

ASYMPTOMATIC BACTERIURIA IN PREGNANT WOMEN
ATTENDING ANTENATAL CLINICS IN THREE HOSPITALS IN KANO,
NIGERIA

BY

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APRIL, 2012

DECLARATION

I hereby declare that the work in this thesis titled “Asymptomatic bacteriuria in pregnant women attending antenatal clinics in three hospitals in Kano, Nigeria” was performed by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria under the supervision of Prof. J.A. Onaolapo and Dr (Mrs). A.R. Oyi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any Institution.

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CERTIFICATION

This thesis entitled 'ASYMPTOMATIC BACTERIURIA IN PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN THREE HOSPITALS IN KANO, NIGERIA' by Durowaiye, Mojirayo. T. meets the regulations governing the award of the degree of Master of Science (Pharmaceutical Microbiology) of the Ahmadu Bello University, Zaria, and is approved for the scholarly contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty and to my lovely family.

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ABSTRACT

Asymptomatic bacteriuria in pregnancy is the significant presence of bacteria in the urine of pregnant women without the clinical symptoms of urinary tract infection (UTI). This study was designed to highlight the prevalence of asymptomatic bacteriuria among pregnant women in Kano, Nigeria. Samples of urine were randomly obtained from 310 women attending antenatal clinics in 3 major hospitals. These were subjected to microbiological and cultural tests. The bacteria isolated were characterized using standard methods. Using 10^5 cfu/ml as significant level of bacteriuria, prevalence was found to be 15.2%. Isolated organisms include *Proteus spp.*, 23 (49%), *Escherichia coli*, 17 (36%), *Staphylococcus aureus*, 3 (6.4%), *Pseudomonas aeruginosa*, 2 (4.3%), and *Klebsiella spp.*, 2 (4.3%). The age group 25-30 years had the highest prevalence (24.2%) with respect to age; the housewives had the highest prevalence (57.4%) with respect to occupational status. Pregnant women in the last trimester had the highest prevalence (18.2%). The result of antimicrobial susceptibility of *Proteus spp.* and *E. coli* to some of the commonly prescribed antibiotics showed resistance to amoxicillin, ciprofloxacin, ceftriaxone, nitrofurantoin, gentamicin, levofloxacin, cotrimoxazole, amoxicillin/clavulanic acid, nalidixic acid and tetracycline (47-100%). *S. aureus* showed resistance to ceftriaxone, nitrofurantoin, amoxicillin/clavulanic acid, nalidixic acid and tetracycline (66.7-100%). The multiple antibiotic resistance index (MARI) observed in this study with reference to the tested antibiotics showed that 99.96% of the *Proteus spp.* isolates have MAR index of 0.2 to 1.0, while 99.97% of the *E. coli* isolates have MAR index

of 0.2 to 1.0. *Proteus spp.* isolates were found to express more MR/P⁺,MR/K⁻ hemagglutinins 12(52.2%) than *E. coli* isolates 1(5.9%) and showed significant urease activity ranging from 24-110 ($\mu\text{mol NH}_3/\text{min}/\text{mg}$ protein). They also produced measurable hemolytic activity with titres ranging from 1:2 to 1:256. The *E. coli* isolates were observed to express more MR/P⁺,MR/K⁺ hemagglutinins 9(52.9%) compared to *Proteus spp.* isolates 8(34.8%). The hemagglutination pattern MR/P⁻,MR/K⁺ was also more observed in *E. coli* isolates 3(17.6%) than in *Proteus spp.* isolates 2(8.7%). MR/P⁻,MR/K⁻ hemagglutinins were expressed by 2(11.8%) isolates of *E. coli* and 1(4.3%) isolate of *Proteus spp.* *E. coli* isolates showed higher range of 1:2 to 1:512 hemolytic activities. The geometric mean of reciprocal hemolytic titres for *Proteus spp.* isolates was 29.23, while that for *E. coli* isolates was 31.99. Majority of the *E. coli* isolates were associated with multi-antibiotic resistance strains, but only 5(five) out of the 17(seventeen) *E. coli* isolates were seen to harbor plasmids Ec1, Ec2, Ec3, Ec11, Ec13: 3700bp, 4000bp, 4000bp, 2800bp, and 3500bp respectively. There was no amplification of CTX-M2, CTX-M9 (for β -lactam antibiotics), pCT (for IncK plasmid encoding bla_{CTX-M} ESBL genes), qnrC and qnrS (for fluoroquinolones) genes in all the *Proteus spp.* and *E. coli* isolates. However amplification of gyrB gene (for fluoroquinolones) was observed in 5 *Proteus spp.* isolates (P1, P2, P3, P4 and P5 respectively). Only one *Proteus spp.* isolate (P3) harboured a plasmid (3500bp), the other four amplified genes in *Proteus spp.* isolates P1, P2 P4 and P5 might not be plasmid-mediated. In 5 *E. coli* isolates (Ec1, Ec2, Ec3, Ec4 and Ec5), amplification of gyrB gene was seen, but only 3 of these isolates (Ec1, Ec2, and Ec3) harboured plasmids of 3700bp, 4000bp, and 4000bp molecular sizes

respectively. These amplified genes in Ec4 and Ec5 can be said to be chromosomally mediated and not extra-chromosomal. The implications of these findings in clinical practice are highlighted.

TABLE OF CONTENTS

TITLES	PAGES
Title Page	ii
Declaration	iii
Certification	iv
Dedication.....	v
Acknowledgement	vi
Abstract.....	vii
Table of Contents.....	ix
List of Tables.....	xiv
List of Figures.....	xvi
List of Chemical Structures.....	xvii
List of Appendices.....	xviii
 CHAPTER ONE	
1.0 INTRODUCTION.....	1
1.1 Pregnancy and the Urinary tract	1
1.2 Asymptomatic Bacteriuria in Pregnancy	3
1.3 Statement of the Problem.....	5
1.4 Aim	6
1.5 Specific Objectives.....	6

1.6	Hypothesis.....	7
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CHAPTER TWO

2.0	LITERATURE REVIEW.....	8
2.1	Symptoms of Urinary Tract Infection.....	10
2.2	Microbiological Diagnostic Tests for Urinary Tract Infection	10
2.2.1	Asymptomatic bacteriuria.....	11
2.2.2	Acute Cystitis.....	11
2.2.3	Pyelonephritis.....	11
2.3	Urine Microscopy, Culture and Sensitivity.....	12
2.4	Chemotherapy of Asymptomatic bacteriuria.....	12
2.4.1	Oral antibiotics for treatment of pregnant women with asymptomatic bacteriuria.....	14
2.5	WHO's Antibiotic Prescription recommendation.....	14
2.6	Antibiotics commonly prescribed in Asymptomatic bacteriuria.....	15
2.6.1	Fluoroquinolones	16
2.6.1.1	Nalidixic acid.....	16
2.6.1.2	Ciprofloxacin.....	17
2.6.1.3	Ofloxacin.....	20
2.6.1.4	Levofloxacin.....	22
2.6.2	Ceftriaxone.....	23
2.6.3	Gentamicin.....	25
2.6.4	Nitrofurantoin.....	26

2.6.5	Tetracycline.....	28
2.6.6	Cotrimoxazole.....	29
2.6.7	Amoxicillin.....	31
2.6.8	Amoxicillin/Clavulanic acid.....	33
2.7	Development of Antibiotic resistance.....	36
2.8	Factors that influence Development of Antibiotic resistance in Nigeria.....	38
2.9	Uropathogens.....	39
2.9.1	<i>Proteus spp</i>	40
2.9.2	<i>Escherichia coli</i>	41
2.9.3	<i>Staphylococcus aureus</i>	41
2.9.4	<i>Klebsiella spp</i>	42
2.9.5	<i>Pseudomonas aeruginosa</i>	43
2.9.6	<i>Candida spp</i>	44
2.10	Other markers of Urinary Tract Infection.....	45
2.10.1	Yeast cells.....	45
2.10.2	Epithelial Cells.....	45
2.10.3	Crystals.....	46
2.10.4	Red blood cells.....	46
2.10.5	White blood cells.....	46
2.10.6	Casts.....	47
2.11	Virulence factors of the Uropathogens	47
2.11.1	Potential virulence factors of <i>Proteus spp</i>	48

2.11.1.1	Surface structures.....	48
2.11.1.2	Flagella and Swarming motility.....	49
2.11.1.3	Outer membrane proteins.....	49
2.11.1.4	Lipopolysaccharides (O-antigen, endotoxin).....	50
2.11.1.5	Capsule antigen.....	50
2.11.1.6	Urease.....	50
2.11.1.7	Hemolysin.....	51
2.11.1.8	Protease IgA & IgG.....	51
2.11.2	Virulence factors of <i>E. coli</i>	51
2.11.2.1	The Aerobactin system.....	52
2.11.2.2	Hemolysin.....	53
2.11.2.3	Capsular polysaccharide (K-antigen).....	53
2.11.2.4	Serum resistance.....	54
2.11.2.5	Adhesin.....	54
2.11.3	Virulence factors of <i>Staphylococcus aureus</i>	55
2.11.3.1	Toxins.....	55
2.11.3.1.1	Pyrogenic toxin super antigens	55
2.11.3.1.2	Exfoliative toxins.....	55
2.11.3.1.3	Other toxins.....	55
2.11.3.2	Protein A.....	56
2.11.3.3	Pigments.....	56
2.11.4	Virulence factors of <i>Klebsiella spp.</i>	57
2.11.4.1	Capsular antigen.....	57

2.11.4.2	Pili (fimbriae).....	57
2.11.4.3	Serum resistance and lipopolysaccharide.....	58
2.11.4.4	Siderophores.....	58
2.11.5	Virulence factors of <i>Pseudomonas aeruginosa</i>	59
2.12	Molecular characterization of resistant isolates.....	59
2.12.1	Plasmid Extraction.....	59
2.12.2	Polymerase Chain Reaction (PCR).....	60

CHAPTER THREE

3.0	MATERIALS AND METHODS.....	62
3.1	Materials.....	62
3.1.1	Laboratory Media.....	62
3.1.2	Sensitivity Discs	62
3.1.3	Reagents and Chemicals.....	63
3.1.4	GeneJET™ Miniprep Plasmid Extraction kit (Fermentas UK).....	63
3.2	Methods.....	64
3.2.1	Collection of Specimen.....	64
3.2.2	Specimen Processing.....	64
3.2.3	Identification/ Biochemical Test.....	64
3.2.4	Urine Microscopy.....	65
3.2.5	Significant bacteriuria determination with respect to age group, gestational age, and occupational status.....	65

3.3	Susceptibility Test.....	65
3.4	Determination of some virulence factor of <i>Proteus spp.</i>	66
3.4.1	Hemolysin.....	66
3.4.2	Urease.....	67
3.4.3	Adherence factor (Hemagglutinin).....	67
3.5	Determination of some virulence factors of <i>E. coli</i>	68
3.5.1	Hemolysin assay.....	68
3.5.2	Hemagglutinin (adherence factor).....	68
3.6	Molecular characterization of resistant isolates.....	69
3.6.1	Plasmid Analysis (extraction)	69
3.6.2	Amplification of antibiotic resistance genes (CTX-M2, CTX-M9,..... qnrC, qnrS, pCT, and gyrB) by polymerase chain reaction (PCR)	71

CHAPTER FOUR

4.0	RESULTS.....	73
4.1	Isolation and identification.....	73
4.2	Urine Microscopy.....	76
4.3	Prevalence of Significant Asymptomatic bacteriuria.....	77
4.3.1	Prevalence of Uropathogens.....	78
4.3.2	Prevalence with respect to Trimester (Gestational age).....	78
4.3.3	Prevalence with respect to age group.....	79
4.3.4	Prevalence with respect to occupational status.....	80

4.4	Susceptibility testing	80
4.5	Multiple antibiotic resistance index.....	86
4.6	Some virulence factors of <i>Proteus spp.</i>	93
4.6.1	Hemagglutinin.....	93
4.6.2	Hemolysin	94
4.6.3	Urease.....	95
4.7	Some virulence factors of <i>Escherichia coli</i>	97
4.7.1	Hemolysin.....	97
4.7.2	Hemagglutinin.....	99
4.8	Molecular characterization of resistant isolates.....	101
4.8.1	Plasmid Extraction/PCR Amplification.....	101

CHAPTER FIVE

5.0	DISCUSSION.....	118
5.1	Summary and Conclusion.....	126
5.2	Public Health Implication(s).....	130
5.3	Recommendation.....	130
	References.....	132

LIST OF TABLES

TABLES	TITLES	PAGES
1	Antimicrobial agents relatively safe in Pregnancy.72 with the genes of interest conferring antibiotic resistance, and their primer sequence	72
2	Summary of the morphological/biochemical reaction.....73 schemes	73
3	Biochemical test to differentiate <i>Proteus species</i> type.....75	75
4	Pus cells distribution in urine samples of pregnant women.....76 attending antenatal clinics	76
5	Epithelial cells distribution in urine samples of pregnant.....76 women attending antenatal clinic.	76
6	Crystals distribution in urine samples of pregnant women.....77 attending antenatal clinics	77
7	Prevalence of significant bacteriuria Prevalence of significant.....77 bacteriuria in urine samples pregnant women attending	77

	antenatal clinic.	
8	Prevalence of Uropathogens in urine samples of pregnant women attending antenatal clinics.	78
9	Prevalence of uropathogens with respect to trimester (gestational age)	79
10	Prevalence of uropathogens with respect to Age group.....	79
11	Prevalence of uropathogens with respect to occupational status.....	80
12	Interpretative chart according to NCCLS (2006).....	80
13	Summary of percentage sensitivity of <i>Proteus spp.</i> , <i>E. coli</i> , and <i>S. aureus</i> isolates from antenatal urine samples in Kano, Nigeria to eleven (11) commonly prescribed antibiotics	81
14	Percentage antibiotics resistance profile of <i>Proteus spp.</i> isolates from 3 Hospitals in Kano, Nigeria	83
15	Percentage antibiotics resistance profile of <i>E. coli</i> isolates from 3 Hospitals in Kano, Nigeria	84

16	Percentage antibiotics resistance profile of <i>S. aureus</i>85 isolates from 3 Hospitals in Kano, Nigeria.
17	Multiple antibiotic resistance index (MARI) and resistance87 pattern of isolated <i>Proteus spp.</i> isolates
18	Multiple Antibiotic Resistance Index and profile occurrence89 of <i>Proteus spp.</i> isolates
19	Multiple antibiotic resistance index (MARI) and resistance.....90 pattern of isolated <i>E. coli</i>
20	Multiple Antibiotic Resistance Index and profile of91 phenotypic occurrence of <i>E. coli</i> isolates
21	Plasmid profile of <i>Proteus spp.</i> isolates harbouring plasmids.....92
22	Plasmid profile of <i>E. coli</i> isolates harbouring plasmids92
23	Hemagglutinins expressed by <i>Proteus spp.</i> isolates from93 urine samples of pregnant women attending antenatal clinics
24	Reciprocal of hemolytic titres of 23 (twenty three) <i>Proteus</i>94 <i>spp.</i> isolates

25	Urease activity shown by 23 <i>Proteus spp.</i> isolates from urine96 samples of pregnant women attending antenatal clinics.
26	Reciprocal of Hemolytic titre of 17 (seventeen) <i>E. coli</i> isolates.....98 from pregnant women with asymptomatic bacteriuria
27	Hemagglutinins expressed by <i>E. coli</i> isolates from urine.....99 samples of pregnant women with asymptomatic bacteriuria

LIST OF FIGURES

FIGURES	PAGES
1 The Urinary Tract.....	2
2 Agarose gel electrophotogram of 10 <i>E. coli</i> isolates (Ec1-Ec10) and PCR amplification using primers for CTX-M2, qnrC genes.	101
3 Agarose gel electrophotogram of 7 <i>E. coli</i> (Ec11-Ec17) and 3 <i>Proteus spp.</i> isolates (P1-P3) and PCR amplification using Primers for CTX-M2 and qnrC genes.	103
4 Agarose gel electrophotogram of 10 <i>Proteus spp.</i> isolates..... (P4-P13) and PCR amplification using primers for CTX-M9 and qnrS (1-2) genes.	106
5 Agarose gel electrophotogram of 10 <i>Proteus spp.</i> isolates..... (P14-P23) and PCR amplification using primers for CTX-M9 and qnrS (1-2) genes.	109

- 6 Agarose gel electrophotogram of 5 *E. coli* (Ec1 -Ec5) and112
5 *Proteus spp.* isolates (P1-P5) and PCR amplification
using primers for gyrB and pCT genes.
- 7 Agarose gel electrophotogram of 5 *E. coli* (Ec1-Ec5) and115
5 *Proteus spp.* isolates (P1-P5) and PCR amplification using
primers for CTX-M9 and qnrS (1-2) genes.

LIST OF CHEMICAL STRUCTURES

STRUCTURES	PAGES
1 Nalidixic acid.....	16
2 Ciprofloxacin.....	18
3 Ofloxacin.....	20
4 Levofloxacin.....	22
5 Ceftriaxone.....	23
6 Gentamicin.....	25
7 Nitrofurantoin.....	27
8 Tetracycline.....	28
9 Sulphamethoxazole.....	30
10 Trimethoprim.....	30
11 Amoxicillin.....	32
12 Amoxicillin/Clavulanic acid.....	34

LIST OF APPENDICES

APPENDIX	PAGES
1	Zone of inhibition of commonly prescribed antibiotics.....152 against isolated <i>Proteus spp.</i> , <i>E. coli</i> and <i>S. aureus</i>
2	Bar chart of percentage sensitivity of <i>Proteus spp.</i> isolates.....155 to eleven commonly prescribed antibiotics
3	Bar chart of percentage sensitivity of <i>E. coli</i> isolates.....156 to eleven commonly prescribed antibiotics
4	Bar chart of percentage sensitivity of <i>S. aureus</i> isolates.....157 to eleven commonly prescribed antibiotics
5	Bar Chart of Average Zone of Inhibition of <i>Proteus spp.</i> ,.....158 <i>E. coli</i> and <i>S. aureus</i> isolates
6	Graph showing Urease activity of <i>Proteus spp.</i> isolates.....159
7	Graph showing Reciprocal Hemolytic titres of <i>Proteus spp.</i>160

and *E. coli* isolates

8	Primers Synthesis Report by Inqaba Biotech., South Africa.....	161
9	Preparation of Reagents.....	164
10	Plasmid DNA curve of the molecular weight of the DNA..... marker/ladder <i>versus</i> the distance travelled by the plasmid DNA on agarose gel electrophotogram of 10 <i>E. coli</i> isolates (Ec1-Ec10).	168
11	Plasmid DNA curve of the molecular weight of the..... DNA marker/ladder <i>versus</i> the distance travelled by the plasmid DNA on agarose gel electrophotogram of 7 <i>E. coli</i> isolates (Ec11-Ec17) and 3 <i>Proteus spp.</i> isolates (P1-P3).	169
12	Plasmid DNA curve of the molecular weight of the..... DNA marker/ladder <i>versus</i> the distance travelled by the amplified <i>gyrB</i> genes on agarose gel electrophotogram of 5 <i>E. coli</i> isolates (Ec1-Ec5) and 5 <i>Proteus spp.</i> isolates (P1-P5).	170
13	Statistical significance between percentage colonized and age..... group of pregnant women.	171
14	Statistical significance between percentage colonized and.....	171

gestational age (trimester) of pregnant women.

CHAPTER ONE

1.0 INTRODUCTION

The urine is ideally sterile in healthy individuals and constantly flushes the urinary tract. Organisms invading the urinary tract (Fig. 1) must avoid being washed out during urination. Bacteria must be introduced directly into the bladder in order to initiate an infection. In males, the ureter is long (about 20 cm), while in females, it is much shorter and is more readily traversed by micro-organisms. Bladder infections are therefore more common in females. Spread of infection from the bladder to the kidney can occur through reflux of urine into the ureter. Long-term catheterization of the bladder can promote the occurrence of bacteriuria and associated complications. Lactic acid in the vagina gives it an acidic pH (5.0) which inhibits other products of metabolism and colonization by most bacteria, except some lactobacilli which constitute the commensal flora (Saidi *et al.*, 2005).

1.1 PREGNANCY AND THE URINARY TRACT

The urinary tract consists of the urethra, prostate glands (in males), bladder, ureter and kidneys. The urinary tract is normally sterile except for the urethra which may contain few commensals such as *Acinetobacter* species and diptheroids. Yeasts may also be found in the female urethra. It is now documented that women in the child bearing age have the highest incidence of urinary tract infection. During pregnancy, vagina secretion is much more increased and is a medium for bacteria growth. Also, due to increased progesterone activity, the glucose level of the vagina is also increased which also aids

bacteria growth. (Saidi *et al.*, 2005). Vaginal contamination is often indicated by the presence of epithelial flora.

An elevated blood pressure is often encountered also during pregnancy. In the case of hypertension, the spread of the infection is through the hematogenous route mainly. This is based on the pressure of the pumping blood in the nephrons; any bacteria present will be rapidly distributed through the urinary tract. Sexual intercourse has been observed to be a major predisposing factor to urinary tract infections (Dominic, 2006). At present, bacteriologic examination of the urine is done mainly when signs and symptoms point to urinary tract infection, renal insufficiency or hypertension. Bacteriologic examination of the urine should always be done in persons with suspected systemic infections or fever of unknown origin. It is desirable for every woman in the first trimester of pregnancy (Jawetz, 1982).

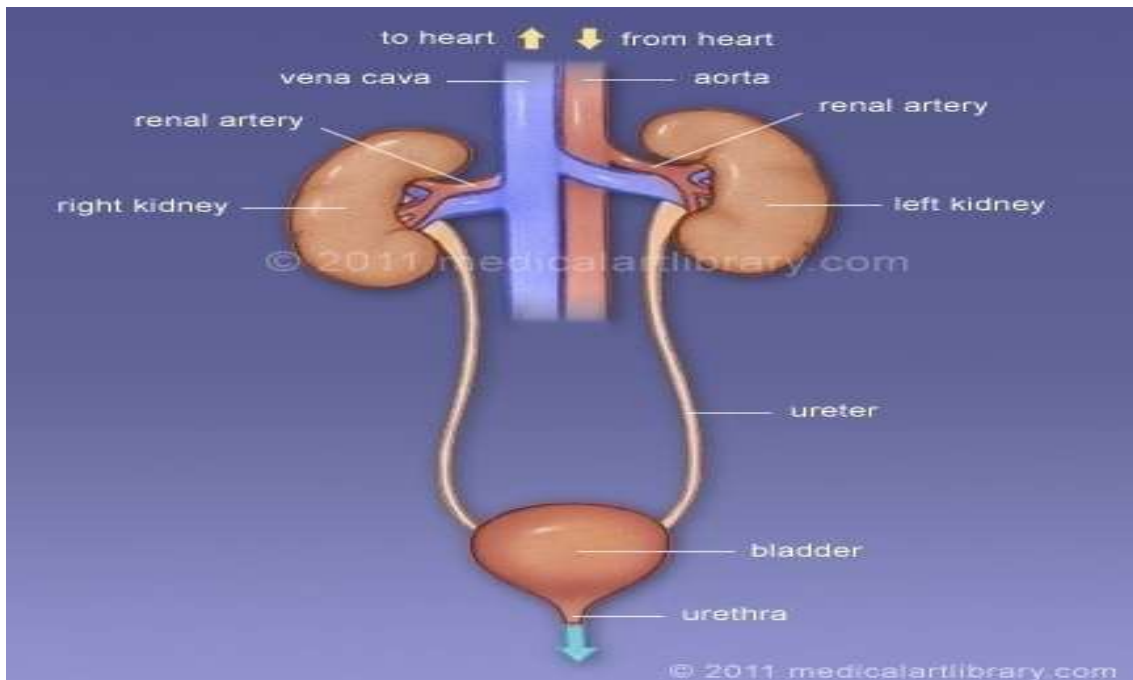


Fig 1: The Urinary Tract (2011 medicalartlibrary.com)

1.2 ASYMPTIOMATIC BACTERIURIA IN PREGNANCY

Bacteriuria refers to the presence of bacteria in voided urine. Asymptomatic is a condition in which urine culture reveals a significant growth of pathogens that is greater than 10^5 bacteria/ml but without the patient showing symptoms of urinary tract infection (UTI) such as burning during micturition or frequent urination (Imade *et al.*, 2010). This is common during pregnancy (Gilbert *et al.*, 2005). The apparent reduction in the immunity of pregnant women appears to encourage the growth of both commensal and non-commensal micro-organisms (Scott *et al.*, 1990). The physiological increase in plasma volume during pregnancy decrease urine concentration and up to 7%. Pregnant women develop glucosuria, which encourage bacterial growth in the urine (Patterson and Andriole, 1987; Lucas and Cunningham, 1993).

Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric out comes such as premature birth, low birth weight (Connolly and Thorp 1999) and higher foetal mortality rates (Nicole, 1994; Delzell and Leferre, 2000). Acute pyelonephritis, foetal growth restriction and still birth in pregnant women have been associated with asymptomatic bacteriuria (Zhao and Wu, 2004; Ryan *et al.*; 1990; Hill *et al.*, 2005). Asymptomatic bacteriuria has also been implicated in post partum endometritis (Lindsay, 2003). Although asymptomatic bacteriuria may not require treatment because the bacteria may not be causing harm, certain group of people such as pregnant women and their unborn feotuses may be at risk of complications (Guyton, 1996; Lindsay, 2003; Bloomberg *et al.*, 2005).

Bacteria are typically introduced into the urinary tract during intercourse or when wiping after a bowel movement (Dominic, 2006). The bacterium *E. coli* is responsible for

at least 75-80% of asymptomatic bacteriuria. *Klebsiella pneumoniae*, *Proteus spp.*, *Enterococci* and *group B Streptococci* can also establish colonization. Because asymptomatic bacteriuria occurs without symptoms, it is important to know what increases infection risk. This can help avoid the consequences of untreated asymptomatic bacteriuria by early detection and treatment. Researchers have identified a number of risk factors which include; immunosuppressive disorders, urinary tract obstructions (from stones) loss of bladder control due to neuromuscular disease, need for chronic instrumentation of the bladder (self-catheterization).

In non-pregnant women, asymptomatic bacteriuria rarely causes serious problem. However, in pregnant women, this infection can progress upward causing acute urethritis, acute cystitis and acute pyelonephritis (kidney infection). Pyelonephritis, in turn can lead to adverse outcomes such as preterm labour which is the most common cause of serious complications including death in newborn babies. A kidney infection can lead to sepsis (pathogenic organisms or toxins invading the blood or tissue) and adult respiratory distress syndrome (ARDS) both can be life-threatening (Dominic, 2006). Some of the complications of bacteriuria in pregnancy include maternal anaemia (some of these uropathogens like *Proteus*, *Escherichia coli*, *Klebsiella* etc are known to possess pili/fimbriae which agglutinate human erythrocytes resulting in anaemia), increase in mid-trimester abortion, low neonatal birth weight, hypertension, growth retardation and preterm delivery (Mc Grady *et al.*, 1985). Approximately 25-30% of asymptomatic bacteriuria cases in pregnancy will progress to symptomatic infection, 3 to 4 times as great a progression as in non-pregnant women, (Dominic, 2006).

1.3 STATEMENT OF THE PROBLEM

Asymptomatic bacteriuria has been observed to be a major risk factor in developing symptomatic urinary tract infection (UTI). In pregnant women, this infection can progress upward causing acute urethritis, acute cystitis and acute pyelonephritis, which in turn can lead to adverse outcomes such as preterm labour, sepsis (pathogenic organisms or toxins invading the blood or tissues), adult respiratory distress syndrome etc.

Untreated asymptomatic bacteriuria during pregnancy is followed by active pyelonephritis in about 40% of cases (Kass, 1959) and this is associated with an increased prenatal mortality. It is now documented that women in the child bearing age have the highest incidence of urinary tract infection.

Recent epidemiologic studies in Nigeria have shown high prevalence of asymptomatic bacteriuria among pregnant women: 86.6% (Akerele *et al.*, 2001), 78.7% (Amadi *et al.*, 2007) and 45.3% (Imade *et al.*, 2010). This research work is important because majority of our hospitals in Nigeria do not carry out routine culture test for their antenatal patients; instead the strip urinalysis method is utilized by most clinicians for assessing the urine of these pregnant women. This method cannot quantify the extent of infection and cannot provide adequate antimicrobial therapy as in the case of a culture and sensitivity test. Asymptomatic bacteriuria occurs without the usual clinical symptoms of UTI. Therefore, most of these pregnant women might be infected with some uropathogens without their knowledge which could lead to adverse neonatal outcome.

1.4 AIM

The aim of this work is to determine the prevalence of asymptomatic bacteriuria in pregnant women (not presenting with the clinical symptoms of urinary tract infection) attending antenatal clinics in three major Hospitals in Kano, Nigeria; to ascertain the antibiotic susceptibility pattern of the uropathogens isolated, to determine some of their pathogenic traits (virulence factors), and also to molecularly characterize the resistant isolates (plasmid extraction and antibiotic resistance genes amplification through Polymerase Chain Reaction) .

1.5 SPECIFIC OBJECTIVES

1. To determine the prevalence of asymptomatic bacteriuria among pregnant women attending antenatal clinics in three Hospitals in Kano, Nigeria namely: Aminu Kano Teaching Hospital, Mohammed Abdullahi Wase Specialist Hospital, and Murtala Mohammed Specialist Hospital.
2. To determine the prevalence of the various uropathogenic isolates. Prevalence of bacteriuria will be observed with respect to Gestational age (trimester), Age group, and Occupational status.
3. To determine the susceptibility pattern of the various uropathogenic isolates to commonly prescribed antibiotics at the various antenatal clinics.

4. To determine some of the virulence factors (pathogenicity) of the major uropathogenic isolates.
5. To molecularly characterize the resistant isolates and to determine whether the resistance was plasmid mediated or not.

1.6 HYPOTHESIS

There is asymptomatic bacteriuria in pregnant women attending antenatal clinics in Aminu Kano Teaching (AKTH), Mohammed Abdullahi Wase Specialist (MAWSH), and Murtala Mohammed Specialist Hospital, all in Kano, Nigeria.

CHAPTER TWO

2.0 LITERATURE REVIEW

Asymptomatic bacteriuria or asymptomatic urinary tract infection is the isolation of a specified quantitative count of bacteria (usually 10^5 cfu/ml) in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary tract infection (Gilbert *et al.*, 2005). Bacteriuria occurs in 2 to 7 percent of pregnancies, particularly in multiparous women, a similar prevalence is seen in non-pregnant women (Smaill and Vazquez, 2007). The organisms are also similar in species and virulence factors in pregnant and non-pregnant women. Thus the basic mechanism of entry of bacteria into the urinary tract is likely to be the same for both groups (Stenqvist *et al.*, 1987).

Bacteriuria often develops in the first month of pregnancy and is frequently associated with a reduction in concentrating ability, suggesting involvement of the kidney (Kaitz, 1961). The smooth muscle relaxation and subsequent urethral dilatation that accompany pregnancy are thought to facilitate the ascent of bacteria from the bladder to the kidney. As a result, bacteriuria during pregnancy has a greater propensity to progress to pyelonephritis (up to 40 percent) than in non-pregnant women (Kass, 1960; Sweet, 1977; Smaill and Vazquez, 2007). *Escherichia coli* is the commonest cause of urinary tract infection, followed by *Staphylococcus spp.* Other microbial contaminants that lead to infection include *Proteus spp.* and *Pseudomonas spp.* (Gilman *et al.*, 1996). *Escherichia coli* remains the single most common organism isolated from bacteriuric women (Evans *et al.*, 1978 Kunin and

Mc Cormack, 1968; Bengtsson *et al.*, 1998), although this happens proportionally less frequently than for women with acute uncomplicated urinary tract infection. Those with asymptomatic bacteriuria are characterized by fewer virulence characteristics than those isolated from women with symptomatic infection (Svanborg and Godaly, 1997). Other Enterobacteriaceae (such as *Klebsiella pneumoniae*) and other organisms (including coagulase-negative *Staphylococci*, *Enterococcus species*, group *B streptococci*, and *Gardnerella vaginalis*) are common as well. Men and women with long-term urologic device in place usually have polymicrobial bacteriuria, which often includes *Pseudomonas aeruginosa* and urease-producing organisms, such as *Proteus mirabilis*, *Providencia stuartii*, and *Morganella morganii* (Warren *et al.*, 1982; Nicole, 1997).

Bacteriuria has been associated with an increased risk of preterm birth, low birth weight, and perinatal mortality (Kass, 1960; Naeye, 1979; Millar and Cox, 1997; Patterson and Andriole, 1997; Delzell and Leferre, 2000). As an example, a review of over 50,000 pregnancies between 1959 and 1966 showed that women with bacteriuria and or pyuria (no comment on the presence or absence of symptoms) in the last two weeks of pregnancy had a higher rate of perinatal mortality from a variety of causes than non-infected women (Naeye, 1979). Studies have shown that the treatment of bacteriuria during pregnancy reduces the incidence of these complications (Kass, 1960; Whalley *et al.*, 1965; Rouse *et al.*, 1995; Mittendorf *et al.*, 1992; Gratacos *et al.*, 1994; Villar *et al.*, 1998) and lowers the longterm risk of sequelae following asymptomatic bacteriuria (Zinner and Kass, 1971).

2.1 SYMPTOMS OF URINARY TRACT INFECTION (UTI)

The symptoms of urinary tract infection include dysuria, polyuria, urgency, hematuria, pus in urine, cramps or pain in the lower abdomen, chills or fever, foul smelling urine, nausea, vomiting and malaise, urinary incontinence, urinary hesitancy (Cornolly and Thorp, 1999).

2.2 MICROBIOLOGICAL DIAGNOSTIC TESTS FOR URINARY TRACT INFECTION

Asymptomatic bacteriuria is a microbiological diagnosis determined with a urine specimen that has been collected in a manner to minimize contamination and transported to the laboratory in a timely fashion to limit bacterial growth. The usual quantitative definition is $\geq 10^5$ cfu/ml in two consecutive urine specimens (Rubin *et al.*, 1992). This was initially proposed after studies performed in the 1940s and 1950s (Kass, 1957 and Kass, 1962). When the screenings of asymptomatic women using multiple voided specimens were evaluated, bacteriuria documented in an initial voided urine specimen was confirmed in a second voided specimen, obtained several days later. If two successive bacteriuric voided specimens had similar positive culture results, a third consecutive specimen also yielded consistent results in 95% of cases (Kass, 1962 and Kunin, 1966).

Some studies involving women have used a more restrictive criterion of three consecutive voided urine specimens collected over three weeks with consistent bacteriologic results (Evans *et al.*, 1978; Kunin and Mc Cormack, 1968) whereas other studies have used a more permissive criterion of a single positive urine

specimen yielding $\geq 10^5$ cfu/ml (Hooton *et al.*, 2000; Kunin *et al.*, 1980 and Nicolle *et al.*, 1982). The prevalence will be lower if >1 specimen is required for identification of bacteriuria (Hooton *et al.*, 2000). Pregnant women should be screened for bacteriuria by urine culture at least once in early pregnancy, and they should be treated if the results are positive. Quantitative midstream culture is the only gold standard for diagnosis of all suspected urinary infections.

2.2.1 Asymptomatic bacteriuria:

- $> 10^5$ bacteria/ml with < 20 white cells generally indicates asymptomatic bacteriuria.
- A count $>10^5$, with two or more organisms, indicates a contamination rather than bacteriuria.

2.2.2 Acute Cystitis:

In addition midstream urine specimen, clinical diagnosis is based on symptoms such as:

- Dysuria, urinary frequency
- Lower abdominal pain or supra-pubic pain without fever
- Pyuria may also be present.

2.2.3 Pyelonephritis:

Pyelonephritis usually presents as an acute episode in addition to midstream urine specimen, clinical diagnosis should include:

- Full maternal clinical history and examination

- Assessment of foetal well being
- Blood cultures (aerobic and anaerobic)
- Low and high vaginal swabs
- Complete blood count, renal function test including creatinine, urea and electrolytes
- Urinalysis for Proteinuria
- Women with pyelonephritis often have pyuria or leukocyte casts; symptoms include; pyrexia, chills, rigor, flank or renal angle pain, nausea and vomiting, dehydration, less commonly dysuria, frequency, foetal tachycardia may also be present.

2.3 URINE MICROSCOPY, CULTURE AND SENSITIVITY (MCS)

The urine culture test detects and identifies bacteria and yeast in urine. In a urine culture test, a small sample of urine is placed on one or more agar plates (a thin layer of a nutrient media) and incubated at body temperature. Any micro-organism present in the urine sample grows over 24 to 48 hours as small circular colonics. The size, shape, and colour of these colonies observed under a microscope help to identify which bacteria are present, and the number of colonies indicates the quantity of bacteria originally present in the urine sample. Biochemical tests are used to identify which bacteria present and susceptibility (or sensitivity) are testing is done to identify the antimicrobial agents that inhibit the growth of bacteria. The results of these laboratory tests allow for proper selection of antibiotic treatment to resolve infection(s).

2.4 CHEMOTHERAPY OF ASYMPTOMATIC BACTERIURIA

Antimicrobial treatment of women with asymptomatic bacteriuria during pregnancy improves foetal outcomes. A variety of antibiotics has been used to treat asymptomatic bacteriuria and seem to have similar efficacy (Christensen, 2000) as seen in meta-analysis of various regimens in the Cochrane Database (Smaill, 2001). Treatment is empiric because causative bacteria are predictable. Increasing antimicrobial resistance among uropathogens poses a challenge to therapy. Although the susceptibility of these pathogens to antimicrobial therapy has changed, their prevalence has not. The pattern of resistance varies geographically. This should be taken into account when determining appropriate therapy. Other factors to be considered in the selection of appropriate antimicrobial therapy include the spectrum of activity of the agent, potential side effects, duration of therapy, cost, and pharmacokinetics (Nicolle, 2002). β -Lactam antibiotics, including ampicillin, are among the oldest antibiotics that are used to treat bacterial infection; however, the pharmacokinetic changes of pregnancy decrease plasma concentrations of β -lactams by up to 50% (Christensen, 2000).

The Infectious Disease Society of America (IDSA) recommends a course of three to seven days of antimicrobial therapy for pregnant women with asymptomatic bacteriuria (Nicolle *et al.*, 2005). Leukocyte esterase and nitrite tests have low sensitivity for identifying bacteriuria in women who are pregnant, hence they should be screened with urine cultures (Bachman *et al.*, 1993), and however the optimal frequency of urine culture screening has not been established. A single urine culture at the end of the first trimester generally is recommended based on clinical outcomes

and cost-effectiveness (Stenqvist *et al.*, 1989; Wadland and Plante, 1989). Women with asymptomatic bacteriuria or symptomatic UTI during pregnancy should be treated and should undergo periodic screening for the duration of their pregnancy.

2.4.1 Oral antibiotics for treatment of pregnant women with asymptomatic bacteriuria

The United States have categorized oral antibiotics for treatment of pregnant women with asymptomatic bacteriuria. FDA (United States Food and Drug Administration) Pregnancy Category B; safety for use in Pregnancy has not been established: Amoxicillin, Amoxicillin/Clavulanate, Ampicillin, Cefuroxime, Cephalexin, Nitrofurantoin.

Pregnancy Category C; No adequate well-controlled studies have been performed in women; should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus: Ciprofloxacin, Gatifloxacin, Levofloxacin, and Trimethoprim/Sulfamethoxazole.

2.5 W.H.O'S ANTIBIOTIC PRESCRIPTION RECOMMENDATION

Antibiotics are effective for the treatment of asymptomatic bacteriuria in pregnancy, decreasing the incidence of pyelonephritis in the treated women. Antibiotic therapy also appears to reduce the incidence of low-birth-weight and preterm birth. The Cochrane review revised in 2011 revealed that antibiotic treatment is effective in reducing the risk of pyelonephritis in pregnancy. Fourteen studies were included in the review. Antibiotic treatment compared to placebo or no treatment was

effective in clearing asymptomatic bacteriuria [risk ratio (RR) 0.25, 95% confidence interval (CI) 0.14 to 0.48]. Antibiotic treatment was also associated with a reduction in the incidence of low birth weight (RR 0.66, 95% CI 0.49 to 0.89), but a difference in preterm delivery was not seen. If a second urine culture confirms asymptomatic bacteriuria, treatment should be for 7 days. Preferred options when sensitivities are known (in order of preference) are:

- Amoxicillin, 250mg, three times daily for 7 days
- Nitrofurantoin, 50mg, four times daily for 7 days
- Trimethoprim, 200mg, twice daily (unless the woman is folate deficient or taking a folate antagonist)
- Cephalexin, 500mg twice daily for 7 days
- After treatment, urine should be sent for culture to screen. For asymptomatic bacteriuria on every antenatal visit until delivery.

2.6 ANTIBIOTICS COMMONLY PRESCRIBED IN ASYMPTOMATIC BACTERIURIA

In practice, the choice of antibiotic to treat asymptomatic bacteriuria is more likely to be guided by national patterns of practice and local resistance patterns, than by evidence from clinical trials. There has been no systematic review of suitable antibiotic best for the treatment of asymptomatic bacteriuria. An optimal drug is required to have favorable pharmacokinetics, however, commonly prescribed antibiotics include: The flouroquinolones such as Nalidixic acid, Ciprofloxacin, Levofloxacin and Ofloxacin. Amoxicillin/Clavulanic acid, Amoxicillin,

Nitrofurantoin, Tetracycline, Cotrimoxazole, Gentamicin, Ceftriaxone, and Cephalexin. The properties of some of these antibiotics are detailed below:

2.6.1 Fluoroquinolones

These are the second- generation quinolones with increased potency and spectrum of activity compared to the first-generation quinolones (e.g nalidixic acid). They are the new derivatives of 4-quinolone antibiotics in which their C-6 is substituted with fluorine. Examples of the fluoroquinolones are ciprofloxacin, sparfloxacin, levofloxacin, and ofloxacin.

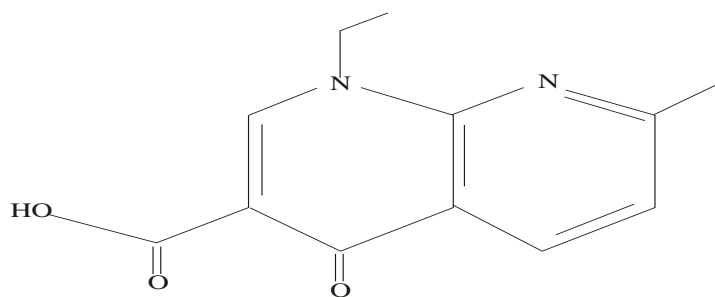
2.6.1.1 Nalidixic acid

Nalidixic acid is the first of the synthetic quinolone antibiotics. Synthetic quinolone antibiotics were discovered by George Leshner and coworker as a byproduct of chloroquine manufacture in the 1960s. It is especially used in treating urinary tract infections caused by *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, and *Klebsiella*.

a. **Chemistry:** $C_{12}H_{12}N_2O_3$

It has a molecular weight of 232.235 g/mol

b. **Chemical Structure:**



Structure 1: Nalidixic Acid

c. **Systematic (IUPAC) name:**

1-ethyl-7-methyl-4-oxo-[1, 8] naphthyridine -3-carboxylic acid

d. **Administration:**

Nalidixic acid is available as 250mg in the oral form. It is taken every 6 hours i.e. four times daily.

e. **Antimicrobial activity:**

The drug is effective against both gram-positive and gram-negative bacteria which include *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, *Klebsiella* etc. In lower concentrations it's bacteriostatic, while it's bactericidal at higher concentrations.

f. **Mechanism of Action:**

It acts by selectively and reversibly blocking DNA replication in susceptible bacteria. Nalidixic acid inhibits a subunit of DNA gyrase.

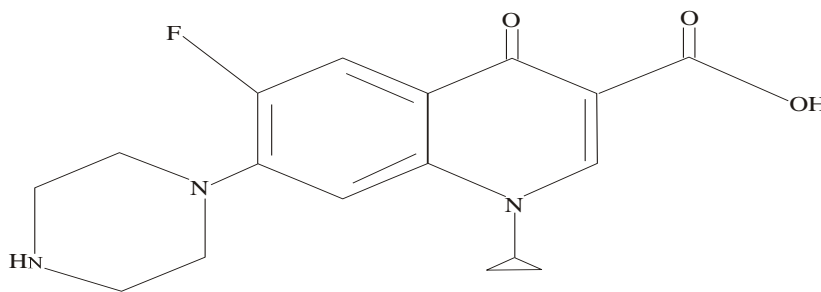
2.6.1.2 Ciprofloxacin

a. **Chemistry:** $C_{17}H_{18}FN_3O_3$.

Its molecular weight is 331.4 g/mol

It is a faintly yellowish to light yellow crystalline substance.

b. **Chemical structure:**



Structure 2: Ciprofloxacin

c. **Systematic (IUPAC) Name:**

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid.

Ciprofloxacin has a bioavailability of 69% with no significant first pass effect.

Metabolism is mainly hepatic including the cytochrome CYP1A2 System. Its

half-life is 4 hours; and its major route of excretion is renal.

d. **Administration:**

Ciprofloxacin is given by mouth or intravenously. The adult oral or intravenous dose ranges from 250mg to 500mg twice daily depending on the severity and nature of the infection. In pregnancy, the fluoroquinolones rapidly

cross the blood-placenta and blood-milk barriers, and are extensively distributed into the foetal tissues. For this reason, the fluoroquinolones are contraindicated during pregnancy due to the risk of spontaneous abortions and birth defects except for cases where the benefit outweighs the risk.

e. **Antimicrobial Activity:**

Ciprofloxacin is a broad-spectrum antibiotic active against both Gram-positive and Gram negative bacteria.

f. **Mechanism of Action:**

It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV (Drlica and Zhao, 1997) enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. The mechanism can also affect mammalian cell replication. In particular, some congeners of this drug family (for example those that contain the C-8 fluorine) (Robinson *et al.*, 1992), display high activity not only against bacterial topoisomerases but also against eukaryotic topoisomerase and are toxic to cultured mammalian cells and *in-vitro* tumor models.

Although quinolones are highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone-induced DNA damage was first reported in 1986 (Hussy *et al.*, 1986).

g. Resistance development of bacteria to Quinolones in the treatment of asymptomatic bacteriuria:

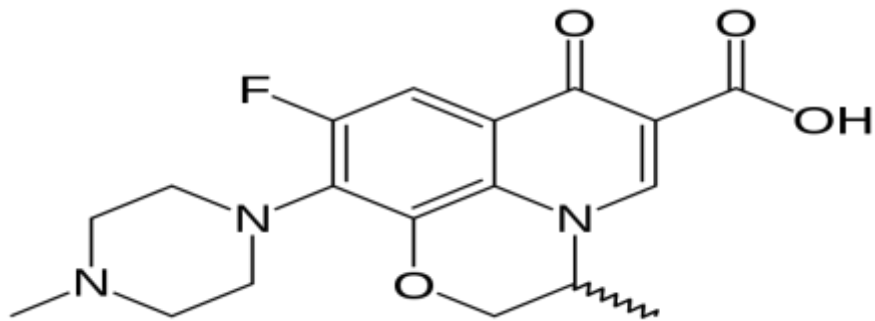
Resistance to Ciprofloxacin and other fluoroquinolones have evolved rapidly. Numerous pathogens including *Staphylococcus aureus*, *Enterococci* and *Streptococcus pyogenes* now exhibit resistance worldwide. Widespread veterinary usage of the fluoroquinolones, particularly in Europe has been implicated. In recent years there have been reports of reduced susceptibility and treatment failure to quinolones (Brown *et al.*, 1996). Due to emergence of multi-drug resistant strains. Some bacteria strains have acquired resistance to quinolones due to chromosomal mutations in the gene coding for DNA gyrase (Hooper and Wolfson, 1993).

2.6.1.3 Ofloxacin

a. **Chemistry:** $C_{18}H_{20}FN_3O_4$

Ofloxacin has a molecular weight of 361.368 g/mol. It is a pale yellow crystalline powder, slightly soluble in water, methyl alcohol and glacial acetic acid.

b. **Chemical Structure:**



Structure 3: Ofloxacin

c. **Systematic (IUPAC) name:**

(RS)-7-fluoro-2-methyl-6-(4-methyl piperazin-1-yl)-10-oxo-4-oxa-1-aza tricyclo
[7.3.1.0 5, 13] trideca-5(13), 6, 8, 11-tetraene-11-carboxylic acid.

d. **Administration:**

Ofloxacin is given by mouth as the base or intravenously as the hydrochloride. The adult oral or intravenous dose ranges from 200mg daily to 400mg twice daily depending on the severity and nature of infection.

e. **Antimicrobial Activity:**

Susceptible bacteria include: Aerobic Gram-positive micro-organisms e.g. *Staphylococcus aureus* (methicillin-susceptible strains), *Streptococcus pneumoniae* (penicillin-susceptible strains), *Streptococcus pyogenes*. Aerobic Gram-negative micro-organisms e.g. *Citrobacter (diversus) koseri*, *Enterobacter aerogenes*, *Haemophilus influenza*, *Klebsiella pneumonia*, *Neisseria gonorrhoeae*, *Proteus*

mirabilis, *Pseudomonas aeruginosa*. Other micro-organisms are *Chlamydia trichomatis*.

f. **Mechanism of Action:**

Ofloxacin acts by inhibiting the A-subunit of DNA gyrase (topoisomerase I) which is essential in the reproduction of bacterial DNA. The enzyme is not found in mammalian cells, it is capable of catalyzing a variety of changes in DNA topology. Which include introduction of negative super-coiling and removal of linked structures. These activities ensure that the daughter chromosome produced during replication can segregate in cytoplasm prior to cell division.

2.6.1.4 Levofloxacin

a. **Chemistry:** $C_{18}H_{20}FN_3O$

Levofloxacin has a molecular weight of 361.3675 g/mol.

b. **Chemical Structure:**

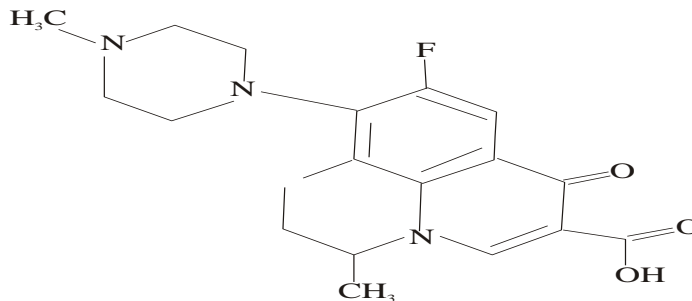


Fig. 2.4 Levofloxacin

Structure 4: Levofloxacin

c. **Systematic (IUPAC) name:**

(2S)-7-fluoro-2-methyl-6-(14-methyl piperazin-1-yl)-10-oxa-1-aza tricycle
[7.3.1.0^{5, 13}] trideca-5, 7, 9 (13), 11-tetraene-11-carboxylic acid.

d. **Administration:**

Levofloxacin is either taken intravenously or orally. Intravenously the strength is 125mg/5ml. The oral preparation is available as 250mg, 500mg, and 750mg film coated tablets.

e. **Antimicrobial Activity:**

This includes susceptible strains of *Corynebacterium species*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus epidermidis*, *Streptococcus (Groups C/F/G)*, *Viridians group Streptococci*, *Acinetobacter lwoffii*, *Haemophilus influenzae*, *Serratia marcescens* e.t.c

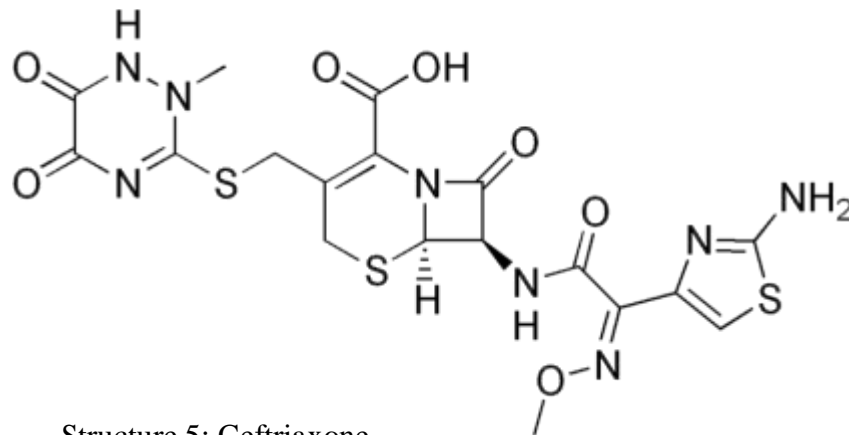
f. **Mechanism of Action:**

Levofloxacin inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. Levofloxacin, like other fluoroquinolones, inhibits the A-subunits of DNA gyrase; two subunits encoded by the *gyr A* gene. This results in strand breakage on a bacterial chromosome, supercoiling, and resealing; DNA replication and transcription are inhibited.

2.6.2 Ceftriaxone

Ceftriaxone is a third generation cephalosporin. Cephalosporins are semi synthetic antibacterial agents produced by the mould *Cephalosporium acremonium*. The third generation cephalosporins are more stable to hydrolysis by beta-lactamase than the second generation cephalosporins e.g. cefuroxime. Comparing ceftriaxone, a third generation cephalosporin with earlier generations, it has a wider spectrum of activity and greater potency against both gram-positive and gram-negative bacteria, hence very useful in the chemotherapy of asymptomatic bacteriuria. Penicillin and Cephalosporins are generally the antibiotics of choice for use in asymptomatic bacteriuria in pregnancy (Schaefer *et al.*, 2007).

a. **Chemical Structure:**



Structure 5: Ceftriaxone

b. **Mechanism of Action:**

Ceftriaxone acts by inhibiting the action of transpeptidase enzyme preventing cell wall formation in susceptible organisms.

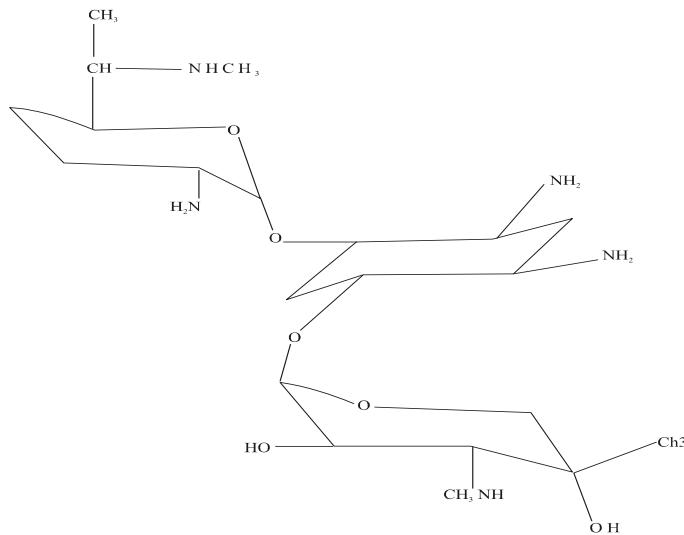
c. **Resistance Pattern:**

Resistance to Ceftriaxone (a Cephalosporin) is mainly due to the production of beta-lactamase by bacteria (Perry, 2001)

2.6.3 Gentamicin

Gentamicin is an aminoglycoside antimicrobial agent used often with other antibiotics to treat severe systemic infections due to Gram-negative and other organisms. Gentamicin sulphate was first isolated from *Micromonospora purpurea* in 1963. Gentamicin is a white hygroscopic powder, freely soluble in water, insoluble in alcohol, in acetone, in chloroform, in ether and in benzene.

a. **Chemical Structure:**



Structure 6: Gentamicin

b. **Administration:**

Gentamicin is commonly administered intramuscularly every 8 hours to provide a total daily dose of 3-5mg/kg. In some cases, a dose of 80mg twice daily in association with penicillin or vancomycin has been suggested for treatment. Gentamicin is available in two parenteral strengths: 80mg and 280mg.

c. **Antimicrobial Activity:**

Gentamicin is a broad spectrum antibiotic. Many strains of Gram-negative bacteria (including *Escherichia coli*, *Proteus spp.* and *Klebsiella*) are reported sensitive (Mingeot-Leclerq, 1999).

d. **Mechanism of Action:**

The drug (gentamicin) is taken up into sensitive bacterial cells by an active transport process within the cell. Gentamicin binds to the 30S and to some extent to the 50S, subunits of the bacterial ribosome thereby inhibiting protein synthesis and generating errors in the transcription of the genetic code.

e. **Development of Resistance:**

Bacteria resistance to gentamicin has been reported to be due to inactivation by enzymatic modification. Three major classes of enzymatic inactivations confer resistance to Gentamicin: phosphorylation, acetylation, or addition of a nucleotide group usually adenyl. These enzymes are plasmid-determined and the observed resistance can therefore be transferred between bacteria (Mingeot-Leclerq, 1999).

2.6.4 Nitrofurantoin

Nitrofurantoin is a synthetic nitrofuran derivative which is produced by the condensation of 5-Nitro-2-furaldehyde with 1-aminohydantoin (Gennaro, 1985).

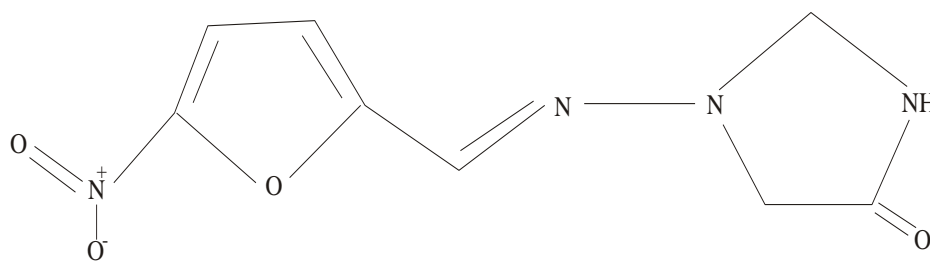
a. **Chemistry:** C₈ H₆ N₄ O₅

Molecular weight is 238.2 g/mol. It is a lemon-yellow solid crystal, very soluble in water and alcohol.

b. **Systematic (IUPAC) name:**

1-(5-nitrofurfurylideneamino) hydantoin.

c. **Chemical Structure:**



Structure 7: Nitrofurantoin

d. **Administration:**

50 mg to 100 mg four times a day/administered with food or milk to minimize anorexia, nausea, and vomiting. A usual prophylactic dose is 50 to 100 mg at bed time. A cure rate of 86% was achieved with a 7- day course in a multicentre study under taken by the World Health Organization (WHO) (Lumbiganon *et al.*, 2009).

d. **Antimicrobial Activity:**

Nitrofurantoin is used in the treatment of initial or recurrent urinary tract infections caused by susceptible gram positive and gram negative bacteria including most strains of *E. coli*, *Enterobacter* and *Klebsiella spp.* which are less susceptible. *Pseudomonas* and most strains of *Proteus* are more resistant to nitrofurantoin (Reynolds, 1993). Nitrofurantoin is ineffective in systemic bacterial infection in blood or tissues outside the urinary tract (American Society of Hospital Pharmacists-AHFS Drug information, 1995).

e. **Mechanism of Action:**

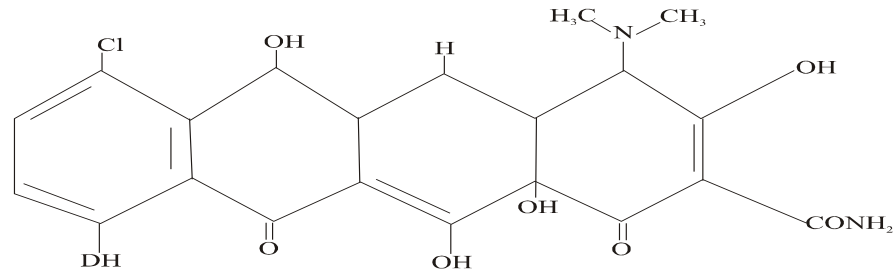
Nitrofurantoin is bacteriostatic or bactericidal depending on the concentration and the susceptibility of the micro-organism.

2.6.5 Tetracycline

Tetracyclines are a group of antibiotics obtained as a by-product from the metabolism of various species of *Streptomyces* for example *Streptomyces aureofaciens*. Tetracyclines are relatively hydrophilic (Hugo and Russel, 2000).

a. **Chemistry:** C₂₁ H₂₁ Cl N₂ O₈

b. **Chemical structure:**



Structure 8: Tetracycline

c. **Administration:**

The usual adult dosage of tetracycline hydrochloride is 500mg by mouth every 6 hours. Tetracycline generally produces a steady-state plasma concentration of 4-5ug/ml.

d. **Antimicrobial Activity:**

Tetracyclines have a wide spectrum of activity and have been used in the treatment of a large number of infections caused by susceptible organisms. However, the emergence of bacterial resistance coupled with the development of other antibacterials, their use has become more restricted (Martindale, 2005).

e. **Development of Resistance:**

Resistance to the tetracyclines appear to be associated with the ability to prevent accumulation of the antibiotic within the bacterial cell, both by decreasing active transport of the drug into the cell and by increasing tetracycline efflux. It is usually plasmid mediated and transferable.

2.6.6 Cotrimoxazole

Cotrimoxazole is a mixture of five parts of Sulphamethoxazole and one part of Trimethoprim. This combination has a synergistic bactericidal effect.

a. Chemistry:

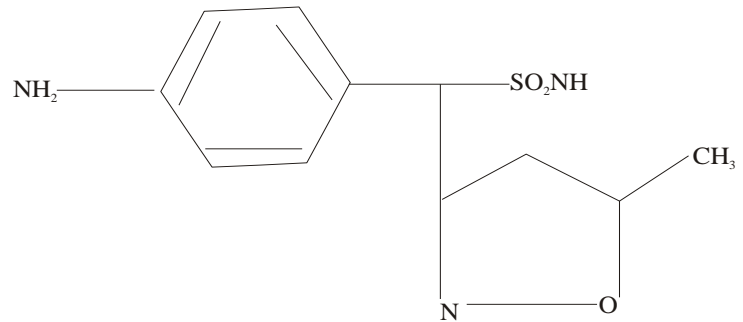
$C_{10}H_{11}N_3O_3S$ (Sulphamethoxazole)

Sulphamethoxazole is a white crystalline powder which is insoluble in water.

$C_{14}H_{18}N_4O_3$ (Trimethoprim)

Trimethoprim is a white-cream coloured crystalline powder very soluble in water, slightly soluble in alcohol. It has a pH of 7.5-8.5 in 0.05% solution of water.

b. Chemical Structure:



NI - (5 - methylisoxazole - 3 - yl) sulphanilamide

Structure 9: Sulphamethoxazole

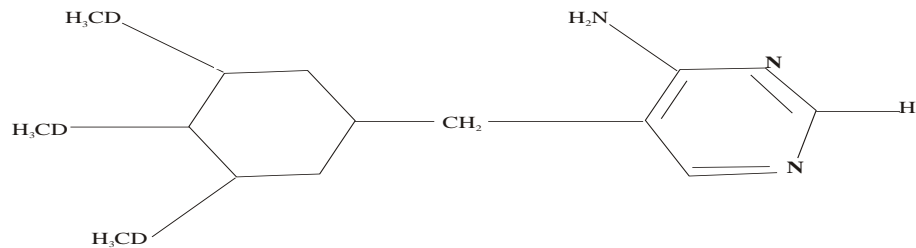


Fig. 2.10 Trimethoprim

Structure10: Trimethoprim

c. **Administration:**

Cotrimoxazole is usually given by mouth in adult dose of 960mg i.e., Trimethoprim 160mg and Sulphamethoxazole 800mg, twice daily. In severe infections, 2880mg daily in two divided doses (Martindale, 2005).

d. **Antimicrobial Activity:**

Cotrimoxazole has a broad spectrum of activity against susceptible organisms, but the rapid emergence of resistance has limited its use.

e. **Mechanism of Action:**

Cotrimoxazole has combined (synergistic) effect of its components. Sulphamethoxazole inhibits the conversion of para-aminobenzoic acid to the co-enzyme dihydrofolic acid the reduced form of folic conversion of bacteria dihydrofolic acid to tetrahydrofolic acid which is essential for the synthesis of DNA precision.

f. **Resistance Development:**

Resistance to Cotrimoxazole has been reported to be due to plasmid-mediated dihydrofolate reductase and dihydropterate synthetase and of the target site of the drug.

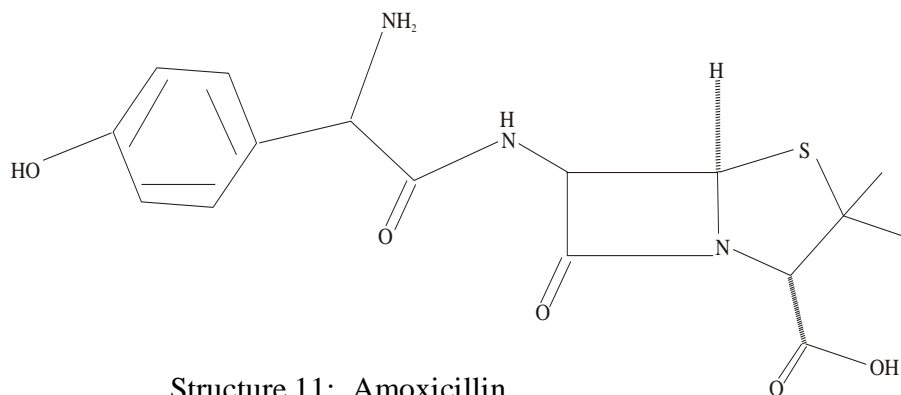
2.6.7 Amoxicillin

Amoxicillin is a moderate-spectrum, bacteriolytic, β -lactam antibiotic used to treat bacterial infections (including urinary tract infections) caused by susceptible micro-organisms. It is usually the drug of choice within the oral administration, than other β -lactam antibiotics.

- a. **Chemistry:** $C_{16}H_{19}N_3O_5S$

Amoxicillin has a molecular weight of 365.4 g/mol.

- b. **Chemical Structure:**



- c. **Systematic (IUPAC) name:**

(2S,5R,6R)- 6- {[[(2R)-2 amino-2-(4-hydroxy phenyl)-acetyl] amino}-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid.

Amoxicillin has a bioavailability of 95% when taken orally. Less than 30% of it is biotransformed in the liver. It has a half life of 61.3 minutes, and is excreted renally (Martindale, 2005).

d. **Administration:**

Amoxicillin in trihydrate form is available as capsules, for oral use, and as sodium salt for intravenous administration. Amoxicillin is usually taken in adult doses of 500mg three to four times daily.

e. **Antimicrobial Activity:**

Amoxicillin is bactericidal against aerobic gram positive bacteria: *Staphylococcus spp.* (with exception of penicillase-producing strains), *Streptococcus spp.*, as well as aerobic gram-negative bacteria: *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Escherichia coli*, *Shigella spp.*, *Klebsiella spp.*, *Salmonella spp.* etc. Micro-organisms which produce penicillase are resistant to amoxicillin.

f. **Mechanism of Action:**

Amoxicillin acts by inhibiting the synthesis of bacterial cell wall. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram-negative bacteria.

2.6.8 Amoxicillin/Clavulanic acid (Co-Amoxiclav[®])

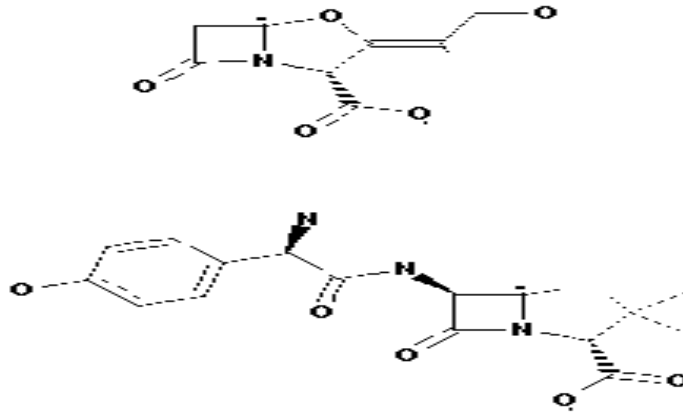
Co-amoxiclav[®] is a combination antibiotic consisting of amoxicillin trihydrate, a β -lactam antibiotic, and potassium clavulanate, a β -lactamase inhibitor. This combination results in an antibiotic with increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β -lactamase.

It is used in urinary tract infections caused by susceptible strains of gram-negative and gram-positive organisms.

a. **Chemistry:** $C_{24}H_{28}N_4O_{10}S$

Co-amoxiclav has a molecular weight of 564.56 g/mol.

b. **Chemical Structure:**



Structure 12: Amoxicillin/Clavulanic acid

c. **Systematic (IUPAC) name:**

(2R, 5R, S)-3-(2-hydroxyethylidene)-7-oxo-4,4-oxa-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid.

d. **Administration:**

Adult daily dose is 375 mg-1g in two divided doses. Intravenously over 3-4 minutes. Co-amoxiclav can be given 1g every 8 hours to 1g every 6 hours.

e. **Antimicrobial Activity:**

Clavulanic acid is a competitive β -lactamase inhibitor; it exhibits negligible antimicrobial activity. It acts solely by preventing the inactivation of the β -lactam antibiotics by binding to the β -lactamases. It used to overcome bacteria resistance in β -lactamase producing organisms.

Co-amoxiclav (the combination of amoxicillin and clavulanic acid) have a synergistic effect with a broad spectrum of activity against susceptible organisms. Despite this, some bacterial strains that are resistant to such combination have emerged.

f. **Mechanism of Action:**

Clavulanic acid has negligible intrinsic antimicrobial activity, despite sharing the β -lactam ring that is characteristic of beta-lactam antibiotics. However, the similarity in chemical structure allows the molecule to interact with the enzyme beta-lactamase secreted by certain bacteria to confer resistance to beta-lactam antibiotics. Clavulanic acid is a competitive inhibitor, covalently bonding to a serine residue in the active site of the beta-lactamase. This restructures the clavulanic acid molecule, creating a much more reactive species that is attacked by another amino acid in the active site, permanently inactivating it, and thus inactivating the enzyme. This inhibition restores the antimicrobial activity of beta-lactam antibiotics against lactamase- secreting-resistant bacteria.

Amoxicillin and other β -lactam antibiotics are bactericidal; they act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell wall which is

important for cell wall structural integrity, especially in Gram-positive organisms. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin binding proteins (PBPs). β -lactam antibiotics block not only the division of bacteria, including cyanobacteria, but also the division of cyanelles, the photosynthetic organelles of the glaucophytes, and the division of chloroplasts of bryophytes.

g. **Resistance development:**

There are two main modes of bacterial resistance to β -lactam antibiotics:

- (i) Enzymatic hydrolysis of the β -lactam ring: β -lactamases or penicillinases produced by the organisms break open the β -lactam ring of the antibiotic, rendering it ineffective. The genes encoding these enzymes may be inherently present on the bacterial chromosome or may be acquired via plasmid transfer (plasmid mediated resistance), induced by exposure to β -lactams.

- (ii) Possession of altered penicillin-binding proteins (PBPs):

β -lactam antibiotics cannot bind effectively to these altered PBPs. Examples of this mode of resistance includes methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin resistant *Streptococcus pneumoniae*. Altered PBPs do not necessarily rule out treatment options with β -lactam antibiotics.

2.7 DEVELOPMENT OF ANTIBIOTIC RESISTANCE

Almost as soon as antibiotics were introduced into clinical circulation, cases where their ability to effectively stop infection were observed. But as the use of antibiotics became more widespread the prevalence of resistance increased.

Sir Alexander Fleming in 1947 reported that *Staphylococcus aureus* was resistant to penicillin (the then wonder drug), just few years after the drug was mass-produced. He predicted that imprudent antibiotic usage could lead to clinical failure in the future. Many gram-negative organisms have been reported to be ineffective to Penicillin due to intrinsic bacteria resistance by species originally considered sensitive, resulting in a wide chemotherapeutic failure worldwide (Iruka *et al.*, 1999).

Antibiotic resistance can be as a result of horizontal gene transfer (HGT) and also of unlinked point mutations in the pathogen genome. The four main mechanisms by which organisms exhibit resistance to antimicrobials are:

- Drug inactivation or modification, for example, enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β -lactamases.
- Alteration of target site: For example, alteration of the Penicillin- binding-protein (PBP); this is the binding target site.
- Alteration of metabolic pathway: for example, some sulphonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids inhibited by sulphomamides.

- Reduced drug accumulation; by decreasing drug permeability and or increasing active efflux of the drugs permeability and or increasing active efflux of the drug across the cell surface.

Bacteria may acquire genes conferring resistance in any of the following way:

- Spontaneous mutation, bacterial DNA may mutate spontaneously.
- Transformation: A bacterium may take up DNA from another bacterium, penicillin-resistant gonorrhoea results from transformation.
- Plasmids: Resistance is acquired from a small circle of DNA called plasmid. A single plasmid can provide number of different resistances. Carbenicillin resistance for example is associated with resistance to tetracycline, kanamycin, cephaloridine and ampicillin (Roe *et al*, 1971; Lowbury *et al*; Onaolapo, 1986). This resistance is known to be coded for by R-plasmid RPI (Lowbury *et al*, 1972, Onaolapo, 1986).

2.8 FACTORS THAT INFLUENCE DEVELOPMENT OF ANTIBIOTIC RESISTANCE

Acquired bacterial resistance is common in isolates from healthy persons and from patients with community- acquired infections in most developing countries where the need for antibiotics is driven by the high incidence of infectious disease (Kunin, 1993). The selection and spread of resistant organisms in developing countries which can often be traced to complex socio-economic and behavioral antecedents, contribute to the escalating problem of antibiotic resistance worldwide.

Some of the factors influencing development of antibiotic resistance in Nigeria include:

- Patient's non-compliance to recommended treatment.
- Irrational use of antibiotics in humans. The use of antibacterial agents without proper sensitivity test should therefore be discouraged. Organisms which harbor antibiotic resistant plasmids have been shown to have ability to compete better in the presence of non-plasmid carrying strains under various environmental conditions since they have the ability to adhere more to prostheses and glass materials (Onaolapo, 1986).
- Mis-use of antibiotics by physicians in clinical practice. Due to indiscriminate use of antibiotics, the activity of many valuable antibacterials has been diminished. The sensitive organisms would be continuously eliminated and replaced by a resistant population particularly in hospital wards (Onaolapo and Olorunfemi, 1988).
- Poor quality of antibiotics
- Poor infection control practices in hospitals
- Irrational use of antibiotics in animals
- Inadequate surveillance and susceptibility testing
- Crowding/movement of people and unhygienic conditions, e.g residents of developing countries often carry antibiotic-resistant fecal commensal organisms; visitors to these developing countries passively acquire antibiotic resistant *E. coli* even if they are not taking prophylactic antibiotics, which suggest that they encounter a reservoir of antibiotic-resistant strains while travelling.

Some of the measures to be taken to combat the growing problem of antibiotic resistance include:

- Developing prescription guidelines for practicing physicians
- Emphasizing on public awareness
- Emphasizing quality compliance and monitoring of antibiotics manufactured or dispensed
- Improved public sanitation
- Improved hospital control etc.

2.9 UROPATHOGENS

These are the organisms commonly invading the urinary tract to initiate an infection. They include: *Proteus spp.*, *Escherichia coli*, *Staphylococcus*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, etc.

2.9.1 *Proteus spp.*

Proteus spp. are part of the Enterobacteriaceae family of gram-negative bacilli. *Proteus* organisms are implicated in serious infections in human, along with *E. coli*, *Klebsiella*, *Enterobacter*, and *Serratia spp.* *Proteus mirabilis* causes 90% of *Proteus* infections, and can be considered as a community acquired infection. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infections were acquired in the hospital have an increased frequency of infection caused by *Proteus* and other organisms (e.g *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Enterococci*, *Staphylococci*) (Mobley and Warnen 1988, Mobley *et al.*, 1990). *P. mirabilis* is one of the most common causes of UTIs among individuals with long term indwelling catheters,

complicated urinary tract infections, and bacteremia among the elderly (Mobley and Warren, 1987; Mobley *et al.*, 1991).

Infection depends on the interaction between the infecting organism and the host defence mechanisms. Certain virulence factors have been identified in the bacteria. The first step in the infection process is adherence of the microbe to host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to attach. Attachment of *Proteus spp.* to uroepithelial cells initiates several events in the mucosal endothelial cells. Production of urease has also been shown to increase the risk of pyelonephritis in experimental animals. Urease production, together with the presence of bacterial motility and fimbriae may favour the production of upper urinary tract infections (UTIs).

Enterobacteriaceae (of which *Proteus* is a member) are organisms most commonly responsible for gram-negative bacteremia. When these organisms invade the bloodstream, an endotoxin (a component of gram-negative bacteria cell walls) apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects. The ability of *Proteus* to produce urease and to alkalinize the urine by hydrolyzing urea to ammonia makes it effective in producing an environment in which it can survive. This leads to precipitation of organic stone formation (Antoni *et al.*, 1997).

2.9.2 *Escherichia coli*

E. coli is a facultative anaerobic gram-negative bacilli. The urinary tract is the most common site of *E. coli* infection, and more than 80% of all uncomplicated UTIs

are caused by *E. coli* infection. Uropathogenic strains of *E. coli* have an adherence factor called P-fimbriae, or pili which bind to the P-blood group antigen (Warren, 1996). These P-fimbriae mediate the attachment of *E. coli* to uroepithelial cells. *E. coli* bacteremia is usually associated with UTI, especially in cases of urinary tract obstruction of any origin. The systemic reaction to endotoxins (cytokines) or lipopolysaccharides can lead to disseminated intravascular coagulation and death. *E. coli* is the leading cause of both communities acquired and nosocomial UTI, up to 50% of females eventually experience at least one episode of UTI (Kahlmeter, 2003 and Ronald, 2003).

2.9.3 *Staphylococcus*

Staphylococci are non-motile, non-spore forming and catalase positive bacteria. The ability to clot plasma is the most widely used and generally accepted criterion for the identification of *S. aureus* (coagulase positive). *S. aureus* is ubiquitous, and may be a part of human flora found in the axillae the inguinal and perineal areas, and the anterior nares. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) is less often found in the anterior nares than are methicillin-sensitive *S. aureus* (MSSA) and hospital-acquired methicillin resistant *S. aureus* (HA-MRSA); rather, it colonizes the skin, particularly in the perineal area and the rectum. It also colonizes the pharynx, gut and vagina. The organism may cause disease through tissue invasion and toxin production. The toxins liberated by the organism may have effects at sites distant from the focus of infection or colonization. *S. aureus* bacteriuria can lead to subsequent invasive infection. The efficacy of antistaphylococcal therapy in

preventing late-onset staphylococcal infection in patients with persistent staphylococcal bacteriuria should be tested in controlled trials (Muder *et al*, 2006).

2.9.4 *Klebsiella*

The genus *Klebsiella* is a member of the family Enterobacteriaceae. *Klebsiella* are non-motile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first is a lipopolysaccharide (O-antigen); the other is a capsular polysaccharide (K-antigen). Capsules and lipopolysaccharides are essential to the virulence of *Klebsiella* (Highsmith and Jarvis, 1985). Both of these antigens contribute to pathogenicity. The bacteria also produce multiple adhesins. These may be fimbrial or non-fimbrial, each with distinct receptor specificity. These help the micro-organism to adhere to host cells, which is critical to the infectious process. *Klebsiella* are also ubiquitous in nature, gastro intestinal tract, and the urinary tract. They are opportunistic pathogens; the principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel. *Klebsiella* can rapidly spread leading to nosocomial outbreaks. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus (Benneth *et al*, 1995 and Lye *et al.*, 1992). *Klebsiella* can cause a variety of clinical syndromes. Common *Klebsiellae* infections in humans include;

- Community-acquired pneumonia
- Urinary tract infection

- Nosocomial infection
- Rhinoscleroma and ozena
- Colonization.

Klebsiella urinary tract infections are clinically indistinguishable from UTIs caused by other common organisms. Clinical features include frequency, urgency, dysuria, hesitancy, low back pain and supra-pubic discomfort. Systemic symptoms such as fever and chills are usually indicative of concomitant pyelonephritis or prostratitis.

2.9.5 *Pseudomonas aeruginosa*

Pseudomonas is a gram-negative rod that belongs to the family Pseudomonadaceae. It is a frequent cause of nosocomial infections (UTIs), and bacteremia. Pseudomonal infections are complicated and can be an opportunistic pathogen. It rarely causes disease in healthy persons. The pathogenesis of pseudomonal infections is multifactorial and complex. *Pseudomonas spp.* is both invasive and toxigenic. The three stages according to Pollack (2000) are:

- Bacterial attachment and colonization
- Local infection and
- Bloodstream dissemination and systemic disease.

Production of extra cellular proteases adds to the organism's virulence by assisting in bacterial adherence and invasion. Pseudomonal urinary tract infections are usually hospital- acquired and are associated with catheterization, instrumentation, and surgery. These infections can involve the urinary tract through

an ascending infection or through bacteremic spread. In addition, these infections are a frequent source of bacteremia. No specific characteristics distinguish this type of infection from other forms of UTI. *P. aeruginosa* is the third most common pathogen associated with hospital-acquired catheter-associated UTIs (Jarvis and Martone, 1992).

2.9.6 *Candida*

Candida species are unusual causes of urinary tract infection (UTI) in healthy individuals, but common in the hospital setting or among patients with predisposing diseases and structural abnormalities of the kidney.

Candidiasis or thrush is a fungal infection (mycosis) of any of the *Candida* species (all yeasts), of which *Candida albicans* is the most common. *Candida albicans* is the most significant pathogenic species.

The urinary tract may be invaded in either an anterograde fashion from the blood stream or retrograde via the urethra and bladder. *Candida* species employ a repertoire of virulence factors, including phenotypic switching, dimorphism, galvanomorphism and thigmotropism, and hydrolytic enzymes, to colonize and then invade the urinary tract. In addition to asymptomatic candiduria, recognized clinical forms of candidal urinary tract infections include bladder infection, renal parenchymal infection, and infections associated with fungus ball formation (John *et al.*, 1982).

2.10 OTHER MARKERS OF URINARY TRACT INFECTION (UTI)

2.10.1 Yeast cells

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals, but are distinguished by their tendency to bud. Most often they are *candida*, which may colonize bladder, urethra, or vagina. The pathogenic yeasts of candidiasis in probable descending order of virulence for humans are: [*C. albicans*](#), [*C. tropicalis*](#), [*C. stellatoidea*](#), [*C. glabrata*](#), [*C. krusei*](#), [*C. parapsilosis*](#), [*C. guilliermondii*](#), [*C. viswanathii*](#), [*C. lusitaniae*](#), and [*Rhodotorula mucilaginosa*](#). [*Candida glabrata*](#) is the second most common *Candida* pathogen after *C. albicans*, causing infections of the urogenital tract, and of the bloodstream (Clark, 2002 and Pfaller *et al.*, 2003).

2.10.2 Epithelial cells

Epithelial cells from the bladder (transitional epithelial cells) or from the external urethra (squamous epithelial cells) can be found in urine sediment. Cells from the kidney (kidney cells) are less common. Epithelial cells are usually reported as “few”, “moderate” or many” present per low power field (lpf) (Schrier, 2007).

2.10.3 Crystals

Urine contains many dissolved substances (solutes). These waste chemicals (solutes) can solidify to form crystals. Some examples of crystals that can be found in the urine of healthy individuals include amorphous urates, crystalline uric acid,

calcium oxalates, amorphous phosphates and calcium carbonate (Coe *et al.*, 1992). Very uncommon crystals include: cystine crystals in urine of neonates with congenital cystinuria or severe liver disease, tyrosine crystals with congenital tyrosinosis or marked liver impairment, or leucine crystals in patients with severe liver disease.

2.10.4 Red blood cells

Normally, a few RBCs are present in urine sediment. Hematuria is the presence of abnormal number of red cells in urine due to glomerular damage, tumors which erode the urinary tract, kidney trauma, urinary tract stones, renal infarcts, upper and lower urinary tract infections, nephrotoxins, and stress (Stacy *et al.*, 2008). RBCs can also be a contaminant due to improper sample collection and blood from hemorrhoids or menstruation.

2.10.5 White blood cells

Pyuria refers to the presence of abnormal number of leukocytes that may appear with infection in either the upper or lower urinary tract or with acute glomerulonephritis. White cells from the vagina, especially in the presence of vaginal and cervical infections may contaminate the urine. Leukocytes have lobed nuclei and granular cytoplasm. The number of WBCs in urine sediment is normally low. When the number is high, it indicates an infection or inflammation somewhere in the urinary tract (Jarvis and Martone, 1992).

2.10.6 Casts

Casts are cylindrical particles sometimes found in urine that are formed from coagulated protein secreted by kidney cells. They are formed only in the distal convoluted tubule or the collecting duct (distal nephron). Different types of casts are associated with different kidney disease, and the type of casts found in the urine may give clues as to which disorder is affecting the kidney. Examples of casts include: granular casts, fatty casts, waxy casts, hyaline casts, cellular casts, (such as WBC and RBC casts) which usually is indicative of a kidney infection (Schrier, 2007).

2.11 VIRULENCE FACTORS OF THE UROPATHOGENS

Virulence is the degree of pathogenicity of bacteria specie. Pathogenicity defines the ability of a bacterium to cause disease. The more virulence factors a strain of bacteria expresses, the more severe an infection it is able to cause. Certain virulence factors specially favour the development of pyelonephritis, others favour cystitis, and others favour asymptomatic bacteriuria (Baylan *et al.*, 2011).

The currently defined virulence factors clearly contribute to the virulence of wild-type strains but are usually insufficient in themselves to transform an avirulent organism into a pathogen, demonstrating that others as-yet-undefined virulence factor testing is a useful epidemiological and research tool. Immunological and biochemical antivirulence factor interventions are effective in animal models of UTI and hold promises for the prevention of UTI in humans. Bacterial virulence is not related to

resistance to antimicrobial drugs. As an example, the most adherent strain of *E. coli* in a patient's intestinal flora is usually sensitive to most antibiotics.

2.11.1 POTENTIAL VIRULENCE FACTORS OF *PROTEUS SPP.*

2.11.1.1 Surface structures

The cell surface of *Proteus* is very important to their virulence, particularly their adherence ability, colonization of the urinary tract, and formation of stones.

Bacterial adhesion capacity is most frequently associated with the presence of fimbriae on bacterial cells. It has been shown that fimbriae are indeed responsible for the attachment of *Proteus* to uroepithelial cells (Silverblatt, 1974).

MR/P Fimbriae: The MR/P Fimbriae (mannose-resistant *Proteus*-like) were isolated and purified for the first time by Sareneva *et al.*, 1990. It is also known as type III fimbriae, and it contributes to pathogenicity by colonization of the upper part of the urinary tract (Bahrani *et al.*, 1994). MR/P Fimbriae or hemagglutinins are strongly immunogenic. Mice infected transurethrally with MR/P *Proteus mirabilis* strains produced specific antibodies whose level increased in the chronically infected animals (Bahrani *et al.*, 1993).

MR/K Hemagglutinins: These are the mannose-resistant klebsiella-like fimbriae; they are completely different from the MR/P fimbriae in the tissue-binding pattern. MR/K hemadhesins bound strongly to the Bowman's capsule of the glomeruli and to the tubular basement membranes and did not adhere to the epithelial cells of the urinary sediment. MR/K hemadhesins are more characteristic of *Proteus penneri*

strains than of *Proteus mirabilis* strains (Yakubu *et al.*, 1989). MR/K hemagglutinin contributes to pathogenicity through association with adhesion of strains to catheters (Mobley *et al.*, 1988 and Yakubu *et al.*, 1989).

PMF Fimbriae: The *Proteus mirabilis* fimbriae contribute to pathogenicity through colonization of the bladder, but not the kidneys (Massad *et al.*, 1994). Other surface structures of *Proteus* bacilli include: Ambient- temperature fimbriae, uroepithelial cell adhesion (UCA), *Proteus mirabilis* P-like fimbriae (non-agglutinating fimbriae).

2.11.1.2 Flagella and swarming motility

The presences of flagella on the surface of pathogenic and opportunistic bacteria facilitate the colonization and dissemination from the initial site. *Proteus* bacilli are dimorphic bacteria. When grown in a liquid medium, these cells display swimming behavior and a distinct morphology, i.e., they are motile, peritrichously flagellated. When transferred to a solid medium, *Proteus* bacilli undergo morphogenesis to swarmer cells (swarming phenomenon). The ability of *Proteus mirabilis* to invade human uroepithelial cells is coupled to motility and swarming differentiation (Allison *et al.*, 1994).

2.11.1.3 Outer membrane proteins

In general, outer membrane proteins (OMP) possess immunogenic properties and mitogenic activity.

2.11.1.4 Lipopolysaccharide (O-antigen, endotoxin)

Proteus is an antigenically heterogeneous genus, principally because of structural difference of its O-specific polysaccharide chain of LPS (O-antigen), as well as its H-antigen. Biologically, LPS are endotoxins, well-known pathogenic factors of gram-negative bacteria, which cause a broad spectrum of pathophysiological effects such as fever, hypotension, disseminated intravascular coagulation, and lethal shock (Rietschel *et al.*, 1994).

2.11.1.5 Capsule antigen

The capsule structures, also termed as slime material or glycocalyx (highly hydrated polymers present on the surface of bacteria), was demonstrated to be a potential pathogenic factor of *Proteus* strains because of its positive effect on struvite growth and stone formation (Beynon *et al.*, 1992 and Dumanski *et al.*, 1994).

2.11.1.6 Urease

Urease is a metalloenzyme containing the catalytic center of an atom of nickel. It catalyzes the hydrolysis of Urea (a major excretory product) to yield ammonia and carbon dioxide which results in increase in urine pH (Clapham *et al.*, 1990). This enzyme has been implicated as a factor contributing to the pathogenicity of *Proteus* bacilli. The role of urease in infections has been studied by several authors (Musher *et al.*, 1975 and Mac Laren, 1969). Increase in the urine pH leads to crystallization and aggregation of crystals (struvite) resulting in urinary stones formation, complicating

urinary tract infection caused by *Proteus* bacilli. Urinary stones are good habitat for bacteria, and they block the flow of urine.

2.11.1.7 Hemolyins

Proteus bacilli produce two types of hemolysin-HpmA associated with the cell (intracellular) and HlyA which is extracellular. Both hemolysins Hly A and HpmA are classified as toxins forming channels in the cell membrane (pore forming toxins), not only in the erythrocytes. Hemolysin HlyA is a potent cytotoxin that causes lysis of the epithelial cells of the urinary tract, and certain immune cells. Hemolysin increases the invasiveness of *Proteus* bacilli.

2.11.1.8 Protease IgA and IgG

Proteus proteinases are metalloenzymes that are similar in some respect to metalloproteinases of *Pseudomonas aeruginosa* and *Serratia marcescens*. *Proteus mirabilis* strains synthesize urease which degrades urea, resulting in the production of alkaline conditions optimal for the action of Ig A (and Ig G) proteases. Allison *et al.*, (1994) have demonstrated that the differentiation of *Proteus mirabilis* short vegetative rods into filamentous multinucleate, and hyperflagellate swarmer cells is accompanied by substantial increases in the activities of virulence factors, including proteases.

2.11.2 VIRULENCE FACTORS OF *E. COLI*

Uropathogenic strains of *Escherichia coli* are characterized by the expression of distinctive bacterial properties, products, or structures referred to as virulence factors because they help the organism overcome host defense and colonize or invade the urinary tract. Virulence factors of recognized importance (UTI) include: Adhesins (P-Fimbriae, Certain other mannose-resistant adhesion, and type 1- Fimbriae), the Aerobactin system, Hemolysin, K-capsule, and Resistance to serum killing. The virulence factors of *E. coli* are mainly responsible for promoting progression of the organism from the fecal reservoir into the bladder and occasionally the kidney.

2.11.2.1 The Aerobactin system

The aerobactin system and P-fimbriae are commonly found together in isolates from patients with UTI and urosepsis (Jacobson *et al.*, 1988). In *E. coli*, the hydroxamate siderophore aerobactin is the most effective of the several iron chelation systems employed by enteric bacteria for iron acquisition (used for oxygen transport and storage, DNA synthesis, electron transport, and metabolism of peroxides) (Williams and Carbonetti, 1986). Aerobactin is a small molecule formed from the condensation of two lysine molecules and one citrate. Following secretion by *E. coli* cells, aerobactin extracts Fe³⁺ from host iron-binding proteins. The aerobactin system is associated with *E. coli* isolates from serious UTI and other serious because it promotes bacterial growth in the limiting iron concentrations encountered during infection. The chromosomal aerobactin system is associated with other uropathogenic

virulence determinants, whereas the plasmid aerobactin system is often carried by plasmids encoding multiple antimicrobial agent resistance.

2.11.2.2 Hemolysin

Hemolysins are cytolytic proteinous endotoxins. Most hemolytic *E. coli* strains secrete α - hemolysin (Cavalieri *et al.*, 1984) although cell-bound (β) hemolysins and secreted hemolysins other than α - hemolysin have been described (Beutin *et al.*, 1988). Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI. It is likely that the provirulence activity of hemolysin is multifactorial, including release of iron from erythrocytes, disruption of phagocyte function, and direct toxicity to host tissues. Anti-hemolysin immunity protects animals from infection with hemolytic strains and should be explored for human use.

2.11.2.3 Capsular polysaccharide (k-antigen)

Capsular polysaccharides, of which *E. coli* has > 80 types, are linear polymers of repeating carbohydrate subunits that sometimes also include a prominent amino acid or lipid component (Jann and Jann, 1983). They coat the cell, interfering with O-antigen detection and protecting the cell from host defense mechanisms (Jann and Jann, 1983). The K1 capsule in particular contribute to virulence by shielding bacteria from phagocytosis and possibly from serum killing in part by blocking activation of the alternative complement pathway. Activation of complement via the classic is impaired as well because many K-antigens, particularly K1 are poor immunogens,

resulting in low or absent anti-K-anti-body levels in most individuals. Since a limited number of capsular types account for most cases of human pyelonephritis and since anticapsular immunity is protective in animal models of UTI, anticapsular immunity (possibly stimulated through the use of protein conjugate vaccines) would be of particular benefit in preventing human pyelonephritis.

2.11.2.4 Serum resistance

Bacterial susceptibility to serum killing is measured by assessing regrowth after incubation in serum (Olling, 1977), or growth rates in dilute serum (Moll *et al.*, 1979). Bacterial resistance to killing by serum results from the individual or combined effects of capsular polysaccharide, O-polysaccharide side chains, and surface proteins (Montenegro *et al.*, 1985). Serum resistance is often multifactorial, with no one bacterial property satisfactorily accounting for serum resistance in the majority of resistant strains. Isolates from patients with pyelonephritis, cystitis, and especially bacteria are typically serum resistant, whereas asymptomatic bacteriuria patient strains are characteristically even more serum sensitive than fecal strains.

2.11.2.5 Adhesins

Type 1 fimbriae are common among *E. coli* strains from all clinical categories of UTI and among fecal strains. The adherence of type 1 fimbriated strains to host cells in the urinary tract may promote the development of cystitis, their adherence to and stimulation of human polymorphonuclear leukocytes (hPMNLS) may promote bacteria killing but may also contribute to renal scarring, and their binding to Tamm-

Horsfall protein (THP): a uromucoid urinary slime, may allow the host to eliminate them from the urinary tract before they can initiate colonization or infection. P-fimbriae are important in the pathogenesis of UTI, primarily because they mediate Gal-Gal specific bacterial adherence to epithelial cells within the human urinary tract, thereby permitting bacterial colonization and stimulating inflammation.

2.11.3 VIRULENCE FACTORS OF *STAPHYLOCOCCUS*

2.11.3.1 TOXINS

Depending on the strain, *Staphylococcus aureus* is capable of secreting several toxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases.

2.11.3.1.1 Pyrogenic toxin Superantigens

(PTS Ags) have superantigen activities that induce toxic shock syndrome (TSS). This group includes the toxin TSS-1, which cause TSS associated with tampon use. The staphylococcal enterotoxins, which cause a form of food poisoning, are included in this group

2.11.3.1.2 Exfoliative toxins

Exfoliative toxins are implicated in the disease staphylococcal scaled-skin syndrome (SSSS), which occurs most commonly in infants and young children. It may also occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins cause peeling of the skin observed with SSSS.

2.11.3.1.3 Other toxins

Staphylococcal toxins that act on cell members include alpha-toxin, beta-toxin, delta-toxin, and several bicomponent toxins. The bicomponent toxin panton-valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community associated MRSA strains.

2.11.3.2 PROTEIN A

‘Protein A’ is a protein that is anchored to staphylococcal peptidoglycan pentaglycine bridges by the transpeptidase Sortase A. Protein A” is an IgG-binding which binds to the Fc region of an antibody. Studies involving mutation of genes coding for Protein A resulted in a lowered virulence of *S. aureus* as measured by survival in blood, and this has led to speculation that protein A contributed virulence requires binding of antibody to the Fc region. Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases such as the sortases which are responsible for anchoring factors like Protein A to the staphylococcal peptidoglycan are being studied with hope of developing new antibiotics to target MRSA infections.

2.11.3.3 PIGMENTS

Some strains of *S. aureus* are capable of producing “Staphyloxanthin” a carotenoid pigment that acts as a virulence factor. It has an antioxidant action that helps the microbe to evade killing with reactive oxygen used by the host immune system. It is

thought that staphyloxanthin is responsible for *S. aureus*' characteristic golden colour. When comparing a normal strain of *S. aureus* with a strain modified to lack the yellow coloration, the pigmented strain was more likely to survive dousing with an oxidizing chemical such as hydrogen peroxide than the mutant strain was. This pigment may be key to the ability of *S. aureus* to survive immune system attacks. Drugs designed to inhibit the bacterium's production of the staphyloxanthin may weaken it and renew its susceptibility to antibiotic; because of similarities in the pathways for biosynthesis of staphyloxanthin and human cholesterol, a drug developed in the context of cholesterol-lowering therapy was shown to block *S. aureus* pigmentation and disease progression in a mouse infection model.

2.11.4 VIRULENCE FACTORS OF *KLEBSIELLA* SPECIES

2.11.4.1 Capsular antigens

Capsules are essential to the virulence of *Klebsiella* (Highsmith and Jarvis, 1985). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers. This protects the bacterium from phagocytosis by polymorphonuclear granulocytes (Podschun and Ullmann, 1992), and prevents killing of the bacteria by bactericidal serum factors (Williams *et al.*, 1983).

2.11.4.2 Pili (Fimbriae)

A critical step in the infectious process is adherence. The adhesive properties in the Enterobacteriaceae are generally mediated by different types of pili. Pili are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of

different animal species. Depending on whether the reaction is inhibited by D-mannose, these adhesions are designated as mannose-sensitive or mannose-resistant hemagglutinins. There are two predominant types in *Klebsiella spp.* (Old *et al.*, 1985): type I (common pili and type 3 pili.)

2.11.4.3 Serum resistance and lipopolysaccharide

The first line of defense by host against invading micro organisms includes, in addition to phagocytosis by polymorphonuclear granulocyte, the bactericidal effect of serum. The serum bactericidal activity is mediated primarily by complement proteins. In response to this host defense, pathogenic micro organisms have developed strategies to counter the serum bactericidal effect. Most commensal gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas pathogenic strains often exhibit serum resistance properties (Olling, 1977). Thus, clinical isolates of enterobacteria often show resistance to serum (Vosti and Randall, 1970), and the features “ Serum resistance “ has been correlated with the onset of infection (Olling, 1977).

2.11.4.4 Siderophores

Iron is an essential factor in bacterial growth, functioning mainly as a redox catalyst in proteins participating in oxygen and electron transport processes (Griffiths, 1987). The growth of bacteria in host tissue is limited not only by the supply of available iron. Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity low molecular weight iron chelators called siderophores that

are capable of competitively taking up iron bound to host proteins (Griffiths *et al.*, 1988). The marked effect of the iron supply in the host body on the pathogenesis of infections has been demonstrated for *Klebsiella*. After parenteral administration of iron in a guinea pig model, the susceptibility to *Klebsiella pneumonia* infections increased dramatically (Khimji and Miles, 1978).

2.11.5 VIRULENCE FACTORS OF *PSEUDOMONAS AERUGINOSA*

The virulence factors of *Pseudomonas aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate (biofilms), lipopolysaccharide (LPS). Flagellum, pilus and non-pilus adhesions as well as with exoenzymes or secretory virulence factors like protease, elastase, phospholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins, (rhamnolipids) and siderophores (Matheson *et al.*, 2006; Yates *et al.*, 2006 ; Zulianello *et al.*, 2006 ; Veessenmeyer *et al.*, 2009).

2.12 MOLECULAR CHARACTERIZATION OF RESISTANT ISOLATES

2.12.1 Plasmid extraction

The Gene JET™ Plasmid Miniprep Kit will be utilized. This is designed for rapid and cost-effective small-scale preparation of quality plasmid DNA from recombinant bacterial cultures. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. Each Gene JET™ spin column can recover up to 20µg of plasmid DNA. The kit can be successfully used for efficient purification of

any size plasmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

2.12.2 Polymerase chain reaction (PCR)

The Polymerase chain reaction is a scientific technique used to analyze a short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce (amplify) selected sections of DNA or RNA.

It makes use of:

- Two “primers”, short single-stranded DNA sequences that are synthesized to correspond to the beginning and ending of the DNA stretch to be copied.
- An enzyme called Polymerase that moves along the segment of DNA, reading its code and assembling a copy.
- A pile of DNA building blocks that the polymerase needs to make that copy.

Three major steps are involved in a Polymerase chain reaction (PCR): Denaturation, Annealing, and Extension. These steps are repeated for 30-40 cycles. The cycles are done on an automated cycler, a device which rapidly heats and cools the test tubes containing the reaction mixture. Each step, denaturation (alteration of structure), annealing (joining), and extension takes place at a different temperature:

Denaturation: At 94 C (201.2 F), the double-stranded DNA melts and opens into two pieces of single-stranded DNA.

Annealing: At medium temperatures, around 54 C (129.2 F), the primers pair up (anneal) with the single-stranded "template" (The template is the sequence of DNA to be copied.) On the small length of double-stranded DNA (the joined primer and template), the polymerase attaches and starts copying the template.

Extension: At 72 C (161.6 F), the polymerase works best, and DNA building blocks complementary to the template are coupled to the primer, making a double stranded DNA molecule.

With one cycle, a single segment of double stranded DNA template is amplified into two separate pieces of double stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the templates is increased exponentially.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Laboratory media

The various Laboratory Media were prepared according to Manufacturer's

Specification:

- Mac Conkey Agar, Nutrient Agar, Mannitol Salt Agar (Biotech Laboratory Ltd., UK)
- Urea Agar (Oxoid Ltd., England)
- Cetrimide Agar, Brain Heart Infusion Broth (Lab M Ltd., UK)
- Sabouraud Dextrose Agar, Cystein Lactose Electrolyte Deficient Agar (Antec diagnostic Product, UK)
- Nutrient Broth, Luria Broth, Eosin Methylene Blue Agar (Fluka Spain)
- Blood Agar Base (Oxoid Ltd, England).

3.1.2 Sensitivity discs (Oxoid Ltd., England)

The antibiotic sensitivity discs and their disc strengths are as follows:

Ofloxacin (5ug), Nalidixic acid (30ug), Amoxicillin/Clavulanic acid (30ug), Gentamicin (10ug), Amoxicillin (10ug), Nitrofurantoin (300ug), Tetracycline (30ug), Cotrimoxazole (25ug), Ceftriaxone (30ug), Levofloxacin (5ug) and Ciprofloxacin (5ug).

3.1.3 Reagents and chemicals

- Phosphate buffered saline, Mannose, Sodium phosphate, Sodium phosphate, Tris-Acetate EDTA (TAE), Ethyidium bromide dye, and Tris-HCl, (Sigma Chemical Ltd., England). Crystal violet, Tannic acid, Urea, Phenol red, Urea, and Ethanol (BDH Chemical Ltd., England).

- Dettol, Jik (Reckit Benckiser Ltd., Nigeria).

- Agarose (Schwarz/Mann, England)

3.1.4 GeneJET™ Miniprep Plasmid Extraction kit (Fermentas UK).

3.2 METHODS

3.2.1 Collection of specimen

A total of three hundred and ten (310) urine samples of pregnant women (not showing clinical symptoms of urinary tract infection) attending antenatal clinic in three (3) major hospitals (Aminu Kano Teaching Hospital, Mohammed Abdullahi Wase Hospital, and Murtala Mohammed Specialist Hospital) in Kano, Nigeria were randomly collected over a period of three (3) months.

On each routine antenatal visit, a clean-catch mid-stream urine specimen was collected from each patient (Hooton *et al.*, 2002) into sterile universal bottles. These were transported over an ice-bath and refrigerated before analysis was carried out within 24 hours.

3.2.2 Specimen processing

From each specimen, using a calibrated loop, 0.01ml of urine was inoculated on blood agar plates and cystein lactose electrolyte deficient (CLED) agar plates. The plates were allowed to dry and then incubated aerobically at 37⁰C for 24 hours (Cheesbrough, 2002) and observed for formed colonies. Colonies formed were counted on the CLED agar plates, this was multiplied by the inoculum volume (0.01ml). Bacterial count of 10⁵ cfu/ml was considered as significant for bacteriuria.

3.2.3 Identification test/Biochemical test

Each of the sub-cultured formed colonies were inoculated into nutrient broth accordingly, and then streaked on the surface of already prepared selective media i.e.,

Mac Conkey agar, Mannitol salt agar, Eosin methylene blue agar, Urea agar, and Sabouraud dextrose agar respectively. The streaked plates were then incubated at 37⁰C for 24 hours. The colonies that developed were observed, noting their characteristics. The isolates were also identified morphologically and biochemically (Cowan, 1974; Collee and Miles, 1989). The Ornithine decarboxylase and Indole tests were used to differentiate *Proteus species* type. Each patient's personal information (e.g. age, gestational and occupation were derived from their hospital files).

3.2.4 Urine microscopy

From each urine sample, 10ml was transferred into sterile centrifuge tubes and then centrifuged at about 3000rpm for 15 minutes. The supernatant was discarded and the sediment examined using a microscope at high performance field (hpf) for pus cells, epithelial cells, crystals and yeast-like cells (Cheesbrough, 2002). Pus cells >5/hpf were also considered for urinary tract infection (UTI).

3.2.5 Significant bacteriuria determination with respect to age group, gestational age, and occupational status

Using 10⁵cfu/ml as significant level of bacteriuria, the prevalence of asymptomatic bacteriuria was determined with respect to age group, gestational age (trimester), and occupational status (Amadi *et al.*, 2007 and Imade *et al.*, 2010).

3.3 SUSCEPTIBILITY TEST

The susceptibility of the various isolates to eleven (11) commonly prescribed antibiotics at the antenatal clinics were determined according to Bauer *et al.* (1966), and Wolf (1975) with modifications by CLSI -Clinical Laboratory Standards Institute (2006) :

1. An overnight culture of each isolate was prepared in nutrient broth and incubated at 37⁰ C for 18 hours.
2. Dry sterile plates of prepared nutrient agar were swabbed with the standardized inoculums of the culture test isolate; adjusting the turbidity to McFarland 0.5 standard.
3. The plates were allowed to dry for 3-4 minutes in a properly disinfected incubator, before placing the sensitivity discs (oxid) of the various antibiotics aseptically in duplicate on the dried inoculated agar surface.
4. The plates were then allowed to stay for one hour before they were incubated at 37⁰ C for 18 hours. After incubation, the plates were examined for the zones of inhibition and measured.
5. The result was interpreted using the interpretation criteria published by CLSI (2006). The isolates were reported as sensitive, moderately sensitive or intermediate, and resistant to the various antibiotics depending on the sizes of the zones of inhibition.

3.4 DETERMINATION OF SOME VIRULENCE FACTORS OF *PROTEUS* *SPP.*

3.4.1 Hemolysin assay

Bacteria were grown at 37⁰ C with aeration in brain-heart infusion broth prepared in Phosphate buffered saline (PBS) at pH 7.2. Turbidity was monitored at optical density 550nm. Samples were taken at various time intervals, and 0.1ml of two fold dilutions of bacterial suspension in PBS, pH 7.2 mixed with 0.05ml of a 3% suspension of erythrocytes. Hemolytic titres were defined as the last dilution in which no visible erythrocyte pellet was observed i.e., complete lysis (Mobley and Chippendale, 1990).

3.4.2 Urease assay

Bacteria cells derived from exponentially growing Luria broth (LB) cultures of about 30ml supplemented with 0.1% urea was harvested by centrifugation when the optical density at 650nm equaled 0.40-0.45 absorbance units (2.3×10^8 cells/ml). The cells were then suspended in 1.5ml of 20mM sodium phosphate, pH 6.8 and ruptured by passage through a pressure cell at 1407kg/cm³. The lysates were then centrifuged in a micro centrifuge (15600g, 3mins, 4°C). Soluble protein was then assayed for urease activity by a calibrated spectrophotometer assay, using 3mM sodium phosphate, pH 6.8, 120mM urea, and phenol red (7µg/ml). Optical density was then monitored at 650nm (Mobley and Chippendale, 1990).

3.4.3 Adherence factor (Hemagglutinin)

Bacteria was passaged (subcultured) statically three times for 48 hours in 4ml nutrient broth at 37⁰ C, and harvested by centrifugation (8000g, 10min, 4⁰ C). The cell pellets were suspended in 0.5ml of phosphate buffered saline (PBS), pH 7.2, and 0.05ml of the suspension were then mixed with 0.1ml of erythrocyte suspension on white ceramic tiles at 23⁰ C.

Hemagglutination was defined as visible clumping of erythrocytes as follows:

Mannose-sensitive (MS) hemagglutination was demonstrated by agglutination of erythrocytes in the absence, but not presence of 50mM mannose.

Mannose-resistant/*Klebsiella*-like (MR/K) hemagglutination was shown by agglutination of tannic-acid treated but not untreated erythrocytes; the reaction was not inhibited by 50mM mannose.

Mannose-resistant/*Proteus*-like (MR/P) hemagglutination was demonstrated by agglutination of untreated and tannic-acid treated erythrocytes. The reaction was not inhibited by 50mM mannose (Mobley and Chippendale, 1990).

3.5 DETERMINATION OF SOME VIRULENCE FACTORS OF *E. COLI*

3.5.1 Hemolysin assay

Bacteria were grown at 37⁰ C with aeration in brain-heart infusion broth prepared in Phosphate buffered saline (PBS) at pH 7.2. Turbidity was monitored at optical density 550nm. Samples were taken at various time intervals, and 0.1ml of two fold dilutions of bacterial suspension in PBS, pH 7.2 mixed with 0.05ml of a 3% suspension of erythrocytes. Hemolytic titres were defined as the last dilution in

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3.5.2 Hemagglutinin (Adherence factor)

Bacteria was passaged (subcultured) statically three times for 48 hours in 4ml nutrient broth at 37⁰ C, and harvested by centrifugation (8000g, 10min, 4⁰ C). The cell pellets were suspended in 0.5ml of phosphate buffered saline (PBS), pH 7.2, and 0.05ml of the suspension were then mixed with 0.1ml of erythrocyte suspension on white ceramic tiles at 23⁰ C. Hemagglutination was defined as visible clumping of erythrocytes as follows:

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Mannose-resistant/*Proteus*-like (MR/P) hemagglutination was demonstrated by agglutination of untreated and tannic-acid treated erythrocytes. The reaction was not inhibited by 50mM mannose (Mobley and Chippendale, 1990).

3.6 MOLECULAR CHARACTERIZATION OF RESISTANT ISOLATES

3.6.1 Plasmid analysis (extraction)

Plasmid extraction was carried out by the alkaline lysis technique. The extracted plasmid was then isolated using horizontal 1% agarose gel electrophoresis. The pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis to liberate the plasmid DNA. The resulting lysate is neutralized to create appropriate condition binding of plasmid DNA on the silica membrane in the spin column. Cell debris and SDS precipitate are pelleted by centrifugation, and the supernatant containing the plasmid DNA is loaded onto the spin column membrane. The absorbed DNA is washed to remove contaminants, and is then eluted with a small volume of the elution buffer (10 mM Tris-HCl, pH 8.5). The purified plasmid DNA is ready for immediate use in all molecular biology procedures such as PCR, automated sequencing, etc.

Bacterial cell preparation: Single colonies were picked from freshly streaked isolates on selective plate to inoculate 1-5ml Luria Bertani (LB) broth medium. This was incubated overnight at 37⁰ C, with loose capping and shaking at 200-250 rpm. Bacterial cells were then harvested by centrifugation at 8000rpm (6800xg) in a micro centrifuge for 2 minutes at room temperature in an Eppendorff's tube. The supernatant was discarded.

Plasmid Extraction procedure: 250ul of resuspension solution was added to the pelleted cells and vortexed. The lysis solution (250ul) was added also and the Eppendorff tube inverted 4-6 times. This was then centrifuged for 5 minutes. The

supernatant was transferred to the GeneJET™ spin column and centrifuged for 1 minute to bind the DNA. Wash solution (500ul) was added to the column and this procedure was repeated to increase plasmid yield. The empty column was then centrifuged for 1 minute.

Elution of purified DNA: The column was transferred into a new Eppendorff's tube, and 50ul of elution buffer was added to the column and incubated for 2 minutes. This was centrifuged for 2 minutes, and the flow-through (purified plasmid DNA) collected.

Separation of Plasmid DNA by Agarose gel electrophoresis:

Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gel in a Tris-Acetate EDTA (TAE) buffer at room temperature at 80 volts (50mA) for 40 mins. About 15ul of plasmid DNA solution was mixed with 3ul of loading dye and loaded into the wells of the gel. The gel (5mm thick) was stained with 7ul ethidium bromide for 15minutes at room temperature, and destained with distilled water for 10 minutes. DNA bands were visualized using a UV trans-illuminator and photographed with an ultraviolet Polaroid camera. The molecular weights of the unknown plasmid DNA was determined on the basis of its mobility through the agarose gel by comparing with a standard 100bp DNA marker.

3.6.2 Amplification of antibiotic resistance genes (CTX-M2, CTX-M9, qnrC, qnrS, pCT, and gyrB) by polymerase chain reaction.

DNA extraction was performed on the isolates and CTX-M2, CTX-M9, qnrC, qnrS, pCT and gyrB genes were amplified using specific primers for each gene of interest. The master-mix containing PCR buffer, dNTP mix, primer, Taq DNA Polymerase and magnesium chloride and template DNA were subjected to hot start PCR. The PCR conditions used are as follows:

For pCT and gyrB: 35 cycles of initial denaturation at 95⁰ C for 5 minutes, denaturation at 95⁰C for 30 seconds, annealing at 51⁰ C for 1 minute, and extension at 72⁰ C for 1 minute, final extension 72⁰C for 5 minutes.

For CTX-M9, CTX-M2, qnrS, and qnrC: 30 cycles of initial denaturation at 95⁰ C for 5 minutes, denaturation at 95⁰ C for 30 seconds, annealing 60⁰C for 1 minute, extension at 72⁰C for 1 minute, and final extension at 72⁰C for 5 minutes. PCR products were visualized on 1% agarose gel with ethidium bromide dye under UV trans-illuminator.

Table 1: Antimicrobial agents relatively safe in Pregnancy with the genes of interest conferring antibiotic resistance, and their primer sequence

Antimicrobial Agent	Gene No	Primer name	Primer Sequence	Expected molecular weights
Group(s)				
Beta lactams	CTX-M2	CTX-M2 Fw	5'-ATGATGACTCAGAGCATTCG-3'	70-160kb
		CTX-M2 Rev	5'-GAAACCGTGGGTTACGATTT-3'	
	CTX-M9	CTX-M-9 Fw	5'-GTGACAAAGAGAGTGCAACGG-3'	70-160kb
		CTX-M-9 Rev	5'-ATGATTCTCGCCGCTGAAGCC-3'	
	pCT	pCT008Fw	5'-CATTGTATCTATCTTGTGGG-3'	428bp
		pCT009Rev	5'-GCATTCAGAAAGATGACGTT-3'	
Quinolones	qnr C	qnrC Fw	5'-GGGTTGTACATTTATTGAATC-3'	7-154kb
		qnrC Rev	5'-TCCACTTACGAGGTTCT-3'	
	qnr S	qnrS(1-2)Fw	5'-TCGACGTGCTAACTTGCG-3'	7-154kb
		qnrS(1-2)Rev	5'-GATCTAAACCGTCGAGTTCGG-3'	
	gyr B (Gene Accession) No D87842	gyr B Fw	5'-GCGCGTGAGATGACCCGCCGT-3	415bp
		gyr B Rev	5'-CTGGCGGTAGAAGGTCAG-3'	

Key:

CTX-M2: for beta-lactam antibiotics

CTX-M9: for beta-lactam antibiotics

pCT: for IncK plasmid encoding bla_{CTX-M} ESBL genes

qnr C: for fluoroquinolones

qnr S: for fluoroquinolones

gyrB: for fluoroquinolones

Fw: forward primer

Rev: reverse primer

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation and identification

The results of the morphological and biochemical studies are shown in Table 2:

Table 2: Summary of the morphological/biochemical reaction schemes

Isolate Code	cat	coa	oxi	<u>Acid from Sugars</u>				MR	VP	IP	UP	GS	Inference
				L	G	S	M						
002				-	-	+	-				+	-	<i>Proteus mirabilis</i>
003	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
021				-	-	+	-				+	-	<i>Proteus mirabilis</i>
023	+			+	+	+	-	+	-	+	-	-	<i>E. coli</i>
025	+	+	-	+	+	+	+	+	+	-		+	<i>S. aureus</i>
031				-	-	+	-				+	-	<i>Proteus mirabilis</i>
032	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
034	+	+	-	+	+	+	+	+	+	-		+	<i>S. aureus</i>
037	+			+	+	-	+	+	-	+	-	-	<i>E. coli</i>
038	+	+	-	+	+	+	+	+	+	-		+	<i>S. aureus</i>
040				-	+-	+	-				+	-	<i>Proteus vulgaris</i>

Isolate Code	cat	coa	oxi	<u>Acid from Sugars</u>				MR	VP	IP	UP	GS	Inference
				L	G	S	M						
042											+	-	<i>Klebsiella</i>
046				+-	-	+	-				+	-	<i>Proteus mirabilis</i>
049				-	-	+	-				+	-	<i>Proteus mirabilis</i>
051				-	-	+	-				+	-	<i>Proteus mirabilis</i>
052	+			+	+	+	-				-	-	<i>E. coli</i>
059				-	-	+	+-				+	-	<i>Proteus vulgaris</i>
060				+-	-	+	-				+	-	<i>Proteus mirabilis</i>
064	+			+	+	+	+-	+	-	+	-	-	<i>E. coli</i>
069				-	-	+	-				+	-	<i>Proteus mirabilis</i>
071				-	-	+	-				+	-	<i>Proteus mirabilis</i>
081	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
084	+			+	+-	+	+	+	-	+	-	-	<i>E. coli</i>
088				-	+-	+	-				+	-	<i>Proteus mirabilis</i>
089	+		+					-	-	-		-	<i>Pseudomonas</i>

<hr/>													
Isolate		cat	coa	oxi	Acid from Sugars				MR	VP	IP	UP	GS
Inference													
Code				L	G	S	M						
<hr/>													
095				-	-	+	-			+	-		<i>Proteus mirabilis</i>
113				-	-	+	+-			+	-		<i>Proteus mirabilis</i>
116	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
117				-	-	+	-			+	-		<i>Proteus mirabilis</i>
118				-	+-	+	-			+	-		<i>Proteus mirabilis</i>
123				-	-	+				+	-		<i>Proteus mirabilis</i>
129	+			+	-	+	+	+	-	+	-	-	<i>E. coli</i>
131				-	-	+	-			+	-		<i>Proteus mirabilis</i>
187	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
192											+	-	<i>Klebsiella</i>
207	+		+					-	-	-		-	<i>Pseudomonas</i>
219	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
229	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>

Isolate Code	cat	coa	oxi	<u>Acid from Sugars</u>				MR	VP	IP	UP	GS	Inference
				L	G	S	M						
243				-	-	+	-				+	-	<i>Proteus mirabilis</i>
250				-	-	+	-				+	-	<i>Proteus mirabilis</i>
264	+			+	+	+	+-	+	-	+	-	-	<i>Proteus mirabilis</i>
266	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
271				-	-	+	-				+	-	<i>Proteus vulgaris</i>
284	+			+	+-	+	+	+	-	+	-	-	<i>E. coli</i>
288	+			+	+	+	+	+	-	+	-	-	<i>E. coli</i>
291				-	-	+	-				+	-	<i>Proteus mirabilis</i>
301				-	-	+		-			+	-	<i>Proteus mirabilis</i>

cat: catalase

coa: coagulase

oxi: oxidase

MR: methyl red

IP: indole production

L: lactose

S: sucrose

GS: gram stain

VP: voges proskauer

G: glucose

M: mannose

+: positive

-: negative

L: lactose

+-: neither positive nor negative

UP: urea production

Table 3: Biochemical test to differentiate *Proteus spp.* type

Organism	Ornithine decarboxylase	Indole production
<i>Proteus mirabilis</i>	+	-
<i>Proteus vulgaris</i>	-	+

+: positive

- : negative

4.2 URINE MICROSCOPY

Yeast cells were observed in thirty three (33) samples, while *Candida spp.* (fungus) was isolated from five (5) samples. One hundred and twelve (112) samples had 1-4 pus cells/hpf and > 5pus cells/hpf with twenty (20) showing significant bacteriuria. One hundred and ninety eight (198) samples had no pus cells with twenty four (24) showing significant bacteriuria (Table 4). Two hundred and two (202) samples had epithelial cells with thirty three (33) showing significant bacteriuria (Table 5). Twenty six (26) samples had calcium oxalate crystals, fourteen samples (14) had calcium phosphate crystals, and seven (7) samples had amorphous phosphate crystals (Table 6).

Table 4: Pus cells distribution in urine samples of pregnant women attending antenatal clinic.

Samples	Number of samples showing significant bacteriuria	
with pus cells	112	20
without pus cells	198	24

1-4pus cells/hpf and 5>pus cells/hpf were considered for UTI

Table 5: Epithelial cells distribution in urine samples of pregnant women attending antenatal clinic

Sample(s) with epithelial cells	Sample without epithelial cells	Number of samples showing significant bacteriuria
202	-	33

Table 6: Crystals distribution in urine samples of pregnant women

Crystals	Number
Calcium oxalate	26
Calcium phosphate	14
Amorphous phosphate	7
Total	47

4.3 PREVALENCE OF SIGNIFICANT ASYMPTOMATIC BACTERIURIA

Fourty seven (47) out of the three hundred and ten (310) urine samples of the pregnant women analyzed were positive for significant bacteriuria, with a prevalence of 15.2(%) (Table 7). These fourty seven (47) positive culture yielded single bacterial isolates.

Table 7: Prevalence of significant bacteriuria in urine samples of pregnant women attending antenatal clinic

Urine sample(s)	Number (Percentage)
With significant bacterial growth	47(15.2%)
Without significant bacterial growth	263(84.8%)
Total	310

Significant level of bacteriuria = 10^5 cfu/ml

4.3.1 Prevalence of uropathogens

Proteus spp. was isolated from twenty three (23) urine samples: twenty (20) were *Proteus mirabilis* and three (3) were *Proteus vulgaris* (Table 7). *Escherichia coli* was isolated from seventeen (17) samples, *Staphylococcus aureus* was isolated from three (3) samples. *Pseudomonas aeruginosa* and *Klebsiella spp.*, were both isolated from two (2) samples each (Table 8).

Table 8: Prevalence of Uropathogens in urine samples of pregnant women attending antenatal clinic.

Organism	Number	Percentage (%) aprevalence
<i>Proteus mirabilis</i>	20	42.6
<i>Proteus vulgaris</i>	3	6.4
	} 23	} 49.0
<i>Escherichia coli</i>	17	36.0
<i>Staphylococcus aureus</i>	3	6.4
<i>Klebsiella spp.</i>	2	4.3
<i>Pseudomonas aeruginosa</i>	2	4.3

4.3.2 Prevalence with respect to gestational age (trimester)

Pregnant women in the last (third) trimester had the highest bacteriuria prevalence of 18.2% with respect to gestational age (Table 9).

Table 9: Prevalence of uropathogens with respect to gestational age (trimester).

Trimester	Number of patients	Number colonized	Percentage(%) colonized
First	87	11	12.6
Second	104	14	13.5
Third	119	22	18.2

There is significant difference in percentage colonized with respect to gestational age, using χ^2 , at $P= 0.005$.

4.3.3 Prevalence with respect to age group

The age group 25-30 years had the highest bacteriuria prevalence of 24.2% with respect to age (Table 10). The mean age of study of the pregnant women is 26.7 years (S.D: 5.2 years, range 17-41years).

Table 10: Prevalence of uropathogens with respect to age group of pregnant women attending antenatal clinic.

Age group(years)	Number of patients	Number colonized	Percentage(%)colonized
15-20	30	2	6.7
20-25	84	3	3.6
25-30	124	30	24.2
30-35	49	10	20.4
35-40	23	2	8.7
Total	310	47	15.2

There is significant difference in percentage colonized with respect to age groups using χ^2 at P=0.05

4.3.4 Prevalence with respect to occupational status

Pregnant women classified as housewives had the highest bacteriuria prevalence of 57.4% with respect to occupational status (Table 11). Information on their occupational status was derived from their hospital files.

Table 11: Prevalence of uropathogens with respect to occupational status

Occupational status	Number	Percentage (%)
Housewives	27	57.4
Working class	20	42.6

4.4 SUSCEPTIBILITY TESTING

Table 12: Interpretative chart according to CLSI (2006)

S/No	Antibiotics	Zones of inhibition (mm)		
		Intermediate	Susceptible	Resistance
1	Amoxicillin/Clavulanic acid	14-17	≥ 18	≤ 13
2	Amoxicillin	14-16	≥ 17	≤ 13
3	Ceftriaxone	20-22	≥ 23	≤ 19
4	Ciprofloxacin	16-20	≥ 21	≤ 15
5	Ofloxacin	13-15	≥ 16	≤ 12
6	Levofloxacin	14-16	≥ 17	≤ 13
7	Nalidixic acid	14-18	≥ 19	≤ 13
8	Nitrofurantoin	15-16	≥ 17	≤ 14
9	Gentamicin	14-15	≥ 15	≤ 12
10	Tetracycline	12-14	≥ 15	≤ 11
11	Cotrimoxazole	11-15	≥ 16	≤ 10

Susceptibility of twenty three (23) *Proteus spp.*, seventeen (17) *Escherichia coli*, and three (3) *Staphylococcus aureus* isolates to antibiotics commonly prescribed in the various antenatal clinics was determined using the disc (oxid) diffusion method. The results are presented in Table 13. The results were interpreted using NCCLS (2006) (Table 12).

Table 13: Summary of Percentage sensitivity of *Proteus spp.*, *E. coli* and *S. aureus* isolates from urine samples of pregnant women attending antenatal clinic in Kano, Nigeria to 11 commonly prescribed antibiotics.

Isolate		AMX	CIP	CEF	LEV	NIT	OFL	GEN	COT	ACA	NAL	TET
<i>Proteus spp.</i>	S	8.7	39.1	13.1	30.4	8.7	60.9	13.0	4.3	0.0	13.0	0.0
	I	26.1	43.5	30.4	30.4	8.7	13.0	13.0	0.0	0.0	39.1	0.0
	R	65.2	17.4	56.5	39.2	82.6	26.1	74.0	95.7	100.0	47.9	100.0

Isolate		AMX	CIP	CEF	LEV	NIT	OFL	GEN	COT	ACA	NAL	TET
<i>E. coli</i>	S	11.8	23.5	5.9	17.6	5.9	17.6	0.0	0.0	5.9	17.6	0.0
	I	41.2	17.6	35.3	17.6	11.8	11.8	11.8	11.8	0.0	29.4	0.0
	R	47.0	58.8	58.8	58.8	29.4	70.6	88.2	88.2	94.1	53.0	100.0

Isolate		AMX	CIP	CEF	LEV	NIT	OFL	GEN	COT	ACA	NAL	TET
<i>S. aureus</i>	S	33.3	33.3	0.0	66.7	33.3	33.3	100.0	33.3	33.3	33.3	0.0
	I	33.3	66.7	33.3	33.3	0.0	33.3	0.0	33.3	0.0	0.0	0.0
	R	33.3	0.0	66.7	0.0	66.7	33.3	0.0	33.3	66.7	66.7	100.0

S: sensitive R: resistant CEF: ceftriaxone NAL: nalidixic acid GEN: gentamicin
 I: intermediate OFL: ofloxacin TET: tetracycline AMX: amoxicillin ACA:
 Amoxicillin/Clavulanic acid CIP: ciprofloxacin COT: cotrimoxazole LEV:
 levofloxacin NIT: nitrofurantoin

Proteus spp. isolates were completely resistant to Co-amoxiclav® (Beta-lactam antibiotic) and tetracycline. Other antibiotics with high resistance profile are Cotrimoxazole, Nitrofurantoin, Gentamicin, Amoxicillin and Ceftriaxone with percentage resistance of 95.7%, 82.6%, 74.0%, 65.2%, and 56.5% respectively. The quinolones and fluoroquinolones displayed the highest antimicrobial activity of 82.6% (ciprofloxacin), 60.8% (levofloxacin), 73.9% (ofloxacin), and 52.1% (nalidixic acid).

E. coli isolates were totally resistant to what was observed for tetracycline, while a high resistance of 94.1%, 88.2%, 82.4% and 70.6% was observed to co-amoxiclav®, cotrimoxazole, nitrofurantoin and gentamicin respectively. A relatively high percentage resistance of 58.8% was observed to the fluoroquinolones (ciprofloxacin and levofloxacin) and also ceftriaxone (a cephalosporin). Nalidixic acid and amoxicillin displayed a resistance profile of 53.0% and 47.0% respectively. Ofloxacin showed the highest antimicrobial activity (potency) of 70.6%, and a percentage resistance of 29.4%.

In *S. aureus* isolates, absolute resistance was observed to tetracycline also. The fluoroquinolones (ciprofloxacin and levofloxacin) displayed the highest antimicrobial activity of 100% respectively. Ofloxacin and cotrimoxazole both showed antimicrobial activity of 66.7%. High resistance profile of 66.7% was observed to ceftriaxone (a cephalosporin), nitrofurantoin, co-amoxiclav® and nalidixic acid respectively.

The data presented in Table 14 shows the distribution of antibiotic resistance profile of *Proteus spp.* isolated from pregnant women from three (3) different major hospitals. The susceptibility of the test bacterial isolates from AKTH was more

resistant to co-amoxiclav®, tetracycline, cotrimoxazole and nitrofurantoin. *Proteus spp.* isolate from MAWSH and MMSH were more resistant to tetracycline, co-amoxiclav®, cotrimoxazole, nitrofurantoin, amoxicillin and gentamicin.

Table 14: Percentage antibiotics resistance profile of *Proteus species* isolates from urine samples of pregnant women in 3 major hospitals in Kano, Nigeria.

S/No	Antibiotics	AKTH (n=8)	MAWSH (n=9)	MMSH (n=6)
		%Resistance	%Resistance	%Resistance
1	Amoxicillin	5(62.5)	6(66.7)	4(66.7)
2	Ciprofloxacin	1(12.5)	2(22.2)	1(16.7)
3	Ceftriaxone	4(50.0)	5(55.6)	3(50.0)
4	Levofloxacin	3(37.5)	3(33.3)	2(33.3)
5	Nitrofurantoin	6(75.0)	7(77.8)	5(83.3)
6	Ofloxacin	2(25.0)	2(22.2)	1(16.7)
7	Gentamicin	5(62.5)	6(66.7)	4(66.7)
8	Cotrimoxazole	7(87.5)	8(88.9)	5(83.3)
9	Amoxicillin/Clavulanic acid	8(100.0)	9(100.0)	6(100.0)
10	Nalidixic acid	3(37.5)	4(44.4)	3(50.0)
11	Tetracycline	8(100.0)	9(100.0)	6(100.0)

Zone of Inhibition Interpretation as susceptible according to CLSI (2006).

COT= ≥ 16 mm, GEN= ≥ 15 mm, NAL = ≥ 19 mm, NFT= ≥ 17 mm, OFL= ≥ 16 mm, TET= ≥ 15 mm, ACA = ≥ 18 mm, AMO= ≥ 17 , LEV= ≥ 17 mm, CIP= ≥ 21 mm, CEF= ≥ 23 mm, n=number of isolates. $P > 0.05$ by student T-test. AKTH: Aminu kano teaching hospital, MAWSH: Mohammed abdullahi specialist hospital, MMSH: Murtala mohammed specialist hospital.

Table 15 shows the percentage distribution of antibiotic resistance profile of *E. coli* isolates from urine samples of pregnant women attending antenatal clinics in three (3) major hospitals in Kano, Nigeria.

Table 15: Percentage antibiotic resistance profile of *E. coli* isolates from urine samples of pregnant women in 3 major hospitals in Kano, Nigeria.

		AKTH (n=7)	MAWSH (n=4)	MMSH (n=6)
S/No	Antibiotics	%Resistance	%Resistance	%Resistance
1	Amoxicillin	3(42.6)	2(50.0)	2(33.3)
2	Ciprofloxacin	4(57.1)	2(50.0)	3(50.0)
3	Ceftriaxone	4(57.1)	1(25.0)	3(50.0)
4	Levofloxacin	3(42.6)	2(50.0)	2(33.3)
5	Nitrofurantoin	5(71.4)	3(75.0)	4(66.7)

S/No	Antibiotics	%Resistance	%Resistance	%Resistance
6	Ofloxacin	2(28.6)	1(25.0)	1(16.7)
7	Gentamicin	5(71.4)	3(75.0)	4(66.7)
8	Cotrimoxazole	6(85.7)	3(75.0)	5(83.3)
9	Amoxicillin/Clavulanic acid	6(85.7)	3(75.0)	5(83.3)
10	Nalidixic acid	3(42.6)	2(50.0)	3(50.0)
11	Tetracycline	7(100.0)	4(100.0)	6(100.0)

Zone of Inhibition Interpretation as susceptible according to CLSI (2006).

Table 16 shows the percentage distribution of antibiotic resistance profile of *S. aureus* isolates from urine samples of pregnant women attending antenatal clinics in three (3) major hospitals in Kano, Nigeria. The susceptibility of the test bacteria isolates from MAWSH was more resistant to ceftriaxone, tetracycline, co-amoxiclav®, nitrofurantoin, and nalidixic acid

Table 16: Percentage antibiotic resistance profile of *S. aureus* isolates from 3 major hospitals in Kano, Nigeria.

MAWSH (n=3)		
S/No	Antibiotics	(%) Resistance
1	Amoxicillin	1(33.3)
2	Ciprofloxacin	-
3	Ceftriaxone	2(66.7)
4	Levofloxacin	-
5	Nitrofurantoin	2(66.7)
6	Ofloxacin	1(33.3)
7	Gentamicin	-
8	Cotrimoxazole	1(33.3)
9	Amoxicillin/Clavulanic acid	2(66.7)
10	Nalidixic acid	2(66.7)
11	Tetracycline	1(100.0)

- : No resistance

Zone of Inhibition Interpretation as susceptible according to CLSI (2006).

4.5 MULTIPLE ANTIBIOTIC RESISTANCE INDEX

The multiple antibiotic resistance index (MARI) is calculated based on the ratio of the number of antibiotics to which the isolates were resistant to out of a total of eleven (11) commonly prescribed antibiotics in the various hospitals. The resistance pattern of the multiple antibiotics resistance indices of *Proteus spp.* and *E. coli* isolated from pregnant women attending antenatal clinics is shown in Table 17. The isolates have multiple antibiotics resistance index > 0.2 . This means resistance of isolates to more than two antibiotics simultaneously.

Table 17: Multiple antibiotic resistance index (MARI) and resistance pattern of isolated *Proteus spp.* isolates

S/No	Resistance Pattern	Isolate Lab No	MAR Index
1	AMO,CEF,NFT,COT,ACA,TET	P1	0.5
2	AMO,CEF,LEV,NFT,OFL,GEN,COT,ACA	P2	0.7
3	CEF,LEV,NFT,GEN,COT,ACA ,TET	P3	0.6
4	AMO,CEF,NFT,COT,,GEN,NAL, ACA,TET,OFL,LEV	P4	1.0
5	CEF,NFT,COT,ACA ,GEN,NAL,OFL,LEV	P5	0.7
6	AMO,CIP,ACA ,TET	P6	0.3
7	NFT,GEN,COT,ACA	P7	0.3

S/No	Resistance Pattern	Isolate Lab No	MAR Index
8	CEF,NFT,GEN,COT,ACA,NAL,TET	P8	0.6
9	AMO,CEF,GEN,COT,ACA,NAL,TET	P9	0.6
10	AMO,NFT,COT,ACA ,TET	P10	0.4
11	AMO,CEF,LEV,NFT,COT,ACA ,NAL,TET	P11	0.7
12	AMO,CEF,LEV,NFT,GEN,COT,NAL,TET	P12	0.7
13	AMO,CIP,LEV,NFT,OFL,GEN,COT,ACA,TET	P13	0.8
14	CEF,LEV,OFL,NFT,GEN,COT,ACA,TET,NAL	P14	0.8
15	CEF,NFT,GEN,COT,ACA,NAL,TET	P15	0.6
16	CEF,NFT,GEN,COT,ACA,NAL,TET	P16	0.6
17	AMO,COT,ACA,TET	P17	0.3
18	AMO,NFT,COT,ACA,TET	P18	0.4
19	AMO,NFT,GEN,COT,ACA,NAL,TET	P19	0.6
20	AMO,OFL,GEN,COT,ACA,NAL,TET	P20	0.6
21	AMO,LEV,NFT,GEN,COT,ACA,NAL,TET	P21	0.7
22	CEF,NFT,GEN,COT,ACA,TET	P22	0.5
23	AMO,NFT,GEN,COT,ACA,TET	P23	0.5

AMO: Amoxicillin, LEV: Levofloxacin, NFT: Nitrofurantoin, GEN: Gentamicin,
 NAL: Nalidixic acid, COT: Cotrimoxazole, CEF: Ceftriaxone, ACA:
 Amoxicillin/Clavulanic acid, TET: Tetracycline, OFL: Ofloxacin, CIP: Ciprofloxacin

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which an isolate is resistant}}{\text{Total number of antibiotics tested}}$$

Table 18: Multiple Antibiotic Resistance Index and profile of occurrence of *Proteus spp.* isolates

MAR Index	No of <i>Proteus Isolates</i>	Percentage
0.2	-	-
0.3	3	13.04
0.4	1	4.34
0.5	4	17.39
0.6	7	30.43
0.7	3	13.04
0.8	3	13.04
0.9	1	4.34
1.0	1	4.34
Total	23	99.96

The Multiple Antibiotic Resistance Index (MARI) of the *Proteus spp.* isolates shows that 99.96% were resistant to 3 or more antibiotics.

Table 19: Multiple antibiotic resistance index (MARI) and resistance pattern of isolated *E. coli*

S/No	Resistance pattern	Isolate Lab No	MAR Index
1	COT, ACA, TET	Ec1	0.2
2	AMO,CEF,LEV,OFL,GEN,COT,TET	Ec2	0.6
3	AMO,CIP,LEV,NFT,COT,ACA,TET	Ec3	0.6
4	CIP,CEF,LEV,GEN,COT,ACA,TET	Ec4	0.6
5	CIP,CEF,LEV,NFT,OFL,GEN,COT,ACA,NAL,TET	Ec5	0.9
6	NFT,COT,ACA,NAL,TET	Ec6	0.4
7	CIP,CEF,LEV,NFT,OFL,GEN,COT,ACA,TET	Ec7	0.8
8	AMO,CIP,CEF,LEV,NFT,GEN,COT,ACA,NAL,TET	Ec8	0.9
9	AMO,CIP,NFT,GEN,COT,ACA,NAL,TET	Ec9	0.7
10	AMO,NFT,COT,ACA,NAL,TET	Ec10	0.5
11	AMO,NFT,ACA,TET	Ec11	0.3
12	CEF,NFT,GEN,COT,ACA,TET	Ec12	0.5
13	NFT,GEN,COT,ACA,TET	Ec13	0.4
14	AMO,CIP,CEF,TET,NAL,COT,LEV,GEN,ACA,NFT	Ec14	0.9

S/No	Resistance Pattern	Isolate Lab No	MAR Index
15	CIP,CEF,LEV,NFT,OFL,GEN,COT,ACA,TET	Ec15	0.8
16	CIP,CEF,LEV,NFT,OFL,GEN,COT,ACA,NAL,TET	Ec16	0.9
17	AMO,CIP,CEF,LEV,NFT,OFL,GEN,COT,NAL COT,ACA	Ec17	1.0

AMO: Amoxicillin, LEV: Levofloxacin, NFT: Nitrofurantoin, GEN: Gentamicin, NAL: Nalidixic acid, COT: Cotrimoxazole, CEF: Ceftriaxone, ACA: Amoxicillin/Clavulanic acid, TET: Tetracycline, OFL: Ofloxacin, CIP: Ciprofloxacin.

Table 20: Multiple Antibiotic Resistance Index and profile of phenotypic occurrence of *E. coli* isolates

MAR Index	No of <i>E.coli</i> Isolates	Percentage (%)
0.2	1	5.88
0.3	1	5.88
0.4	-	-
0.5	4	23.52
0.6	3	17.64
0.7	1	5.88
0.8	1	5.88
0.9	5	29.41

1.0	1	5.88
Total	17	99.97

The Multiple Antibiotic Resistance Index (MARI) of the *E. coli* isolates shows that 99.97% were resistant to 2 or more antibiotics.

Table 21: Plasmid profile of *Proteus spp.* isolates harbouring plasmids

Lab No	<i>Proteus</i> isolate	No of plasmids	Estimated mol sizes of plasmids(bp)	Phenotypic resistance pattern
P3	<i>Proteus mirabilis</i> 031	1	5000	CEF,LEV,NFT,GEN,COT ACA,TET

Table 22: Plasmid profile of *E. coli* isolates harbouring plasmids

Lab No	<i>Proteus</i> isolate	No of plasmids	Estimated mol sizes of plasmids(bp)	Phenotypic resistance pattern
Ec1	<i>E.coli</i> 003	1	4500	ACA,COT,TET
Ec2	<i>E.coli</i> 023	1	5000	AMO,CEF,LEV,OFL,GEN,COT,TET
Ec 3	<i>E.coli</i> 032	1	5000	AMO,CIP,LEV,NFT,COT,ACA,TET
Ec 11	<i>E.coli</i> 187	1	4000	AMO,NFT,ACA,TET
Ec 13	<i>E.coli</i> 229	1	5000	NFT,GEN,COT,ACA,TET

4.6 SOME VIRULENCE FACTORS OF *PROTEUS SPP.*

4.6.1 Hemagglutinins

Hemagglutination patterns were determined for all the *Proteus spp.* isolates, 12 (52.2%) were found to express only the MR/P⁺ hemagglutinins while a combination of both MR/P⁺ MR/K⁺ hemagglutinins were observed in 8(34.8%) isolates. MR/P⁻, MR/K⁺ pattern were observed in 2(8.7%). MR/P⁻, MR/K⁻ hemagglutination pattern was expressed by only 1(4.3%). Mannose-sensitive adhesions were not observed in any of the *Proteus spp.* isolates.

Table 23: Hemagglutinins expressed by *Proteus spp.* isolates from urine samples of pregnant women attending antenatal clinics

Hemagglutination pattern	Number (%)
MR/P ⁺	12(52.2)
MR/K ⁻	
MR/P ⁺	8(34.8)
MR/K ⁺	
MR/P ⁻	2(8.7)
MR/K ⁺	
MR/P ⁻	1(4.3)
MR/K ⁻	
MS	0
N	23

MR/P+: Mannose resistant *Proteus*-like hemagglutination present
 MR/P-: Mannose resistant *Proteus*-like hemagglutination absent
 MR/K+: Mannose resistant *Klebsiella*-like hemagglutination present
 MR/K-: Mannose resistant *Klebsiella*-like hemagglutination absent
 MS: Mannose sensitive hemagglutination
 N: Total number of *Proteus species* isolates (23)

4.6.2 Hemolysin

Hemolysin activities of all the *Proteus spp.* isolates were measured as a function of growth. Hemolytic activity that peaked during the late exponential phase of growth was produced by most isolates. *Proteus spp.* isolates produced measurable hemolytic activity with titres ranging from 1:2 to 1:512. Geometric mean of reciprocal hemolytic titres for *Proteus spp.* isolates was 29.23.

Table 24: Reciprocal of hemolytic titres of 23 (twenty three) *Proteus spp.*

Urine isolate(s)	Isolate Lab No	Reciprocal Hemolytic titre
<i>Proteus mirabilis</i> 002	P1	128
<i>Proteus mirabilis</i> 021	P2	32
<i>Proteus mirabilis</i> 031	P3	8
<i>Proteus vulgaris</i> 040	P4	32
<i>Proteus mirabilis</i> 046	P5	64
<i>Proteus mirabilis</i> 049	P6	4

Urine isolate (s)	Isolate Lab No	Reciprocal Hemolytic titre
<i>Proteus mirabilis</i> 051	P7	32
<i>Proteus vulgaris</i> 059	P8	256
<i>Proteus mirabilis</i> 060	P9	128
<i>Proteus mirabilis</i> 069	P10	32
<i>Proteus mirabilis</i> 071	P11	8
<i>Proteus mirabilis</i> 088	P12	64
<i>Proteus mirabilis</i> 095	P13	8
<i>Proteus mirabilis</i> 113	P14	4
<i>Proteus mirabilis</i> 117	P15	128
<i>Proteus mirabilis</i> 118	P16	16
<i>Proteus mirabilis</i> 123	P17	32
<i>Proteus mirabilis</i> 131	P18	2
<i>Proteus mirabilis</i> 243	P19	64
<i>Proteus mirabilis</i> 250	P20	256
<i>Proteus mirabilis</i> 271	P21	8
<i>Proteus mirabilis</i> 291	P22	16
<i>Proteus mirabilis</i> 301	P23	128

4.6.3 Urease assay

Urease has been implicated as a factor contributing to the pathogenicity of *Proteus spp.* All the *Proteus* isolates showed significant urease activity ranging from 24-110 ($\mu\text{mol NH}_3/\text{min}/\text{mg}$ protein).

Table 25: Urease activity shown by 23 *Proteus spp.* isolates from urine samples of pregnant women attending antenatal clinics.

Urine isolates	Isolate Lab No	Urease activity($\mu\text{mol}/\text{NH}_3$ /min/mg protein)
<i>Proteus mirabilis</i> 002	P1	48
<i>Proteus mirabilis</i> 021	P2	70
<i>Proteus mirabilis</i> 031	P3	24
<i>Proteus vulgaris</i> 040	P4	36
<i>Proteus mirabilis</i> 046	P5	52
<i>Proteus mirabilis</i> 049	P6	63
<i>Proteus mirabilis</i> 051	P7	81
<i>Proteus vulgaris</i> 059	P8	32
<i>Proteus mirabilis</i> 060	P9	42
<i>Proteus mirabilis</i> 069	P10	110
<i>Proteus mirabilis</i> 071	P11	76
<i>Proteus mirabilis</i> 088	P12	61
<i>Proteus mirabilis</i> 095	P13	58
<i>Proteus mirabilis</i> 113	P14	93

Urine isolates	Isolate Lab No	Urease activity($\mu\text{mol}/\text{NH}_3$ /min/mg protein)
<i>Proteus mirabilis</i> 117	P15	85
<i>Proteus mirabilis</i> 118	P16	67
<i>Proteus mirabilis</i> 123	P17	46
<i>Proteus mirabilis</i> 131	P18	55
<i>Proteus mirabilis</i> 243	P19	73
<i>Proteus mirabilis</i> 250	P20	28
<i>Proteus vulgaris</i> 271	P21	87
<i>Proteus mirabilis</i> 291	P22	53
<i>Proteus mirabilis</i> 301	P23	61

4.7 SOME VIRULENCE FACTORS OF *ESCHERICHIA COLI*

4.7.1 Hemolysin assay

Hemolysin activity of all the 17 (seventeen) *E. coli* isolates were also measured as a function of growth. Hemolytic activity that peaked during the late exponential phase of growth was produced by most of the isolates. *E. coli* isolates produced measurable hemolytic activity with titres ranging from 1:2 to 1:512. Geometric mean of reciprocal hemolytic titres for the isolates was 31.99.

Table 26: Reciprocal of Hemolytic titre of 17 (seventeen) *E. coli* isolates from pregnant women with asymptomatic bacteriuria

Urine Isolates	Isolate Lab No	Reciprocal Hemolytic titre
<i>E. coli</i> 003	Ec 1	256
<i>E. coli</i> 023	Ec 2	64
<i>E. coli</i> 032	Ec 3	8
<i>E. coli</i> 037	Ec 4	32
<i>E. coli</i> 052	Ec 5	2
<i>E. coli</i> 064	Ec 6	32
<i>E. coli</i> 081	Ec 7	64
<i>E. coli</i> 084	Ec 8	8
<i>E. coli</i> 116	Ec 9	256
<i>E. coli</i> 129	Ec 10	4
<i>E. coli</i> 187	Ec 11	64
<i>E. coli</i> 219	Ec 12	512
<i>E. coli</i> 229	Ec 13	32
<i>E. coli</i> 264	Ec 14	4
<i>E. coli</i> 266	Ec 15	128
<i>E. coli</i> 284	Ec 16	8
<i>E. coli</i> 288	Ec 17	64

4.7.2 Hemagglutinins

Hemagglutination patterns were also determined for all the *E. coli* isolates. MR/P+ hemagglutination pattern was expressed by only 1(5.9%) *E. coli* isolate. *E. coli* isolates were observed to express the combination of MR/P+, MR/K+ hemagglutinins 9 (52.9%). The hemagglutination pattern MR/P-, MR/K+ was more observed in *E. coli* isolates 3(17.6%). MR/P-, MR/K- hemagglutinins were expressed by 2 (11.8%) isolates of *E. coli*. Mannose-sensitive adhesions were not observed in all the *E. coli* isolates. 2 (111.8%) isolates failed to hemagglutinate with human erythrocytes tested.

Table 27: Hemagglutinins expressed by *E. coli* isolates from urine samples of pregnant women with asymptomatic bacteriuria

Hemagglutination pattern	Number (%)
MR/P+	
MR/K-	1(5.9)
MR/P+	
MR/K+	9(52.9)
MR/P-	
MR/K+	3(17.6)
MR/P-	
MR/K-	2(11.8)
MS	0(0)
No Hemagglutination	2(11.8)

MR/P+: mannose resistant *Proteus*-like hemagglutination present

MR/K-: mannose resistant *Klebsiella*-like hemagglutination absent

MR/P-: mannose resistant *Proteus*-like hemagglutination absent

MR/K+: mannose resistant *Klebsiella*-like present

MS: mannose sensitive hemagglutination

4.8 MOLECULAR CHARACTERIZATION OF RESISTANT ISOLATES

4.8.1 Plasmid Extraction/PCR Amplification

AGAROSE GEL ELECTROPHOTOGRAM OF 10 *E. coli* ISOLATES (Ec1-Ec10) AND PCR AMPLIFICATION USING PRIMERS FOR CTX-M2, qnrC GENES.

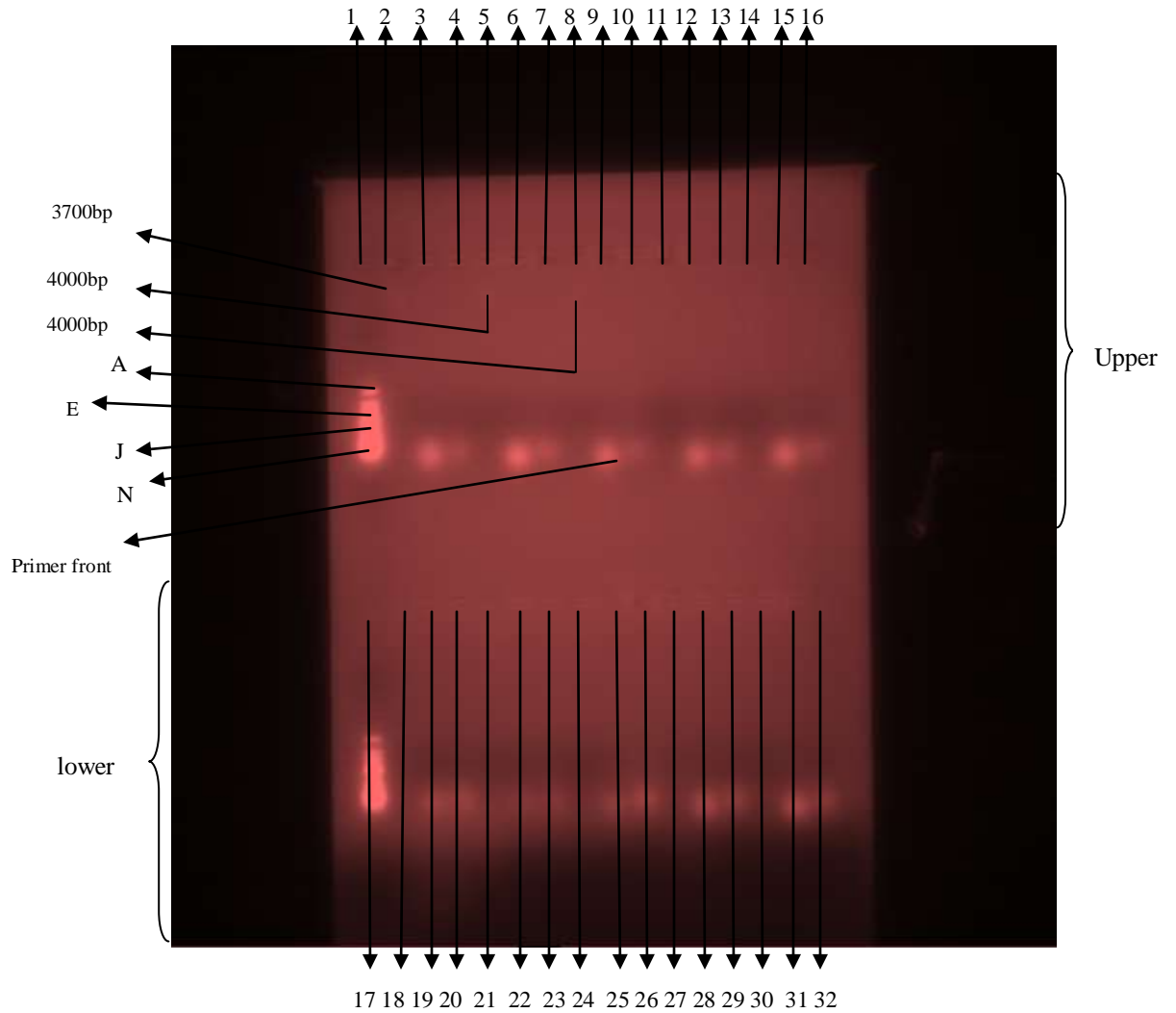


Fig.2: 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *E. coli* isolates (Ec1-Ec10) from pregnant

women in Kano, Nigeria and PCR amplification of CTX-M2 and qnrC genes
from these isolates.

Lane 1 and 17: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 2,3,4: Resistant *E. coli* (Ec1) with Primer 1 and 2 respectively

Lane 5,6,7: Resistant *E. coli* (Ec2) with Primer 1 and 2 respectively

Lane 8,9,10: Resistant *E. coli* (Ec3) with Primer 1 and 2 respectively

Lane 11,12,13: Resistant *E. coli* (Ec4) with Primer 1 and 2 respectively

Lane 14,15,16: Resistant *E. coli* (Ec5) with Primer 1 and 2 respectively

Lower:

Lane 18,19,20: Resistant *E. coli* (Ec6) with Primer 1 and 2 respectively

Lane 21,22,23: Resistant *E. coli* (Ec7) with Primer 1 and 2 respectively

Lane 24,25,26: Resistant *E. coli* (Ec8) with Primer 1 and 2 respectively

Lane 27,28,29: Resistant *E. coli* (Ec9) with Primer 1 and 2 respectively

Lane 30,31,32: Resistant *E. coli* (Ec10) with Primer 1 and 2 respectively

Primer 1: CTX-M2 (for β -lactam antibiotics)

Sequence: forward: 5'-ATGATGACTCAGAGCATTCG-3'

reverse: 5'-GAAACCGTGGGTTACGATTT-3'

Primer 2: qnrC (for fluoroquinolones)

Sequence: forward: 5'-GGGTTGTACATTTATTGAATC-3'

reverse: 5'-TCCACTTTACGAGGTTCT-3'

Observation from the above showed single plasmid DNA bands in *E. coli* isolates Ec1, Ec2 and Ec3 of estimated molecular sizes 37000bp, 4000bp and 4000bp respectively. There was no amplification of the PCR products of CTX-M2 and qnrC.

AGAROSE GEL ELECTROPHOTOGRAM OF 7 *E. coli* (Ec11-Ec17) AND 3 *Proteus spp.* ISOLATES (P1-P3) AND PCR AMPLIFICATION USING PRIMERS FOR CTX-M2 and qnrC GENES.

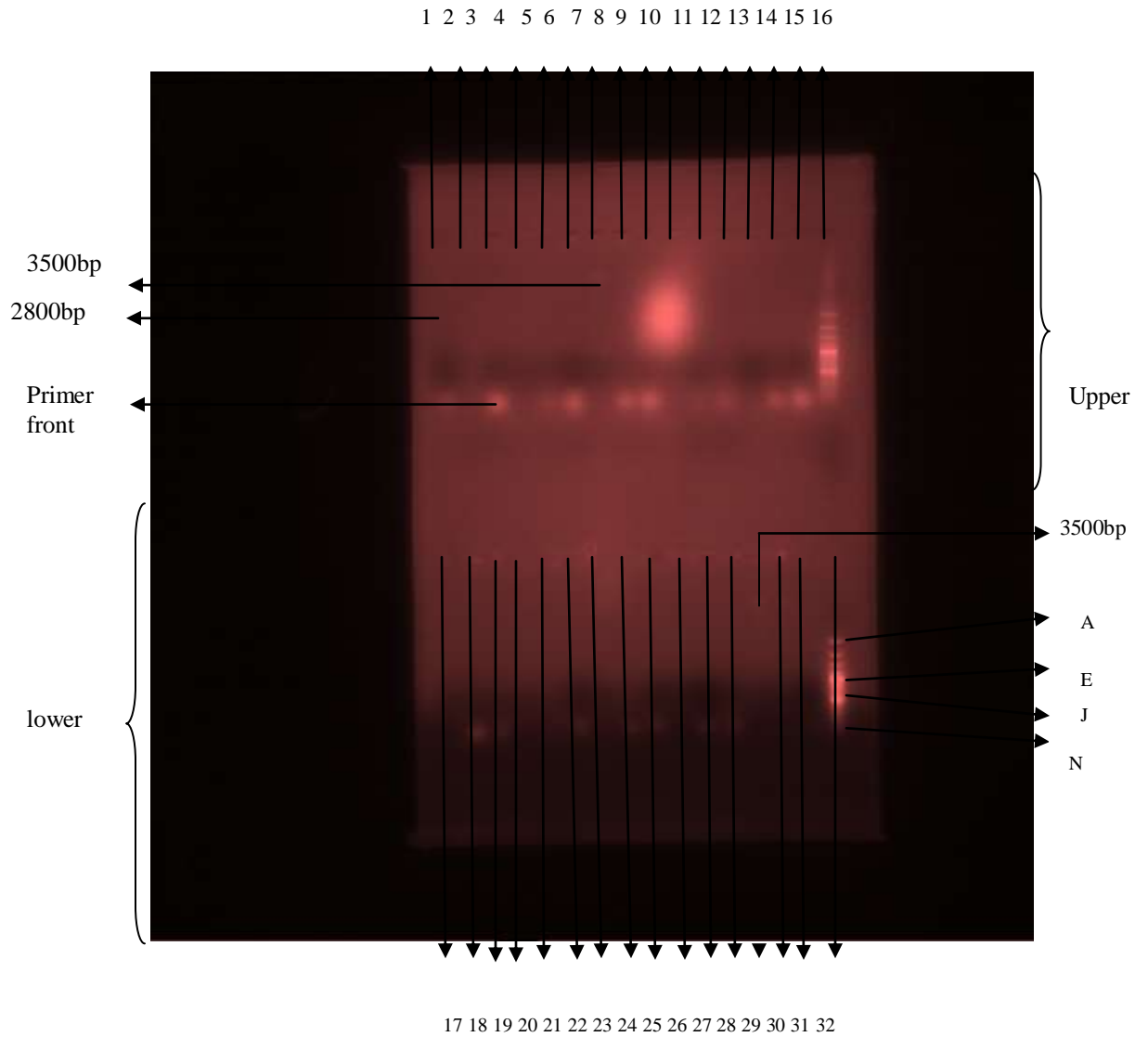


Fig.3: 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *E. coli* isolates Ec11-Ec17, and *Proteus spp.* isolates (P1-P3) from pregnant women in Kano, Nigeria and PCR amplification of CTX-M2 and qnrC genes from these isolates.

Lane 16 and 32: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 1,2,3,: Resistant *E. coli* (Ec11) with Primer 1 and 2 respectively

Lane 4,5,6,: Resistant *E.coli* (Ec12) with Primer 1 and 2 respectively

Lane7,8,9,: Resistant *E. coli* (Ec13) with Primer 1 and 2 respectively

Lane 10,11,12: Resistant *E. coli* (Ec14) with Primer 1 and 2 respectively

Lane 13,15,16: Resistant *E. coli* (Ec15) with Primer 1 and 2 respectively

Lower:

Lane 17,18,19: Resistant *E. coli* (Ec16) with Primer 1 and 2 respectively

Lane 20,21,22: Resistant *E. coli* (Ec17) with Primer 1 and 2 respectively

Lane 23,24,25: Resistant *Proteus spp.* (P1) with Primer 1 and 2 respectively

Lane26,27,28,: Resistant *Proteus spp.* (P2) with Primer 1 and 2 respectively

Lane 29,30,31: Resistant *Proteus spp.* (P3) with Primer 1 and 2 respectively

Primer 1: CTX-M2 (for β -lactam antibiotics)

Sequence: forward: 5'-ATGATGACTCAGAGCATTCG-3'

reverse: 5'-GAAACCGTGGGTTACGATTT-3'

Primer 2: qnrC (for fluoroquinolones)

Sequence: forward: 5'-GGGTTGTACATTTATTGAATC-3'

reverse: 5'-TCCACTTTACGAGGTTCT-3'

Observation from the above showed single plasmid DNA bands in *E. coli* isolates Ec11, Ec13 and *Proteus spp.* isolate P3 of estimated molecular sizes 2800bp, 3500bp and 3500bp respectively. There was no amplification of the PCR products of CTX-M2 and qnrC.

AGAROSE GEL ELECTROPHOTOGRAM OF 10 *Proteus spp.* ISOLATES (P4 - P13) AND PCR AMPLIFICATION USING PRIMERS CTX-M9 and qnrS (1-2).

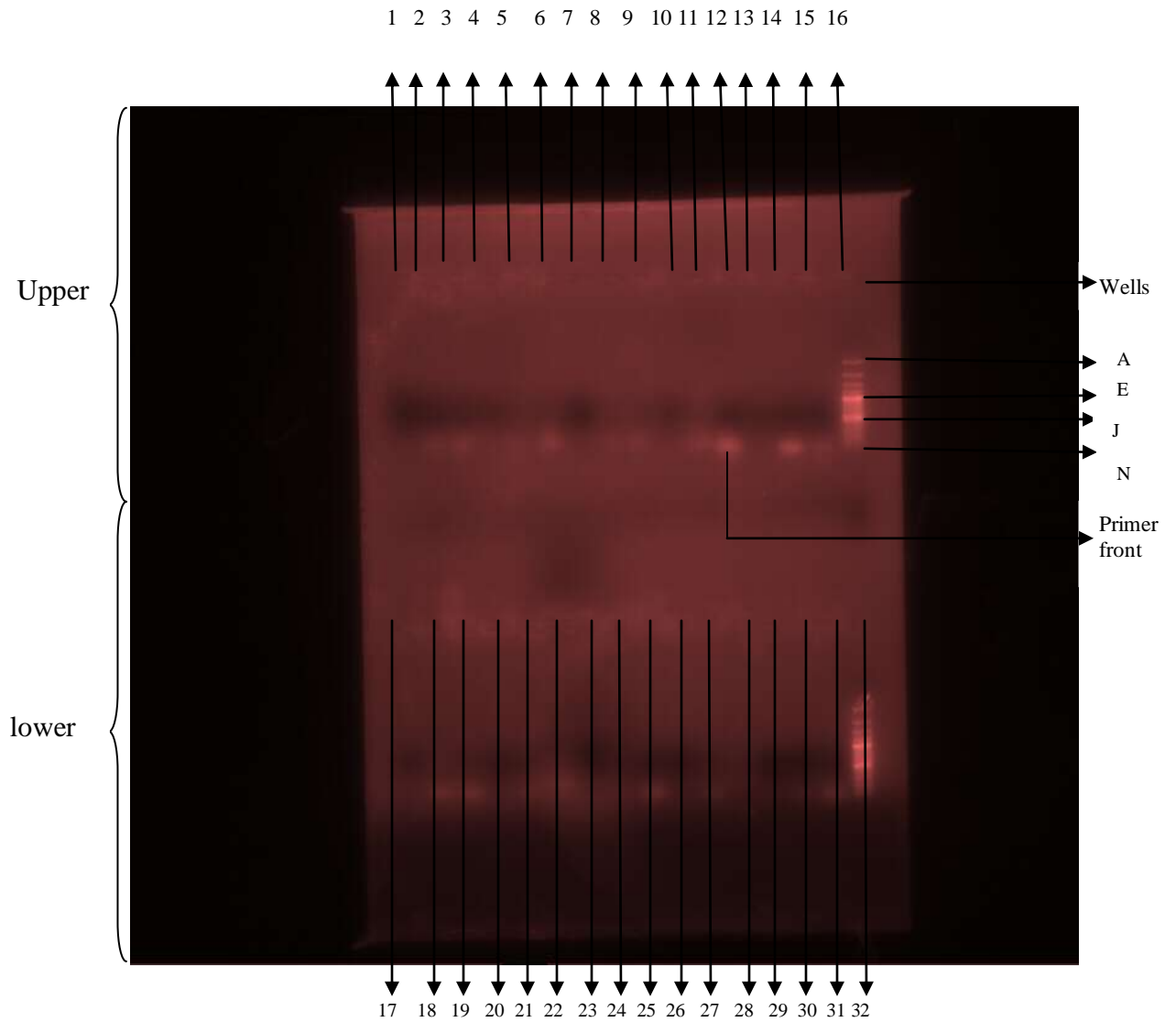


Fig.4: 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *Proteus spp.* isolates (P4-P13) from pregnant women in Kano, Nigeria and PCR amplification of CTX-M9 and qnrS(1-2) genes from these isolates.

Lane 16 and 32: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 1,2,3,: Resistant *Proteus spp.* (P4) with Primer 3 and 4 respectively

Lane 4,5,6,: Resistant *Proteus spp.* (P5) with Primer 3 and 4 respectively

Lane 7,8,9,: Resistant *Proteus spp.* (P6) with Primer 3 and 4 respectively

Lane 10,11,12: Resistant *Proteus spp.* (P7) with Primer 3 and 4 respectively

Lane 13,15,16: Resistant *Proteus spp.* (P8) with Primer 3 and 4 respectively

Lower:

Lane 17,18,19: Resistant *Proteus spp.* (P9) with Primer 3 and 4 respectively

Lane 20,21,22: Resistant *Proteus spp.* (P10) with Primer 3 and 4 respectively

Lane 23,24,25: Resistant *Proteus spp.* (P11) with Primer 3 and 4 respectively

Lane 26,27,28,: Resistant *Proteus spp.* (P12) with Primer 3 and 4 respectively

Lane 29,30,31: Resistant *Proteus spp.* (P13) with Primer 3 and 4 respectively

Primer 3: CTX-M9 (for β -lactam antibiotics)

Sequence: CTX-M-9 P1: 5'-GTGACAAAGAGAGTGCAACGG-3'

CTX-M-9 P2: 5'-ATGATTCTCGCCGCTGAAGCC-3'

Primer 4: qnrS(1-2) [for fluoroquinolones]

Sequence: qnrS(1-2)F: 5'-TCGACGTGCTAACTTGCG-3'

qnrS(1-2)R: 5'-GATCTAAACCGTCGAGTTCGG-3'

Observation from the above showed no plasmid DNA bands in these *Proteus spp.* isolates (P4-P13). No amplicons of the PCR of CTX-M9 and qnrS (1-2) were observed. Only the primer fronts and DNA ladder were visible.

AGAROSE GEL ELECTROPHOTOGRAM OF 10 *Proteus spp.* ISOLATES (P14-P23) AND PCR AMPLIFICATION USING PRIMERS FOR CTX-M9 and qnrS GENES.

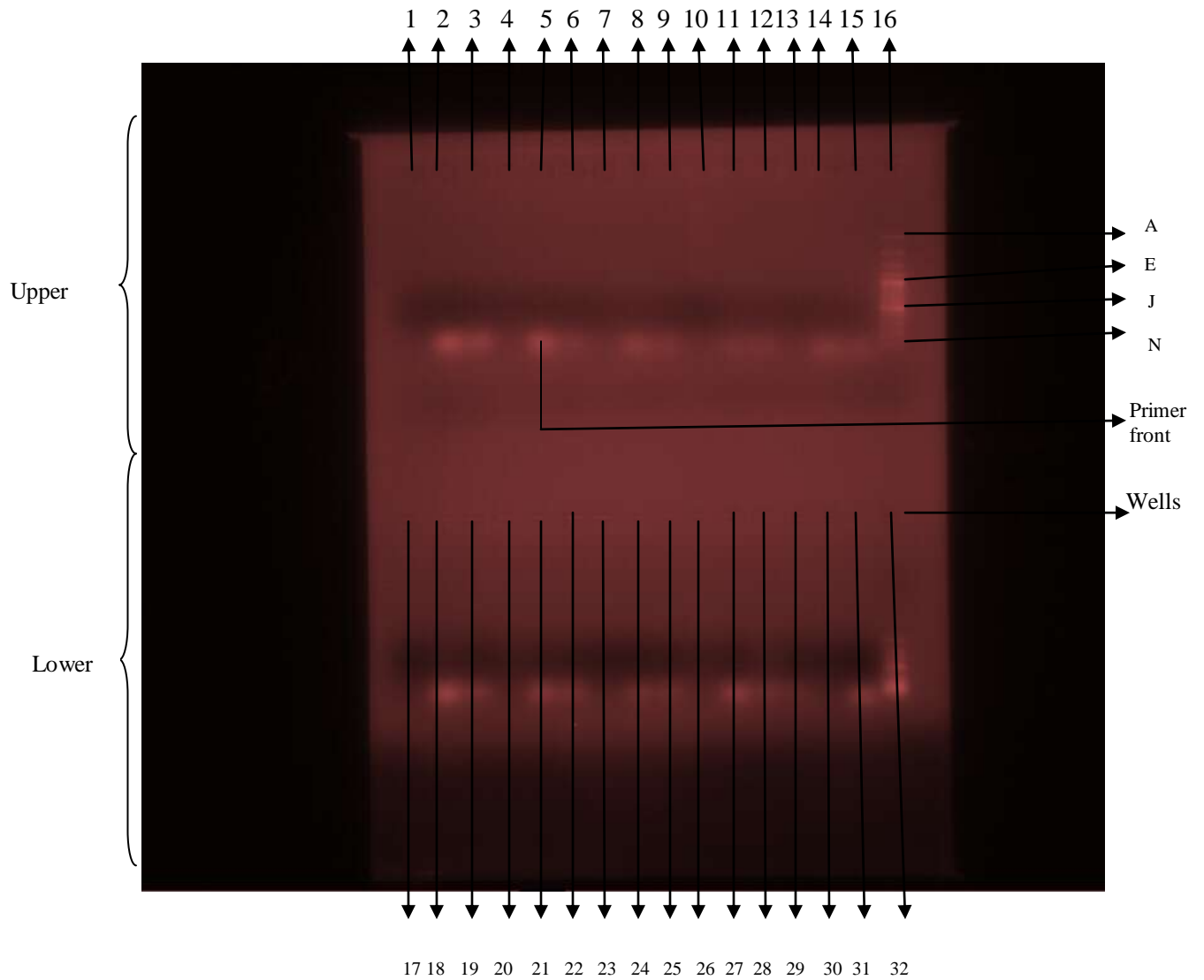


Fig. 5: 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *Proteus spp.* isolates (P14-P23) from pregnant women in Kano, Nigeria and PCR amplification of CTX-M9 and qnrS (1-2) genes from these isolates.

Lane 16 and 32: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 1,2,3,: Resistant *Proteus spp.* (P14) with Primer 3 and 4 respectively

Lane 4,5,6,: Resistant *Proteus spp.* (P15) with Primer 3 and 4 respectively

Lane 7,8,9,: Resistant *Proteus spp.* (P16) with Primer 3 and 4 respectively

Lane 10,11,12: Resistant *Proteus spp.* (P17) with Primer 3 and 4 respectively

Lane 13,15,16: Resistant *Proteus spp.* (P18) with Primer 3 and 4 respectively

Lower:

Lane 17,18,19: Resistant *Proteus spp.* (P19) with Primer 3 and 4 respectively

Lane 20,21,22: Resistant *Proteus spp.* (P20) with Primer 3 and 4 respectively

Lane 23,24,25: Resistant *Proteus spp.* (P21) with Primer 3 and 4 respectively

Lane 26,27,28: Resistant *Proteus spp.* (P22) with Primer 3 and 4 respectively

Lane 29,30,31: Resistant *Proteus spp.* (P23) with Primer 3 and 4 respectively

Primer 3: CTX-M9 (for β -lactam antibiotics)

Sequence: CTX-M-9 P1: 5'-GTGACAAAGAGAGTGCAACGG-3'

CTX-M-9 P2: 5'-ATGATTCTCGCCGCTGAAGCC-3'

Primer 4: qnrS(1-2) [for fluoroquinolones]

Sequence: qnrS(1-2)F: 5'-TCGACGTGCTAACTTGCG-3'

qnrS(1-2)R: 5'-GATCTAAACCGTCGAGTTCGG-3'

Observation from the above showed no plasmid DNA bands in these *Proteus spp.* isolates (P14-P23). No amplicons of Polymerase Chain Reaction product of CTX-M9 and qnrS (1-2) were observed. Only the primer fronts and DNA ladder were visible.

AGAROSE GEL ELECTROPHOTOGRAM OF 5 *E. coli* (Ec1-Ec5) and 5 *Proteus spp.* ISOLATES (P1-P5) AND PCR AMPLIFICATION USING PRIMERS FOR *gyrB* and *pCT* GENES.

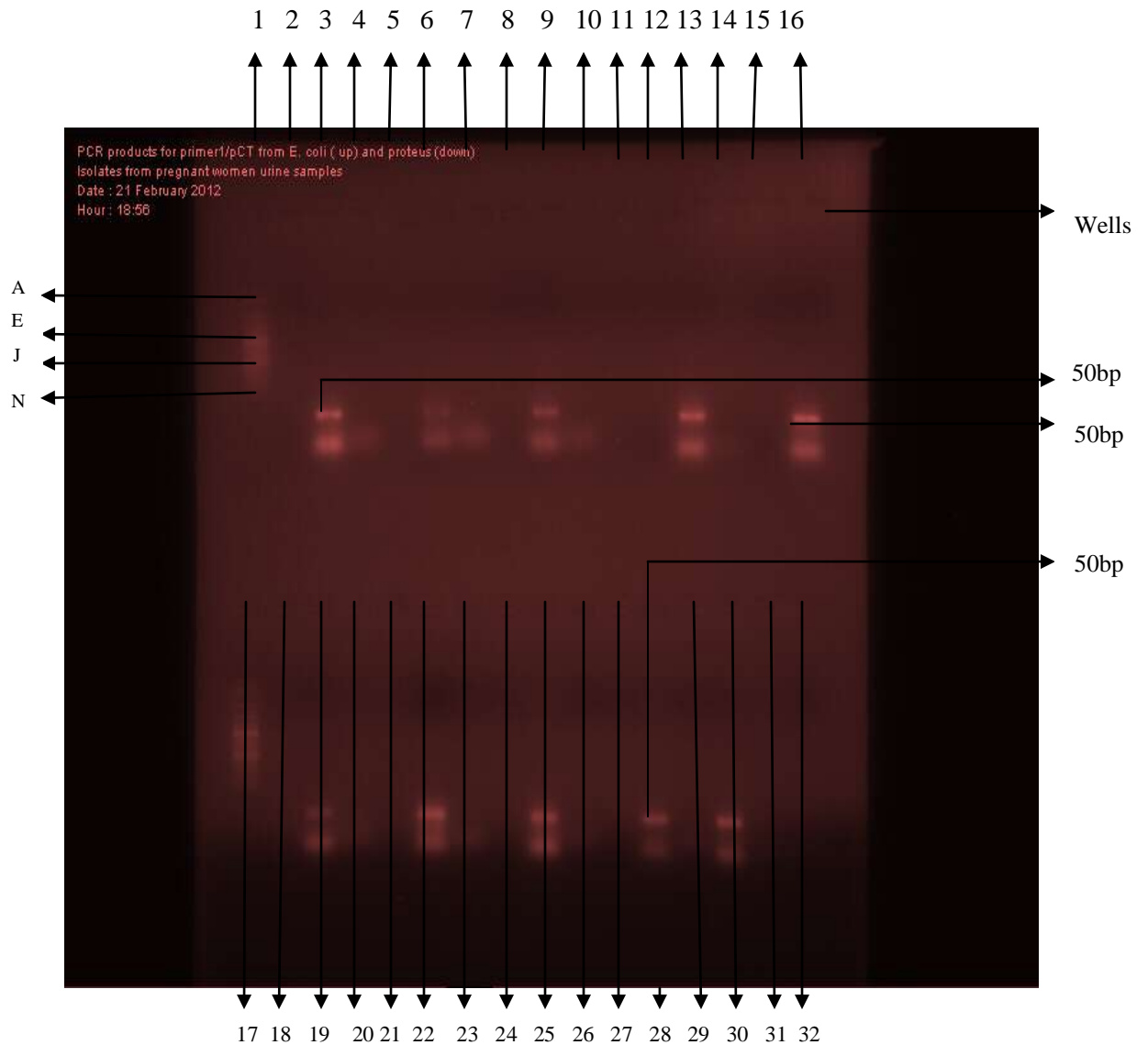


Fig.6: 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *E. coli* isolates (Ec1-Ec5) and *Proteus spp.* isolates (P1-P5), from pregnant women in Kano, Nigeria and PCR amplification of *gyrB* and *pCT* genes from these isolates.

Lane 1 and 17: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 3 and 4: PCR product of *E. coli* (Ec1) with Primer 5 and 6 respectively

Lane 6 and 7: PCR product of *E. coli* (Ec2) with Primer 5 and 6 respectively

Lane 9 and 10: PCR product of *E. coli* (Ec3) with Primer 5 and 6 respectively

Lane 12 and 13: PCR product of *E. coli* (Ec4) with Primer 5 and 6 respectively

Lane 15 and 16: PCR product of *E. coli* (Ec5) with Primer 5 and 6 respectively

Lanes 2, 5, 8, 11 and 14: Empty

Lower:

Lane 19 and 20: PCR product of *Proteus spp.* (P1) with Primer 5 and 6 respectively

Lane 22 and 23: PCR product of *Proteus spp.* (P2) with Primer 5 and 6 respectively

Lane 25 and 26: PCR product of *Proteus spp.* (P3) with Primer 5 and 6 respectively

Lane 28 and 29: PCR product of *Proteus spp.* (P4) with Primer 5 and 6 respectively

Lane 30 and 31: PCR product of *Proteus spp.* (P5) with Primer 5 and 6 respectively

Lanes 18, 21, 24, 27 and 32: Empty

Primer 5: gyrB [to amplify the 448bp fragment of QRDR (quinolone resistance determining region) of gyrB, from nucleotides 995 to 1442] (Gene Bank Accession number D87842).

Sequence: forward: 5'-GCGCGTGAGATGACCCGCCGT-3'

reverse: 5'-CTGGCGGTAGAAGGTCAG-3'

Primer 6: pCT (for IncK plasmid encoding bla_{CTX-M} ESBL genes)

Sequence: pCT 008 (F): 5'-CATTGTATCTATCTTGTGGG-3'

pCT 009(R): 5'-GCATTCCAGAAGATGACGTT-3'

Observation from the above showed amplification of gyrB genes (50bp) in Ec1, Ec2, Ec3, Ec4 and Ec5 respectively. No amplicons of pCT were visible to either the PCR products of *Proteus spp.* or *E. coli*. Amplification of gyrB genes was also observed to the PCR products of 5 *Proteus spp.* isolates

AGAROSE GEL ELECTROPHOTOGRAM OF 5 *E. coli* (Ec1-Ec5) and 5 *Proteus* spp. ISOLATES (P1-P5) AND PCR AMPLIFICATION USING PRIMERS CTX-M9 AND qnrS (1-2)

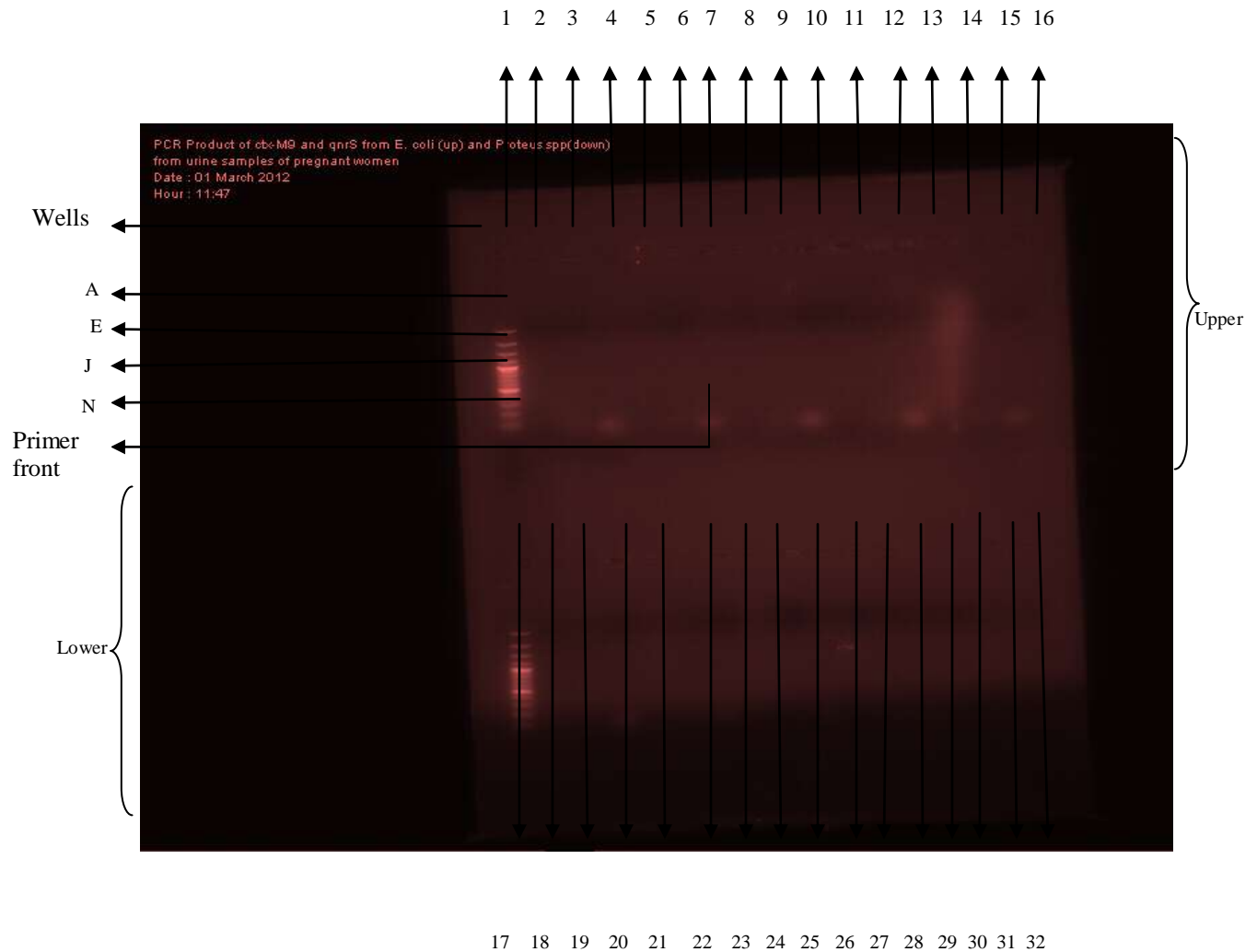


Fig.7 : 1.0% agarose gel electrophoresis (100v, 45min) of plasmid DNAs isolated from multiple antibiotic resistant *E. coli* isolates (Ec1-Ec5) and *Proteus* spp. isolates (P1-P5), from pregnant women in Kano, Nigeria and PCR amplification of CTX-M9 and qnrS (1-2) genes from these isolates.

Lane 1 and 17: 100bp plus DNA ladder showing plasmid sizes of 3000(A), 2000, 1500, 1200, 1000(E), 900, 800, 700, 600, 500(J), 400, 300, 200, 100(N).

Upper:

Lane 3 and 4: PCR product of *E. coli* (Ec1) with Primer 3 and 4 respectively

Lane 6 and 7: PCR product of *E. coli* (Ec2) with Primer 3 and 4 respectively

Lane 9 and 10: PCR product of *E. coli* (Ec3), with Primer 3 and 4 respectively

Lane 12 and 13: PCR product of *E. coli* (Ec4) with Primer 3 and 4 respectively

Lane 15 and 16: PCR product of *E. coli* (Ec5) with Primer 3 and 4 respectively

Lanes 2,5,8,11 and 14: Empty

Lower:

Lane 19 and 20: PCR product of *Proteus spp.* (P1) with Primer 3 and 4 respectively

Lane 22 and 23: PCR product of *Proteus spp.* (P2) with Primer 3 and 4 respectively

Lane 25 and 26: PCR product of *Proteus spp.* (P3) with Primer 3 and 4 respectively

Lane 28 and 29: PCR product of *Proteus spp.* (P4) with Primer 3 and 4 respectively

Lane 31 and 32 PCR product of *Proteus spp.* (P5) with Primer 3 and 4 respectively

Lanes 18, 21,24,27,and 30: Empty

Primer 3: CTX-M9 (for β -lactam antibiotics)

Sequence: CTX-M-9 P1: 5'-GTGACAAAGAGAGTGCAACGG-3'

CTX-M-9 P2: 5'-ATGATTCTCGCCGCTGAAGCC-3'

Primer 4: qnrS(1-2) [for fluoroquinolones]

Sequence: qnrS(1-2)F: 5'-TCGACGTGCTAACTTGCG-3'

qnrS(1-2)R: 5'-GATCTAAACCGTCGAGTTCGG-3'

Observation from the above showed no amplification of both CTX-M9 and qnrS(1-2) in Ec1, Ec2, Ec3, Ec4 and Ec5 respectively. No amplicons of either CTX-M9 or qnrS(1-2) were also visible in *Proteus spp.* P1, P2, P3, P4 and P5.

CHAPTER FIVE

5.0 DISCUSSION

From the results obtained, urine is seen to be a medium in which various micro-organisms thrive, some of which are pathogenic and infectious. In this study, the prevalence of asymptomatic bacteriuria was found to be 15.2%. This is higher than 8.0% from the study in a hospital in Kano (Omole *et al.*, 2008), lower than 21.0% from the study in Ibadan (Ogbolu *et al.*, 2006), lower than 23.9% from the study in Sagamu (Olusanya *et al.*, 1993), 45.3% reported in Benin City (Imade *et al.*, 2010), 78.9% in Abakaliki (Amadi *et al.*, 2007), and 86.6% in Benin (Akerere *et al.*, 2001) all in Nigeria. The lower prevalence obtained in this work may be associated with the regular antenatal teachings (which often lay emphasis on good personal hygiene practice like regular hand-washing, etc) given at each routine antenatal clinic in the three major hospitals used in this study. Also majority of the patients in this study practice the religion in which they often wash their hands and other body parts before saying their regular daily prayers. This could have helped to reduce bacteria load and transmission. The organisms isolated from the urine samples of these pregnant women were *Proteus spp.* (*Proteus mirabilis* and *Proteus vulgaris*), *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella spp.*

Proteus spp. (a major soil contaminant) was observed to be the most prevalent uropathogen (49%). This may have been introduced into the urinary tract of these women (especially the house-wives who are more busy with house chores exposing them to soil contact like sweeping, farming, etc) while they clean up using their

hands after defecating or urinating. In the past *Proteus spp.* was observed to be among the least organism likely to cause asymptomatic bacteriuria (Esmaeili, 2005). This study has shown that *Proteus spp.* (especially *Proteus mirabilis*) could be a major uropathogen causing urinary tract infection; this is at variance with some studies in the country and beyond (Girishbabu *et al.*, 2011), where *E. coli* and *S. aureus* were the most prevalent uropathogen (Nerissa *et al.*, 2003; Eyal *et al.*, 2009; Obirikorang *et al.*, 2012; Perera *et al.*, 2012). This suggests a changing pattern in the prevalence of organisms causing infection in the population. This is followed by *Escherchia coli* (36%). This organism is observed to thrive well in the urinary stasis condition found in most pregnant women, and also poor hygiene practice after defecating and urinating.

Women in the third trimester were observed to have the highest prevalence (18.2%) than those in their second and first trimesters. This agrees with the findings of Lindsay (2003), that asymptomatic bacteriuria increases with increase in gestational period. There is usually an increased hormonal activity with increase in gestational age, and this aids the growth of bacteria. In terms of occupational status, the housewives were observed to have a higher bacteriuria prevalence of 57.4% than women of the working class, which could be as a result of reduced social exposure and poor hygiene. These women might have been exposed to these bacteria from the house chores like sweeping, cleaning, and handling of dirty surfaces, etc. The working class women are more educated, and enlightened about precautionary measures (which includes personal hygiene, etc) to take to avoid being infected. The age group 25-30 years had the highest prevalence of 24.2%. Women in this age group

are more sexually active and multiparity is also a risk factor in acquiring asymptomatic bacteriuria (Antoni *et al.*, 1997).

Kidney stones were also observed to be a major risk factor in developing bacteriuria in pregnancy. Forty seven (47) urine samples were observed in this study to have crystals which could aid the progression of bacteriuria. In this study, *Proteus mirabilis* was observed to be the most predominant isolate, and some of the virulence (degree of pathogenicity) factors of *Proteus spp.* include: urease, hemolysin, hemagglutinin, adherence factors e.g fimbriae (pili), flagella/swarming phenomenon, etc. Urease production has been observed to result in the elevation of pH in the surrounding of bacterial growth resulting in stone formation and cytotoxicity (Antoni *et al.*, 1997).

The occurrence and apparent prevalence of these microorganisms (some of which are pathogenic) in a vast number of women, especially those pregnant, gives cause for concern as they are prone to several complications as well as foetal risks. Due to the increase in sex hormones, the anatomic and physiologic changes during pregnancy, bladder and kidney infection is more likely and may result in hypertension, preeclampsia, low birth weight, prematurity, septicemia, and maternal death.(Klein and Gibbs, 2004; Delzell and Leferre, 2000; Christensen, 2000). Asymptomatic bacteriuria must be treated in pregnancy in order to prevent complications such as pyelonephritis, premature labour, still birth, hypertension, maternal anaemia, preeclampsia and septicemia. (Raz, 2003).

Forty-seven (47) of the pregnant women were observed to have crystals (Calcium Oxalate, Calcium Phosphate and Amorphous Phosphate) in their urine samples. This

signifies an underlying renal abnormality (kidney stones), which may be associated with repeated infections caused by *Proteus spp.* (Hugo and Russel, 1993). The pus cells, epithelial cells, and yeast cells observed also signifies an infection. A kidney infection can also lead to sepsis-pathogenic organism or toxin invading the blood or tissues and adult respiratory distress syndrome (ARDS) both of which can be life threatening (Dominic, 2006). Some of these pathogens isolated have been observed to bring about miscarriages, prevent future conception (infertility), blindness in newborn and several other damages to the foetus (Dominic, 2006).

The result of antimicrobial susceptibility of *Proteus spp.* to eleven (11) commonly prescribed antibiotics showed high resistance to eight antibiotics such as Amoxicillin, Ceftriaxone, Nitrofurantoin, Gentamicin, Cotrimoxazole, Amoxicillin/Clavulanic acid (Co-Amoxiclav®), Nalidixic acid and Tetracycline (47.9-100%). The result of susceptibility of *Escherichia coli* to eleven commonly prescribed antibiotics showed high resistance to ten antibiotics such as Amoxicillin, Ciprofloxacin, Ceftriaxone, Levofloxacin, Nitrofurantoin, Gentamicin, Cotrimoxazole, Co-Amoxiclav®, Nalidixic acid and Tetracycline (47-100%). *Staphylococcus aureus* showed high resistance to five antibiotics out of eleven: Ceftriaxone, Nitrofurantoin, Co-Amoxiclav®, Nalidixic acid and Tetracycline (66.7-100%).

The multiple antibiotic resistance index (MARI) observed in this study with reference to the tested antibiotics showed that 99.96% of the *Proteus spp.* isolates have MAR index of 0.2 to 1.0, while 99.97% of the *E. coli* isolates have MAR index of 0.2 to 1.0. This observation suggests that the isolates in this study may probably have originated from an environment where antibiotics are often used irrationally

(Paul *et al.*, 1997). Broad-spectrum antibiotics are sometimes reported to be given in place of narrow-spectrum antibiotics as a substitute for culturing and sensitivity testing, with the consequent risk of selection of antibiotic-resistant mutants (Krumperman, 1983).

MR/P fimbriae also known as type III fimbriae first isolated and purified by Sareneva *et al.* (1990) contributes to pathogenicity by colonization of the upper part of the urinary tract; they are strongly immunogenic (Bahrani *et al.*, 1994). MR/K hemagglutinins are different from the MR/P fimbriae in the tissue binding pattern. MR/K hemadhesins bind strongly to the Bowman's capsule of the glomeruli and to the tubular basement membranes and do not adhere to the epithelial cells of the urinary sediment. MR/K hemagglutinins contribute to pathogenicity through association with adhesion of strains to catheters (Mobley *et al.*, 1988 and Yakubu *et al.*, 1989).

Twelve (52.2%) *Proteus spp.* isolates were found to express a combination of MR/P+ and MR/K- hemagglutination pattern (i.e., more of type III fimbriae), 8 (34.8%) expressed a combination of MR/P+ and MR/K+ fimbriae. MR/P- and MR/K+ hemagglutination pattern was observed in 2 (8.7%) *Proteus spp.* isolates. One (4.3%) expressed a combination of MR/P- and MR/K- hemagglutination pattern. Some of these fimbriae are immunogenic and they contribute to pathogenicity by colonization of the upper part of the urinary tract of these pregnant women.

Type I fimbriae are common among *E. coli* strains from all clinical categories of Urinary tract infection. The *E. coli* isolates were found to have more of type I fimbriae, with the following hemagglutination pattern: MR/P+ and MR/K- 1(5.9%), MR/P+ and MR/K+ 9(52.9%), MR/P- and MR/K+ 3(17.6%), MR/P- and MR/K-

2(11.8%), Mannose Sensitive 0(0%), No hemagglutination of human erythrocytes tested, 2 (11.8%).

Urease catalyzes the hydrolysis of urea (a major excretory product) to yield ammonia and carbon dioxide which results in increase in urine pH. Urease production has been observed to result in the elevation of pH in the surrounding of bacterial growth resulting in stone formation and cytotoxicity (Antoni *et al.*, 1997). This enzyme has been implicated as a factor contributing to the pathogenicity of *Proteus spp.* Increase in urine pH leads to crystallization and aggregation of crystals (struvites) resulting in urinary stone formation, complicating urinary tract infection caused by *Proteus spp.* Urinary stones are good habitat for bacteria, and they block the flow of urine. Urease has been implicated as a factor contributing to the pathogenicity of *Proteus spp.* All the *Proteus spp.* isolates showed significant urease activity ranging from 24-110 ($\mu\text{mol NH}_3/\text{min}/\text{mg}$ protein). Forty seven (47) urine samples were observed in this study to have crystals which could aid the progression of bacteriuria in these pregnant women.

Hemolysin (a potent cytolytic) causes lysis of the epithelial cells of the urinary tract, and certain immune cells. Hemolysin increases the invasiveness of *Proteus spp.* Most hemolytic *E. coli* strains secrete α -hemolysin (Cavalieri *et al.*, 1984). Hemolysin production is associated with human pathogenic strains of *E. coli* especially those causing more severe forms of UTI. It is likely that the prevalence activity of hemolysin is multifactorial: including release of iron from erythrocytes, disruption of phagocyte function and direct toxicity to host tissues (the pregnant women). Hemolysin activity of all the isolates was measured as a function of growth.

Hemolytic activity that peaked during the late exponential phase of growth was seen in both the *Proteus spp.* and *E. coli* isolates. *E. coli* isolates had a higher geometric mean of hemolytic titre of 31.99, than the *Proteus spp.* isolates 29.23.

Bacterial plasmids are key vectors of horizontal gene transfer (HGT). These genes might alter the virulence of the host, confer metabolic benefits, or enable the bacteria to colonize new environments (Johnson *et al.*, 2007). Agarose gel electrophoretic analysis revealed the presence of single plasmid bands of approximately 2800bp in Ec11, 3500 bp in Ec13, 3700bp in Ec1, and 4000bp in *E. coli* isolates Ec2, and Ec3 respectively. Majority of the *E. coli* isolates are associated with multi-antibiotic resistance strains, but only 5(five) out of the 17(seventeen) *E. coli* isolates were seen to harbor plasmids. The virulence factors observed in these *E. coli* isolates (hemolysin production and their hemagglutination patterns) might have been responsible for the multiple drug resistance observed in the isolates that are not harbouring plasmids (Baylan *et al.*, 2011). Most of the *Proteus spp.* isolates were also resistant to multiple antibiotics, but only 1(one) was seen to harbor plasmid of 3500bp molecular size. The virulence determinants observed in these *Proteus spp.* isolates also might have contributed to the multiple antibiotic resistance in the *Proteus spp.* isolates (Soto *et al.*, 2009).

The β - lactams and cephalosporins are a group of antibiotics considered as safe in pregnancy, In *Proteus spp.* isolates, a high percentage of resistance of 100%, 65.2% and 56.5% were observed to Co-amoxiclav®, Amoxicillin and Ceftriaxone respectively. In *E. coli* isolates, a high percentage of resistance of 94.1%. 58.8%, and 47.0% was also observed to Co-Amoxiclav®, Ceftriaxone and Amoxicillin

respectively. The fluoroquinolones are contraindicated in pregnancy, however in a life threatening situation, the risk-benefit ratio can be considered and administered. In *Proteus spp.* isolates, resistance was observed to Levofloxacin (39.2%), Ofloxacin (26.1%), Ciprofloxacin (17.4) and Nalidixic acid (47.9%). In *E. coli* isolates, resistance was observed to Levofloxacin (58.8%), Ciprofloxacin (58.8%), Ofloxacin (29.4), and Nalidixic acid (53.0%).

There was no amplification of CTX-M2 genes (for β -lactam antibiotics) as was found by Park *et al.* (2006) and qnrC (for fluoroquinolones) as was also found by Wang *et al.* (2009) in all the *Proteus spp.* and *E. coli* isolates i.e., the genes coding for resistance to these antibiotics were not present in the isolates. There was also no amplification of CTX-M9 genes (for β -lactam antibiotics) as was found by Brinas *et al.* (2005) and qnrS (for fluoroquinolones) as was found by Cavaco *et al.* (2008) in all the *Proteus spp.* and *E. coli* isolates. However amplification of gyrB gene [to amplify a 448bp fragment of QRDR (quinolone resistance determining region) of gyrB, from nucleotides 995 to 1442] (Gene Bank Accession number D87842) was observed in 5 *Proteus spp.* isolates (P1, P2, P3, P4 and P5 respectively) and might be responsible for the resistance observed to the fluoroquinolones by these isolates. Only 1 (one) *Proteus spp.* isolate (P3) harboured a plasmid (5000bp), the other four amplified genes in *Proteus spp.* isolates P1, P2 P4 and P5 might not be plasmid-mediated. In 5 *E. coli* isolates (Ec1, Ec2, Ec3, Ec4 and Ec5), amplification of gyrB gene above was also seen (this might also responsible for the resistance observed to the fluoroquinolones by these isolates), and only *E. coli* isolates Ec1, Ec2, and Ec3 out of these 5 *E. coli* isolates harboured plasmids of 3700bp, 4000bp, and 4000bp molecular sizes

respectively. These amplified genes in Ec4 and Ec5 might also not be plasmid-mediated.

a. Summary and conclusion

- This study was designed to determine the prevalence of asymptomatic bacteriuria among pregnant women in Kano, Nigeria. Using 10^5 cfu/ml as significant level of bacteriuria, the prevalence of asymptomatic bacteriuria was found to be 15.2% (n=310).
- One hundred and ninety eight (198) samples had no pus cells with 24 showing significant bacteriuria. One hundred and twelve (112) samples has 1-4 pus cells/hpf (high power field) and > 5 pus cells/ hpf, with 20 showing significant bacteriuria. Two hundred and two (202) samples had epithelial cells, with 33 showing significant bacteriuria. Twenty six (26) samples had calcium oxalate crystals, 14 samples had calcium phosphate crystals, and 7 samples had amorphous phosphate
- The isolated organisms include *Proteus spp.* 23 (49%), *Escherichia coli*, 17 (36%), *Staphylococcus aureus*, 3 (6.4%), *Pseudomonas aeruginosa* 2 (4.3%), *Klebsiella spp.*, 2 (4.3%). *Proteus spp.*, [*Proteus mirabilis* 20 (87%), *Proteus vulgaris* 3 (13%)] was found to be the most predominant bacteria isolated, followed by *Escherichia coli*.
- There is also a changing pattern in the prevalence of the organisms causing asymptomatic bacteriuria; *Proteus spp.* is seen to be the most prevalent

uropathogen isolated, while previously reported organisms include *S. aureus*, and *E. coli*.

- The age group 25-30 years had the highest prevalence (24.2%) with respect to age, while the housewives had the highest prevalence (57.4%) with respect to occupational status. Pregnant women in the last trimester had the highest prevalence of asymptomatic bacteriuria (18.2%), followed by those in the second trimester (13.5%), then women in the first trimester (12.6%).
- The result of antimicrobial susceptibility of *Proteus spp.* to eleven (11) commonly prescribed antibiotics showed resistance to eight antibiotics such as Amoxicillin, Ceftriaxone, Nitrofurantoin, Gentamicin, Cotrimoxazole, Co-Amoxiclav®, Nalidixic acid and Tetracycline (47.9-100%).
- The result of susceptibility of *Escherichia coli* to eleven commonly prescribed antibiotics showed resistance to ten antibiotics such as Amoxicillin, Ciprofloxacin, Ceftriaxone, Levofloxacin, Nitrofurantoin, Gentamicin, Cotrimoxazole, Co-Amoxiclav®, Nalidixic acid and Tetracycline (47-100%).
- *S. aureus* showed resistance to five antibiotics out of eleven: Ceftriaxone, Nitrofurantoin, Co-Amoxiclav®, Nalidixic acid and Tetracycline (66.7-100%).
- The β -lactams and cephalosporins are a group of antibiotics considered as safe in pregnancy, In *Proteus spp.* isolates, resistance of 100%, 65.2% and 56.5% were observed to Co-amoxiclav®, Amoxicillin and Ceftriaxone respectively. In *E. coli* isolates, resistance of 94.1%, 58.8%, and 47.0% was also observed to Co-Amoxiclav®, Ceftriaxone and Amoxicillin respectively.

- The fluoroquinolones are contraindicated in pregnancy, however in a life threatening situation, the risk-benefit ratio can be considered and administered. In *Proteus spp.* isolates, resistance was observed to Levofloxacin (39.2%), Ofloxacin (26.1%), Ciprofloxacin (17.4) and Nalidixic acid (47.9%). In *E. coli* isolates, resistance was observed to Levofloxacin (58.8%), Ciprofloxacin (58.8%), Ofloxacin (29.4), and Nalidixic acid (53.0%).
- *Proteus spp.* isolates were found to express much more MR/P⁺, MR/K⁻ hemagglutinins 12(52.2%) than *E. coli* isolates 1(5.9%).
- *E. coli* isolates were observed to express MR/P⁺, MR/K⁺ hemagglutinins 9(52.9%) more than *Proteus spp.* isolates 8(34.8%).
- The hemagglutination pattern MR/P⁻,MR/K⁺ was more observed in *E. coli* isolates 3(17.6%), than in *Proteus spp.* isolates 2(8.7%).
- MR/P⁻, MR/K⁻ hemagglutinins were expressed by 2(11.8%) isolates of *E. coli* and 1(4.3%) isolate of *Proteus spp.*
- Mannose-sensitive (MS) adhesins were not observed in all the *Proteus spp.* and *E. coli* isolates.
- Two (11.8%) *E. coli* isolates failed to agglutinate with the human erythrocytes tested in this study.
- All the *Proteus* isolates showed significant urease activity ranging from 24-110 (μmol NH₃/min/mg protein).
- *Proteus spp.* isolates produced measurable hemolytic activity with titres ranging from 1:2 to 1:256, while that of *E. coli* showed a higher range of 1:2 to 1:512.

Geometric mean of reciprocal hemolytic titres for *Proteus spp.* isolates was 29.23, and for *E. coli* isolates 31.99.

- Only one (1) *Proteus spp.* isolate (P3) was seen to harbor plasmid of 5000bp molecular size. The virulence determinants observed in these *Proteus spp.* isolates also might have contributed to the multiple antibiotic resistance observed in the *Proteus spp* isolates.
- Most of the *E. coli* isolates are associated with multi-antibiotic resistance strains, but only 5 (five) out of the 17 (seventeen) *E. coli* isolates were seen to harbor plasmids. The virulence factors observed in these *E. coli* isolates (hemolysin production and their hemagglutination patterns) might have been responsible for the multiple drug resistance observed in the isolates that are not harbouring plasmids.
- Five *E. coli* isolates and 5 *Proteus spp.* isolates were found to amplify the *gyrB* genes with molecular weight of 50bp on polymerized chain reaction.
- None of the *Proteus spp.* isolates or the *E. coli* isolates was found to harbor the resistant plasmid (IncK) encoding *bla*_{CTX-M} ESBL genes, i.e., there was no amplification of pCT.
- It could be concluded that some of the multiple antibiotic resistant *Proteus spp.* and *E. coli* isolates observed in this study were plasmid-mediated, while some were not. Some of the virulence factors observed in these isolates especially those that don't harbor plasmids might have contributed to the multiple antibiotic resistance.

5.2 PUBLIC HEALTH IMPLICATION(S)

- The occurrence of these uropathogenic isolates in pregnant women gives cause for concern as they are prone to several complications as well as foetal risks.
- Complications of bacteriuria in pregnancy include maternal anaemia, increase in mid trimester abortion, low neonatal birth weight, hypertension, growth retardation and preterm labour.
- Urease production by *Proteus spp.* isolates will lead to kidney stones formation, complicating bacteriuria in pregnancy.
- Hemolysins are potent cytolysins which cause lysis of uroepithelial cells and some immune cells. They increase the invasiveness of uropathogens.
- MR/P and MR/K hemagglutinins (fimbriae) agglutinates human erythrocytes leading to maternal anaemia. They are also responsible for adherence to and colonization of the upper part of the urinary tract.

5.3 RECOMMENDATION(S)

- Approximately 25-30% of asymptomatic bacteriuria in pregnancy will progress to symptomatic infection, 3-4 times as great progression as in non-pregnant women (Dominic, 2006), hence there is need for early routine screening of all antenatal patients presenting or not presenting with clinical symptoms of urinary tract infection in order to prevent adverse maternal and neonatal outcome.
- Proper routine culture test should be carried out by clinicians for their antenatal patients; the strip urinalysis method which is been utilized by most clinicians for

assessing the urine of these pregnant women which cannot quantify the extent of infection as well provide adequate antimicrobial therapy as in the case of a culture test should be discouraged in antenatal clinics. Asymptomatic bacteriuria occurs without the usual clinical symptoms of UTI, most of these pregnant women might be infected with some uropathogens without their knowledge; and this could lead to adverse maternal and neonatal outcome.

- Asymptomatic bacteriuria must be treated in pregnancy in order to prevent complications such as pyelonephritis, premature labour, still birth, hypertension, preeclampsia and septicemia. (Raz, 2003).
- Rational use of antibiotics should be encouraged in all antenatal clinics to help fight the war against the emergence and transmission of multiple antibiotic resistant strains.
- There is need for the development of new and effective antibiotics that are safe in pregnancy.

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APPENDIX 1: Zone Inhibition of Commonly Prescribed Antibiotics against

Proteus spp., E. coli and S. aureus isolated at 37⁰ C for 18 hours

(Diameter Measurement in mm)

S/No	URINE ISOLATES CODE	AMOXICILLIN10µG	CIPROFLOXACIN 5µG	CEFTRIAXONE 30µG	LEVOFLOXACIN 5µG	NITROFURANTOIN 300µG	OFLOXACIN 5µG	GENTAMICIN 10µG	COTRIMOXAZOLE 25µG	CO-AMOXICLAV® 30µG	NALIDIXIC ACID 30 µG	TETRACYCLINE 30µG
1.	Pm 002	0.0	16.0	0.0	26.0	1.0	20.0	30.0	0.0	1.0	6.0	4.0
2.	Pm 021	2.0	18.0	12.0	12.0	9.0	14.0	10.0	0.0	10.0	2.0	0.0
3.	Pm 031	4.0	22.0	4.0	2.0	10.0	16.0	10.0	0.0	3.0	10.0	1.0
4.	Pv 040	0.0	0.0	2.0	0.0	14.0	0.0	8.0	0.0	2.0	1.0	2.0
5.	Pm 046	0.0	0.0	6.0	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
6.	Pm 049	1.0	12.0	22.0	16.0	16.0	20.0	14.0	16.0	9.0	12.0	0.0
7.	Pm 051	0.0	20.0	18.0	26.0	9.0	20.0	10.0	1.0	4.0	4.0	4.0
8.	Pv 059	2.0	22.0	12.0	22.0	1.0	24.0	8.0	0.0	0.0	8.0	1.0
9.	Pm 060	24.0	18.0	0.0	18.0	22.0	18.0	12.0	0.0	14.0	2.0	2.0
10.	Pm 069	0.0	26.0	14.0	18.0	0.0	20.0	14.0	0.0	0.0	0.0	3.0
11.	Pm 071	1.0	18.0	10.0	14.0	10.0	28.0	16.0	1.0	12.0	0.0	2.0
12.	Pm 088	2.0	20.0	12.0	10.0	6.0	22.0	9.0	0.0	10.0	2.0	0.0
13.	Pm 095	0.0	10.0	16.0	8.0	10.0	8.0	12.0	0.0	0.0	12.0	1.0
14.	Pm 113	0.0	18.0	8.0	12.0	9.0	12.0	9.0	0.0	16.0	2.0	1.0

S/No	URINE ISOLATE CODE	AMOXICILLIN 10µG	CIPROFLOXACIN 5µG	CEFTRIAZONE 30µG	LEVOFLOXACIN 5µG	NITROFURANTOIN 300µG	OFLOXACIN 5µG	GENTAMICIN 10µG	COTRIMOXAZOLE 25µG	CO-AMOXICLAV® 30µG	NALIDIXIC ACID 30µG	TETRACYCLINE 30µG
15.	Pm 117	3.0	24.0	4.0	22.0	10.0	24.0	4.0	1.0	8.0	2.0	0.0
16.	Pm 181	0.0	22.0	8.0	20.0	8.0	20.0	12.0	0.0	9.0	0.0	0.0
17.	Pm 123	0.0	18.0	20.0	16.0	18.0	18.0	16.0	0.0	6.0	1.0	0.0
18.	Pm 131	4.0	26.0	20.0	22.0	1.0	18.0	14.0	1.0	20.0	0.0	0.0
19.	Pm 243	2.0	20.0	22.0	18.0	4.0	22.0	8.0	0.0	12.0	1.0	0.0
20.	Pm 250	0.0	24.0	28.0	20.0	16.0	10.0	12.0	0.0	14.0	4.0	6.0
21.	Pv 271	1.0	20.0	18.0	14.0	8.0	20.0	4.0	2.0	10.0	8.0	0.0
22.	Pm 291	3.0	24.0	12.0	16.0	9.0	16.0	6.0	0.0	8.0	6.0	0.0
23.	Pm 301	0.0	22.0	14.0	18.0	2.0	18.0	10.0	0.0	6.0	1.0	0.0
24.	Ec 003	1.0	28.0	24.0	18.0	16.0	20.0	16.0	1.0	8.0	0.0	1.0
25.	Ec 023	1.0	20.0	12.0	14.0	16.0	12.0	8.0	1.0	22.0	10.0	0.0
26.	Ec 032	1.0	10.0	16.0	10.0	9.0	20.0	14.0	0.0	4.0	8.0	2.0
27.	Ec 037	0.0	0.0	0.0	0.0	20.0	24.0	0.0	0.0	14.0	8.0	0.0
28.	Ec 052	0.0	0.0	6.0	0.0	2.0	0.0	0.0	0.0	6.0	2.0	1.0
29.	Ec 064	0.0	24.0	14.0	18.0	0.0	16.0	14.0	0.0	0.0	10.0	1.0
30.	Ec 081	12.0	12.0	10.0	12.0	0.0	12.0	6.0	2.0	10.0	2.0	0.0
31.	Ec 084	1.0	0.0	1.0	0.0	8.0	16.0	1.0	1.0	1.0	1.0	0.0

S/No	URINE ISOLATES CODE	AMOXICILLIN 10µG	CIPROFLOXACIN 5µG	CEFTRIAXONE 30UG	LEVOFLOXACIN 5UG	NITROFURANTOIN 300UG	OFLOXACIN 5UG	GENTAMICIN 10UG	COTRIMOXAZOLE 25UG	CO-AMOXICLAV® 25UG	NALIDIXIC ACID 30UG	TETRACYCLINE 30UG
32.	Ec 116	3.0	10.0	14.0	18.0	6.0	22.0	4.0	2.0	18.0	12.0	4.0
33.	Ec 129	14.0	22.0	16.0	24.0	14.0	24.0	16.0	4.0	14.0	1.0	2.0
34.	Ec 187	0.0	20.0	20.0	22.0	12.0	18.0	18.0	12.0	16.0	0.0	0.0
35.	Ec 219	6.0	18.0	8.0	20.0	8.0	22.0	12.0	2.0	18.0	2.0	1.0
36.	Ec 229	12.0	24.0	14.0	18.0	10.0	26.0	10.0	0.0	10.0	4.0	1.0
37.	Ec 264	2.0	14.0	12.0	12.0	12.0	20.0	12.0	2.0	8.0	2.0	2.0
38.	Ec 266	6.0	12.0	0.0	4.0	1.0	0.0	12.0	0.0	8.0	0.0	0.0
39.	Ec 284	1.0	1.0	6.0	1.0	6.0	0.0	4.0	1.0	6.0	8.0	1.0
40.	Ec 288	0.0	0.0	0.0	0.0	14.0	30.0	0.0	0.0	14.0	2.0	1.0
41.	Sa 025	18.0	18.0	12.0	20.0	20.0	16.0	18.0	16.0	2.0	1.0	11.0
42.	Sa 034	18.0	18.0	10.0	18.0	12.0	14.0	20.0	14.0	20.0	0.0	0.0
43.	Sa 038	12.0	22.0	18.0	20.0	1.2	22.0	18.0	0.0	16.0	4.0	0.0

Pm: *Proteus mirabilis*

Pv: *Proteus vulgaris*

Ec: *Escherichia coli*

Sa: *Staphylococcus aureus*

APPENDIX 2: Bar chart of percentage sensitivity of *Proteus spp.* isolates to eleven commonly prescribed antibiotics

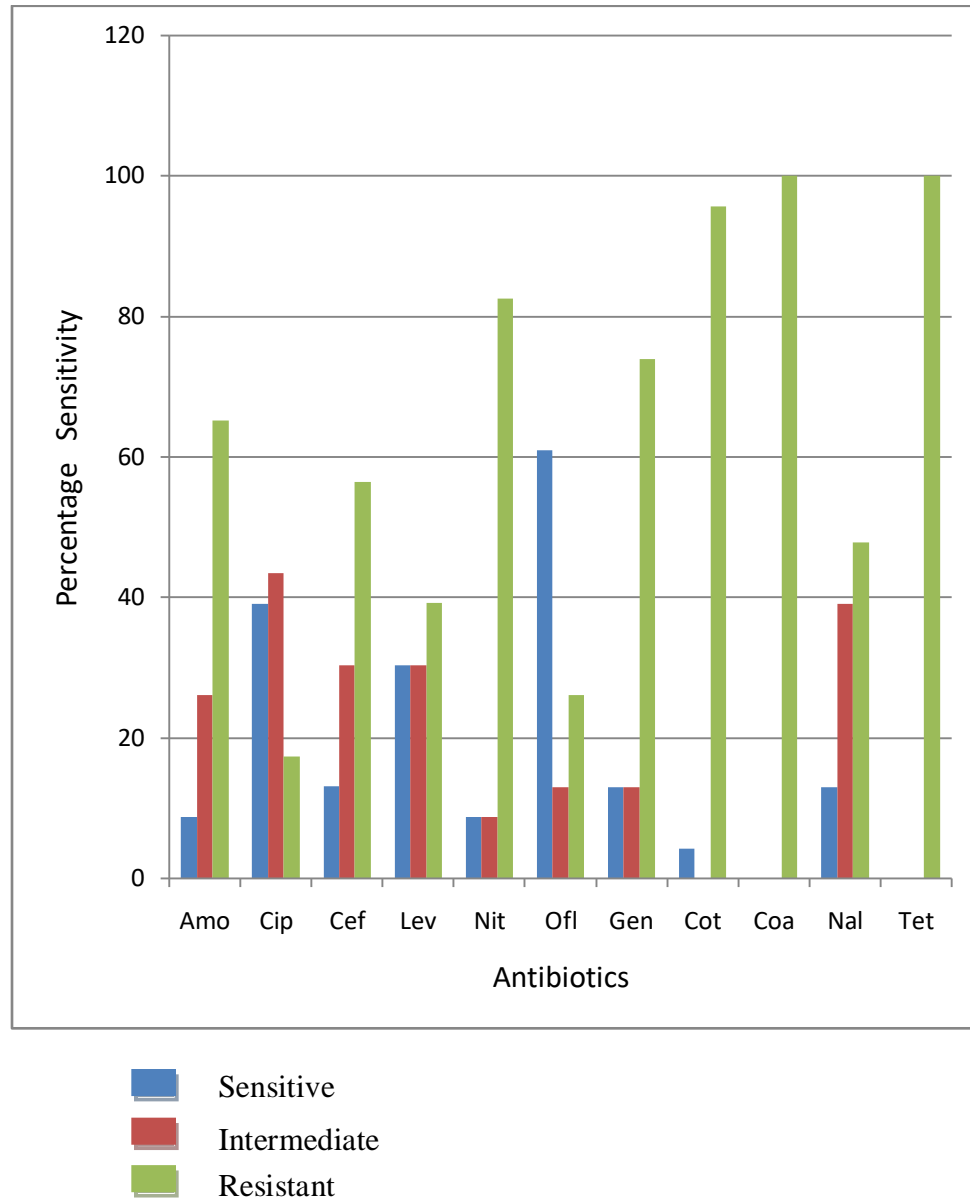


Fig. 8: Percentage Sensitivity of *Proteus spp.* isolates to 11 commonly prescribed antibiotics.

APPENDIX 3: Bar chart of percentage sensitivity of *E. coli* isolates to eleven commonly prescribed antibiotics

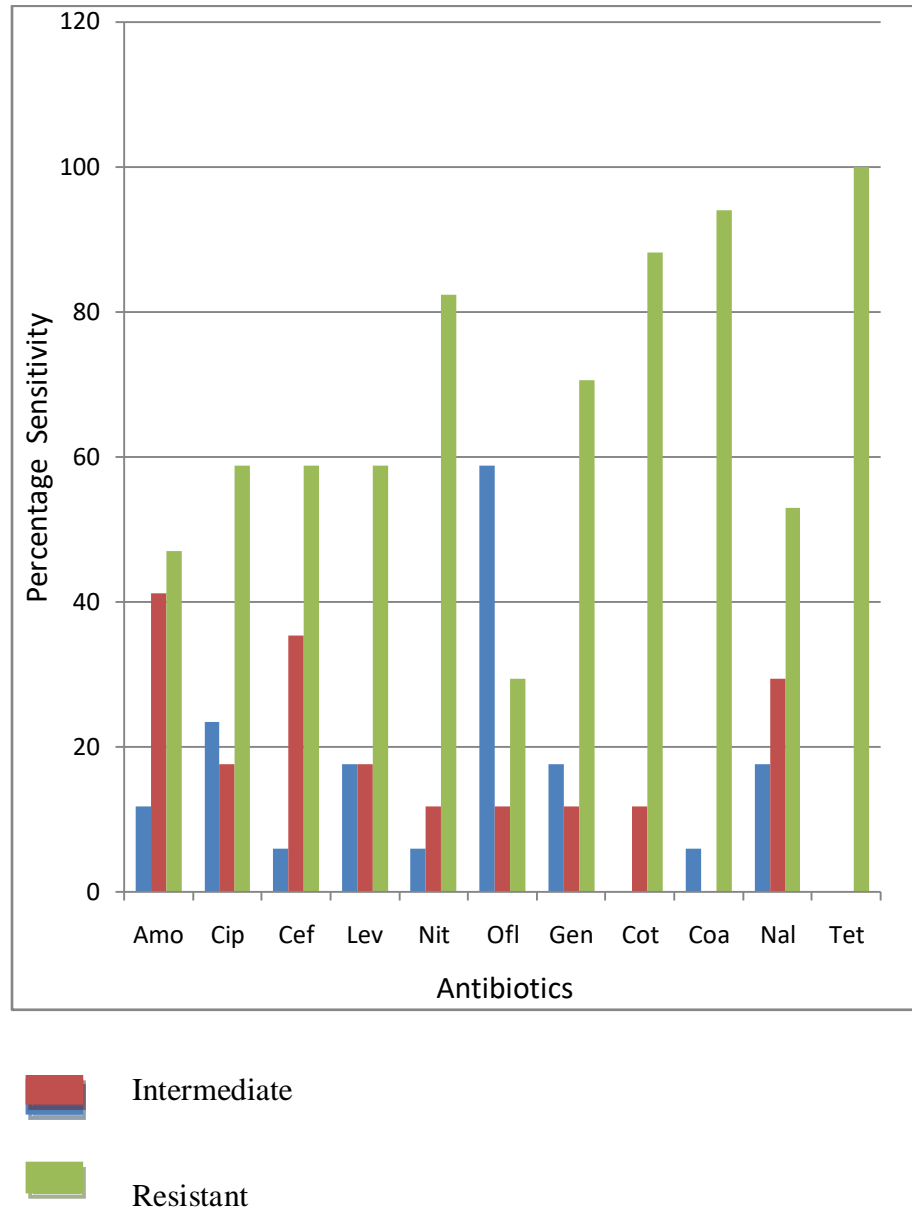


Fig. 9: Percentage Sensitivity of *E. coli* isolates to 11 commonly prescribed Antibiotics

APPENDIX 4: Bar chart of percentage sensitivity of *S. aureus* isolates to eleven commonly prescribed antibiotics

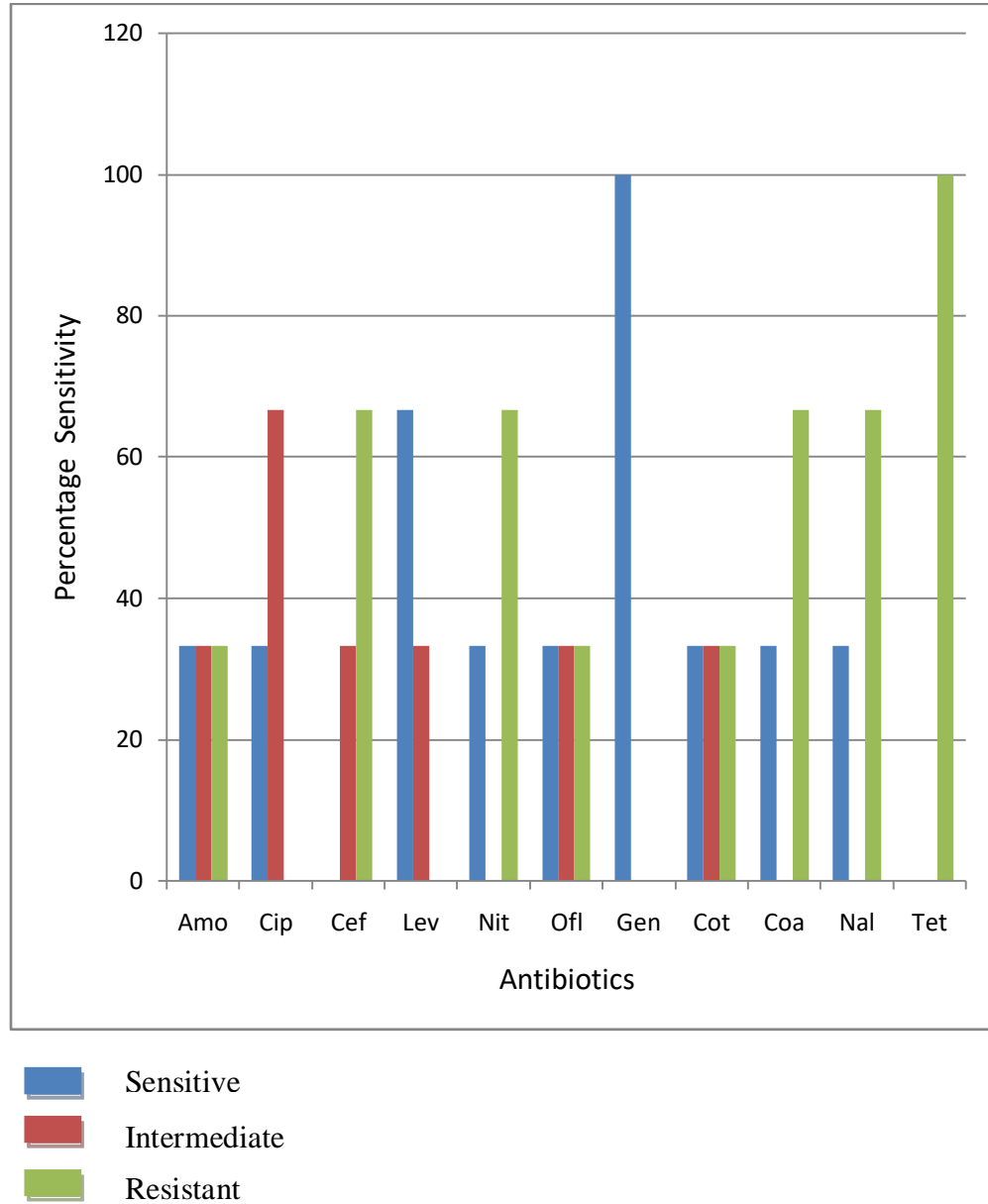


Fig. 10: Percentage Sensitivity of *S. aureus* isolates to 11 commonly prescribed antibiotics

APPENDIX 5: Bar Chart of Average Zone of Inhibition of *Proteus spp.*,
E. coli and *S. aureus* isolates

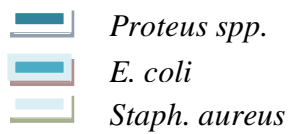
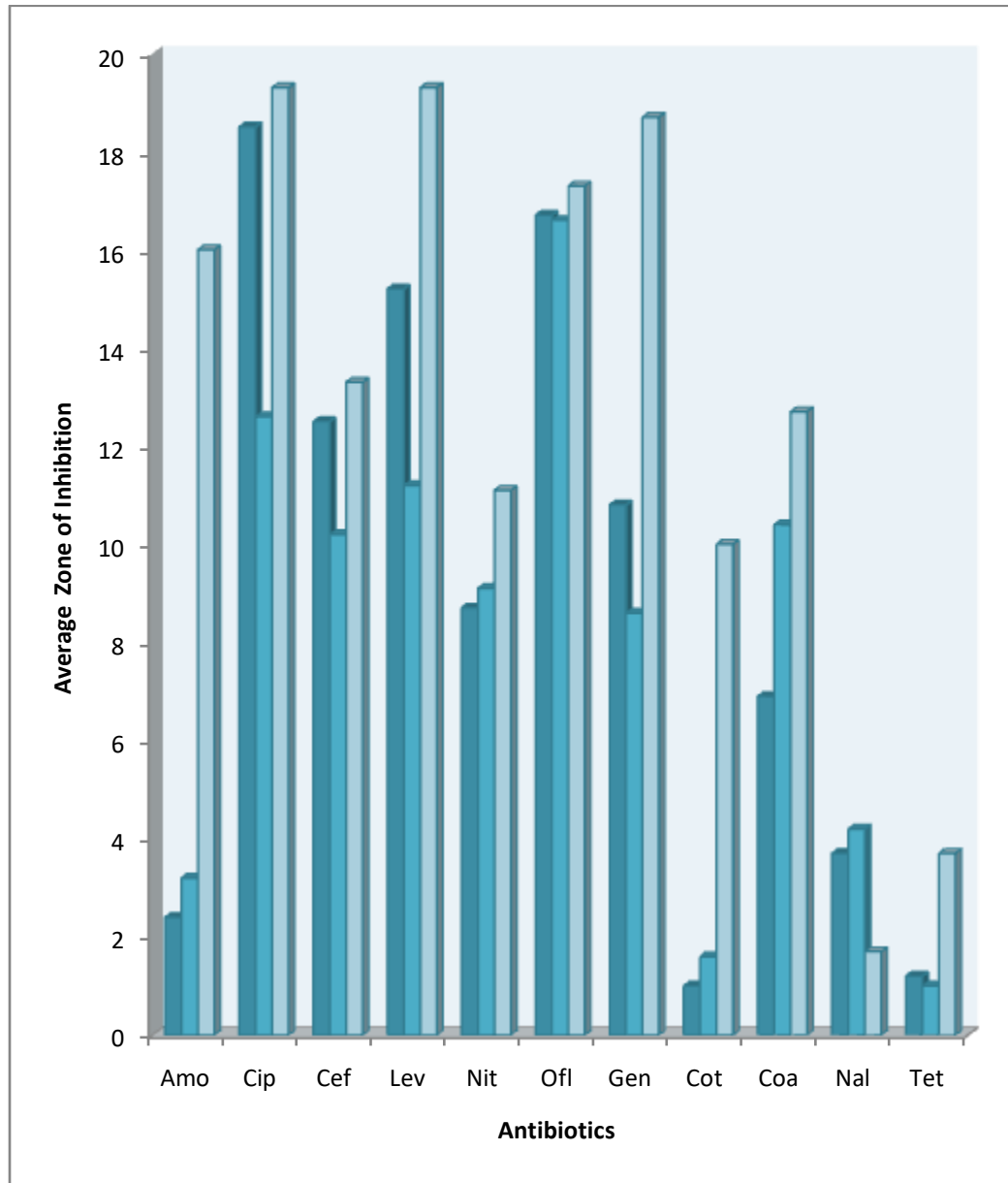


Fig. 11: Average Zone of inhibition of *Proteus spp.*, *E. coli* and *S. aureus* isolates

APPENDIX 6: Graph showing Urease activity of *Proteus spp.* isolates

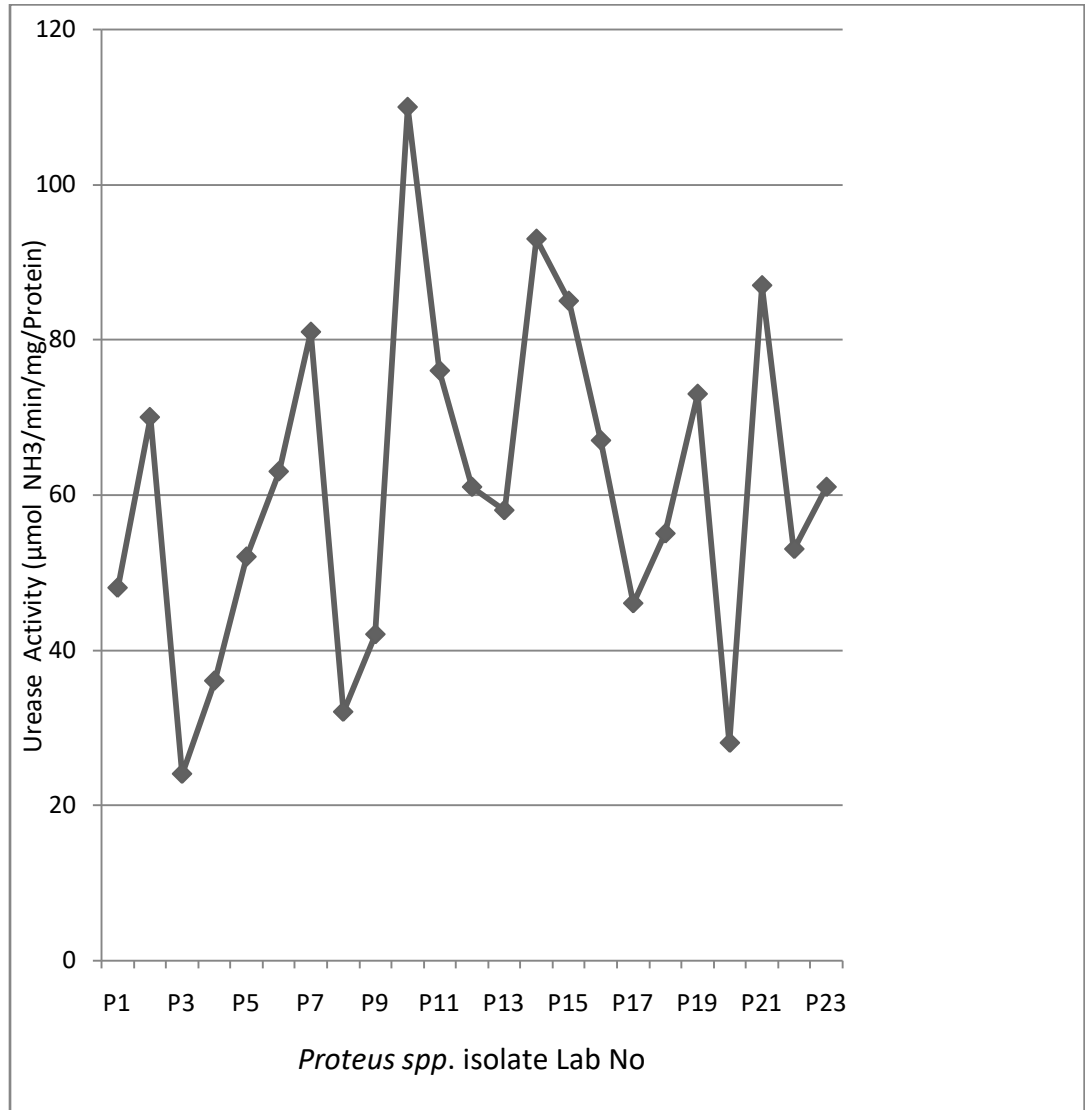


Fig. 12: Urease Activity of *Proteus spp.* isolates

APPENDIX 7: Graph showing Reciprocal Hemolytic titres of *Proteus spp.*
and *E. coli* isolates

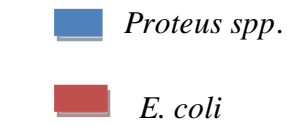
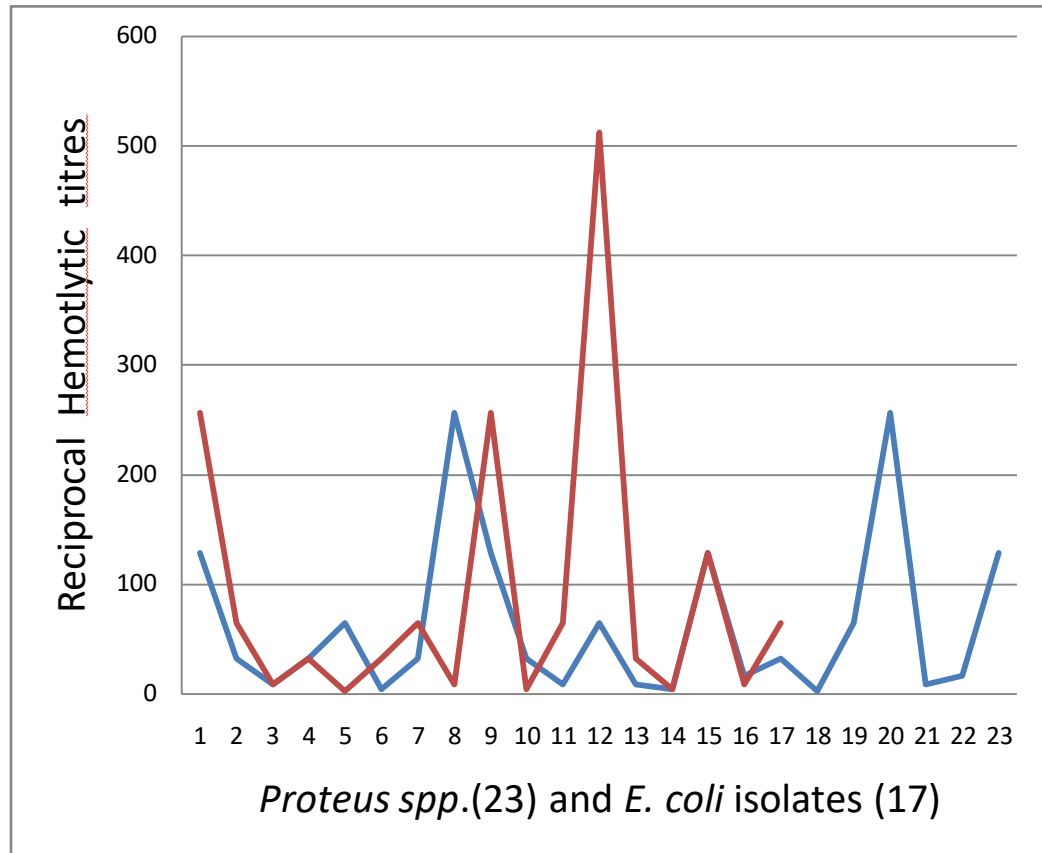


Fig. 13: Reciprocal Hemolytic Titres of 23 *Proteus spp.* and 17 *E. coli* isolates

APPENDIX 8: Primers Synthesis Report by Inqaba Biotech., South Africa.



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SYNTHESIS REPORT

02 Nov 2011

Client Detail: Elijah Ella
 Ahmadu Bello University
 Dept. of Microbiology / Lab no: 43-1st Floor
 Zaria
 Nigeria


Name:	ctx-M2-group Fwd		Barcode: S28E0	Length: 20 bases		
Sequence:	ATGATGACTCAGAGCATTCCG					
OD	8.9262	MW min \ max	6140.5/6140.5	5' Mod		None
nmoles	39.83	GC % min \ max	45/45	3' Mod		None
Tm min \ max	58.35/58.35		Purification	Standard		
For a 100 µM stock solution add 398.31 µl water or buffer				PAGE QC Image >>		
Comments						


Name:	ctx-M2-group rev		Barcode: S28E1	Length: 20 bases		
Sequence:	GAAACCGTGGGTTACGATTT					
OD	6.6184	MW min \ max	6171.6/6171.6	5' Mod		None
nmoles	38.84	GC % min \ max	45/45	3' Mod		None
Tm min \ max	58.35/58.35		Purification	Standard		
For a 100 µM stock solution add 388.39 µl water or buffer				PAGE QC Image >>		
Comments						

Name:	ctx-M-9 P1		Barcode: S28E2	Length: 21 bases
Sequence:	GTGACAAAGAGAGTGCAACGG			
OD	9.4012	MW min \ max	6552.3\6552.3	5' Mod None
nmoles	36.68	GC % min \ max	52.38\52.38	3' Mod None
Tm min \ max	62.57\62.57		Purification	Standard
For a 100 µM stock solution add 366.8 µl water or buffer			PAGE QC Image >>	
Comments				

Name:	ctx-M-9 P2		Barcode: S28E3	Length: 21 bases
Sequence:	ATGATTCTCGCCGCTGAAGCC			
OD	7.7843	MW min \ max	6381.6\6381.6	5' Mod None
nmoles	36.17	GC % min \ max	57.14\57.14	3' Mod None
Tm min \ max	64.52\64.52		Purification	Standard
For a 100 µM stock solution add 361.72 µl water or buffer			PAGE QC Image >>	
Comments				

Name:	qnrC fw		Barcode: S28E4	Length: 21 bases
Sequence:	GGGTTGTACATTTATTGAATC			
OD	8.1358	MW min \ max	6466\6466	5' Mod None
nmoles	35.48	GC % min \ max	33.33\33.33	3' Mod None
Tm min \ max	54.76\54.76		Purification	Standard
For a 100 µM stock solution add 354.81 µl water or buffer			PAGE QC Image >>	
Comments				

Name:	qnrC rev	Barcode: S28E5	Length: 18 bases			
Sequence:	TCCACTTTACGAGGTTCT					
OD	7.6285	MW min \ max	5440.5\5440.5		5' Mod	None
nmoles	42.52	GC % min \ max	44.44\44.44		3' Mod	None
Tm min \ max	55.34\55.34		Purification		Standard	
For a 100 µM stock solution add 425.22 µl water or buffer			PAGE QC Image >>			
Comments						

Name:	qnrS(1-2)F	Barcode: S28E6	Length: 18 bases			
Sequence:	TCGACGTGCTAACTTGCG					
OD	7.6456	MW min \ max	5490.3\5490.3		5' Mod	None
nmoles	41.28	GC % min \ max	55.56\55.56		3' Mod	None
Tm min \ max	59.9\59.9		Purification		Standard	
For a 100 µM stock solution add 412.83 µl water or buffer			PAGE QC Image >>			
Comments						

Name:	qnrS(1-2)R	Barcode: S28E7	Length: 21 bases			
Sequence:	GATCTAAACCGTCGAGTTCGG					
OD	10.1308	MW min \ max	6445.6\6445.6		5' Mod	None
nmoles	44.49	GC % min \ max	52.38\52.38		3' Mod	None
Tm min \ max	62.57\62.57		Purification		Standard	
For a 100 µM stock solution add 444.92 µl water or buffer			PAGE QC Image >>			
Comments						

APPENDIX 9: Preparation of Reagents

Agarose gel electrophoresis

Preparation of reagents:

Stock solution 20 times concentration (20x) of Tris Acetate Ethylenediamine

Tetra acetate (TAE) buffer: 0.8M Tris (hydroxymethyl) aminomethane, 0.4M sodium acetate, and 0.04 M disodium ethylenediamine tetraacetate (Na_2EDTA), and glacial acetic acid to pH 8.3 in distilled water.

96.9g	Tris base
32.8g	$\text{NaOAc}\cdot 3\text{H}_2\text{O}$
14.9g	Na_2EDTA

96.9g Tris base, 32.8g Sodium acetate and 14.9g disodium ethylenediamine tetra acetate were dissolved in 700ml of double distilled water, and the pH was adjusted to 8.3 with glacial acetic acid and the volume was made up to 1 litre with sterile distilled water. One time concentration of Tris Acetate Ethylenediamine tetra acetate (TAE) buffer was used for electrophoresis.

0.5M Na_2EDTA (disodium ethylenediamine tetra acetate) pH 8.0:

18.6g Na_2EDTA was dissolved in 60ml distilled H_2O , and the pH to 8.0 with 1M NaOH, the total volume was then made up to 100ml with distilled water.

Ethydium bromide:

5mg/ml ethyidium bromide (EtBr)

500mg Ethyidium bromide (Sigma E-8751)

Sterile distilled water to 100ml.

0.1% urea:

0.1g urea

Sterile distilled water to 100ml.

Phosphate Buffered Saline (PBS):

10x stock solution

80g NaCl

2g KCl

11g Na₂HPO₄·7H₂O

2g KH₂PO₄

Distilled water to 1Litre

50Mm Mannose:

Molar mass mannose=180.2g/mol

1000 mM=1M

9.01g Mannose

Distilled water to 1Litre

20m M Sodium Phosphate:

Molar mass Sodium phosphate=163.94g/mol

1000mM=1M

3.23g Sodium Phosphate

Distilled water to 1Litre.

3mM Sodium Phosphate:

Molar mass Sodium phosphate=163.94g/mol

1000mM=1M

0.49g Sodium phosphate

Distilled water to 1Litre.

120mM Urea:

Molar mass Urea=60.055g/mol

1000m M=1M

7.20g Urea

Distilled water to 1 Litre.

10M m Tris:

Molar mass of Tris=121.14g/mol

1000m M=1M

1.2g Tris

Distilled water to 1Litre.

Blood agar:

1. Suspend 42 g of the medium (**Blood agar base**) in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated **Sheep blood** to melted sterile agar medium, cooled to $45 - 50^{\circ}\text{C}$.

MacFarland Standard:

A BaSO_4 0.5 McFarland standard may be prepared as follows:

A 0.5-ml aliquot of 0.048 mol/L BaCl_2 (1.175% w/v $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

APPENDIX 10: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the plasmid DNA on agarose gel electrophotogram of 10 *E. coli* isolates (Ec1-Ec10).

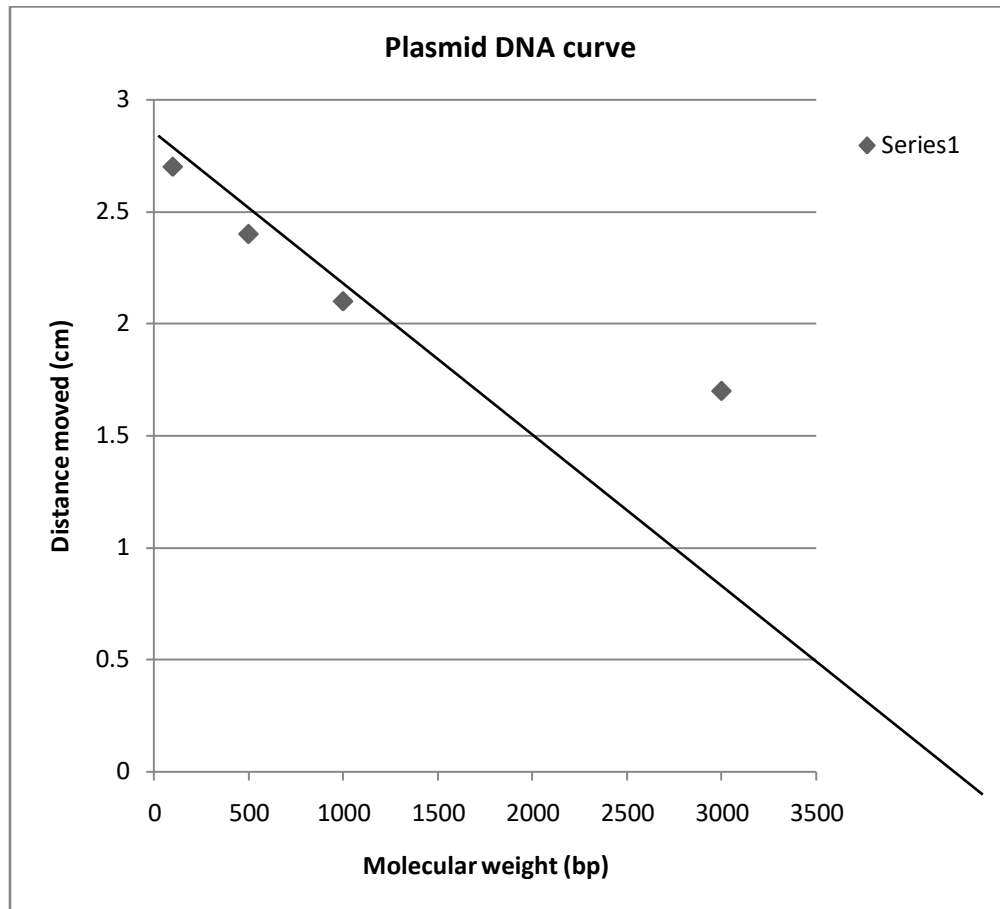


Fig. 14: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the plasmid DNA on agarose gel electrophotogram of 10 *E. coli* isolates (Ec1-Ec10). This was used to estimate the molecular weights of the isolated plasmids.

Lane 2 (Ec1): 0.4cm (distance moved); 3700bp (estimated molecular weight)

Lane 5 (Ec2): 0.3cm (distance moved); 4000bp (estimated molecular weight)

Lane 8 (Ec3): 0.3cm (distance moved); 4000bp (estimated molecular weight)

APPENDIX 11: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the plasmid DNA on agarose gel electrophotogram of 7 *E. coli* isolates (Ec11-Ec17) and 3 *Proteus spp.* isolates (P1-P3).

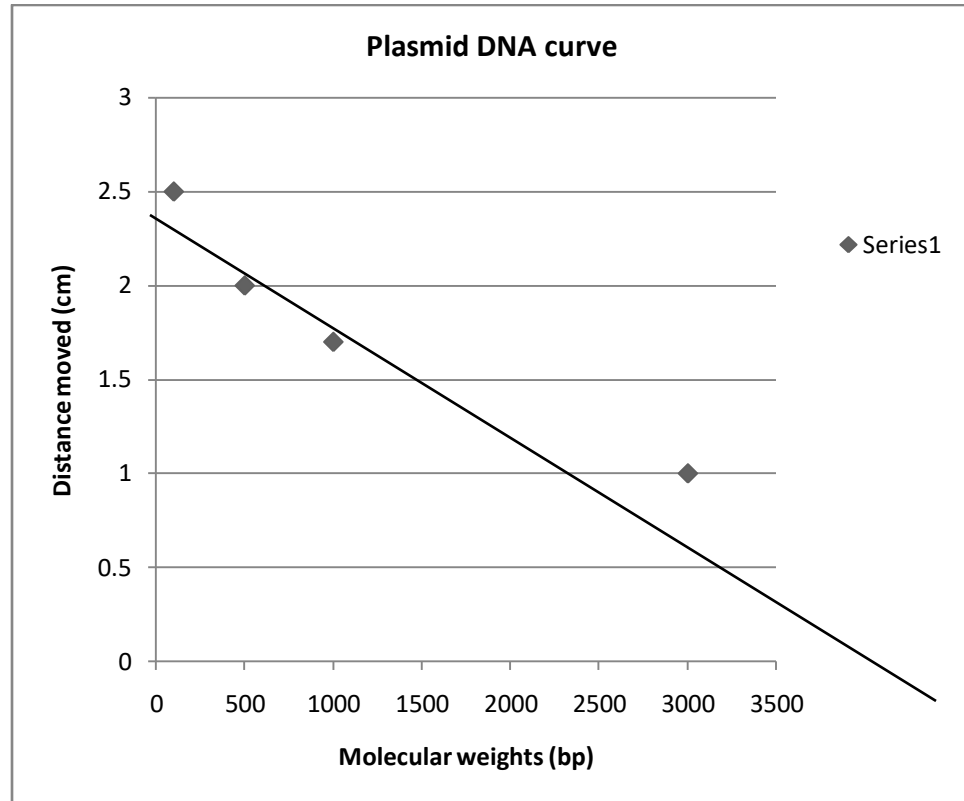


Fig. 15: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the plasmid DNA on the agarose gel electrophotogram of 7 *E. coli* isolates (Ec11-Ec17) and 3 *Proteus spp.* isolates (P1-P3). This was used to estimate the molecular weights of the isolated plasmids.

Lane 1 (Ec11): 0.9cm (distance moved); 2800bp (estimated molecular weight)
 Lane 7 (Ec13): 0.6cm (distance moved); 3500bp (estimated molecular weight)
 Lane 29 (P3): 0.6cm (distance moved); 3500bp (estimated molecular weight)

APPENDIX 12: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the amplified *gyrB* genes on agarose gel electrophotogram of 5 *E. coli* isolates (Ec1-Ec5) and 5 *Proteus spp.* isolates (P1-P5).

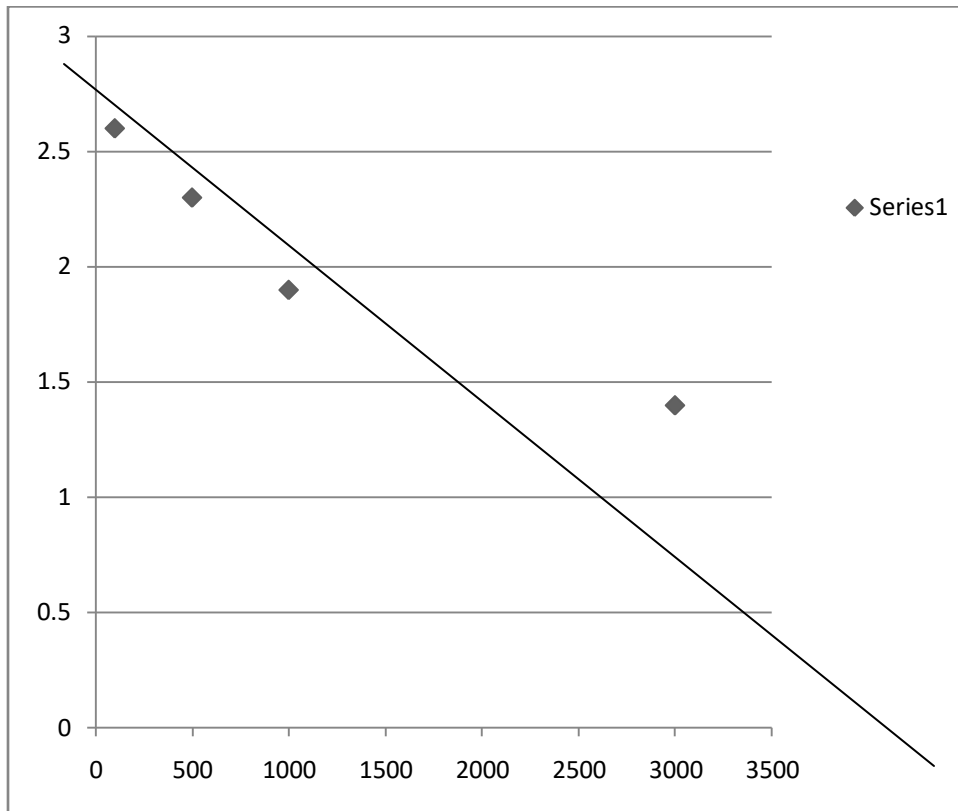


Fig. 15: Plasmid DNA curve of the molecular weight of the DNA marker/ladder *versus* the distance travelled by the amplified *gyrB* genes on the agarose gel electrophotogram of 5 *E. coli* isolates (Ec1-Ec5) and 5 *Proteus spp.* isolates (P1-P5). This was used to estimate the molecular weights of the *gyrB* genes.

Lanes 3, 6, 9, 12 and 15 (Ec1, Ec2, Ec3, Ec4 and Ec5 respectively): 2.8cm (distance moved); 50bp (estimated molecular weight)

Lanes 19, 22, 25, 28 and 30 (P1, P2, P3, P4 and P5 respectively): 2.8cm (distance moved); 50bp (estimated molecular weight).

APPENDIX 13: Statistical significance between percentage colonized and age group of pregnant women.

Using the χ^2 , at $P=0.05$, there is significant difference between the percentage colonized and the age groups of the pregnant women.

$$\chi^2 \text{ (calculated)} = 25.62$$

$$\chi^2 \text{ (table)} = 11.07$$

APPENDIX 14: Statistical significance between percentage colonized and gestational age (trimester) of pregnant women.

Using the χ^2 , at $P=0.005$, there is significant difference between the percentage colonized and gestational age (trimester) of the pregnant women.

$$\chi^2 (\text{calculated}) = 1.22$$

$$\chi^2 (\text{table}) = 0.071$$