp*, *E*. *coli*, *Proteus spp* |  |
|  | and *Pseudomonas aeruginosa*- - - - - | 98 |

**LIST OF FIGURES**

Figure 2.1: The Urinary System - - - - - - 9

Figure 2.2: The Processes of Biofilm Formation and Maturation; Adapted

from (Leid, 2009) - - - - - - 23

|  |  |  |
| --- | --- | --- |
| Figure 4.1: | The Retrospective Study of the most Prevalent Uropathogens |  |
|  | Isolated in ABUTH from May-July, 2012 - - - | 73 |
| Figure. 4.2: | Percentage of the Uropathogens isolated - - - | 75 |
| Figure 4.3: | The Percentage Biofilm Production by Uropathogens - | 77 |
| Figure 4.4: | Percentage Resistance of isolated *Klebsiella spp, E. coli* and |  |
|  | *Proteus spp.* (Enteric bacteria) - - - - | 83 |
| Figure 4.5: | Percentage Susceptibility and Resistance of isolated *P. aeruginosa* | 85 |
| Figure 4.6: | Percentage Susceptibility and Resistance of isolated *S. aureus* | 86 |
| Figure 4.7: | The Distribution of the Uropathogens into AS, NON-MDR, MDR, |  |
|  | XDR and PDR - - - - - - | 91 |

# LIST OF PLATES

Plate 2.1a: D-test Negative, *S. aureus* Sensitive to Clindamycin and

Resistant to Erythromycin - - - - - 57

Plate 2.1b: D- test Positive, flattening of zone of inhibition of

Clindamycin (Inducible Clindamycin Resistance) - - 57

|  |  |  |
| --- | --- | --- |
| Plate 4.1: | 1.0% agarose gel electrophoresis of 100 base pair and genomicDNA isolated from quinolones resistant uropathogenic *Klebsiella* |  |
|  | *spp* and *E. coli. - - - - - -* | 100 |
| Plate 4.2: | 1.0% agarose gel electrophoresis of 100 base pair and |  |
|  | genomic DNA isolated from quinolones resistant uropathogenic |  |
|  | *E. coli*, *Proteus spp,* and *Pseudomonas aeruginosa. - -* | 101 |
| Plates 4.3: | 2.0% agarose gel electrophoresis of 100 bp DNA ladder and |  |
|  | PCR amplification of genomic DNA with qnrA genes of |  |
|  | uropathogens isolated from A.B.U Teaching Hospital, Zaria. - | 103 |
| Plates 4.4: | 2.0% agarose gel electrophoresis of 100 bp DNA ladder |  |
|  | and PCR amplification of genomic DNA with qnrB genes of |  |
|  | uropathogens isolated from A.B.U Teaching Hospital, Zaria. - | 104 |
| Plates 4.5: | 2.0% agarose gel electrophoresis of 100 bp DNA ladder |  |
|  | and PCR amplification of genomic DNA with qnrC genes of |  |
|  | uropathogens isolated from A.B.U Teaching Hospital, Zaria- | 105 |
| Plates 4.6: | 2.0% agarose gel electrophoresis of 100 bp DNA ladder |  |
|  | and PCR amplification of genomic DNA with qnrS genes of |  |
|  | uropathogens isolated from A.B.U Teaching Hospital, Zaria- | 106 |

# LIST OF APPENDICES

Appendix I: The Retrospective Study on the Prevalence of Uropathogens Isolated from ABUTH, Zaria**- - - - - - -** 141

Appendix II: The Summary of the Morphological/Biochemical Reaction

Schemes of the Uropathogens- - - - - 142

Appendix III: Biofilm Production by Uropathogens Isolated from ABUTH- 152

Appendix IV: The Zones of Inhibition of the Antibiotic Discs against the Uropathogens (mm) - - - - - - 160

Appendix V: The Antibiotic Susceptibility pattern of the Uropathogens - 167

Appendix VI: The Percentage Susceptibility Pattern of Uropathogens *-* 174

Appendix V11: The Statistical Analysis of the Relationship between Multidrug Resistance and Biofilm Formation among the Uropathogens- 177

Appendix VIII: qnr genes protocol from Zymo Research Corporation, UK.- 179

**ABBREVIATIONS**

A.B.U: Ahmadu Bello University

ABUTH: Ahmadu Bello University Teaching Hospital AHL: Acyl Homoserine Lactone

AIs: Author Inducers

[APA](http://en.wikipedia.org/wiki/6-APA): Amino Penicillanic Acid AS: All Susceptible

CAT: Computerized Axial Tomography

CLSI: [Clinical and Laboratory Standards Institute](http://en.wikipedia.org/wiki/Clinical_and_Laboratory_Standards_Institute) CSP: Competence Signal Peptides

DNA: Deoxyribose Nucleotide

ELISA: Enzyme Linked ±Immunosorbent Assay

EUCAST: European Committee on Antimicrobial Susceptibility Testing IUPAC: International Union of Pure and Applied Chemistry

MAR: Multiple Antibiotic Resistance MDR: Multiple Drug Resistance MFG: Manufacturing

MICs: Minimum Inhibitory Concentrations

MRSA: Methicillin Resistant *Staphylococcus aureus* MSSA: Methicillin Sensitive *Staphylococcus aureus* NAPCO: Northwestern Autor Parts Company

NCCLS: National Committee For Clinical Laboratory Standards NICE: National Institute of Health and Care Excellence

NON-MDR: Non Multiple Drug Resistance OD: Optical Density

PCR: Polymerase Chain Reaction PDR: Pandrug Resistance

PMNs: Polymorphonucleotides Qnr: Quinolone Resistance QS: Quorum Sensing

RNA: Ribose Oxynucleotide rRNA: Ribosomal RNA

STD: Sexually Transmitted Diseases TSI: Triple Sugar Iron

TSS: Toxic Shock Syndrome USA: United State of America USP: United State pharmacopoeia UTIs: Urinary Tract Infections UV: Ultra Violet light

WHO: World Health Organisation XDR: Extensively Drug Resistance ȝO l0iteLrs FUR

ȝP 0LFURJUDP

# ABSTRACT

Biofilm Production on surfaces (animate and inanimate object) by microorganisms has become a major concern now in medical settings since it poses threat of induction and spread of resistance among pathogens. Uropathogens as associated with catheterization, ureter and bladder surfaces is of a major interest as far as biofilm production is concerned. This study determined the biofilm production and antibiotic susceptibility pattern of uropathogens isolated from Ahmadu Bello University Teaching Hospital, Zaria. Isolates from urine samples submitted to the Medical Microbiology Laboratory of the hospital were collected over a period of five months and identified using standard microbiological techniques. Alongside, a three months retrospective study within the period of the sample collections was carried out to give a prevalent rate of the occurrence of urinary tract infections in the study area. Further more, the isolates were evaluated for their biofilm production potentials and their susceptibility to antibiotics were determined. The Cefoxitin Disc Test for MRSA and the D-test for erythromycin resistant *S. aureus* were also carried out. From the retrospective study, *Klebsiella spp* were the most common bacteria isolated (36.40%), followed by *E*. *coli* (34.98%), *S. aureus* (7.77%)*, Proteus spp* (2.47%), *Pseudomonas spp* (1.77%) and others including: *Enterococcus spp, Citrobacter spp, Entrobacter spp, Alkaligines spp, Providencia spp, Streptococcus spp, Acenobacter spp* and *Candida spp.* An overall prevalence of 19.71% of the uropathogens was obtained from the retrospective study. *Klebsiella spp:* 55 (37.93%), *E.coli:* 35 (24.14%), *Proteus spp*: 12 (8.28%), *P. earuginosa:* 20 (13.79%) and *S. aureus*: 23 (15.86%), were identified as uropathogens from the urine isolates. The result showed high percentage of biofilm production by all the five species of the uropathogens with

*Proteus spp* (100%) being the highest, followed by *P. aeruginosa* (90%), *S. aureus* (82.61%), *E. coli* (71.43%) and *Klesiella spp* (63.64%). Antibiotic susceptibility testing recorded that, amikacin (87.97%), meropenem (84.04%), p-tazobactam (83.11%) and tigecycline (81.75%) were the most effective against the uropathogens whereas, amoxicillin (26.79%), cotrimoxazole (36.75%), tetracycline (38.12%), ceftazidime

(38.97%), ceftriaxone (52.615%), clindamycin (21.74%), and cefoxitin (47.83%) were generally less effective. There was no significant difference between the percentage of the multidrug resistant isolates of the biofilm producers and the non biofilm producers using chi square at < 0.05 significance level. Ciprofloxacin was less effective against *P*. *aeruginosa* (75.00% were resistant) compared to other uropathogens. Among the *S*. *aureus* isolates, 56.52% were MRSA, 21.74% were erythromycin resistant and these were constitutive not inducible clindamycin resistance. The molecular work identified one of the *Klesiella spp* (K10) to harbor *qnr*S gene, indicating the presence of this mobile genetic element in the study area.

# CHAPTER ONE

**INTRODUCTION**

# : Background of the Study

Several pathogens associated with urinary tract infections have been linked to chronic infections and diseases as a result of biofilm production. This has been seen of *P. aeruginosa* in cystic fibrosis, S*. pneumonia* in chronic otitis media, *S. aureus* in chronic rhinosinusitis and enteropathogenic *E. coli* in recurrent urinary tract infections (Hall- Stoodley and Stoodley, 2009). The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake drugs of questionable quality in circulation (El- Astal, 2005). This antimicrobial resistance among uropathogens is said to have increased over the past 30 years (Zhanel, 2000). The resistance is mostly encountered with the antibiotics frequently used for the treatment of these infections and it varies among communities depending on their prescription pattern (Tessema *et al.,* 2007; Moges and Genetu, 2002). The worldwide data generally, is showing an increasing resistance among the uropathogens (Hryniewicz *et al.*, 2001).

These problematic uropathogens include: *E. coli, Klebsiella spp*, *Enterobacter spp, Citrobacter spp, Proteus spp, Providencia stuart, Ps. aeruginosa, Staphylococcus spp,* etc. The uropathogens are mostly bacteria from the bowel that enter the urinary tract through the urethra, invading and multiplying throughout the urinary system (Gonzalez

and Schaeffer, 1999). The relative frequency of the pathogens varies depending upon SDWLaHgeQ, seWx,¶hoVspitalization and catheterization (Sefton, 2000).

Despite the challenge of increasing resistance at hand as a result of the social and behavioral factor of abusing and misusing antibiotics, the environmental factor of biofilm production have been seen to worsen the situation by making these uropathogens 1000 times more resistant to antibacterial compounds than planktonic bacteria (Gilbert, *et al.,* 1997). The intrinsic and acquired resistance of uropathogens is due to several mechanisms, including active efflux systems, reduced cell wall permeability, plasmid acquisition, expression of various enzymes and biofilm formation which on its own housed the above and other mechanisms of resistance. Many virulence factors are expressed through a cell density-dependent mechanism known as biofilm and quorum sensing. Biofilm can cause significant problems in many areas, both in the medical settings such as persistent infections, recurrent infections, device-related infections and in the industrial settings such as biofouling in the drinking water distribution systems and in the food processing environments. Biofilms have major medical significance as they decrease the susceptibility to the antimicrobial agents. Furthermore, the proximity of cells within biofilms can facilitate plasmid exchange and hence enhance the spread of antimicrobial resistance (Watnick and Kotler, 2000).

In contrast to the commonly perceived idea that most bacteria are planktonic (free floating cells); many bacterial species tend to irreversibly attach to surfaces (Donlan and Costerton, 2002). The attachment of bacteria to surfaces is dependent on the nutritional signals as well as on the number of bacteria present (Serralta *et al*., 2001). When these

bacteria attach and aggregate, they start to recruit other microorganisms like bacteria from the same or from different species, as well as fungi and protozoa (Serralta *et al*., 2001). These aggregates called biofilms, are encased in a self-secreted three-dimensional extracellular polymeric substance called matrix (Donlan and Costerton, 2002; Percival and Bowler, 2004).

Biofilm communities of microorganisms are different from their planktonic counterparts in very many important ways. When microorganisms live as a community, they become much less susceptible to antibiotics, even if highly susceptible as individual cells. Thus, when they form a community, they are protected against a variety of antibiotics that clinicians commonly prescribe for their patients. This is made possible via several mechanisms, including: decreased penetration or diffusion of antimicrobial agents into biofilms, increased activity of multidrug efflux pumps, quorum sensing systems, starvation or stress responses, and genetic switches that turn susceptible planktonic cells into antibiotic-resistant persisters (Leid, 2009).

In uropathogens implicated in biofilm formation has resulted to the evolution of resistant strains (Patel, 2005), persistent infections (Shirtliff and Leid, 2009), recurrent infections and it serves as a training ground that metamorphosizes a susceptible organisms to resistant strains (Shigemura *et al*., 2005)

# : Statement of Research Problem

The resistance of pathogens implicated in urinary tract infections is a global problem and rapidly increasing (Adedeji and Abdulkadir, 2009)

The resistance development and widespread distribution of urinary tract infections has been attributed to the production of biofilm. Shirtliff and Leid (2009) approximates 60% of all hospital-associated infections per year to be due to biofilms that are formed on indwelling medical devices.

Biofilm production by uropathogens evolves resistant organisms that are very difficult to eradicate with standard antibiotic regimens and inherently resistant to the host immune system, thereby resulting to persistent and recurrent infections (Patel, 2005)

Biofilms on many medical implants such as catheters, artificial hips and contact lenses, can only be treated by their removal, thus increasing the trauma to the patient (Saleh *et al,* 2011)

Bacteria in biofilms also coordinate cell±cell communication using secreted chemical signals thereby transferring resistant plasmid among themselves (Hall-Stoodley and Stoodley, 2009).

Biofilm implicated urinary tract infections considerably add to the cost of hospitalization and longer stay in the hospital (Schachter, 2003)

Biofilms serve as reservoir and training ground that disseminates not only infectious but the most resistant organisms round the body (Shigemura *et al*., 2006).

# : Justification

Urinary tract infections remain the most common infections that affect all age groups worldwide (Oluremi *et al*, 2011)

The increasing rate of multidrug resistance among the uropathogens, especially with the conventional antibiotics has been reported by several researchers (Adedeji and Abdulkadir, 2009; Inabo and Obanibi, 2005; Ehinmidu, 2003)

It is estimated that 65% of all chronic bacterial infections in humans involve biofilms (Bezerra *et al*., 2009)

Catheterization and implanted medical devices will continue to increase especially with the aging populations; hence the incidence of biofilm implicated infections will continue to rise (Kojic *et al*, 2004).

It is necessary to optimize the treatment of urinary tract infections through antibiotic susceptibility testing to reduce the emergence of antimicrobial resistance, which is responsible for the increasing number of therapeutic failure (Adedeji and Abdulkadir, 2009).

Not many researches have documented biofilm formation especially among uropathogens in this environment.

# Research Aim

To determine biofilm production and antibiotic susceptibility of uropathogens isolated from Ahmadu Bello University Teaching Hospital, Zaria.

# Specific Objectives

1. To isolate and identify uropathogens from urine samples submitted to Medical Microbiology Laboratory of ABUTH
2. To determine and quantify biofilm production of the uropathogens by the Microtiter Plate Biofilm Assay method.
3. To determine the antimicrobial susceptibility pattern of the uropathogens.
4. To determine the relationship between biofilm production and multi drug resistance among uropathogens using Chi-square.
5. To detect MRSA among the uropathogenic *S. aureus*.
6. To determine inducible clindamycin resistance among the uropathogenic *S. aureus*

using D-Test.

1. To check for quinolone resistance genes (qnr) among the uropathogens

# Null Hypothesis

There is no biofilm production and multidrug resistance in uropathogens isolated from Ahmadu Bello University Teaching Hospital Zaria.

# Alternate Hypothesis

There is biofilm production and multidrug resistance in uropathogens isolated from Ahmadu Bello University Teaching Hospital Zaria, produce biofilms.

# CHAPTER TWO LITERATURE REVIEW

**2.1.0 The urinary system**

The urinary system ±also known as the renal system ±produces, stores and eliminates urine, the fluid waste excreted by the kidneys. The urinary system includes two kidneys, two ureters, the bladder, two sphincter muscles and the urethra. The urinary system works with the lungs, skin and intestines to maintain the balance of chemicals and water in the body. Adults eliminate about a quart and a half (1.42 liters) of urine each day, depending on the amount of fluid consumed and fluid lost through perspiring and breathing. Certain types of medications, such as diuretics that are sometimes used to treat high blood pressure, can also affect the amount of urine a person produces and eliminates. Certain beverages, such as coffee, can also cause increased urination in some people. The primary organs of the urinary system are the kidneys, which are bean-shaped organs that are located just below the rib cage in the middle of the back. The kidneys remove urea ² waste product formed by the breakdown of proteins ² from the blood through small filtering units called nephrons. Each nephron consists of a ball formed of small blood capillaries, called a glomerulus, and a small tube called a renal tubule. Urea, together with water and other waste substances, forms the urine as it passes through the nephrons and down the renal tubules of the kidney. From the kidneys, urine travels down two thin tubes, called ureters, to the bladder. The ureters are about 8 to 10 inches long (Zimmermann, 2013).

The female and male urinary systems are very similar, differing only in the length of the urethra. Urine is formed in the kidneys through a filtration of blood. The urine is then

passed through the ureters to the bladder, where it is stored (David, 2011). During urination the urine is passed from the bladder through the urethra to the outside of the body. Urologic disease can involve congenital or acquired dysfunction of the urinary system. Urinary tract infections (UTIs) occur when bacteria enters the urinary tract and can affect the urethra, bladder or even the kidneys. While UTIs are more common in women, they can occur in men. UTIs are typically treated with antibiotics (Zimmermann, 2013).



**Figure 2.1: The urinary system (2011 medicalartlibrary.com)**

# : Urinary Tract Infection

Urinary tract infection occurs when microorganisms invade the urinary system; the microorganisms multiply within the urinary system causing infections in all age groups and most prominent among young sexually active women ([Najar](http://www.ncbi.nlm.nih.gov/pubmed/?term=Najar%20MS%5Bauth%5D), 2009). Many a times these are bacteria from the bowel that enter the urinary tract through the urethra. Urinary tract infections are classified as either asymptomatic or symptomatic. Asymptomatic bacteriuria is defined as the presence of significant bacteriuria without the symptoms of an acute urinary tract infection. Symptomatic urinary tract infections are accompanied by symptoms of cloudy urine, haematuria, nausea and vomiting depending on the gravity of the infection. The symptomatic urinary tract infections are divided into lower tract (acute cystitis) or upper tract (acute pyelonephritis) infections. Cystitis is defined as significant bacteriuria with associated bladder mucosal invasion, whereas pyelonephritis is the infection of the kidney (Schnarr and Smaill, 2008). Urinary tract infections, if diagnosed early, and with adequate antibiotic coverage is not alarming, however, if not adequately treated, can cause significant morbidity and mortality. Urinary tract infections are the most common bacterial infections in humans both in the community and hospital setting ([Oladeinde,](http://www.ncbi.nlm.nih.gov/pubmed/?term=Oladeinde%20BH%5Bauth%5D) *et al*, 2011). It is one of the most common bacterial infections encountered by clinicians in developing countries (Tessema *et al*., 2007)

Globally it is estimated that, about 150 million people are diagnosed with urinary tract infection each year and symptomatic urinary tract infection result in 7 million visits to outpatient clinics, one million to emergency unit (Wilson and Gaido, 2004).

Urinary tract infections occur more often in women than men, because of the short urethra and the closeness of the system to the anus. Also, sexual activity appears to

increase the chances of bacterial contamination of the female urethra (Adedeji and Abdulkadir, 2009; Akram *et al.*, 2007)

# : Catheter Associated Urinary Tract Infection

Urinary tract infections are persistent and recurrent which attribute to their wide spread especially during catheterization. Catheter associated urinary tract infections result to biofilm formation on the surfaces of the catheter which are said to make pathogens more resistant than their planktonic counterparts. Biofilm formation on surfaces of indwelling catheters is central to the causes of urinary tract infection (Stamm, 1991).

# : Risk Factors for Urinary Tract Infections

Women are more at risk of urinary tract infections than men (Hunjak *et al*., 2007).

)UHTXHQW VH[XDO LQWHUFRXUVH DOVR LQFUHDVHV

infections. The use of contraceptive spermicides and diaphragm by women has also been seen as a risk factor. When women reach menopause, the decrease in estrogen thins the lining of the urinary tract, which increases susceptibility to bacterial infections. Pregnancy does not necessarily increase the risk of getting a urinary tract infection but it can increase the risk of developing a serious infection that could potentially harm the mother and fetus. Women should get screened for asymptomatic bacteriuria during pregnancy. Any abnormality of the urinary tract that obstructs or slows the flow of urine makes it easier for bacteria to grow in the bladder. A stone in the kidney or any part of the urinary tract can form such a blockage, creating the condition for urinary tract infections. In men, an enlarged prostate gland can obstruct urine flow and make infection difficult to treat. One of the most common sources of infection is catheter placed in the

bladder. People who have diabetes are prone to urinary tract infection. Immuno suppressed patients are at high risk for this infection. Urinary tract infections occur in a small percentage of infants due to congenital abnormalities that sometimes require surgery. Patients with a neurogenic bladder or bladder diverticulum are at high risk. Postmenopausal women with bladder or uterine prolapsed are also at the risk of contracting these infections (Chamberlain, 2009)

# : The symptoms of a Urinary Tract Infection

The symptoms of urinary tract infections as stated by National Kidney Foundation include the followings: An urgent need to urinate, often with only a few drops of urine to pass, an aching feeling, pressure or pain in your lower abdomen, cloudy urine, Haematuria, a strong odor to urine, Dysuria, Suprapubic tenderness. If the infection spreads to the kidneys and becomes more severe, you may also have: pain in your lower back and chills nausea and vomiting (National Kidney Foundation, 2010).

# : Epidemiology of Urinary Tract Infection in Nigeria

Urinary tract infections are among the most common bacterial infections in humans both in the community and hospital settings, and they occur in all age groups, and usually required urgent treatment (Orrett and Davis, 2006). Urinary tract infection remains a leading cause of health care expenditure for people of all age groups (Vasquez and Hand, 2004). In Nigeria, malnutrition, poor hygiene and low socio-economic status are associated with urinary tract infections; these factors are rife in rural settings (Ahmed and Avasara, 2008)

The prevalence rate of UTI from a study that was carried out in Abuja the Capital of Nigeria was 13%, with the highest percentage yield among the age bellow one and above 57 age

groups and with more cases among females. The most common etiological agents were *E. coli* (37%) and *Klebsiella spp* (25%). In Yola, Adamawa State, Northern Nigeria a similar work recorded a prevalence rate of 67.2% with higher occurrence among women than men.

*E. coli* were also seen as the most frequently occurring uropathogens, In the Eastern part of Nigeria, Enugu State a prevalence of 77.9% was reported (Iregbu and Nwajiobi, 2013). In Okada, a town in Edo state, the southern region of Nigeria. A prevalence of 39.69% was observed in the study. Females also had the higher prevalence rate than the male counterpart. *E. coli* were the predominant isolates causing the UTI (Oladeinde *et al*, 2011). In Abeokuta, Ogun State, Western Nigeria, the overall prevalence of UTI was 47%, with more of the cases among females, also *E. coli* occurring the most prevalent (Ojo *et. al.,* 2004).

With the little data gathered above, we can conclude that there is high prevalent rate of UTI in Nigeria, occurring higher among females than males with *E. coli* as the most commonly implicated uropathogens.

# : Diagnosis of Urinary tract infection

UTI is usually diagnosed based on symptoms, physical examination, and laboratory examination of the urine. In men, physical examination is important for detecting possible infection of the genitals and enlargement of the prostate gland, which may be a sign of serious disease. A clean-catch urine sample, in which urine is collected in midstream to prevent contamination with organisms present at the opening of the urethra, is necessary for laboratory analysis. Analysis may involve simple detection for the presence of bacteria, or it may involve culture and identification of the specific organism

that is causing infection. Over-the-counter dipstick tests performed at home are useful for women who experience recurrent UTIs. These tests are based on the detection of nitrates such as those of ammonia in the urine. In severe infections, laboratory culture of urine is required to identify the organism involved. Infections that extend into the kidneys may require examination using ultrasound or other visualization techniques, such as X-ray or computerized axial tomography (CAT). Blood analysis also may be performed to determine if infection has spread into the bloodstream, placing other tissues at risk. Recurrent infections may necessitate cystoscopy, in which an instrument called a cystoscope is inserted into the urethra and bladder to view the tissues and to collect samples for biopsy. In many cases, the extent of pyelonephritis (inflammation in the kidney and the lining of the renal pelvis) that is a direct result of recurrent UTI is not known with certainty. However, it is known that, in the presence of urinary tract obstruction, which disrupts the flow of urine, infection is likely to ascend the urinary tract and cause infection within the renal pelvis and kidney tissue (Rogers, 2014)

# : Treatment of Urinary tract infection

The choice of antibiotics for treatment of urinary tract infections depends mainly on the susceptibility of the infecting pathogens to the drug, the seriousness of the infection, the age, pregnancy status, the doctor's experience and knowledge of local antibiotic resistance patterns of commonly infecting bacteria (Davis and Balentine, 2014)

The following antibiotics are used to treat Urinary tract infections according to Davis and Balentine (2014). Beta-lactams, including penicillins and cephalosporins (for example, Amoxicillin, Augmentin, Keflex (R), Duricef (R), Ceftin, Lorabid, Rocephin (R), Cephalexin, Suprax (R), and others); many organisms have resistance to some of these

drugs. Trimethoprim-sulfamethoxazole combination antibiotic (for example, Bactrim DS and Septrin); many organisms may show resistance. Fluoroquinolones (for example, Ciprofloxacin, Levaquin, and Floxacin) resistance is developing; also these should not be used in pregnancy or in the pediatric population. Tetracyclines (for example, tetracycline, doxycycline or minocycline) used most often for Mycoplasma or Chlamydia infections; like fluoroquinolones, they should not be used in pregnancy or by the pediatric population. Aminoglycosides (for example, gentamycin, amikacin, and tobramycin) used usually in combination with other antibiotics to combat severe urinary tract infections.

Macrolides (for example, clarithromycin, azithromycin, and erythromycin), used more often with some STD-caused urinary problems. There are other antibiotics that are used occasionally, such as nitrofurantoin, but its use is limited to cystitis and should not be used to treat more serious (kidney) urinary tract infections (Davis and Balentine, 2014).

# : Prevention of Urinary Tract Infection

A number of measures such as personal hygiene can affect urinary tract infections, these include: The use of clean underwear always, proper and adequate cleaning after urinating or defecating (Nicolle, 2008). In those with frequent urinary tract infections who use [spermicide](http://en.wikipedia.org/wiki/Spermicide) or a diaphragm as a method of contraception, they should use alternative methods (Salvatore *et al.*, 2011). The use of condom should be without spermicide (Nicolle, 2008; Engleberg *et al.,* 2007). Shortening the time usage of catheter as possible and appropriate care of the catheter when in use prevents infections ([Nicolle, 2008).](http://en.wikipedia.org/wiki/Urinary_tract_infection#cite_note-Nic2001-27) They should be inserted using sterile technique in hospital however none sterile technique may be appropriate in those who self catheterize (Gould *et al.,* 2010).

# Uropathogens

The microorganisms implicated in urinary tract infections include, bacteria, fungi, viruses, parasitic protozoan. Bacteria are recognized as the most causative agents of more than 95% of all the urinary tract associated infections (Bonadio *et al.*, 2001; Amdekar *et al.,* 2011). Most times, *Escherichia coli* is the most common cause of both complicated and uncomplicated urinary tract infections, (Yüksel *et al*., 2006; Tessema *et al*., 2007) with *Enterococcus species, Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumanii, Citrobacter species., Serratia species.*, coagulase-negative *Staphylococci* and *Klebsiella spp* being the next most frequently encountered species. Most bacteria found in the urinary tract emanate from the intestine (Ozumba, 1993)

## Klebsiella species

*Klebsiella spp* are [genus](http://en.wikipedia.org/wiki/Genus) of [non-motile](http://en.wikipedia.org/wiki/Motility), [Gram-negative,](http://en.wikipedia.org/wiki/Gram-negative) [oxidase-negative](http://en.wikipedia.org/wiki/Oxidase_test), rod-shaped [bacteria](http://en.wikipedia.org/wiki/Bacteria) with a prominent [polysaccharide](http://en.wikipedia.org/wiki/Polysaccharide)-based [capsule](http://en.wikipedia.org/wiki/Capsule_%28microbiology%29), members of of the family *enterobacteriaceae* (Ryan and Ray, 2004). *Klebsiella species* are ubiquitous in nature. This is thought to be due to their distinct sublineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects, animals and humans (Brisse *et al.*, 2006). They are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. *Klebsiella species* are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens (Podschun and Ullmann, 1998)

*Klebsiella spp* can lead to a wide range of disease states, notably [pneumonia](http://en.wikipedia.org/wiki/Pneumonia), [urinary tract](http://en.wikipedia.org/wiki/Urinary_tract_infection) [infections,](http://en.wikipedia.org/wiki/Urinary_tract_infection) [septicemia,](http://en.wikipedia.org/wiki/Septicemia) [meningitis](http://en.wikipedia.org/wiki/Meningitis), [diarrhea,](http://en.wikipedia.org/wiki/Diarrhea) and soft tissue infections (Podschun and Ullmann, 1998).

## Escherichia coli

*E. coli* is a [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative), [facultative anaerob](http://en.wikipedia.org/wiki/Facultative_anaerobic_organism)e, [rod-shaped](http://en.wikipedia.org/wiki/Bacillus_%28shape%29) [bacterium](http://en.wikipedia.org/wiki/Bacterium) of the genus [*Escherichia*](http://en.wikipedia.org/wiki/Escherichia) that is commonly found in the lower [intestine](http://en.wikipedia.org/wiki/Gastrointestinal_tract) of [warm-blooded](http://en.wikipedia.org/wiki/Warm-blooded) organisms (Singleton, 1999). Most *E. coli* [strains](http://en.wikipedia.org/wiki/Strain_%28biology%29) are harmless, but some [serotypes](http://en.wikipedia.org/wiki/Serotype) can cause serious [food poisoning](http://en.wikipedia.org/wiki/Foodborne_illness) in their hosts, and are occasionally responsible for [product recalls](http://en.wikipedia.org/wiki/Product_recall) due to [food contamination](http://en.wikipedia.org/wiki/Food_contamination) (Vogt and Dippold, 2005). It is [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative_bacteria), [facultative](http://en.wikipedia.org/wiki/Facultative_anaerobic_organism) [anaerobic](http://en.wikipedia.org/wiki/Facultative_anaerobic_organism) and [non-sporulating](http://en.wikipedia.org/wiki/Endospore) member of *Enterobacteriaceae*. Cells are typically rod-

shaped, and are about 2.0 [micrometers](http://en.wikipedia.org/wiki/Micrometers)  ȝP ORQ±J1. 0 ȝDPQ GL Q G L DPHWHU cell volume of 0.6±0.7 ȝP3 (Britannica Online Encyclopedia, 2011). Virulent strains of

*E. coli* can cause [gastroenteritis](http://en.wikipedia.org/wiki/Gastroenteritis), [urinary tract infections](http://en.wikipedia.org/wiki/Urinary_tract_infection), and [neonatal](http://en.wikipedia.org/wiki/Neonatal) [meningitis](http://en.wikipedia.org/wiki/Meningitis). In rare cases, virulent strains are also responsible for [hemolytic-uremic syndrome](http://en.wikipedia.org/wiki/Hemolytic-uremic_syndrome), [peritonitis](http://en.wikipedia.org/wiki/Peritonitis), [mastitis](http://en.wikipedia.org/wiki/Mastitis), [septicemia](http://en.wikipedia.org/wiki/Septicemia) and Gram-negative [pneumonia](http://en.wikipedia.org/wiki/Pneumonia) (Todar, 2007). UPEC (uropathogenic

*E. coli*) is one of the main causes of [urinary tract infections](http://en.wikipedia.org/wiki/Urinary_tract_infection) (Nova publishers, 2013). It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices (Nova publishers, 2013)

## Proteus species

*Proteus spp* are of genus of [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative) [Proteo bacteria,](http://en.wikipedia.org/wiki/Proteobacteria) bacilli and are widely distributed in nature as saprophytes, being found in decomposing animal matter, in

sewage, in manure soil, and in human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often [nosocomial](http://en.wikipedia.org/wiki/Nosocomial). There are three species, which are, [*P. vulgaris*,](http://en.wikipedia.org/wiki/Proteus_vulgaris) [*P. mirabilis*](http://en.wikipedia.org/wiki/Proteus_mirabilis), and [*P. penneri*](http://en.wikipedia.org/wiki/Proteus_penneri)*,* which are [opportunistic](http://en.wikipedia.org/wiki/Opportunistic_infection) human [pathogens](http://en.wikipedia.org/wiki/Pathogen) (Guentzel, 1996). *Proteus* spp do not usually ferment [lactose,](http://en.wikipedia.org/wiki/Lactose) but have shown to be capable lactose fermenters depending on the species in a triple sugar iron ([TSI](http://en.wikipedia.org/wiki/TSI_slant)) test. Since it belongs to the family of [*Enterobacteriaceae*](http://en.wikipedia.org/wiki/Enterobacteriaceae), general characters are applied on this genus. It is [oxidase](http://en.wikipedia.org/wiki/Oxidase_test)-negative but [catalase](http://en.wikipedia.org/wiki/Catalase_test)- and [nitrate](http://en.wikipedia.org/wiki/Nitrate)- positive. Specific tests include positive [urease](http://en.wikipedia.org/wiki/Urease) (which is the fundamental test to differentiate *Proteus* from [*Salmonella*](http://en.wikipedia.org/wiki/Salmonella)) and [phenylalanine deaminase](http://en.wikipedia.org/wiki/Phenylalanine_deaminase) tests. On the species level, [indole](http://en.wikipedia.org/wiki/Indole) is considered reliable, as it is positive for [*Proteus vulgaris*](http://en.wikipedia.org/wiki/Proteus_vulgaris) but negative for [*Proteus mirabilis*](http://en.wikipedia.org/wiki/Proteus_mirabilis). Most strains produce a powerful urease enzyme, which rapidly hydrolyzes urea to ammonia and carbon monoxide. Species can be [motile](http://en.wikipedia.org/wiki/Motility) (Ryan and Ray, 2004) and have characteristic "[swarming](http://en.wikipedia.org/wiki/Swarming_motility)" patterns (Matsuyama *et al.,* 2000)

## Pseudomonas aeruginosa

*P. aeruginosa* is a [Gram-negative,](http://en.wikipedia.org/wiki/Gram-negative) [aerobic,](http://en.wikipedia.org/wiki/Aerobic_organism) [coccobacillus](http://en.wikipedia.org/wiki/Coccobacillus) [bacterium](http://en.wikipedia.org/wiki/Bacterium) with [unipolar](http://en.wikipedia.org/wiki/Flagellum) [motility](http://en.wikipedia.org/wiki/Flagellum) (Ryan and Ray, 2004), an [opportunistic human pathogen](http://en.wikipedia.org/wiki/Opportunistic_infection). *P. aeruginosa* is also an opportunistic pathogen of plants. *P. aeruginosa* is the [type species](http://en.wikipedia.org/wiki/Type_species) of the genus *Pseudomonas* (Anzai *et al.*, 2000)*.* It is a common [bacterium](http://en.wikipedia.org/wiki/Bacterium) that can cause [disease](http://en.wikipedia.org/wiki/Disease) in animals, including humans. It is citrate, catalase, and oxidase positive from the family *pseudimonadaceae*. It is found in soil, water, [skin flora](http://en.wikipedia.org/wiki/Skin_flora), and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in [hypoxic](http://en.wikipedia.org/wiki/Hypoxia_%28environmental%29) atmospheres, and has thus colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the

organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized [inflammation](http://en.wikipedia.org/wiki/Inflammation) and [sepsis](http://en.wikipedia.org/wiki/Sepsis). If such colonization occur in critical body organs, such as the [lungs,](http://en.wikipedia.org/wiki/Lung) the [urinary tract](http://en.wikipedia.org/wiki/Urinary_tract) and [kidneys](http://en.wikipedia.org/wiki/Kidneys), the results can be fatal (Aldona and Raymond, 1994). Because it thrives on moist surfaces, this bacterium is also found on and in [medical equipment,](http://en.wikipedia.org/wiki/Medical_equipment) including [catheters](http://en.wikipedia.org/wiki/Catheter), causing cross-[infections](http://en.wikipedia.org/wiki/Infection) in [hospitals](http://en.wikipedia.org/wiki/Hospital) and [clinics](http://en.wikipedia.org/wiki/Clinic). It is implicated in [hot-tub rash](http://en.wikipedia.org/wiki/Hot-tub_rash). It is also able to decompose hydrocarbons and has been used to break down [tarballs](http://en.wikipedia.org/wiki/Tarball_%28oil%29) and oil from [oil spills](http://en.wikipedia.org/wiki/Oil_spill) (Itah and Essien, 2005). *P. aeruginosa* secretes a variety of pigments, including [pyocyanin](http://en.wikipedia.org/wiki/Pyocyanin) (blue- green), [pyoverdine](http://en.wikipedia.org/wiki/Pyoverdine) (yellow-green and [fluorescent](http://en.wikipedia.org/wiki/Fluorescence)), and pyorubin (red-brown).

*P. aeruginosa* is an [opportunistic](http://en.wikipedia.org/wiki/Opportunistic_infection), [nosocomial](http://en.wikipedia.org/wiki/Nosocomial_infection) pathogen of [immunocompromised](http://en.wikipedia.org/wiki/Immunodeficiency) individuals, it typically infects the pulmonary tract, [urinary tract](http://en.wikipedia.org/wiki/Urinary_tract_infection), [burns](http://en.wikipedia.org/wiki/Burn_%28injury%29), [wounds](http://en.wikipedia.org/wiki/Wound), and also causes other [blood infections](http://en.wikipedia.org/wiki/Sepsis) (Flores-Encarnación *et al.*, 2014). [Biofilms](http://en.wikipedia.org/wiki/Biofilm) of *P. aeruginosa* can cause chronic [opportunistic infections](http://en.wikipedia.org/wiki/Opportunistic_infection), which are a serious problem for medical care in industrialized societies, especially for immunocompromised patients and the elderly. They often cannot be treated effectively with traditional [antibiotic](http://en.wikipedia.org/wiki/Antibiotic) therapy. Biofilms seem to protect these bacteria from adverse environmental factors. *P. aeruginosa* can cause [nosocomial infections](http://en.wikipedia.org/wiki/Nosocomial_infection) and is considered a [model organism](http://en.wikipedia.org/wiki/Model_organism) for the study of antibiotic-resistant bacteria (Cornelis, 2008)

## Staphylococcus aureus

*S. aureus* is a [facultative anaerobic](http://en.wikipedia.org/wiki/Facultative_anaerobic_organism) Gram-positive coccal bacterium also known as "golden staph" and Oro staphira. *S. aureus* appears as [grape](http://en.wikipedia.org/wiki/Grape)-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies, often with [hemolysis,](http://en.wikipedia.org/wiki/Hemolysis_%28microbiology%29) when grown on [blood agar plates](http://en.wikipedia.org/wiki/Agar_plate) (Ryan and Ray, 2004) *S. aureus* reproduces

[asexually](http://en.wikipedia.org/wiki/Asexually) by [binary fission](http://en.wikipedia.org/wiki/Binary_fission). The two daughter cells do not fully separate and remain attached to one another. This is why the cells are observed in clusters. *S. aureus* is [catalase](http://en.wikipedia.org/wiki/Catalase)-positive (it produces the enzyme catalase). Catalase-activity tests are sometimes used to distinguish *staphylococci* from [*enterococci*](http://en.wikipedia.org/wiki/Enterococcus)and [*streptococci*](http://en.wikipedia.org/wiki/Streptococcus). *S. aureus* can be differentiated from other staphylococci by the [coagulase test](http://en.wikipedia.org/wiki/Coagulase) (Ryan and Ray, 2004)

*S. aureus* can cause a range of illnesses, from minor skin [infections](http://en.wikipedia.org/wiki/Infection), such as [pimples](http://en.wikipedia.org/wiki/Pimple), [impetigo,](http://en.wikipedia.org/wiki/Impetigo) [boils](http://en.wikipedia.org/wiki/Boil) (furuncles), [cellulitis](http://en.wikipedia.org/wiki/Cellulitis) folliculitis, [carbuncles,](http://en.wikipedia.org/wiki/Carbuncle) [scalded skin syndrome,](http://en.wikipedia.org/wiki/Scalded_skin_syndrome) and [abscesses,](http://en.wikipedia.org/wiki/Abscess) to life-threatening diseases such as [pneumonia](http://en.wikipedia.org/wiki/Pneumonia), [meningitis](http://en.wikipedia.org/wiki/Meningitis), [osteomyelitis](http://en.wikipedia.org/wiki/Osteomyelitis), [endocarditis,](http://en.wikipedia.org/wiki/Endocarditis) [toxic shock syndrome](http://en.wikipedia.org/wiki/Toxic_shock_syndrome) (TSS), [bacteremia](http://en.wikipedia.org/wiki/Bacteremia), and [sepsis](http://en.wikipedia.org/wiki/Sepsis). Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular, [wound infections](http://en.wikipedia.org/wiki/Wound_infection) to urinary tract infections. It is still one of the five most common causes of [nosocomial infections](http://en.wikipedia.org/wiki/Nosocomial_infection) and is often the cause of postsurgical wound infections (John, 1999)

* + 1. **Methicillin-Resistant *S. aureus* (MRSA)**

Methicillin-resistant *Staphylococcus aureus* (MRSA) are isolates of *Staphylococcus aureus* which have acquired genes encoding antibiotic resistance to all penicillins including methicillin. This resistance is mediated by an altered penicillin binding protein (PBP2a) which is encoded by the Mec A gene (Ahmed *et al.*, 2012). They were first discovered in the United Kingdom in 1961 but have now become a major clinical problem worldwide (Chambers, 2001). However, information on the prevalence of MRSA in Nigeria is insufficient despite the established fact that MRSA is a significant health threat. Precisely patients with surgical wounds have been reported to be at high risk of MRSA infection (Coello *et al.*, 1994)

# 2.4:0 Microbial Biofilms

These aggregates, called biofilms, are encased in a self-secreted three-dimensional extracellular polymeric substance called matrix (Donlan and Costerton, 2002; Percival and Bowler, 2004). The extracellular polymeric substance is generally composed of secreted polysaccharides, proteins, glycoproteins, glycolipids and extracellular DNA (Flemming *et al*., 2007). Formation of a biofilm begins with the attachment of free- floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible adhesion via Van der waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing using such products as N-Acyl homoserine lactones (AHL) (Hall-Stoodley and Stoodley, 2009). Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development, and is the stage in which the biofilm is established and may only change in shape and size.

Figure 2.2 is the depiction of the dynamic nature of a biofilm community. The community starts to form when single cells called planktonic bacteria attach to an animate or inanimate surface that normally is conditioned to enhance attachment. As those individual cells strongly adhere, and expand in number, they are surrounded by an

extracellular matrix. As the community matures, partly by cell to cell communication (signaling), parts of the biofilm can disperse, migrate, or the community can continue to develop into a heterogeneous population of cells that are metabolically, physiologically and genetically distinct from one another. If appropriate attachment reservoirs are available downstream from the dispersion event, the entire cycle can start over again (Leid, 2009).



Figure 2.2: The Processes of Biofilm Formation and Maturation; Adapted from (Leid, 2009)