MOLECULAR CHARACTERIZATION OF MULTIDRUG RESISTANT BACTERIAL UROPATHOGENS FROM PREGNANT WOMEN IN IBADAN METROPOLIS, SOUTH-WEST NIGERIA

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DECLARATION

I hereby declare that the work in this thesis titled "Molecular characterization of multidrug resistant bacterial uropathogens from pregnant women in Ibadan metropolis, Southwest Nigeria" was performed by me in the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria under the supervision of Prof. J.A. Onaolapo, Prof. A.R. Oyi, and Dr R.O. Bolaji. 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 MOLECULAR CHARACTERIZATION OF MULTIDRUG RESISTANT BACTERIAL UROPATHOGENS FROM PREGNANT WOMEN IN IBADAN METROPOLIS, SOUTHWEST NIGERIA by Durowaiye, Mojirayo Titilayo, meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY (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 my family.

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ABSTRACT

Urinary tract infection (UTI) is one of the commonest infectious diseases of women worldwide, and pregnant women are more predisposed. This study aimed to determine the prevalence of bacterial infecting agents in UTI during pregnancy, to establish their antimicrobial susceptibility profiles, identify the socio-economic factors that promote UTI. Virulence factor genes (VFGs) of the multi-drug resistant bacterial uropathogens were determined in order to establish their pathotypes. Clean catch mid-stream urine samples were collected, processed and analyzed from a total of 650 women on their first visit to antenatal clinics of three major health facilities in Ibadan metropolis. Demographic information was collected using structured questionnaires. Dip-stick leucocyte esterase test was performed and urinary isolates were identified using standard conventional biochemical tests and further confirmed to specie level with Microbact® identification kits (Oxoid). Modified Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility profile and interpreted as specified by the Clinical Laboratory Standard Institute (CLSI).Genomic DNA extraction and amplification of specific virulence genes associated with UTI by polymerase chain reaction (PCR) was carried out. Extended spectrum beta-lactamase production by the most prevalent multi-drug resistant Gram negative isolate was determined phenotypically and genotypically using universal primers.Molecular phylogenetic analysis by maximum likelihood method (MLM) was carried out for some sequenced isolates, and an evolutionary analysis was conducted using MEGA 7®. Significant bacteriuria was observed in 246(37.8%) antenatal patients, with 149(60.6%) being asymptomatic. The major bacterial species recovered included *Staphylococcus epidermidis* 51(21.9%), *Escherichia coli* 48(19.5%), *Staphylococcus aureus* 27(10.9%), *Streptococcus*spp

17(6.0%), *Providencia stuartii* 12(4.8%), *Proteus mirabilis* 13(5.2%),*Staphyloccocus saprophyticus* 12(4.9%),*Enterobacter agglomerans* 8(3.2%). Others including *Staphylococcus* sppranged from 0.4% to 2.3%. The highest prevalence of bacteriuria with respect to gestational age, women's age group, occupational status and educational level were 53.2% (n=131) third trimester, 44.2% (n=101) 25-30 years, 35.3% (n=87) housewives, and women with no formal education 32.1% (n=79) respectively and were statistically significant at $P<0.05$. Majority (69.5%) of the isolates were multidrug resistant. The isolates were most resistant to Cotrimoxazole (66.6%), and most susceptible to Nitrofurantoin (11.9% resistance). Phenotypic ESBL production was seen in 35(72.9%) of *E. coli* isolates; the ESBL genes amplified were *bla CTX-M* 10(100%), *blaTEM* 10(100%), *bla OXA* 10(100%), and *bla SHV* 7(70%). Virulence genes amplified in the analyzed MDR ESBL *E. coli* isolates were *fim H* 10(100%), *pap* 10(100%), *cnf* 5(50%), upa Bc 10(100%), *qep* 5(50%), *Int 1* 10(100%), *IS 256* 8(80%), *amp C* 8(80%), *aac* 1(10%), *tsp* 10(100%), *yjaA* 9(90%), but *chuA* gene was absent. Virulent extra-intestinal phylogenetic group B2 strains of uropathogenic *E. coli* (UPEC) 7(70.0%) were identified. In the analyzed MDR *S. aureus* isolates, using simplex PCR, the following were amplified: *mec A* 2(20%), *amp C* 10(100%), *ica D* 8(80%), *ica A* 5(50%), *Int 1* 10(100%), *IS 256* 7(70%), *upa Bc* 10(100%), while *pvl, hly, aer, qep* and *upa Cc* genes were absent. The nucleotide sequence of *16SrRNA* gene of all the MDR isolates used in the molecular analysis in comparison with those from GenBank database revealed 100-99% identity.

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LIST OF ABBREVIATIONS

UTI: urinary tract infection

ASB: asymptomatic bacteriuria

cUTI: complicated urinary tract infection

PCR: polymerase chain reaction

MARI: multiple antibiotic resistance index

VGs: virulence genes

AST: antimicrobial susceptibility testing

ESBL: extended spectrum beta-lactamase

VFs: virulence factors

CLSI: clinical laboratory standard institute

DNA: deoxyribonucleic acid

UPEC: uropathogenic *Escherichia coli*

MEGA: molecular evolutionary genetic analysis

MGEs: mobile genetic elements

Cnf: cytotoxic necrotizing factor

Pvl:panton valentine leukocidin

Ica: intracellular adhesion factor

IS: insertion sequence

Int: intergron/integrase

Qep: quinolone exflux pump

MRSA: methicillin resistant *S. aureus*

MSSA: methicillin sensitive *S. aureus*

GNB: gram negative bacillus

LIST OF ABBREVIATIONS (continued)

CLED: cystein lactose electrolyte deficient agar

NCCLS: national committee for clinical laboratory standards

AT: autotransporter

ATCC: american type culture collection

BLAST: basic local alignment sequence tool

MDR: multi-drug resistance

LET: leucocytes esterase test

NCBI: national center for biotechnology information

MLM: maximum likelihood method

ADRS: adult respiratory distress syndrome

HGT: horizontal gene transfer

PBP: penicillin binding protein

PABA: para-aminobenzoic acid

VFGs: virulence factor genes

SIRS: systemic inflammatory response syndrome

CA-MRSA: community-associated methcillin-resistant *S. aureus*

HA-MRSA: hospital-acquired methicillin resistant *S. aureus*

MSCRAMM: microbial surface component recognizing adhesive matrix molecules

CoNS: coagulase negative *Staphylococci*

hPMNLs: human polymorphonuclear leucocytes

MGEs: mobile genetic elements

PIA: polysaccharide intercellular adhesin

QIR: quiescent intracellular bacterial reservoirs

LIST OF ABBREVIATIONS (continued)

ARSG: Aminoglycoside resistance study groups

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CHAPTER ONE

1.0 INTRODUCTION

Urinary tract infection (UTI) is among the most common health problem affecting women in their reproductive ages (Onyango *et al*., 2018). UTI is defined as significant bacteriuria in the presence of a constellation of symptoms such as dysuria (painful urination), increased urinary frequency and urgency, suprapubic discomfort and costovertebral angle tenderness. Microbial colonization of the urine and tissue invasion of any structure of the urinary tract could occur. Bacteria are most commonly responsible (Onyango *et al*., 2018), although yeast and viruses may also be involved. It is a common cause of infections, particularly among young, sexually active women (Foxman, 2003a). UTI occurs much more frequently in female than male due to the proximity of the urethra to the anus (Foxman, 2003a). Approximately 50% of all women will have at least one UTI in her life time with many women having several infections through their life time (Foxman, 2003a). UTIs reportedly account for more than 8 million outdoor patient visits, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually with a total annual cost of more than $3.5 billion. (Yadav, 2015). Fortunately, this infection is rapidly responsive to modern antibiotic therapy, so early diagnosis is required to prevent the morbidity.

Globally, UTI is the most frequent bacterial infection. (Kudinha, *et al.,* 2012). It is often characterized by significant bacteriuria (asymptomatic or symptomatic). UTIs are one of the most common medical complications of pregnancy (Mittal and Wing, 2005). 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). Pregnant women are

predisposed to UTI due to the physiologic and hormonal changes that occur during pregnancy (Imade *et al*., 2010; Assel *et al*., 2011).

In many hospitals in developing countries, routine urine culture is not carried out even for antenatal mothers. Most patients are treated empirically without culture and antimicrobial susceptibility testing (AST) and treatment is therefore based on empiric guidelines that are rarely updated (Kose *et al*., 2007). Even where UTI tests are done, only dipstick analysis of urine is used, but this test has poor positive and negative predictive values to detect bacteriuria particularly in asymptomatic persons (Nicolle *et al*., 2005). In order to prevent or reduce maternal and fetal morbidities in low resource settings, antibiotics are usually given empirically before the urine culture reports are available (Nithyalakshmi and Vijayalakshmi, 2014). Poor socioeconomic status is reported to be a major risk factor with indigent patients having a fivefold increased risk (Johnson and Wolf, 2013). The emergence of multidrug resistant uropathogens with virulent traits has led to adverse obstetric and neonatal outcomes (Ramos *et al*., 2012).

1.1 Urinary tract infection and pregnancy

Globally, the prevalence of UTI in pregnancy reportedly, ranges between 13% - 33% with symptomatic bacteriuria occurring in 1% - 18% while asymptomatic cases are noted in 2% - 10% of women (Agersew *et al*., 2012). UTIs represent the most common bacterial infection in pregnant and non-pregnant women (Foxman, 2003a; Foxman, 2003b). Eight million women visit a physician annually for evaluation of UTIs (Schappert, 1996) at a direct cost of $659 million and aggregate cost of $1.6 billion (Foxman *et al*., 2000).

UTI is one of the common bacterial infections that have been implicated to complicate pregnancy (Addo, 2002; Cunningham *et al.,* 2001). Pregnant women are more susceptible to

UTIs due to a combination of hormonal and physiologic changes that predispose them to bacteriuria (Aseel *et al*., 2011). Progesterone effects and mechanical compression by the gravid uterus impair emptying of the bladder and lead to increased bladder residual volume and vesicoureteral reflux. Relative stasis of urine in the ureter results in hydronephrosis. Bacterial growth is facilitated by changes in the glomerular filtration rate which increases the urinary glucose concentration and alkalinity. In addition, alterations in maternal immunologic defense mechanisms occur in pregnancy (Christensen, 2000). The signs and symptoms of UTIs vary by the type of infection. UTI in pregnancy is classified by the site of bacterial proliferation as follows: asymptomatic bacteriuria (ASB; urine), cystitis (bladder), pyelonephritis (kidney).

1.2 Asymptomatic bacteriuria in pregnancy

Bacteriuria refers to the presence of bacteria in voided urine. Asymptomatic bacteriuria 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 UTI such as burning during micturition or frequent urination. This is common during pregnancy ((Imade *et al*., 2010; 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). Bacterial growth in the urine is been encouraged by glucosuria, physiological increase in plasma volume and decrease in urine concentration. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as premature birth, low birth weight and higher foetal mortality rates (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; Hill *et al*., 2005). Asymptomatic bacteriuria has also been implicated in postpartum 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 (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 pneumonia*, *Proteus spp.,* Enterococciand 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. 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. 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 Virulence of micro-organisms

Virulence (from the Latin word for poisonous) is defined as the ability of an organism to cause disease in a particular host. A number of biological features known as virulence factors are

common to many bacterial species, although some of these are characteristic only for certain bacteria. Their presence helps in the pathotype classification of some micro-organisms.

1.4 Antibiotic resistance in UTI

The emergence of resistant bacterial uropathogens in UTI has been reported by some researchers (Imade *et al*, 2010, Gravett *et al*., 2012, Perera *et al*., 2012, Bolaji *et al*., 2013, Durowaiye *et al*., 2015, Ezeh *et al*., 2016, Hassan *et al*., 2016, Onyango *et al*., 2018). Many Gram-negative organisms have been reported to be refractory to Penicillin due to intrinsic bacteria resistance by species originally considered sensitive, resulting in a wide chemotherapeutic failure worldwide (Okeke *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); which 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.

Some factors which can affect the mechanisms above include: Spontaneous mutation, bacterial DNA may mutate spontaneously. Transformation: A bacterium may take up DNA from another bacterium, penicillin-resistant gonorrhea 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 ampicilin. This resistance is known to be coded for by R-plasmid RPI (Onaolapo, 1986).

1.5 Statement of research problem

Asymptomatic bacteriuria has been observed to be a major risk factor in developing symptomatic 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 can result in pyelonephritis and is associated with an increased prenatal mortality. Further studies have revealed possible association of asymptomatic bacteriuria with adverse neonatal outcomes (Perera *et al*., 2012; Hassan *etal*., 2016).It is now documented that women in the child bearing age have the highest incidence of urinary tract infection.

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), 45.3% (Imade *et al*., 2010), 58.4% (Bolaji *et al.,* 2013), 15.7% (Durowaiye *et al.,* 2015), 16.0% (Ezeh *et al.,* 2016).

1.6 Justification of study

Majority of the 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 is inadequate. 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.

The pathological agents of UTI and its susceptibility profile vary according to geographical, social and biological settings. Therefore, it is necessary to identify the etiologic agent and establish its susceptibility profile in order to select appropriate antimicrobial drugs for the management of patients suffering from UTIs.

Hence, the present study was carried out to determine the range of bacterial isolates and their antibiotic susceptibility among pregnant women attending antenatal clinic.

1.7 Aim and specific objectives

1.7.1 Aim

This study aimed to molecularly characterize multidrug resistant bacterial uropathogens from pregnant women attending antenatal clinics in Ibadan metropolis, south-west Nigeria.

1.7.2 Specific objectives

In order to achieve the aim stated above, the specific objectives are to:

- isolate the organisms causing bacteriuria in pregnancy.
- determine the prevalence of bacteriuria (asymptomatic and symptomatic) in pregnancy.
- establish the socio-economic factors influencing UTI by determining the prevalence of bacteriuria with respect to gestational age, parity, age group, educational level and occupational status.

- establish the antimicrobial susceptibility profile of the isolates to commonly prescribed antibiotics.
- phenotypically and genotypically determine ESBL production in the most prevalent Gram negative isolates.
- determine the pathotype of multidrug resistant bacterial isolates through specific virulence genes amplification using polymerase chain reaction (PCR).

1.9 Research question(s)

- What is the prevalence of bacteriuria (both symptomatic and asymptomatic) among pregnant women attending antenatal clinics in Ibadan metropolis?
- What are the socio-economic factors affecting bacteriuria among pregnant women in Ibadan metropolis?
- Have multidrug resistant bacterial uropathogens emerged among pregnant women attending antenatal clinics in Ibadan metropolis?
- Do these bacterial uropathogens possessvirulence genes (VGs)?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Urinary tract infection

Urinary tract infection (UTI) is an infection caused by the presence and growth of micro-organisms in the urinary tract. It is one of the common bacterial infection worldwide (Getenet and Wondewosen, 2011), and the second most common clinical indication for empirical antimicrobial treatment in primary and secondary care (Mc Kenzie *et al.,* 1994). The urine is ideally sterile in healthy individuals and constantly flushes the urinary tract. Organisms invading the urinary tract (Fig. 2.1) 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). Urinary tract infections (UTIs) are among the most prevalent infectious diseases and are the cause of more than 7 million physician visits in the United States each year (Azadeh *et al*., 2010). These infections are diagnosed in 1% to 3% of school-aged girls and in 2% to 8% of pregnant women. Symptomatic infections in upper urine system are unusually prevalent in pregnancy, while 20%-30% of pregnant women with asymptomatic bacteriuria complicated with pyelonephritis (Stamn, 2008). Almost, all of women

have at least one episode of UTI in their lifetime (Norrby, 2008) however, 25% of women experience symptomatic UTI at least once in their lifetime (Jack *et al*., 2010).

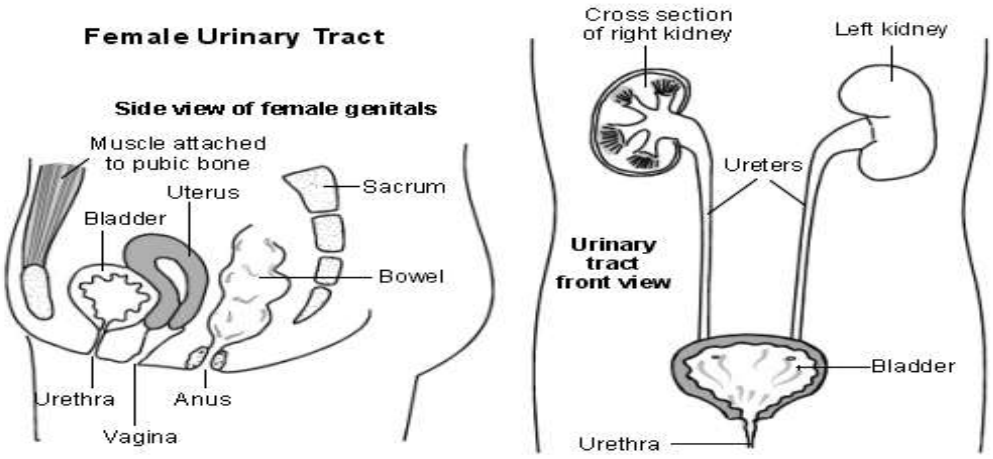


Figure 2.1: The Female urinary tract (Side view and front view)
Dalston medical group (dalstonmedicalgroup.uk)

UTIs can result in sepsis if not recognized and treated rapidly. Failure to treat or a delay in treatment can result in Systemic inflammatory response syndrome (SIRS), which carries a mortality rate of 20% - 50%. The pathological agents for UTI and its susceptibility pattern can vary according to the geographical, social and biological settings. Therefore, it is necessary to identify the etiological agent and its susceptibility pattern in order to select appropriate antimicrobial drugs for the management of patients suffering from UTIs.

2.1.1 The urinary tract

The urinary tract include organs that collect, stores urine and release it from the body such as the kidneys, ureters, bladder and urethra. Anatomically, UTI can be classified as upper UTI involving the bladder and urethra; lower UTI involving the kidney, pelvis and ureter. Infection may involve either only the lower urinary tract or both the upper and lower tracts. The term

cystitis is used to describe the syndrome involving dysuria, suprapubic tenderness with urinary frequency and urgency. These symptoms may also be related to lower tract inflammation without bacterial infection and can be caused by urethritis (ex.gonorrheal or chlamydial urethritis). Acute pyelonephritis refers to the syndrome of cystitis accompanied by significant bacteriuria and acute infection in the kidney; it is characterized by clinical symptoms such as flank pain, fever, dysuria, urinary urgency and frequency. Lower UTI: cystitis, urethritis, prostatitis. Upper UTI: pyelonephritis, intra-renal abscess, perinephric abscess (usually late complications of pyelonephritis), uncomplicated UTI- infection in a structurally and neurologically normal urinary tract. Simple cystitis of short (1-5 day) duration, complicated UTI- infection in a urinary tract with functional or structural abnormalities (ex. indwelling catheters and renal calculi). Cystitis of long duration or hemorrhagic cystitis. The likelihood of treatment failure and serious complications, particularly the development of antimicrobial resistance, is more common in cUTI.

UTIs are among the most common infectious diseases occurring in either the community or healthcare setting (Nicolle, 2005). Uncomplicated UTIs typically occur in the healthy adult non-pregnant woman, while complicated UTIs may occur in all sexes and age groups and are frequently associated with either structural or functional urinary tract abnormalities. The genitourinary tract is the site of disease responsible for Gram-negative bacteremia in approximately 35% of patients. In previously healthy outpatients, *E. coli* is by far the most often implicated cause of UTIs. In contrast, individuals with multiple prior episodes of UTI, multiple antibiotic treatments, urinary tract obstruction, or infection developing after instrumentation frequently become infected with *Proteus* bacteria or other bacteria such as *Enterobacter, Klebsiella, Serratia,* and *Acinetobacter*. Bacteriuria occurs in 10% - 15% of hospitalized patients

with indwelling catheters. The risk of infection is 3% - 5% per day of catheterization. Among long-term care residents, UTIs are the second most common infection responsible for hospital admission, second only to pneumonia.

2.2 Urinary tract infection in pregnancy

UTIs in pregnant women continue to pose a clinical problem and a great challenge for physicians (Matuszkiewicz-Rowińska *et al*., 2015). Although the incidence of bacteriuria in this population is only slightly higher than in non-pregnant women, its consequences for both the mother and the unborn child are more severe. There is a much higher risk (up to 40%) of progression to pyelonephritis, and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight (Schnarr and Smaill, 2008; Farkash *et al*., 2012; Gravett *et al*., 2012; Foxman, 2002; Mazor-Dray *et al*., 2009; Bolton *et al*., 2012). That is related to profound structural and functional urinary tract changes, typical for pregnancy. In about 80% of pregnant women dilation of the urinary tract combined with slight hydronephrosis is observed, caused partly by a reduction in smooth muscle tone with slowing of ureteral peristalsis, and partly by urethral sphincter relaxation. This may be due to high levels of circulating progesterone (Schnarr and Smaill, 2008; Jeyabalan and Lain, 2007). Simultaneously, the enlarged uterus compresses the urinary bladder, thus increasing the intravesical pressure, which may result in vesico-ureteral reflux and urine retention in the bladder after micturition, commonly observed in pregnant women. Urinary stasis and impairment of the physiological anti-reflux mechanism create conditions favorable for bacterial growth and ascending infection (Matuszkiewicz-Rowińska *et al*., 2015).

The additional predisposing factors include pregnancy-specific biochemical changes in urine, with higher amounts of glucose, amino acids and hormone degradation products, which increase urinary pH (Jeyabalan and Lain, 2007; Jolley and Wing, 2010). Similarly as in non-pregnant women, in pregnant women UTIs are classified either as asymptomatic bacteriuria (ASB), when the infection is limited to bacterial growth in urine, or symptomatic infections (acute cystitis, acute pyelonephritis), when bacteria invade urinary tract tissues, inducing an inflammatory response. The UTIs in pregnancy are by definition considered complicated infections and require a special diagnostic approach and management (Matuszkiewicz-Rowińska *et al*., 2015).

2.2.1 Asymptomatic bacteriuria (ASB)

ASB is defined as significant bacterial colonization of the lower urinary tract. Asymptomatic bacteriuria, in which urine culture reveals a significant growth of pathogens, that is greater than 10^5 bacteria/ml (Gilbert *et al*., 2005) but without the patient showing symptoms of UTI, can be found in both pregnant and non pregnant women. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, lowbirth weight, and higher fetal mortality rates (Delzell and Leferre, 2000).

In Nigeria, Olusanya *et al* in Sagamu reported a prevalence rate of 23.9% in 1993. Akerele *et al* also reported 86.6% in Benin city in 2001. Some other studies conducted includes Imade *et al*., (2010), Durowaiye *et al*., (2015), Bolaji *et al*., (2013), Ezeh *et al*., (2016), with prevalence rates of 45.3%, 15.8%, 58.4% and 16.0% respectively. A prevalence rate of 9.2% in pregnant women has been reported in Ethiopia (Getenet and Wondewosen, 2011). In Canada the prevalence rate varies from 4-7%. The prevalence is higher among individuals in lower socioeconomic classes

and those with a past history of asymptomatic urinary tract infection (Nicolle, 2005). Other reports have noted that asymptomatic bacteriuria occurs in 5 to 9% of both non-pregnant and pregnant women and that if left untreated in pregnancy progression to symptomatic UTI including acute cystitis and pyelonephritis occurs in 15 to 45%, or 4-fold higher than in non-pregnant women (Barry *et al*., 1997). Studies have postulated that since asymptomatic bacteriuria, usually caused by aerobic gram-negative bacilli or *Staphylococcus haemolyticus* can lead to UTI, a urine culture should be obtained from all women early in pregnancy, even bacteriuria should be treated with a 3-7 day course of antimicrobials, which reduces the risk of symptomatic UTI by 80 to 90% (Delzell and Leferre, 2000).

The only serious maternal consequence of untreated ASB in pregnant women is a significant risk of acute pyelonephritis in later pregnancy (30–40% vs. 3–4%) in treated patients (Smaill and Vazquez, 2007). The results of the studies on perinatal outcomes of untreated ASB are controversial. Although a number of them demonstrated a relationship of ASB in pregnant mothers and the risk of premature delivery and/or lower birth weight, some other studies failed to prove the association (Sheiner *et al.*, 2009; Chen *et al.*, 2010). The Cochrane Library meta-analysis revealed that antibiotic treatment was effective in reducing the incidence of low-birth-weight infants but not of preterm deliveries (Smaill and Vazquez, 2007).

2.2.2 Symptomatic urinary tract infection

Pyelonephritis presents with flank pain, fever, malaise, WBCs and bacteria in urine, while dysuria, increased frequency and urgency, pain above pubic region are common in cystitis (Figure 2.2). About 15-20% of women with pyelonephritis have bacteremia (Jolley and Wing, 2010; Hill *et al*, 2005). They may develop various complications, such as acute kidney injury,

anemia, hypertension, preeclampsia, sepsis and septic shock, hemolysis, thrombocytopenia, and acute respiratory distress syndrome, particularly if treatment is initiated too late (Hill *et al*., 2005, Smaill and Vazquez, 2007; Adis Data Information, 2011; Galajdova, 2010; Conde-Agudelo, 2008; Mittal and Wing, 2005). Although these associations have not always been proved to be causal, most of the complications seem to be due to renal or other tissue damage caused by bacterial endotoxins and a systemic inflammatory response with endothelial injury (Galajdova, 2010; Neal, 2008). A number of observational studies have demonstrated the relationship between maternal symptomatic UTI and the risk of premature delivery and lower birth weight (Sheiner *et al*., 2009; Gravett *et al*., 2010). The frequency of preterm deliveries in women with acute pyelonephritis is significantly higher than in women free of this complication, and pyelonephritis seems to be an important independent risk factor for delivery before 37 weeks' gestation (Farkash *et al*., 2012; Mazor-Dray *et al*., 2009; Millar *et al*., 2003). However, again, a substantial heterogeneity between these studies, together with many possible biases, makes it difficult to establish the overall contribution of UTI to preterm birth (Beck *et al*., 2010). A rare but severe complication is the transmission of the infection onto the newborn baby (Torres and Moayedi, 2012). Very often the transmitted infection originates from a heavily colonized birth canal, usually with Group B Streptococcus - GBS (Schrag *et al*., 2000).

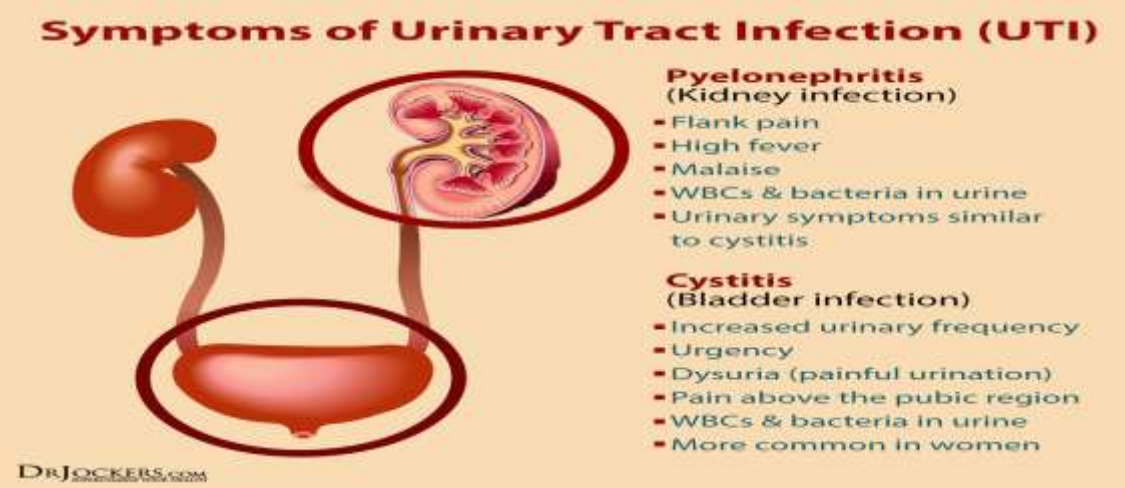


Figure 2.2: Symptoms of urinary tract infection (drjockers.com)

2.3Bacterial factors in urinary tract infection

Symptomatic bacteriuria is highly correlated with the presence of bacteria that mediate attachment to uroepithelial cells. The most common agent implicated in symptomatic and asymptomatic bacteriuria is *Escherichia coli* that are responsible for 70% - 80% of the infections (Masinde *et al*., 2009). Other microorganisms include *Staphylococcus* spp., *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, *Enterococcus* spp.and *Acinetobacter* (Delzell Jr. and Lefevre, 2000). Certain strains of *E. coli* are selected from the fecal - flora by the presence of virulence factors that enhance both colonization and invasion of the urinary tract and the ability to produce infection. Bacteria with enhanced adherence to vaginal and peri-urethral cells would be selected to colonize the anatomic regions adjacent to the urethral orifice. Binding to the uroepithelial surface, in turn, prevents bacterial washout during micturition and is the first step to bacterial invasion.

The adhesive properties of *E. coli*, for example, are facilitated by fimbriae, filamentous surface organelle. The most common ones are Type 1 and P-fimbriae. The attachment of Type 1 is blocked by mannose (mannose-sensitive, MS-adhesins), while the latter is mannose resistant (MR-adhesins). The P-fimbriae augment the virulence of uropathogenic *E. coli* by allowing more efficient spread from the intestinal tract to the urinary tract and thereby causing ascending infection. Once in the urinary tract, P-fimbriated strains adhere, persist and invade the kidney, inducing bacteremia and resulting in pyelonephritis.

Type 1 fimbriae increase susceptibility to polymorphonuclear phagocytosis, while P-fimbriae block phagocytosis. It is hypothesized that there are dual-phase kinetics of bacterial adherence in the pathogenesis of urinary tract infection. After entry into the bladder, MS-adhesins which are present on the majority of the Enterobacteriaceae, facilitate attachment to the bladder epithelium. However, when the bacteria ascend to the renal parenchyma, they undergo phase variation and do not express type 1 fimbriae which, as noted above, enhance phagocytosis. Rather, in the upper tract, P-fimbriae are expressed, allowing attachment to renal parenchymal cells.

Other Gram negative bacterial uropathogens, such as *Proteus mirabilis* and *Klebsiella* species, have demonstrated similar ability to adhere to the vaginal and periurethral cells, thereby enhancing their pathogenicity. Among Gram positive organisms, in contrast, *Staphylococcus aureus* uncommonly causes cystitis and ascending pyelonephritis, whereas *Staphylococcus saprophyticus*, which adheres significantly better to uroepithelium than do *Staphylococcus aureus* or *Staphylococcus epidermidis*, is a frequent cause of lower urinary tract infections.

In an individual with structural abnormalities of the urinary tract or with a catheter, even organisms of low pathogenicity can cause infection of bladder, kidney, or both, and the above-described properties of the bacteria are not essential.

2.4 Bacterial uropathogens

2.4.1 *Staphylococcus* spp.

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

Staphylococcus aureus is facultative anaerobic Gram-positive cocci which occur singly, in pairs, and irregulular clusters. *S. aureus* is non-motile, non-spore forming, catalase and coagulase positive. Typical colonies are yellow to golden yellow in color, smooth, entire, slightly raised, and hemolytic on 5% sheep blood agar. However, many strains may appear dirty white and non-

hemolytic. It also gives a positive mannitol fermentation and deoxyribonuclease test. The major criterion for identification is the organism's ability to clot plasma. There are three coagulase-positive *Staphylococcal* species; *S. aureus* in humans and animals, and *S.intermedius*, and *S. hyicus* in animals. (Turnidge *et al.,* 2008). The presence of the enzyme coagulase separates the virulent pathogen, *S. aureus*, from the less virulent coagulase-negative Staphylococci species.

The Staphylococci are most frequently isolated from clinical specimens in the microbiology laboratory with the exception of the Enterobacteriaceae. These bacteria are widespread in nature and can be recovered from environment or as commensally inhabitants of the skin, mucous membranes and other body sites in humans and animals (Quinn *et al*., 1994).

S. aureus is a more common coagulase positive *Staphylococci* spp widely distributed in nature and carried by 25-33% of normal individuals in the anterior nares and skin. It can colonize and infect both healthy, immunologically competent people in the community and hospitalized patients with decreased host defenses. *S. aureus* is one of the commonest and most important Gram-positive hospital-acquired organisms. It has a high propensity to colonize abnormal skin surfaces and open wounds, where it may merely reside rather than cause active infection.

The nose is the main ecological niche of *S. aureus* and about 20% (range 12–30%) of individuals are persistent *S. aureus* nasal carriers, increasing risk of acquiring an infection with this pathogen (Turnidge *et al*., 2008). *Staphylococcus aureus* adheres to cells and extra cellular matrix components by the joint action of MSCRAMM (microbial surface component recognizing adhesive matrix molecules) and secreted expanded repertoire adhesive molecules. MSCRAMMs bind molecules such as collagen, fibronectin and fibrinogen, and different MSCRAMMs may adhere to the same host-tissue component. Thus, these molecules act in initiation of endovascular infections, endocarditis and bone and joint infections. By the heterogeneity of MSCRAMMs

types, different *S. aureus* strains may be predisposed to causing certain kinds of infections (Clarke and Foster, 2006). Coagulase is an extracellular protein, belonging to the secreted expanded repertoire adhesive molecules class, which binds to prothrombin to form a complex called staphylothrombin. The protease activity of the thrombin complex is activated, resulting in the conversion of fibrinogen to fibrin. Coagulase is produced by almost all clinical isolates of *S. aureus;* thus it is a traditional marker for identifying *S. aureus* in the clinical microbiology laboratory. *Staphylococcus aureus* causes pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, endocarditis and superficial skin lesions such as furunculosis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices.

Coagulase negative Staphylococci (CoNS) are normal and abundant colonizers of humans and become pathogenic only in certain situations. Two main reasons for the increasing rate of CoNS infections are the spreading antibiotic resistance among CoNS and the increasing use of medical devices in recent years (Otto, 2004). The pathogenic potential of classical disease-inducing Staphylococci, such as *S. aureus*, is associated with a number of biochemical functions which may be considered virulence factors. The virulence factors include surface proteins that promote colonization of host tissues, invasions that promote bacterial spread in tissues (leukocidin, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule), biochemical properties that enhance their survival in phagocytes (catalase production), and membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin).

2.4.2*Escherichia coli*

Escherichia coli, the most prevalent facultative gram negative bacillus in the human fecal flora, usually inhabit the colon as an innocuous commensal. According to the special pathogenicity

theory (Orskov and Orskov, 1985), special properties enabling *E. coli* to overcome host defenses in a new environment are necessary in order for it to escape the limitations of the colonic mileu and move into new niches devoid of competition from other bacterial species. *Escherichia coli* is the most prevalent facultative anaerobic species in the human gastrointestinal tract (10^9 cfu/g faeces) but it also colonizes the intestines of animals and is thus used as an indicator of faecal contamination of drinking water and food. *E.coli* is usually a harmless microbe, although it is also the most common cause of community-acquired bacteraemia and the fifth most common cause of nosocomial bacteraemia. (Friedman *et al.*, 2002).

UTI is the most common form of extraintestinal *E. coli* infection, and *E. coli* is the most common cause of UTI. At some point during their lives, at least 12% of men and 10 to 20% of women experience an acute symptomatic UTI, and an even greater number develop asymptomatic bacteriuria. More than 100,000 patients are hospitalized annually in the United States because of renal infection (Johnson and Stell, 2000), with its attendant risk of Gram-negative sepsis and death.

2.4.3*Proteus* spp.

Proteus species are part of the Enterobacteriaceae family of Gram-negative bacilli commonly found in the human intestinal tract as part of normal human intestinal flora, along with *Escherichia coli* and *Klebsiella* species. *Proteus vulgaris* and *Proteus penneri* are easily isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems.

Certain virulence factors have been identified in bacteria. The first step in the infectious process is adherence of the microbe to host tissue via fimbriae. The attachment of *Proteus* species to

uroepithelial cells initiates several events in the mucosal endothelial cells, including secretion of interleukin-6 and interleukin-8. *Proteus* organisms also induce apoptosis and epithelial cell desquamation. Bacterial 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 favor the production of upper UTIs by organisms such as *Proteus.*

The ability of *Proteus* organisms 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 (Struble *et al*., 2011). This leads to precipitation of organic and inorganic compounds, which leads to struvite stone formation. Struvite stones are composed of a combination of magnesium ammonium phosphate (struvite) and calcium oxalate.

UTIs are the most common clinical manifestation of *Proteus* infections. *Proteus* infection accounts for 1%-2% of UTIs in healthy women and 5% of hospital-acquired UTIs. Complicated UTIs (i.e., those associated with catheterization) have a prevalence of 20%-45%.

2.4.4 *Streptococcus* spp.

Streptococci are non-motile, microaerophilic, Gram positive spherical bacteria (cocci). They often occur as chains or pairs and are facultative or strict anaerobes. *Streptococci* give a negative catalase test, while *Staphylococci* are catalase-positive.

Alpha-Hemolytic *Streptococci*: In alpha hemolysis, the red blood cells remain intact, but the hemoglobin is converted to biliverdin. This causes a greening of the blood agar plate around the colonies. Examples include: *S. pneumonia*, and viridans group *Streptococci* (*S. mutans,*

S.viridians, S. Slivarius etc). Causes bacterial pneumonia, otitis media and meningitis. *S.pneumoniae* sensitivity to optochin (ethyl hydrocupreine hydrochloride) is the basis of a diagnostic test.

Beta-Hemolytic *Streptococci*: Beta hemolysis is a true hemolysis of erythrocytes by the enzyme hemolysin. Clear zones will appear around the colonies on the blood agar plate. An example is *S. pyogenes*. Non-Hemolytic or Gamma-Hemolytic *Streptococci*: Gamma hemolysis is a misnomer as there is actually no hemolysis. Non-hemolytic or gamma-hemolytic *streptococci* rarely cause disease.

2.4.5 *Providencia* spp.

Providencia represents a genus of urease producing, Gram negative bacilli which although rare, are quite ubiquitous in the environment (Sagar *et al*., 2017). *Providencia* species closely resemble *Proteus* and *Morganella* species. The genus *Providencia* encompasses five common species i.e., *P. alcalifaciens, P. rettgeri, P. stuartii, P. rustigianii*, and *P. heimbachae*. They are frequently isolated from wounds, respiratory tract and urinary tract (*P. alcalifaciens, P. rettgeri* and *P. stuartii*), stool of humans (*P. alcalifaciens)*, poultry, faeces from reptiles (*P*. rettgeri), throat, perineum, axilla and blood of humans (O'Hara *et al.,* 2000). A report from Nepal in 2014, a cluster of surgical infections with regards to the isolation of *P. rettgeri*, demonstrated the presence and significance of this organism in the Asia-Pacific region (Tada *et al*., 2014). *P. rettgeri* has also been implicated as a causative agent of "purple bag syndrome", where the enzymatic activity gives rise to purple tinged urine (Peters *et al.,* 2011).

2.4.6 *Enterobacter* spp.

Examples of *Enterobacter spp* are *E. agglomerans*, *E. cloacae*, *etc. Pantoea agglomerans* (formerly *Enterobacter agglomerans*) is a motile peritrichous, non-sporforming, Gram-negative aerobic bacilli in the *Enterobacteriaceae* family (Sharma *et al*., 2013). It is commonly found in the ecological niches such as water, soil, sewage, seeds, vegetables, feculent material and foodstuffs, as well as reported as both commercial and opportunistic pathogens of animals and humans (Liberto *et al*., 2009). This opportunistic pathogen isolated from clinical specimens including blood, wounds, urine, throat, and internal organs.

P. agglomerans is the most frequent species associated with human infections (Boszczowski *et al*., 2012). Hospital outbreaks due to contamination of anesthetic agent propofol, blood products, parenteral nutrition, and transference tubes used for intravenous hydration have been demonstrated (Boszczowski *et al*., 2012). *P. agglomerans* has been implicated in pneumonia, wound infections, septicemia, bacteremia, urinary tract infection, meningitis, lung and brain abscess, septicemia, osteomyelitis, septic arthritis, peritonitis and colelithiasis. The organism is generally regarded as opportunistic, of low virulence, low degree of toxicity and with little intrinsic invasiveness but can cause infection even in the healthy individuals with immunocompetent system.

2.4.7 *Klebsiella* spp.

Klebsiella spp. are Gram-negative, non-motile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae (Janda and Abbott, 2006; Abbott, 2007). These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive

in the Voges-Proskauer test. *Klebsiella* spp. often occur in mucoid colonies (Janda and Abbott, 2006; Abbott, 2007). The genus consists of 77 capsular antigens (K-antigens), leading to different serogroups.

Klebsiella spp. have been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), UTIs (6 to 17%), wound infections (2 to 4%), intensive care unit (ICU) infections (4 to 17%), and neonatal septicaemias (3 to 20%) (Abbott, 2007). *Klebsiella spp*. can also cause bacteremias and hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis (Abbott, 2007). They are also important opportunistic pathogens, particularly among the immunocompromised. Pathogenicity factors of *Klebsiella spp*. include adhesins, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs), and toxins, each of which plays a specific role in the pathogenesis of these species. Depending on the type of infection and the mode of infectivity, cells of *Klebsiella spp*. may adhere and attack upper respiratory tract epithelial cells, cells in gastrointestinal tract, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes. *Klebsiella* spp. are known to show resistance to penicillins, especially ampicillin and carbenicillin (Janda and Abbott, 2006). Since more and more strains of *Klebsiella* spp. appear to be developing and harbouring extended-spectrum beta-lactamases (ESBLs), cephalosporinases, and carbapenemases, resistance of *Klebsiella* spp. to current antibiotics appears to be increasing.

2.4.8 *Morganella* spp.

Examples of Morganella spp are M. morganii and *M. sibonii*. *M. morganii* is a gram-negative rod commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora. *Morganella morganii* is facultatively anaerobic and oxidase-negative. *M. morganii* can produce the enzyme catalase, so is able to convert hydrogen peroxide to water and oxygen. This is a common enzyme found in most living organisms. In addition, it is indole test-positive representing this organism can split tryptophan to indole, pyruvate, and ammonia. Methyl red tests positive in *M. morganii*, an indicator dye that turns red in acidic solutions (O'Hara *et al*., 2000). Although a rare human pathogen, *M. morganii* has been reported as a cause of urinary tract infections, nosocomial surgical wound infections, peritonitis, central nervous system infection, endophthalmitis, pneumonia, chorioamnionitis, neonatal sepsis, pyomyositis, necrotizing fasciitis, and arthritis. Numerous cases of nosocomial infection have been described, usually as postsurgical wound infections or urinary tract infections.

Despite its wide distribution, it is an uncommon cause of community-acquired infection and is most often encountered in postoperative and other nosocomial settings. *M morganii* infections respond well to appropriate antibiotic therapy; however, its natural resistance to many beta-lactam antibiotics may lead to delays in proper treatment. The genus Morganella belongs to the tribe Proteeae of the family Enterobacteriaceae. The Proteeae, which also include the genera Proteus and Providencia, are important opportunistic pathogens capable of causing a wide variety of nosocomial infections. *M. morganii* is a member of the tribe Proteeae (normal fecal flora that often causes infection in patients whose normal flora have been disturbed by antibiotic therapy, (Cunha and Burke, 2012), it is of the family Enterobacteriaceae.

2.4.9 *Pseudomonas* spp.

P. aeruginosa is the third most common pathogen associated with hospital-acquired catheter-associated UTIs (Jarvis, 1992). Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores (Matheson *et al*., 2006; Yates *et al*., 2006; Zulianello *et al.,* 2006; Veesenmeyer *et al*., 2009). These factors have been shown to play an important role in pathogenesis of *P. aeruginosa* induced infections like respiratory tract infections; burn wound infections and keratitis (Lysczak *et al*., 2000; Smith *et al*., 2006). However, limited reports are available regarding role of these virulence traits in urinary tract infections. High production of elastase and protease in strains isolated from urinary tract infections compared to isolates from other infections like burn wounds infection, skin wound infection and acute pneumonia have been observed. High levels of elastase and phospholipase C were produced by most isolates obtained from trachea, urinary tract, and wounds. Significantly higher levels of toxin A was produced by wound isolates, while significantly higher level of exoenzyme S was produced by wound and urinary tract isolates. It was observed that persistent infection isolates from different sites produce significantly higher levels of exoenzyme S. These workers concluded that elastase, phospholipase C, toxin A, and exoenzyme S are important virulence traits which help *P. aeruginosa* to cause a variety of persistent infections. Ciragil and Soyletir (2004) investigated relationship between production of virulence traits and site of infection. These workers isolated *P. aeruginosa* strains from cystic fibrosis patients as well as from lungs, urine and blood of non-cystic fibrosis patients. It was observed that urinary isolates produced least amount of alginate

and maximum amount of alkaline protease as compared to other isolates. Significantly lower levels of alkaline protease were observed in cystic fibrosis isolates as compared to other isolates. No significant difference in elastase levels was observed among different strains of *P. aeruginosa*. However these workers observed no correlation between elaboration of virulence factors and site of infection. It was concluded that virulence factors play an important role in pathogenesis of infections caused by *P. aeruginosa*. Mittal *et al*., 2006 also reported that uroisolates of *P. aeruginosa* produce high levels of alginate, siderophores, exoenzymes and hemolysin. However uroisolates possessing high hemolytic property showed significantly high renal bacterial counts and marked tissue damage compared to low producers indicating direct association between hemolysin production and renal colonization. It was suggested that besides considering levels of all extracellular enzymes, high levels of hemolysin production *in vitro* could be used as surrogate information for assessing pyelonephritic potential of *P. aeruginosa*.

2.4.10 *Serratia* spp.

Serratia is a genus of Gram-negative, facultatively anaerobic, rod-shapedbacteria of the Enterobacteriaceae family. The most common and pathogenic of the species in the genus, *S. marcescens*, is normally the only pathogen and usually causes nosocomial infections. However, rare strains of *S. plymuthica*, *S. liquefaciens*, *S. rubidaea*, and *S. odoriferae* have caused diseases through infection. (Ania, 2015). *S. marcescens* is typically found in showers, toilet bowls, and around wetted tiles. Members of this genus produce characteristic red pigment, prodigiosin, and can be distinguished from other members of the Enterobacteriaceae family by their unique production of three enzymes: DNase, lipase, and gelatinase. A pan-genome analysis of the genus *Serratia* revealed that it does not depict exceptional genetic conservation or diversity as compared to other bacterial genera. (Basharat and Yasmin, 2016).

The bacterium is an opportunistic human pathogen, capitalizing on its ability to form tight-knit surface communities called biofilms wherever it can. (Ania, 2015). *S. marcescens* is thought to be transmitted through hand-to-hand transmission by hospital personnel. (Ania, 2015). In the hospital, *Serratia* species tend to colonize the respiratory and urinary tracts, rather than the gastrointestinal tract, in adults. *Serratia* infection is responsible for about 2% of nosocomial infections of the bloodstream, lower respiratory tract, urinary tract, surgical wounds, and skin and soft tissues in adult patients.

2.4.11 *Alcaligenes feacalis*

Alcaligenes faecalis is an aerobic Gram-negative, non-fermentative rod. The microbe has peritrichous flagellar arrangement which allows for motility (Winn *et al*., 1988). Most infections caused by *A. faecalis* are opportunistic and acquired from moist items such as nebulizers, respirators, and lavage fluids. When an infection occurs, it is usually in the form of a urinary tract infection (Winn *et al*., 1988). It is considered as an opportunistic pathogen (Jachna-Sawicka and Gospodarek, 2009), and found to cause urinary tract infections in humans. In addition, *A. faecalis* has been found in corneal ulcers, feces, blood, and other bodily fluids (Kaliaperumal *et al.,* 2006).

2.4.12 *Salmonella* spp.

Salmonella is a genus of rod-shaped gram-negative bacteria of the Enterobacteriaceae family. Although there are many serotypes that are pathogenic to both humans and animals, the vast majority of human *Salmonella* isolates are serotype. *S. enterica* subsp. *Enterica* (Heymann, 2008). The serovars *Typhi* and *Paratyphi* of this serotype, *S. enterica* subsp. *enterica* are the causative agents of typhoid and paratyphoid fevers respectively. These types are generally found

in developing countries such as countries in South America, Africa, and parts of Asia (Heymann, 2008). The procedures recommended by different regulatory bodies, although having some variations in the selection of culture media and method of sample preparation basically are in five steps, which are: pre-enrichment, selective enrichment, plating, biochemical testing and serological testing/confirmation (Mahmoud, 2012).

The PCR detection of *Salmonellae* spp is based on the amplification of a bacterial DNA sequence that is unique to *Salmonellae*. The PCR assay consists of three different steps: denaturation (by heat) of duplex bacterial DNA into single strands (94°C), annealing of synthetic oligonucleotide primers (45-65°C) that are highly specific to *Salmonella* sp. DNA sequences that flank the *Salmonella*-specific DNA target, and a polymerase-dependent extension (72°C) of the single-stranded DNA starting at the primer site where elongation progress from 3' to 5' end of the template DNA strand (Mahmoud, 2012). The most commonly targeted sequence for this amplification lies in the *InvA* gene of *Salmonella spp* (Daoust, 1994). *Inv A* gene, (Invasion protein) is a gene involved in the invasion of the cells of the intestinal epithelium. It could also be involved in the translocation of the *Inv E* protein. One of the first steps in the pathogenic cycle of the facultative intracellular pathogen is the invasion of the cells of the intestinal epithelium.

2.4.13 *Acinetobacter* spp.

Acinetobacter species are saprophytic, ubiquitous and have emerged as an important nosocomial pathogen due to its ability for survival in the hospital environment on a wide range of dry and moist surfaces (Mindolli *et al.,* 2010). Human infections caused by *Acinetobacter* species include pneumonia, which is most often related to endotracheal tubes or tracheostomies, endocarditis,

meningitis, skin and wound infections, peritonitis in patients receiving peritoneal dialysis, UTI and bacteremia (Winn *et al.,* 2006; Oberoi *et al.,* 2009).

2.5 Virulence factors of uropathogens

Bacterial pathogens have developed many strategies for survival in higher organisms, which during their evolution have formed very sophisticated defense mechanisms. This defense system includes non-specific reactions such as mechanical clearing of the mucosa, control of iron transfer, phagocytosis, elimination of bacteria by enzyme attack (e.g., by lysozyme), and activation of complement, as well as specific reactions involving antibodies and cells of the immune system. Pathogenic bacteria have worked out many different ways to overcome the host defense system. Common bacterial properties involved in the infection process include adhesion to epithelial surfaces, invasion (penetration) of host cells, intracellular multiplication of the pathogen, colonization of the cell tissue or transmission to a new susceptible host, production of enzymes which damage the host defense system, and synthesis of toxins.

2.6 *Staphylococcus aureus*

2.6.1 Leukocidins

These toxins consist of two separately secreted and non-associated proteins (class S and class F components) acting synergistically and promoting eukaryotic cell lysis. They are: γ-haemolysin, panton valentine leukocidin (*pvl*) and leukocidins *LukD/E* and *LukM/F*. The γ haemolysin is produced by almost all (≥ 99%) *S. aureus* strains.

The *pvl* is a pore-forming toxin which damages human neutrophils, recovered from less than 2% of *S. aureus* strains. It has been epidemiologically linked to primary skin and soft tissue

infections and to deep seated infections such as necrotizing pneumonia and severe recurrent osteomyelitis occurring in young immunocompetent patients. The genes coding for leukotoxins *LukE-LukD* were detected in a high prevalence (82%) among blood isolates and 61% among nasal isolates (Von Eiff *et al*., 2004).

2.7 *Escherichia coli*

In *E. coli*, virulence results from the cumulative impact of one or several special properties, or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains. The practical goal of investigations into the virulence properties of any pathogen is the development of specific anti-VF interventions (such as vaccines) to prevent infection. The more virulent pathotypes often have a larger genome compared to the non-pathogenic *E. coli*, and there are also many different virulence factors, which are usually encoded on plasmids, chromosomes, or bacteriophages (Welch *et al.*, 2002; Rasko *et al.*, 2008). The serotypes and groups of pathogenic *E. coli* are defined by their lipopolysaccharide (O) and flagellar (H) antigens (Tenaillon *et al*., 2008).

Geographically widespread epidemic clones with the same chromosomal sequence types (STs) have been identified among *E. coli* strains that cause urinary tract infections. Extended-spectrum beta-lactamase (ESBL) producing strains are usually community acquired, and only a few hospital outbreaks of such bacteria have been reported (Nicolas-Chanoine *et al.*, 2008; Alsterlund *et al*., 2009).

UTI is the predominant type of bacterial infection among pregnant women (Dwyer and O'Reill, 2002 ; Nowicki, 2002).As many as 90% of UTIs are caused by *Escherichia coli* (Delzell Jr and Lefevre, 2000). Hormonal and physiological changes in the urinary tract, including ureteral

dilatation and changes in bladder volume and tone, may promote infection in pregnant women (Nowicki, 2002). Furthermore, it has been observed that pregnant women have a propensity to develop recurrent UTIs (Nowicki, 2002). Overall, UTI can be dangerous for both the mother and fetus. Complications that can arise include preterm delivery and increased incidence of intrauterine growth restriction. To a lesser degree, preeclampsia, caesarean delivery, anemia, sepsis, and septic shock may also be associated with UTI in these patients (Mazor-Dray *et al*., 2009). Among patients suffering acute pyelonephritis, those who are pregnant are more likely than nonpregnant women to develop renal scars (Raz *et al*., 2002). The possession of virulence factors that enable colonization is important in the pathogenesis of uropathogenic *E. coli* (UPEC). UPEC is the most common etiologic agent responsible for UTI, resulting in more than 80% of infections (Allsopp *et al*., 2012). UPEC strains possess an array of virulence factors, with no factor solely responsible for the ability to cause UTI (Oelschlaeger *et al*., 2002). However, the ability of UPEC to colonize the urinary tract and cause disease involves the expression of adhesins (e.g., type1 and P-fimbriae), toxins (e.g., hemolysin), and iron acquisition systems that utilize siderophores [e.g., enterobactin, salmochelin, aerobactin] (Klemm and Schembri, 2000). Some virulent inherent properties of *Escherichia coli* include:

2.7.1 Adherence (fim and pap operon)

Adherence to the urinary tract epithelium enables bacteria to resist the hydrodynamic forces of urine flow, to trigger host and bacterial cell signaling pathways, and to establish infection. Among adhesins, P and type 1 fimbriae correlate strongly with uropathogenesis and mediate binding to specific receptors within the urinary tract (Wullt *et al*., 2000).Many pathogenic microorganisms possess properties that enable them adhere/attach themselves to host structures thus preventing them from been swept along by the normal flow of body fluids (blood, urine,

intestinal contents) and eliminated.A necessary first step in the coloinization of host mucosal surfaces and precedence to invasive infection process is attachment.

UPEC expresses a multitude of virulence factors to break the inertia of the mucosal barrier (Bien *et al*., 2012). The ability of these bacteria to adhere to host epithelial cells is considered a prerequisite for the establishment of in-fectious diseases, mainly through expression of fimbriae. UPEC generally possesses type 1 and P fimbriae (Snyder *et al*., 2006). Type 1 fimbriae are characterized as having the ability to agglutinate chicken and guinea pig erythrocytes in the absence of D-mannose. They consist of a major protein, *FimA*, associated with ancillary proteins *FimF*, *FimG*, and the adhesin protein *FimH*, encoded by the *fim* gene cluster. This type of fimbria is common among Enterobacteriaceae, also several variants have been strongly associated with UPEC.

2.7.2 Aerobactin (aer operon)

Iron is essential for bacterial growth but is of limited availability in animal tissues. Therefore, bacteria have evolved several strategies for iron uptake, most of which have been explained in molecular terms.*E. coli* uses iron for oxygen transport and storage, DNA synthesis, electron transport, and metabolism of peroxides. Part of the host response to infection is to further reduce the amount of iron available to the invading pathogen by decreasing intestinal iron absorption, synthesizing additional iron proteins, and shifting iron from the plasma pool into intracellular storage. (Johnson and Russo, 2002). Thus, bacteria face a formidable challenge in meeting their iron needs during infection. In *E. coli*, the hydroxamate siderophore aerobactin is the most effective of the several iron chelation systems employed by enteric bacteria for iron acquisition.

Strains with the aerobactin system have a growth advantage in low-iron conditions, including in serum and dilute urine.

2.7.3 Hemolysin (hly operon)

In human UTI, hemolysin production is most common among strains from patients with pyelonephritis (49%), followed by those from patients with cystitis (40%) and asymptomatic bacteriuria (20%). Hemolysin production is more prevalent in UTI episodes localized to the upper than the lower urinary tract (Brooks *et al*., 1988).The cytolytic protein toxin secreted by most hemolytic *E. coli* strains is known as alpha hemolysin (Bhakdi *et al*., 1989).Although cell-bound (beta) hemolysins and secreted hemolysins other than alpha hemolysin have been described, their prevalence and clinical significance are unknown.

The erythrocytes of all mammals are lysed by alpha hemolysin; which is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses. Exposure of hPMNLs to hemolysin stimulates chemiluminescence, degranulation, and release of leukotrienes and ATP; causes marked morphologic alterations; and impair chemotaxis and phagocytosis. Lysis occurs at higher concentrations(Bhakdi *et al*., 1989).

Hemolysin production is encoded by a four-gene operon termed *hly*: *hlyA, hlyB, hlyC*, and *hlyD*, which is located on the chromosome in human isolates of *E. coli* in contrast to the plasmid location common among animal strains (Johnson *et al*., 1988). Plasmid and chromosomal *hly* regions differ with respect to flanking and regulatory sequences and to the precise sequence of *hlyA*, the gene encoding the structural hemolysin protein (Vogel *et al*., 1988).Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI. Hemolysin is probably produced in vivo during UTI by

uropathogenic strains. 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.

2.7.4 Cytotoxic necrotizing factor (cnf operon)

CNF1 has been found to be associated with UPEC; however, the specific function is unclear and partly conflicting results have been published. A recent study suggested that *cnf1* may promote bacterial attachment to and invasion of uroepithelial cells and induce an inflammatory response in vitro, and another study found *cnf1* to promote UTI in a murine model (Hertting *et al.,* 2008; Rippere-Lampe *et al*., 2001).

The pathogenicity island PAI V for example contains the genes encoding the well known α-hemolysin, the cytotoxic necrotizing factor and the P fimbriae (Kuhnert *et al*., 2000). *HlyA* is often co-expressed with cytotoxic necrotizing factor 1 (*cnf1*), a toxin which activates the Rho GTPases leading to cytoskeleton reorganization, induction of membrane ruffling and modulation of inflammatory signaling pathways (Lemonnier *et al*., 2007). The capability of *cnf1* to cause cytoskeleton reorganization could when unregulated be speculated to disrupt actin network and thus facilitate the intracellular proliferation of UPEC in quiescent intracellular bacterial reservoirs(QIR) that has been described to be restricted by actin structures (Eto *etal*., 2006).

2.8 Extended-spectrum β-lactamase production

In Gram-negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance. (Livermore, 2003). β-lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds. One group of β lactamases, extended-spectrum β lactamases (ESBLs), have the ability to hydrolyse and

cause resistance to various types of the newer β-lactam antibiotics, including the expanded-spectrum (or third-generation) cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime) and monobactams (e.g., aztreonam), but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem) (Bradford, 2001). Organisms that produce ESBLs remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control. (Paterson and Bonomo, 2005). That clinical microbiology laboratories detect and report ESBL-producing organisms is therefore important. Most ESBLs can be divided into three groups: TEM, SHV, and CTX-M types. (Paterson and Bonomo, 2005).

Klebsiella pneumoniae and *Escherichia coli* remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the Enterobacteriaceae family and in certain non-fermentors. (Jacoby and Munoz-Price, 2005). A report from the Infectious Diseases Society of America listed ESBL-producing *Klebsiella* spp and *E. coli* as one of the six drug-resistant microbes to which new therapies are urgently needed (Talbot*et al.*,2006).Because of the increasing importance of multi-resistant ESBL-producing *E. coli* in the community, clinicians should be aware of the potential of treatment failures associated with serious infections caused by these bacteria. Emergence in serious community-onset infections organisms that produce CTX-M enzymes have become the most prevalent type of ESBLs described from certain European and South American countries (Canton and Coque, 2006).The CTX-M β lactamases, now exceeding fifty different types, can be divided into five groups based on their aminoacid identities: CTX-M1, CTX-M2, CTX-M8, CTX-M9, and CTX-M25 (Bonnet, 2004). Of note, organisms producing specific CTX-M enzymes have been isolated from different countries (Canton and Coque, 2006; Livermore *et al.*, 2007; Pitout *et al.*, 2005a**).**

The CTX-M enzymes originated from the *Kluyvera spp* of environmental bacteria, usually have greater activity against cefotaxime than ceftazidime (although certain types also inactivate ceftazidime), and are associated with mobile elements such as ISEcp1 (Walther-Rasmussen and Hoiby, 2004).

The epidemiology of organisms producing CTX-M enzymes is very different from those that produce TEM-derived and SHV-derived ESBLs. CTX-M enzymes are not limited to nosocomial infections caused by *Klebsiella* spp, and their potential for spread beyond the hospital environment serves to exacerbate public-health concerns. *E. coli* is most often responsible for producing CTX-M β lactamases and seems to be a true community ESBL pathogen (Pitout, *et al.,* 2005b). The characteristics and risk factors of community-onset infections caused by ESBL-producing bacteria compared with nosocomial onset infections are summarised in table 2.2. Surveys since 2000 from several European countries (including Spain, Rodriguez-Bano, *et al*., 2004, Italy, Mugnaioli, 2006, Greece, Pournaras*et al*., 2004, the UK, Woodford *et al.,* 2004,and Canada, Pitout, *et al*., 2007) have shown an alarming trend of associated resistance to other classes of antimicrobial agents among ESBL-producing organisms isolated from community sites. These surveys show co-resistance to co-trimoxazole, tetracycline, gentamicin, and ciprofloxacin (up to 66% of isolates were resistant to ciprofloxacin in Canada (Pitout *et al.,* 2007). These studies also showed that strains producing CTX-M enzymes were substantially more resistant to ciprofloxacin than strains lacking PCR evidence for blaCTX-M genes. (Pitout *et al.,* 2007; Pitout *et al.,* 2004). Two reports from Israel and Spain have shown that CTX-M-producing *E coli* is an important cause of community-onset bloodstream infections. Ben Ami, *et al*., 2006, Rodriguez-Bano*et al*., 2006from Tel-Aviv, Israel, investigated patients with community-onset, Gram-negative bacteraemia admitted to their hospital and found that 14%

were caused by ESBL-producing organisms (most commonly CTX-M-producing *E. coli*). These bacteria were multi-resistant, with resistance to cotrimoxazole (64.0%), gentamicin (61.0%), and ciprofloxacin (64.0%) being reporte. The most frequent origin of infection was the urinary tract, and empirical therapy with cephalosporins and fluoroquinolones was associated with higher mortality. (Rodriguez-Bano *et al*., 2006).

2.8.1 ESBL detection

The clinical laboratory acts as an early warning system, alerting the medical community to new resistance mechanisms present in clinically important bacteria. The methods for detection of ESBLs can be broadly divided into two groups: phenotypic methods that use non-molecular techniques, which detect the ability of the ESBL enzymes to hydrolyse different cephalosporins; and genotypic methods, which use molecular techniques to detect the gene responsible for the production of the ESBL. Clinical diagnostic laboratories use mostly phenotypic methods because these tests are easy to do, are cost effective, and have been incorporated in most automated susceptibility systems, making them widely accessible (Wiegand*et al*., 2007). However, phenotypic methods are not able to distinguish between the specific enzymes responsible for ESBL production (SHV, TEM, and CTX-M types). Several research or reference laboratories use genotypic methods for the identification of the specific gene responsible for the production of the ESBL, which have the additional ability to detect low-level resistance (i.e., can be missed by phenotypic methods) (Woodford, 2005). Furthermore, molecular assays also have the potential to be done directly on clinical specimens without culturing the bacteria, with subsequent reduction of detection time. (Tenover, 2007). The detection of ESBL-producing bacteria in laboratories is a crucial step for appropriate management of patients, but genotypic identification of these enzymes provides essential information for infection prevention and control efforts, as well as

the tracking of these organisms in surveillance systems. The clinical diagnostic microbiology laboratory plays a crucial part in the detection and reporting of ESBL-producing bacteria, but many laboratories may not be fully aware of the importance of ESBL-producing organisms and the best phenotypic methods for detecting them (Thomson, 2001). The consequence has been several treatment failures in patients who received inappropriate antibiotics and outbreaks of multidrug-resistant, Gram-negative pathogens, which required expensive control efforts. (Paterson and Bonomo, 2005). Although there are several guidelines available for the phenotypic detection of ESBL-producing bacteria, this remains a contentious issue, and proficiency testing shows compliance varies widely across different parts of the world. (Jacoby and Munoz-Price, 2005). A US study reported that only 8% of clinical laboratories from rural hospitals routinely screened for ESBL-producing organisms, (Stevenson *et al.,* 2003) whereas a recent performance survey of 60 Italian laboratories misidentified up to 50% of known ESBL-producing isolates.(Luzzaro *et al.,* 2006).

2.8.2 Phenotypic detectionof ESBL

The US Clinical and Laboratory Standards Institute (CLSI) and the UK Health Protection Agency (HPA) have published guidelines for ESBL detection in Enterobacteriaceae specifically for *E. coli, Klebsiellaspp,* and *Proteus spp*. (NCCLS, 2002; HPA, 2008)**.** The HPA (UK, Health Protection Agency guidelines also include other species, such as *Salmonella spp.* These guidelines are based on the principle that most ESBLs hydrolyse third-generation cephalosporins although they are inhibited by clavulanate, and recommend initial screening with either 8 mg/L (CLSI) or 1 mg/L (HPA) of cefpodoxime, 1 mg/L each of cefotaxime, ceftazidime, ceftriaxone, or aztreonam, followed by confirmatory tests (including the E-test ESBL strips) with both cefotaxime and ceftazidime in combination with clavulanate at a concentration of 4 µg/mL.

Automated systems that use similar detection principles have proved to be popular in clinical laboratories, especially those in North America and certain European countries. (Spanu**,** 2006).If clinical laboratories adhere to the published guidelines for detecting ESBLs, the CLSI and HPA published methods show high sensitivity of up to 94% and specificity of 98% for detecting ESBLs in *E coli*, *Klebsiella spp*, and *Proteus spp.* (Wiegand, 2007).The phenotypic detection of ESBLs in bacteria other than *E. coli, Klebsiella* spp, and *Proteus spp* remains a problematic and controversial issue. (Thomson, 2001). The reason for this is that the clavulanate effect noticed with these ESBL-producing species is not always present in species such as *enterobacter* and *citrobacter*. This is because the clavulanate inhibition of ESBLs is often masked by other types of β lactamases, such as AmpC enzymes produced by *Enterobacter spp*. Several methods have been described, including modifications of double-disk method with cefepime, (Pitout, 2003; Tzelepi, *et al.*, 2000) chromogenic agar, (Glupczynski, *et al*., 2007) three-dimensional methods, (Ho *et al*., 2005) and microdilution methods that use clavulanate with different β lactams (including fourth-generation cephalosporins, such as cefepime (Thomson, *et al.*, 1999). Unfortunately, most of these tests are technically demanding and difficult to interpret, which has limited their widespread use to detect ESBLs in bacteria such as *Enterobacter, Citrobacter*, and *Serratia spp*.

2.8.3 Genotypic detection of ESBL

The determination of whether a specific ESBL present in a clinical isolate is related to TEM and SHV enzymes is a complicated process because point mutations around the active sites of the TEM and SHV sequences have led to amino acid changes that increase the spectrum of activity of the parent enzymes, such as in TEM1, TEM2, and SHV1. The molecular method commonly used is the PCR amplification of the *bla*TEMand *bla*SHV genes with oligonucleotide primers,

followed by sequencing. Sequencing is essential to discriminate between the non-ESBL parent enzymes (e.g., TEM1, TEM2, or SHV1) and different variants of *TEM* or *SHV* ESBLs (e.g., *TEM3, SHV2,* etc). (Bradford, 2001). Several other molecular methods that do not use sequencing have been developed to characterize ESBLs and included PCR with RFLPs (Arlet, *et al*., 1995)**,** PCR with single-strand conformational polymorphism, (M'Zali *et al*., 1996), ligase chain reaction (Kim *et al.,* 2000), restriction site insertion PCR (Chanawong, *et al*., 2001) and real-time PCR (Randegger and Hachler, 2001). However, the increasing number of additional subtypes within each ESBL family has placed strict limitations on these techniques with regard to their ability to cover the whole range of variants with different point mutations. PCR amplification followed by nucleotide sequencing remains the gold standard for the identification of specific point mutation of *bla*TEM or *bla*SHV ESBL genes (Fluit *et al.,* 2001). However; this is not always straightforward and cost effective because clinical isolates often have multiple copies of ESBL genes. Genetic methods for detection of TEM and SHV types of ESBLs are thus complex and challenging because of the diversity of different point mutations that can cause an ESBL. The use of genetic methods to identify the different types of TEM and SHV ESBLs is mainly restricted to reference laboratories and to molecular surveillance studies. Hopefully, recent molecular developments, such as microarrays and rapid-cycle sequencing, will make genotypic detection more readily available and cost effective for diagnostic laboratories to identify these types of ESBLs in a real-time fashion (Sundsfjord *et al.*, 2004).

The PCR amplification of CTX-M-specific products without sequencing, in an isolate that produces an ESBL, usually provides sufficient evidence that a *bla*CTX-M gene is responsible for this phenotype. This is unlike TEM and SHV types of ESBLs. Several recent studies have described various molecular approaches for the rapid screening of ESBL-positive organisms for

the presence of different *bla*CTX-M genes. This involved a PCR assay that used four sets of primers to amplify group-specific CTX-M β-lactamase genes (Pitout *et al.,* 2004) amplification of a universal DNA fragment specific for most of the different groups of CTX-M β lactamases, (Batchelor *et al*., 2005), duplex PCR, (Pitout *et al.*, 2007) multiplex PCR, (Woodford *et al.*, 2006), real-time PCR, (Birkett *et al.,* 2007) pyrosequencing, (Naas *et al.,* 2007) and reverse-line hybridization (Ensor *et al.,* 2007). Molecular techniques undoubtedly have the potential to play an essential part in the laboratory setting for the screening, tracking, and monitoring of the spread of large numbers of organisms producing CTX-M enzymes from the community and hospital settings in real time.

2.9 Autotransporter genes of uropathogenic *E. coli* (*Upa B, UpaC*)

Autotransporter (AT) proteins are frequently associated with aggregation, cell adherence, adhesion to extracellular membrane (ECM) proteins, and biofilm formation. In addition to fimbrial adhesins, a number of AT proteins (non-fimbrial adhesins) associated with virulence have been characterized from UPEC. These include the secreted toxin *Sat* (Guyer *et al*., 2000; Guyer *et al*., 2002), the phase variable outer membrane protein antigen 43 [*Ag*43] (Ulett *et al*., 2007), the trimeric AT protein *UpaG* (Valle *et al*., 2008), and the surface-located *UpaH* (Allsopp *et al*., 2010). AT proteins represent the largest group of bacterial type V secreted proteins and share several common features: an N-terminal signal sequence, a passenger (α) domain that is either anchored to the cell surface or released into the external milieu, and a translocation (β) domain that resides in the outer membrane (Henderson *et al*., 2000). AT proteins were originally thought to possess structural properties that facilitate their independent transport across the bacterial membrane system and final routing to the cell surface (Henderson *et al*., 2004). However, this classical view has recently been called into question, as accessory factors, such as

the *Bam*-complex (also known as the *YaeT* or *Omp*85 complex), as well as periplasmic chaperones, such as *SurA*, *Skp*, and *DegP*, are required for the secretion of some AT proteins (Ruiz-Perez *et al*., 2010). In general, AT proteins differ substantially in their passenger domain sequence, which determines the unique functional characteristics of the protein and is often associated with virulence (Henderson *et al*., 2004).

Eleven putative AT-encoding genes have been identified in the sequenced genome of the prototype UPEC strain CFT073 (Allsopp *et al*., 2010; Parham *et al*., 2004). The biological significance of these AT proteins and their roles in UPEC pathogens is yet to be fully elucidated. UPEC strains produce a range of fimbrial and nonfimbrial adhesins that play a role in virulence and contribute to persistent infection of the urinary tract. Some UPEC fimbrial adhesins, including type 1 and P fimbriae, have been well characterized with respect to their expression, regulation, receptor-binding target, and role in virulence. Of the nonfimbrial adhesins, the AT family of proteins represents another group of virulence factors that contribute to adhesion, invasion, and biofilm formation. Although UPEC strains possess multiple AT-encoding genes, very little is known about the function of their products and only *Ag*43, *UpaG*, *Sat*, and *UpaH* have been associated with virulence (Allsopp *et al*., 2010; Guyer *et al*., 2000; Guyer *et al*., 2002; Ulett *et al*., 2007; Valle *et al*., 2008).

2.10 Phylogenetic grouping genes of UPEC

Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) (Herzer *et al*., 1990; Selander *et al*., 1987) and that virulent extra-intestinal strains belong mainly to group B2 and, to a lesser extent, to group D (Bingen *et al*., 1998; Boyd and Hartl, 1998; Johnson and Stell, 2000; Picard *et al*., 1999), whereas most commensal strains

belong to group A. A better understanding of how pathogenic strains acquire virulence genes is described by (Bingen *et al*., 1998). Actually, phylogenetic grouping can be done by multilocus enzyme electrophoresis (Herzer *et al*., 1990; Selander *et al*., 1987) or ribotyping (Bingen *et al*, 1994; Bingen *et al*., 1996; Bingen *et al*., 1998; Desjardins *et al*., 1995), but both of these reference techniques are complex and time-consuming and also require a collection of typed strains. Three candidate markers were studied further: (i) *chuA*, a gene required for heme transport in enterohemorrhagic O157:H7 *E. coli* (Bonacorsi *et al*., 2000; Mills and Payne, 1995; Torres and Payne, 1997; Whittam, 1996); (ii) *yjaA*, a gene initially identified in the recent complete genome sequence of *E. coli* K-12, the function of which is unknown (Blattner *et al*., 2000); and (iii) an anonymous DNA fragment designated *TSPE4.C2* from our subtractive library (Bonacorsi *et al*., 2000). Here we describe a rapid technique for determining the phylogenetic groups of *E. coli* strains based on PCR detection of the *chuA* and *yjaA* genes and DNA fragment.

2.10.1 Building phylogenetic trees from molecular data with MEGA®

A phylogenetic tree is an estimate of the relationships among taxa (or sequences) and their hypothetical common ancestors (Nei and Kumar, 2000; Felsenstein 2004; Hall, 2011). Today most phylogenetic trees are built from molecular data: DNA or protein sequences. Originally, the purpose of most molecular phylogenetic trees was to estimate the relationships among the species represented by those sequences, but today the purposes have expanded to include understanding the relationships among the sequences themselves without regard to the host species, inferring the functions of genes that have not been studied experimentally (Hall *et al*. 2009), and elucidating mechanisms that lead to microbial outbreaks (Hall and Barlow, 2006) among many others. Building a phylogenetic tree requires four distinct steps: (Step 1) identify and acquire a set of homologous DNA or protein sequences, (Step 2) align those sequences,

(Step 3) estimate a tree from the aligned sequences, and (Step 4) present that tree in such a way as to clearly convey the relevant information to others.

MEGA 5 (Tamura *et al*., 2011) is an integrated program that carries out all four stepsin a single environment, with a single user interface eliminating the need for interconverting file formats. At the same time, MEGA 5 is sufficiently flexible to permit using other programs for particular steps if that is desired. MEGA 5 is, thus, particularly well suited for those who are less familiar with estimating phylogenetic trees. MEGA 5: Is a molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. The first step, identification of a set of homologous sequences and downloading those sequences, is implemented by MEGA's own browser built on top of the Google Chrome toolkit. For the second step, alignment of those sequences, MEGA offers two different algorithms: Clustal W and MUSCLE. For the third step, construction of a phylogenetic tree from the aligned sequences, MEGA offers many different methods. The maximum likelihood method is illustrated, beginning with MEGA's models feature, which permits selecting the most suitable substitution model (Hall, 2013). Finally, MEGA provides a powerful and flexible interface for the final step, actually drawing the tree for publication.

2.11 Some antibiotic resistance genes of *Staphylococci* and *E. coli* isolates

2.11.1 *AmpC*

AmpC beta-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of the enterobacteriaceae and a few other organisms, where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and beta-lactamase inhibitor-beta-lactam combinations. In many bacteria, *AmpC* enzymes are inducible and can be expressed at high

levels by mutation. Over-expression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone and is a problem especially in infections due to *Enterobacter aerogenes* and *Enterobacter cloacae*, where an isolate initially susceptible to these agents may become resistant upon therapy (Thompson, 2010).

Transmissible plasmids have acquired genes for *AmpC* enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal *bla* (*AmpC*) gene, such as *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis*. Resistance due to plasmid-mediated *AmpC* enzymes is less common than extended-spectrum beta-lactamase production in most parts of the world but may be both harder to detect and broader in spectrum. *AmpC* enzymes encoded by both chromosomal and plasmid genes are also evolving to hydrolyze broad-spectrum cephalosporins more efficiently. Techniques to identify *AmpC* beta-lactamase-producing isolates are available but are still evolving and are not yet optimized for the clinical laboratory, which probably now underestimates this resistance mechanism (Thompson, 2010). Carbapenems can usually be used to treat infections due to *AmpC*-producing bacteria, but carbapenem resistance can arise in some organisms by mutations that reduce influx (outer membrane porin loss) or enhance efflux (efflux pump activation). The most commonly encountered plasmid-mediated *AmpC* β-lactamases belong to the CMY, FOX, and DHA families. Accurate prevalence data are scarce due to lack of testing, but they appear to be less common than ESBLs (Jacoby, 2009).

2.11.2 *Aac(6')-lb*

Plasmid-mediated *aac(6')-Ib-cr* and *qnr* genes confer reduced quinolone susceptibility, facilitating the selection of chromosomal mutations that confer high-level resistance (Jacoby *et*

al., 2006; Robicsek *et al*., 2006b). *Qnr* and *Aac(6')-Ib-cr* have therefore been hypothesized as potential contributors to the increase in prevalence of quinolone resistance among gram-negative bacteria. Epidemiological surveys have found *qnr* genes in various *Enterobacteriaceae* (Robicsek *et al*., 2006a). Findings from a recent study suggest that *qnrA* and *qnrB* may have contributed to the emergence of fluoroquinolone resistance among *Klebsiella pneumoniae* and *Enterobacter* spp. (Strahilevitz *et al*., 2007). This survey also observed an association between ceftazidime resistance and the presence of *qnr* genes, suggesting that in some species, the frequent clinical association of cephalosporin resistance with fluoroquinolone resistance might be due to the genetic linkage of these two elements on plasmids.

The *cr* variant of *aac(6′)-Ib* encodes an aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin by the N-acetylation of its piperazinyl amine (Endimiani *et al*., 2008). *Aac(6')-Ib-cr* has two amino acid changes, Trp102Arg and Asp179Tyr, which together are necessary and sufficient for the enzyme's ability to acetylate ciprofloxacin. Several prior surveys of *aac(6′)-Ib-cr* prevalence (Endimiani *et al*., 2008; Park *et al*., 2006; Pitout *et al*., 2008) were limited to cephalosporin and or ciprofloxacin-resistant isolates, which were collected over relatively short time frames. Hence, little is known about the epidemiological patterns of *aac(6′)-Ib-cr* in population-representative clinical isolates over time. Interestingly, in two of these earlier studies *aac(6′)-Ib-cr* was found predominantly in *Escherichia coli*. A recent finding support earlier suggestions of a linkage between *aac(6′)-Ib-cr* and CTX-M ESBLs (Coque *et al*., 2008; Pitout *et al*., 2008) and raise the possibility that the use of ciprofloxacin; a widely prescribed fluoroquinolone in the world-is a driver of both fluoroquinolone resistance and the emergence of CTX-M ESBLs.

2.11.3 *Qep A* (quinolone exflux pump)

Three mechanisms have been described for plasmid-mediated quinolone resistance (Cattoir *et al*., 2008): *qnr* determinants (Mammeri *et al*., 2005; Nordmann and Poirel, 2005; Robicsek *et al*., 2006; Wang *et al*., 2011), aminoglycoside acetyltransferase *aac(6')- Ib-cr* (Robicsek *et al*., 2006), and the *qepA* and *oqxAB* efflux pump genes, which confer decreased susceptibility to quinolones (Périchon *et al.*, 2007; Yamane *et al*., 2007; Wong *et al*., 2015). The *qepA1* gene was first investigated in 2007 by two groups from Japan and Belgium (Périchon *et al*., 2007; Yamane *et al*., 2007). In 2008, the *qepA2* was discovered by a French research group (Cattoir *et al*., 2008). Currently, both the *qepA1* and *qepA2* genes have been reported worldwide (Liu *et al*., 2008; Park *et al*., 2009; Guillard *et al*., 2011; Ruiz *et al*., 2012; Chen *et al*., 2014). Recently, a new *qepA* allele *qepA3* from a Chinese patient (GenBank with accession number JQ064560) has been identified. Although the human *qepA* has a fairly low prevalence in Korea (Kim *et al*., 2009; Park *et al*., 2009), it is commonly found in *Enterobacteriaceae* isolates from food-producing animals in China (Liu *et al*., 2008; Ma *et al*., 2009; Chen *et al*., 2014). Therefore, more surveillance is needed for *Enterobacteriaceae* harboring the *qnr*, *aac(6')-Ib-cr* and *qepA*.

2.12 Mobile genetic elements (MGES) of *Staphylococci* and *E. coli* isolates

2.12.1Insertion sequences (IS)

Insertion sequencesare among the simplest mobile genetic elements (MGEs) and widespread in all domains of life. They can occur in a wide range of copy numbers in a particular genome and can move within a genome (between different DNA molecules or within an individual DNA molecule) or horizontally between genomes as part of other MGE vectors such as phages and

plasmids. More than 4500 insertion sequences belonging to 29 families have been identified to date (Siguier *et al*. 2006; Siguier *et al*., 2014) These small and compact elements (typically less than 3 kb), capable of independent transposition, are generally flanked by short terminal inverted repeats (IR) and carry one or two open reading frames (ORF) encoding gene products essential for their mobility (Mahillon and Chandler 1998; Siguier *et al*., 2014). DNA cleavage (at the transposon tips) and strand transfer (of the transposon DNA into the target) necessary for transposition are catalyzed by a transposon-specific enzyme, the transposase. The IR are necessary for transposase-binding, donor DNA cleavage and strand transfer. Often, short directly repeated sequences of the target DNA, from 2 to 14 bp long and whose length is specific for a given element, are generated at the point of insertion (Siguier *et al*. 2014).

In *S. aureus* and *S. sciuri*, IS*256* transposition into an upstream region of, respectively, the *llm* and *mecA* gene resulted in a hybrid promoter that enhanced their transcription and elevated methicillin resistance (Couto *et al*. 2003). Although IS classification is based on a variety of characteristics, the amino acid sequence similarity of their transposases is the principal parameter used for classification (Siguier *et al*. 2006). IS elements have been reviewed thoroughly with a focus on their classification, transposition mechanism and structure (Chandler and Mahillon 2002; Bennett, 2004; Nagy and Chandler 2004; Siguier *et al*. 2006; Siguier *et al.*, 2004; Siguier *et al*., 2014; Siguier *et al*., 2015). Studies have revealed that *IS256* can be involved in phase variation of biofilm formation in *S. epidermidis* (Cho *et al*., 2002).

Normally, *IS256* is part of the composite transposon *Tn4001*, which mediates gentamicin resistance by the bifunctional aminoglycoside-modifying enzyme *aac (6') aph (2").* Previous studies have shown that *IS256* can influence the expression of biofilm formation in *S.*

epidermidis. *Staphylococcal* biofilm formation is mainly mediated by the expression of the *icaADBC* operon, which encodes enzymes for the production of the polysaccharide intercellular adhesin PIA.

2.12.2 Plasmids, Integrons and Integrase

Microbial resistance to antibiotics such as beta-lactams, aminoglycosides, trimethoprim and chloramphenicol according to Hall and Collis (1998) is caused by many different acquired genes, and a substantial proportion of these are part of small mobile elements known as gene cassettes. This gene cassette consists of genes and a downstream sequence (59-base element: 59-be), that acts as a specific recombination site. Gene cassettes can move into or out of a specific receptor site in a companion element called an integron, and integration or excision of the cassettes is catalysed by a site-specific recombinase (*Intl*) that is encoded by the integron. The role of integrons in the spread of antibiotic resistance has been well established. At present count there are 40 different cassette-associated resistance genes and three distinct classes of integron, each encoding a distinct Intl integrase. The same cassettes are found in all three classes of integron, indicating that cassettes can move freely between different integrons. Integrons belonging to class I often contain a further antibiotic resistance gene, *sull*, conferring resistance to sulphonamides.

Plasmid is an automously replicating extra-chromosomal circular DNA molecule, distinct from the normal bacterial genome and nonessential for cell survival under nonselective conditions. Some plasmids are capable of integrating into the host genome. A number of artificially constructed plasmids are used as cloning vectors (Plasmid.org, 2012). Plasmids usually contain between 5 and 100 genes. Plasmids are not essential for normal bacterial growth and bacteria

may lose or gain them without harm. They can, however, provide an advantage under certain environmental conditions.

Bacterial plasmids serve as the scaffold on which they assembled arrays of antibiotic resistance genes, by transposition (transposable elements and insertion sequence of common region [ISCR] mediated transposition) and site-specific recombination mechanisms (integron gene cassettes) (Bennett, 2008). According to Todar (2012), ESBLs production is plasmid mediated and the genes encoding these enzymes are easily transferable among different bacteria. Most of these plasmids not only contain DNA encoding ESBL but also carry genes conferring resistance to several non-β-Lactam antibiotics. Plasmids serve a central role, as the vehicles for resistance gene capture and their subsequent dissemination. According to Daniel and Fabian (2011), plasmids in particular have been implicated in the spread of antibiotic resistance genes. However, the selective pressures which favour plasmid-carried resistance genes and other have not been fully established. Transmission of plasmids is a key factor influencing plasmid-borne antibiotic resistance, but the dosage and interval between treatments is also important (Daniel and Fabian 2011).

2.13 Mechanisms of antibiotic resistance in uropathogens

Antibiotic resistance is defined, if bacteria can still grow under achievable therapeutic concentrations of antibiotic substances at the site of infection (Wagenlehner and Naber, 2004). Resistance is distinguished into primary or inherent resistance of bacteria if bacteria are constitutively resistant against an antibacterial substance, and secondary or acquired resistance if resistance emerges in intrinsically susceptible bacteria. Epidemiologically important is transferable resistance located on plasmids (extrachromosomal autonomous mobile genetic

element transferable to other cells) or transposons [mobile genetic element transposable to plasmids or other chromosomal sites] (Wagenlehner and Naber, 2004).

2.13.1 Alterations of permeability and efflux mechanisms

Probably all antibiotics have to get into the bacterial cells in order to act. Intrinsic resistance for example of Gram-negative bacteria against macrolides is due to impermeability of the outer membrane to these hydrophilic compounds. *Enterococcus* spp. shows decreased permeability towards aminoglycosides and is therefore intrinsically low-level resistant to aminoglycosides. On the other hand permeability can also be altered by altered production of outer membrane proteins, for example in *E. coli*, leading to decreased susceptibility to fluoroquinolones or beta-lactam antibiotics. Efflux mechanisms can potentially pump antibiotic substances, such as quinolones or tetracyclines out of the cell. There are five superfamilies of efflux transport systems known so far: ABC (ATP-binding cassette), MFS (major facilitator superfamily), RND (resistance nodulation-division), SMR (small multidrug resistance) and MATE (multidrug and toxic compound extrusion) family. Efflux systems are responsible for low level resistance, and thus may promote selection of mutations responsible for higher level resistance (Lehn, 2004). A powerful efflux mechanism in *Pseudomonas* spp. is one constitutively produced system (MexAB-OprM–RND superfamily) that generates intrinsic resistance against most beta-lactams, quinolones, tetracycline, chloramphenicol, trimethoprim and sulfamethoxazole. On the other side non-constitutive systems (i.e. MexCD-OprJ; MexEF-OprN) can be expressed by mutation. In other species like *S. aureus*, coagulase-negative *staphylococci* or *C. freundii* efflux is also an important mechanism of clinical resistance against quinolones (Linde *et al*., 2001).

2.13.2 Alterations of target structures

Target structures can be altered by mutations, acquisition of genetic material or inactivation of antibiotics by enzymatic modification (Lehn, 2004).

a. **Mutations**: Fluoroquinolone resistance is mediated by target modifications (DNA gyrase and/or topoisomerase IV) and/or decreased intracellular accumulation (Piddock, 1999). Whereas in Gram-negative bacteria like *E. coli* the DNA gyrase is the primary target, in Gram-positive bacteria like *S. aureus* topoisomerase IV is the primary target for some but not all quinolones. With clinically relevant concentrations newer quinolones like moxifloxacin and gemifloxacin inhibit both targets, the DNA gyrase as well as topoisomerase IV. Ampicillin resistance in enterococci is associated with overproduction of a low-affinity penicillin-binding protein (PBP) that is called (PBP)-5. Vancomycin resistance in enterococci is due to the manufacture of a peptidoglycan side chain from D-alanyl-D-lactate, which is incorporated into the peptidoglycan cell wall instead of the vancomycin target D-alanyl-D-alanyl. The D-alanyl-D-lactate chain shows dramatically lowered affinity to vancomycin.

b. **Acquisition of genetic material**: Resistance to TMP/SMX arises from a variety of mechanisms, involving enzyme alteration, cellular impermeability, enzyme overproduction, inhibitor modification or loss of binding capacity. The mechanism of greatest clinical importance is the production of plasmid encoded, trimethoprim resistant forms of dihydrofolate reductase (Park *et al*., 1997). Resistance in methicilline resistant *S. aureus* (MRSA) is mediated by an additional penicillin-binding protein (PBP)-2a, which has unusual low affinity for all beta-lactam antibiotics. The (PBP)-2 and (PBP)-2a belong to a family of bifunctional proteins with an N-terminal transglycosylase and C-terminal transpeptidase

domain. In case of blockage of (PBP)-2 by betalactam antibiotics, (PBP)-2a takes over the enzymatic activity (Lehn, 2004). (PBP)-2a is encoded by a *mecA*-gene that has been incorporated into the chromosomal DNA of *S. aureus* and coagulase negative *staphylococci* strains.

c. **Inactivation of antibiotics**: Beta-lactamases are enzymes produced by bacteria that inactivate beta-lactam antibiotics by cleavage of the beta-lactam ring. More than 80 different enzymes so far are identified and the substrates comprise penicillins, cephalosporins or other beta-lactam antibiotics (Lehn, 2004). Resistance to penicillin is mediated by a penicillinase that hydrolyses the beta-lactam ring of penicillin. More than 90% of *S. aureus* isolates are penicillinase producer. This resistance can be overcome with penicillinase-stable penicillins such as oxacillin (Livermore, 2000). TEM-1 production is a frequent resistance mechanism in *E. coli* and *Proteus spp*. It therefore confers resistance in strains having acquired the resistance plasmid, for example to ampicillin as well as ampicillin/sulbactam. The SHV-1 beta-lactamase of *Klebsiella pneumoniae*, as well as the K1-beta lactamase of *Klebsiella oxytoca* spp. is chromosomally encoded but are inhibitor-sensitive (Chaves *et al*., 2001). It therefore encodes intrinsic resistance in all *Klebsiella* strains, for example to ampicillin but not to ampicillin/sulbactam. *Enterobacter* spp. possesses a chromosomally encoded *ampC* beta-lactamase that inactivates penicillins and cephalosporins and is not inhibitor-sensitive. Resistance, however, results only if the beta-lactamase is hyper-produced. Ampicillin is a strong inducer of this enzyme; Mezlocillin is less suitable to induce hyperproduction of this beta-lactamase (Yang *et al.,* 1988).

The genus *Citrobacter* comprises such species (*Citrobacter freundii* group) that behave like *Enterobacter* spp. and those that produce other less extended beta-lactamases (*Citrobacter*

koseri/*diversus*). In *Proteus* spp. a wide diversity of beta-lactamases can be produced, serving as a possible beta-lactamase encoding reservoir (Bonnet *et al*., 1999). Plasmid encoded extended-spectrum beta-lactamase production (ESBL) is important in *K. pneumoniae, E. coli, Proteus spp.* and *C. diversus.* Other resistances, such as aminoglycoside and trimethoprim-sulfamethoxazole resistance, are often cotransferred on the same plasmid (Paterson, 2000). As indicated above, *Enterobacter* spp., *C. freundii, Serratia* spp., *K. oxytoca, M. morganii and Providencia* spp. possess a chromosomally encoded beta-lactamase that can be induced to hyperproduction by mutation or derepression (Jones, 2001). This hyper-produced beta-lactamase also causes a resistance phenotype, comparable to ESBL, although no ESBL is produced. Other inactivating enzymes for example can inactivate aminoglycosides or macrolides. *Enterococci* are intrinsically low level resistant, in the case of high level resistance, aminoglycoside combination therapy would be ineffective. Among enterobacteriaceae combinations of gentamicin-modifying enzymes are common in *Pseudomonas* spp. the combination of gentamicin-modifying enzymes and decreased permeability is common (ARSG, 1995). Bacteria exhibit an enormous repertoire of different resistance mechanisms. Unspecific mechanisms such as reduced permeability or efflux alter the tolerance to antibiotic substances less than specific mechanisms, such as inactivation of the antbiotic for example; however the antibiotic spectrum targeted is much more extensive. On the other hand unspecific mechanisms can also be induced by non-antibiotic substances such as salicylates. Low level resistance can thus be confered and give bacteria a selection advantage.

2.14 Causes of increasing antimicrobial resistance

Antibiotic resistance in general is related to the amount of application of an antibiotic substance or a related substance or an unrelated substance with an identical resistance mechanism.

2.14.1 Frequent application of antimicrobials in clinical practice

It is generally assumed that the antibiotic prescription policy of a hospital has a significant impact on emergence of bacterial resistance. In a three-year surveillance study it could be shown that the consumption of imipenem correlated significantly with betalactam resistance in nosocomial *Pseudomonas aeruginosa* isolates, while consumption of ceftazidime or piperacillin/tazobactam had no apparent association with resistance (Lepper *et al*., 2002). The authors could show that periods of extensive imipenem use were associated with significant increases in resistance and thus support the concept that a written antibiotic policy which balances the use of various antibiotic classes may be helpful. Another example is the resistance rate of *E. coli* against fluoroquinolones in isolates of hopitalised children, which is ten-fold less than in adults, because fluoroquinolones are generally not used for children.

2.14.2 Pharmacokinetic parameters of antimicrobials

It has been shown that resistant mutants are selectively enriched in the concentration range between the minimal inhibitory concentraton of wild-type cells and the mutant prevention concentration. This concentration window is called mutant selection window and has a function over the time. The longer bacterial cells are exposed to the mutant selection window, the higher is the possibility of selection of resistant mutants (Zhao and Drlica, 2001). Therefore optimal kinetics show those antibiotics that pass rapidly through the mutant selection window at start of therapy, stay above the mutant prevention concentration the entire time of therapy and return

rapidly again through this window at the end of therapy. Antibiotics with extensive long half lives exhibit a slow return through the window and have therefore a higher propensity to select resistant mutants. On the other hand compliance of patients is improved with reduced dosing by antibiotics with longer half lives.

2.14.3 Increasing numbers of patients with severe or chronic diseases or immune disorders

Patients with severe or chronic diseases or immune disorders are continuously becoming more frequent in the hospital landscape. Baquero *et al*. have presented a mathematical model in which the propability of survival of a resistant bacterial population has been calculated in respect to the host immune status (Baquero *et al*., 1998). A resistant bacterial population had a 44% probability to be selected by antibiotics in the immunocompetent host, and of 90% in the immunodepressed patient. Therefore the patient population is a signi ficant factor in the emergence of antibiotic resistance.

2.14.4 Antibacterial substances other than for treatment of human infections

Antibiotics are not only used for treatment of human infections. A great proportion of antibiotic substances or a related compound is used in animal husbandry as food adjunctive or in household products, such as aseptic soaps or lotions. Avoparcin for example is a glycopeptide antibiotic and therefore related to vancomycin, used exclusively in animal husbandry. Avoparcin was prohibited to be used in husbandry in Denmark since 1995. Consecutively the percentage of vancomycin resistant *E. faecium* isolates in broiler decreased from 80% in 1995 to less than 10% in 1999 (Bager *et al*., 1999).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Equipment

Auto clave (Dixons®), incubator 37ºC (Thermo Scientific®), vortex mixer (Votex-1 Genie®), Bunsen burner (Usbeck®), test tubes (Pyrex®), weighing balance (Ohaus®, Pioneer™), 37ºC incubator shaker (New Brunswick Scientific™), microscope (Wild Heerbrugg M11, Switzerland), 3ºC refrigerator (LG®), -20ºC freezer (LG®), -80ºC freezer (Panasonic®), freezer box, oven (Thermo Scientific®), microcentrifuge (Eppendorf®), boiler (Russell Hobbs®), laminar air flow (PCR-8 recirculating laminar flow pre station Labcaire product 220/240 v), spectrophotometer (Eppendorf Biophotometer 8,5 mm, Lichtstrahihohe), Polymerase chain reaction Thermocycler (Veriti®), electrophoresis casting chamber (Cleaver Scientific™), electrophoresis comb, electrodes, electrophoresis power supply (EPS301), UVP® GelMax Imager.

3.1.2 Glasswares and other materials

Petri dishes (Greiner bio-one®), glass pipette (Pyrex®), measuring cylinder (Pyrex®), bijou bottles, glass slides, glass jars (125, 250 and 500 ml) (Schott Duran™), magnetic stirrer (W and T Avery Ltd., England), eppendorf tubes (Sarstedt®), micropipette tips, marker (Sharpie®), gloves (Finite P IndigoAF®), sterile universal bottles, sterile cotton wool (Dele® absorbent Cotton BP), test tube racks (Science ware®), inoculating loops, eppendorf tube racks, aluminium

foil paper (Novena® foil), micropipette (Pipetman®), micropipette tips, cryovials (Greiner bio-one®).

3.1.3 Culture media

The various laboratory media were prepared according to manufacturer's specification: Cystine Lactose Electrolye Deficient Lactose Agar (Oxoid® Ltd., England), Mac Conkey Agar (Oxoid® Ltd., England), Nutrient Agar (Oxoid® Ltd., England), Mannitol Salt Agar (Biotech Laboratory Ltd., UK), Urea Agar (Oxoid Ltd., England), Cetrimide Agar (Sigma aldrich®), Nutrient Broth (Fluka Spain), Eosin Methylene Blue Agar (Oxoid Ltd., England), Blood Agar (prepared), Luria bertani broth (Oxoid® Ltd., England), Peptone water (Bacto™).

3.1.4 Reagents and chemicals

Glycerol (Sigma aldrich®), 70% ethanol (Analar®), Distilled water, Phosphate buffered saline (Sigma®), illustra™ puReTaq ready-to-go™ PCR beads containing recombinant puReTaq Deoxyribosenucleic acid (DNA) polymerase, gibco® DNAase/RNAase free distilled water, 1X TAE (40 mM Tris-edetate; 1 mM Ethylenediaminetetraacetic acid) buffer, EZ® vision IN GEL Solution 10,000X Biotechnology, agarose (AGTC), Quick load® Purple 100 bp DNA ladder, 10 mM Tris-HCl (Sigma chemical Ltd.), ethidium bromide dye (Sigma chemical Ltd., England), Genomic DNA extraction kit (Zymo Research Corp®, USA), DNA primers (Table 3.1, Table 3.2, Table 3.3, Table 3.4, Table 3.5, and Table 3.6).

3.1.5 Sensitivity discs (Oxoid® Ltd., England)

The antibiotic sensitivity discs and their disc strengths are as follows: Ofloxacin (5 *u*g)-fluoroquinolone, Amoxicillin/clavulanic acid-(20 *u*g/10 *u*g)-beta lactam, Nitrofurantoin (300 *u*g)- furan derivative, Amoxicillin (10 *u*g)-penincillins, Gentamicin (10 *u*g)-aminoglycoside, Ceftriaxone (30 *u*g)-third generation cephalosporin, Cefuroxime (30 *u*g)-third generation

cephalosporin, Tetracycline (30 *u*g)- tetracyclines, Chloramphenicol (30 *u*g)-phenicols, Cotrimoxazole (1.25 *u*g/23.75 *u*g)- sulphonamide, Imipenem (10 *u*g)-carbapenem, and Fosfomycin (200 *u*g) - Fosfomycins. These are commonly prescribed antibiotics, except Imipenem and Fosfomycin.

3.1.6 Control strains

The control strains used for PCR include *S. aureus*25923(MRSA positive), *E. coli* 32518 (*ESBL positive), E. coli* 25922 (antimicrobial sensitivity test).

3.1.7 DNA Primers (Inqaba®)

Staphylococcus aureus specific virulence genes:*mec A-P4, mec A-P7; Luk-pvl-F, Luk-pvl-R; ica A-F, ica A-R; ica D-F, ica D-R* (Table 3.1).

Escherichia coli specific virulence genes*: fim H-F, fim H-R; pap 1, pap 2; hly-s, hly-as; cnf-s, cnf-as; aer s, aer as; bla TEM-F, bla TEM-R; bla SHV-F, bla SHV-R; bla CTX M-F, bla CTX M-R ; bla OXA-F, bla OXA-R* (Table 3.2).

Uropathogenetic *E. coli* (UPEC) phylogenetic grouping: *ChuA.1,ChuA.2; YjaA.1, YjaA.2; TspE4C2.1, TspE4C2.2* (Table 3.3).

Antibiotic resistance genes, auto-transporter genes and mobile genetic elements: *amp c AB-1, amp c-2; aacIb-F; aacIb-R; qepA-F, qep A-R; IS 256-F, IS256-R;c0426.s-5, c0426.s-3; c0478.s-5, c0478.s-3; Int 1-F, Int 1-R* (Tables 3.4, 3.5 and 3.6).

Table 3.1 Primers for virulence factor genes in Staphylococcal isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	Annealing temp(°c)	References	Virulence factor
1	mec A-P4 mec A-P7	TCCAGATTACAACTTCACC AGG CCACTTCATATCTTGTAACG	*mec A*	162	53	Milheiric *etal*., 2007	Methicillin resistance *S.aureus* (MRSA)
2	Luk-pvl-F Luk-pvl-R	ATCATTAGGTAAAATGTCT GGACATGATCCA GCATCAAGTGTATTGGATA GCAAAAGC	*pvl*	433	53	Lina *et al*., 1999	Panton valentin leukocidin (pvl)
3	ica A-F ica A-R	TCTCTTGCAGGAGCAATCA A TCAGGCACTAACATCCAGC A	*ica A*	188	55.5	Cramton *et al.*, 1999	Intracellular adhesion factor(ica)
4	ica D-F ica D-R	ATGGTCAAGCCCAGACAGA G CGTGTTTTCAACATTTAATG CAA	*ica D*	198	55.5	Cramton *et al*., 1999	Intracellular adhesion factor(ica)

Table 3.2 Primers for virulence factor genes in *E. coli* isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	Annealing temp(°c)	References	Virulence factor/ Confers resistance to
1	fim H- F fim H-R	AACAGCGATGATTTCCAGTTTGTGTG ATTGCGTACCAGCATTAGCAATGTCC	*fim H*	465	65	Farmer, 1999	Type1 fimbriae
2	pap 1 pap 2	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	*pap C*	328	65	Soto *et al*., 2011	P-fimbriae
3	hly-s hly-as	AGATTCTTGGGCATGTATCCT TTGCTTTGCAGACTGTAGTGT	*hlyC A* region	556	65	Codruta Romanita *et al*., 2001	Hemolysin
4	cnf-s cnf-as	TTATATAGTCGTCAAGATGGA CACTAAGCTTTACAATATTGA	*cnf*	693	58	Licznar *et al*., 2003	Cytotoxic necrotizing factor
5	aer s aer as	AAACCTGGCTTACGCAACTGT ACCCGTCTGCAAATCATGGAT	*iuc C*	269	60	Codruta Romanita *et al*., 2001	Aerobactin

Table 3.3 Primers for ESBL genes in *E. coli* isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	Annealing temp(°c)	References	Virulence factor/ Confers resistance to
1	bla TEM-F bla TEM-R	AAACGCTGGTGAAAGTA AGCGATCTGTCTAT	*bla TEM*	822	45	Paterson *et al*, 2003; Hujer et al,2006	Penincillins, Cephalosphorins
2	bla SHV-F bla SHV-R	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	*bla SHV*	753	45	Paterson *et al*,2003; Hujer *et al*, 2006	Penincillins, Cephalosphorins
3	bla CTX M-F bla CTX M-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	*bla CTX M*	550	45	Paterson *et al*, 2003;Hujer *et al*, 2006	Penincillins, Cephalosphorins
4	bla OXA-F bla OXA-R	ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC	*bla OXA*	619	45	Karami *et al*, 2008	Penincillins, Cephalosphorins

Table 3.4 Primers for autotransporter genes and phylogenetic grouping in *E. coli* isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	Annealing temp(°c)	References	Confers resistance to/virulence factor
1	c0426.s-5 c0426.s-3	GGAAAGGCAAAGTTTCAGGG GGTGGTATGTTTCTGTTTAC	*Upa B* (Autotransporter gene)	250	65	Allsopp *et al.*, 2011	Mediates adherence to several extracellular matrix (ECM) proteins
2	c0478.s-5 c0478.s-3	GGTGGTTGGGTAGATGC GTTCACATCCAGTACACCAC	*Upa C* (Autotransporter gene)	variable	65	Allsopp *et al.*, 2011	Associated with increased biofilm formation
3	ChuA.1 ChuA.2	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	*Chu A* (Phylogenetic grouping)	279	59	Clermont *et al.*, 2000	A virulenec gene that encodes a hem transporter protein in *E. coli* O157: H7
4	YjaA.1 YjaA.2	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	*Yja A* (Phylogenetic grouping)	211	59	Clermont *et a*l., 2000	A virulence gene Initially identified in the genome of *E. coli* K-12
5	TspE4C2.1 TspE4C2.2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	*TspE* Phylogenetic grouping)	152	59	Clermont *et al.*, 2000	One DNA fragment

Table 3.5 Primers for insertion sequence and plasmid integron genes for Staphylococcal and *E.coli*isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	Annealing temp (°c)	Reference
1	IS 256-F	TGAAAAGCGAAGAGATTCAAAGC	*IS256*	1327	55	Kozitskaya *et al*., 2004
	IS256-R	ATGTAGGTCCATAAGAACGGC				
2	5'-CS	GGC ATC CAA GCA GCA AG	Class 1 integron	Variable amplicon size	55	Levesque *et al,* 1995
	3'-CS	AAG CAG ACT TGA CCT GA				
3	Int 1-F	CAG TGG ACA TAA GCC TGT TC	*Int 1*	160	55	Dillion *et al,* 2005
	Int 1-R	CCC GAG GCA TAG ACT GTA				

Table 3.6 Antibiotic resistance genes for Staphylococcal and *E. coli* isolates

S/N	Primer name	Sequence 5'-3'	Target gene	Amplicon size (bp)	References	Confers resistance to
1	amp c AB-1 amp c-2	GATCGTTCT GCCGCTGTG GGGCAGCAAATGTGGAGCAA	*Amp c*	271	Caroff *et al*., 2000	carbapenems
2	aac*Ib*-F aac*Ib*-R	TTG CGA TGC TCT ATG AGT GGC TA CTC GAA TGC CTG GCG TGT TT	*aac(6')-lb*	482	Park *et al*, 2006	Flouroquinolones
3	qepA-F qep A-R	AAC TGC TTG AGC CCG TAG AT GTC TAC GCC ATG GAC CTC AC	*qep A*	596	Kim *et al*, 2009	Flouroquinolones (quinolone exflux pump)

3.2 METHOD

3.2.1 Ethical Approval

Ethical approval (AD/13/376) was obtained from the Oyo State Ministry of Health (Appendix II), and relevant authorities of the various health facilities.

3.2.2 Study area

Three major maternity health facilities in Ibadan metropolis, Oyo State, Nigeria:

- Adeoyo maternity teaching hospital (Ibadan North LGA)
- Jericho Nursing Home (Ibadan North West LGA)
- Our Lady of Apostle Catholic Hospital, Oluyoru (Ibadan North East LGA)

3.2.3 Study design and duration

A prospective experimental study involving 650 pregnant women on their first visit to the antenatal clinic was carried out over a period of 9 months.

3.2.4 Sample size/study population

This was determined using the Leslie Kisch formular (1965): $n=Z^2pq/d^2$

Where:

n= sample size estimate of patients/volunteers; p: previous proportion in population (50%) = 0.50; q= (1-p) = the probability=0.50; Z: standard normal deviation at 95% confidence interval, corresponding to or =1.96; d = 5% level of significance=0.05

p=50%, 1-p=0.50, d=5% level of significance=0.05

Therefore, $n= \frac{1.96^2 \text{ X } 0.50 \text{ X } 0.50}{0.05^2} = \frac{3.8416 \text{ x } 0.25}{0.0025} = \frac{0.9604}{0.0025}$ = 384 patients approximately

A total of 400 pregnant women attending antenatal clinics at these various health facilities were initially used in this study; dip stick leucocyte esterase test was carried out on an additional 250 pregnant women to confirm actual infection and not contamination with normal flora of the skin making a total of 650 pregnant women.

3.2.5 Inclusion criteria

- Pregnant women attending antenatal clinics for the first time.
- Pregnant women who gave their consent to participate in the study.
- Pregnant women who have not been on any antibiotic medication in the past one month before samples were taken.

3.2.6 Exclusion criteria

- Pregnant women not attending antenatal clinic for the first time
- Pregnant women who did not give their consent to participate in the study.
- Pregnant women who have been on antibiotic medication in less than one month.

3.2.7 Data collection instrument

A structured interviewer-administered questionnaire consisting of twelve questions were used in this study (Appendix I). There are three questions on socio-demographic data, three on gravidity/gestational age, one on the clinical symptoms of urinary tract infection, and five questions on antibiotic drug use/ medication adherence. Patients' medical records from their hospital case files were used to obtain information on drug regimen when a patient could not provide such information.

3.2.8 Statement of confidentiality

All data collected from patients in the course of this study were held highly confidential. Names of the patients were not required.

3.2.9 Data analysis

The statistical tools employed in data analysis were Microsoft excel and SPSS version 20 software; comparison were made at a value of <0.05 significance level. Significant bacteriuria with respect to age group, gestational age (trimester), parity, and occupational status were determined as described by Amadi *et al*., (2007) and Imade *et al*., (2010).

3.3 Specimen collection and analysis

Clean-catch mid-stream urine samples were randomly (convenient) collected and processed adequately (Hooton *et al*., 2000) from volunteers who consented. They were counseled on how to collect the specimen appropriately in order to avoid contamination.

3.4 Dip-stick leucocyte esterase test

Dip-stick leucocyte esterase test (Wilkins*et al.,* 1985; Priyanka *et al.,* 2015) was done on the last 250 samples to confirm actual infection; comparison was made with the first batch of 400 samples which did not show variation in isolation pattern. The sensitivity, specificity, positive predictive value and negative predictive values of the test were determined as described by Yuen *et al*., (2001).

3.5 Specimen processing

Using sterile distilled water, 1:100 dilution of each urine sample collected were made, these were further inoculated using the spread plate method unto cystine lactose electrolyte deficient

(CLED) agar plates and spread over the agar plate using a sterile L-shaped glass rod. The plates were allowed to dry and then incubated aerobically at 37 °C for 24 hours and observed for formed colonies (Cheesbrough, 2002). Colonies formed were counted on the CLED agar plates; this was multiplied by the inoculum volume and dilution factor to obtain the bacterial count present in each undiluted urine sample. Bacterial counts of 10^5 cfu/ml and above were considered as significant for bacteriuria (Cheesbrough, 2002).

3.6 Bacterial identification

Few specks were picked from each formed bacterial colonies and then streaked on the surfaces of already prepared selective media i.e. Mac Conkey agar, Cetrimide agar, Mannitol salt agar, Eosin methylene blue agar, Urea agar. The streaked plates were incubated at 37 °C for 24 hours. Characteristics morphology of the resultant colonies were noted (Cowan, 1974; Collee and Miles, 1989).

3.7 Gram Staining

Heat-fixed smears of the bacterial isolates were prepared, and the slides placed on the staining rack. The smears were flooded with crystal violet and let stand for 30 seconds after which it was rinsed with water for 5 seconds. This was then covered with Gram's iodine mordant and let to stand for a minute and rinsed with water shortly afterwards; 95% ethanol was used to decolourise for 15 to 30 seconds and rinsed with water for 5 seconds. Counterstaining with safranin for about 60 to 80 seconds was done and rinsed with water. Blot drying was done and examined under oil immersion. Gram-positive organisms stain blue to purple; Gram-negative organisms stain pink to red. (Prescott, 2002).

3.8 Characterisation of bacterial isolates

The species of the various isolates were later confirmed using the commercial Microbact identification kits: MB1130A Microbact^{TM} GNB 12E, Microbact^{TM} Staph 12S, and MB0266A Microbact^{TM} Oxidase strips (Oxoid, UK). The Biochemical tests carried out using the kit included:

3.8.1 Indole Test

This test is a biochemical test used to differentiate *E. coli* from other members of the family enterobacteriaceae. Using the Microbact GNB 12A kit, the cultures that turned red/deep pink at the surface layer were regarded as *E. coli* while those which didn't turn red were regarded as non *E. coli.*

3.8.2 Catalase Test

This test is to determine the ability of an organism to produce the enzyme catalase that decomposes hydrogen peroxide to give water and oxygen which is given off as a gas. This test is primarily used to distinguish among Gram-positive cocci. Members of the genus *Staphylococcus* are catalase positive and members of the genera *Streptococcus* and *Enterococcus* are catalase-negative.

A microscope slide was placed inside a petri dish. A petri dish cover was made available. Using a sterile inoculating loop a small amount of colony was collected from a well isolated 24 hour colony and placed on a microscope slide. A dropper was then used to place 1 drop of 3% H_2O_2 onto the organism on the microscope slide without mixing.

3.8.3 Coagulase Test

The coagulase test differentiates strains of *S. aureus* from other coagulase-negative species. *S. aureus* are capable of coagulating plasma in the test tube. There are two types of coagulase test which are; slide and tube test. While *S. aureus* is the most commonly isolated coagulase-positive organism, there are several *Staphylococci* spp which are positive for coagulase activity. *S. schleiferi* and *S. lugdunensis* may give positive result in the slide test for bound coagulase, and *S. schleiferi* and *S. intermedius* may give positive results in the tube coagulase test.

Using the tube method, three test tubes were taken and labeled 'test', negative control' and 'positive control'. Each tube was filled with 0.5ml of 1 in 10 diluted rabbit plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test organism was added. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* was added and also to the tube labeled negative control, 0.1 ml of sterile broth was added. All the tubes were incubated at 37˚C, and observed for four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube.

The Microbact kit 12S comprising of various biochemical tests like fermentation of Maltose, Mannitol, Mannose, Sucrose, Trehalose, N-Acetytl glucosamine, Arginine, urease, Beta-Glucosidase, Alkaline Phosphotase, Beta-Glucoronidase and Beta-Galactosidase was also used in this study.

3.8.4 Oxidase Test

MB0266A Microbact^{TM} Oxidase Strips (Oxoid, UK) was used to smear few colonies of isolates; the phenylalanine present on the oxidase strip was then oxidised to give deep purple colour

indicating the production of oxidase enzyme and a positive result. This detects *Pseudomonas spp,* and *Neisseria spp.*

3.8.5 Methyl Red Test

Using a sterile inoculating loop, bacterial isolates were inoculated into sterile nutrient broth in a test tube. Another test tube containing sterile nutrient broth was prepared (as control). The inoculated tube was incubated at 37 °C or 48 hrs. After incubation, 5 drops of Methyl red reagent was added to the broth and observed for colour change. Red colouration signified a positive result, while yellow was negative. Organisms that are normally methyl red positive were: *E. coli, Salmonella,* and *Proteus.*

3.8.6 Voges Proskauer Test

Using the Microbact GNB 12A kit, a pink-red colouration at the surface signifies a positive result, while lack of red is negative.

3.8.7 Citrate utilization Test

The citrate test which is based on the ability of an organism to utilise citrate as its only source of carbon helps in the identification of enterobacteria. Using the Microbact GNB 12S kit, a bright blue colouration signified a positive result.

3.9 Antibiotic sensitivity Testing

The modified Kirby-Bauer disc diffusion (Bauer *et al.,* 1996) method was used to determine the antibiotic susceptibility profile of the isolates to twelve commonly prescribed antibiotics, and interpreted as specified by the Clinical Laboratory Standard Institute (CLSI, 2012).

3.10 Multiple Antibiotic Resistance (MAR) Index

The MAR Index was determined accordingly (Paul *et al.*, 1997). From the result of the antibiotic susceptibility test, MARI was calculated using the following formula:

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

3.11 Phenotypic extended spectrum β-lactamase (ESBLs) production test

Phenotypic determination of ESBL production by the *E. coli* isolates was done by the double disc synergy test as described by Tsering, *et al.,* 2009. The multi-drug resistant *E. coli* isolates were standardized in 5 ml normal saline using McFarland 0.5 turbidity standard. Standardized organisms were then streaked onto prepared Mueller-Hinton agar; this was allowed to dry for 5 mins at room temperature. Using a sterile pair of forceps, cefpodoxime (10 µg) and ceftriaxone (30 µg) discs were gently placed on the agar at a distance of 15 mm, center to center from a combination disc of amoxicillin-clavulanic acid (20:10 µg respectively).

The plates were then incubated for 18-24hrs and extended spectrum in the zone of inhibition were observed and interpreted. *E. coli* ATCC 25922 was used as a negative control. Positive result of ESBLs was interpreted as any one that has the zone around the test antibiotics disc increased towards the center disc of amoxicillin-clavulanic acid. A $\geq$ 5 mm increase in zone diameter for either antimicrobial agent compared to its zone when tested alone signifies positive result. The results were further interpreted using CLSI (2012) guidelines (Appendix VII).

3.12 Detection of *AmpC* production

A presumptive test for *AmpC* enzyme detection was done before the disc confirmatory test. The method described by Cheesbrough, (2000) was used to determine the sensitivity to cefoxitin

using the disc diffusion and interpreted with the CLSI (2016) guideline. Isolates yielding a zone diameter of less than 18mm were screen positive and they were further subjected to confirmatory test.

3.12.1 *AmpC* Confirmatory Test

AmpC confirmatory test was carried out as described by Jarlier, *et al.*, (1988). All confirmed isolates from the presumptive test were standardized in sterile normal saline using 0.5 MacFarland turbidity standards. Prepared Mueller-Hinton agar was streaked with the confirmed standardized isolates of *E. coli* from the presumptive test and allowed to dry for 5 mins. Cefoxitin antibiotics (10 µg) was placed in the middle of the inoculated plate and sterile 6 mm disc impregnated with sterile 20 µl normal saline were placed side by side as if they were to touch each the cefoxitin disc. The plates were incubated for 18 - 24 hrs at 37^{o}C and *E. coli* ATCC 25922 was used as a negative control. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc. A negative test had an undistorted zone.

3.13 Identification of methicillin-resistant *S. aureus* (MRSA)

Sterile Mueller-Hinton agar was prepared and poured in a petri dish, this was allowed to set. Confirmed *S. aureus* isolates were swabbed onto the set agar surface. A disc of cefoxitin antibiotic was placed at the centre of the inoculated agar and was incubated in an inverted position for 18 - 24 hours at 37˚C. The test result was interpreted as sensitive or resistant based on the Clinical and Laboratory Standard Institute (CLSI), 2012 guideline.

3.14 Molecular analysis

3.14.1 Bacteria cell preparation

The method described by Dubey (2009) and Allsopp *et al.,* (2011) was used in the preparation of bacterial cells. Single colonies were picked from freshly streaked isolates on the various selective media plates and inoculated into 5 ml Luria and Bertani (LB) broth (Oxoid®) medium and incubated (in a shaker incubator) overnight at 37^0C for 18 - 24 hrs. Bacterial cells were then harvested by centrifugation at 4^oC, 8000 rpm (6800 x g) in a refrigerated microcentrifuge for 30 seconds in an eppendorff tube. The supernatant was decanted and bacterial cells harvested.

3.14.2 Genomic DNA Extraction

Genomic DNA extraction was carried out using the protocol in DNeasy Blood and Tissue Handbook (2006). The harvested cell pellets were dislodged and 20 µl of proteinase K was added and mixed thoroughly by vortexing. The mixture was further incubated at 56 ^{o}C for 3 hrs and vortexed occasionally using a thermo-mixer until the cells were completely lysed and properly mixed to prevent clogging of the DNeasy Mini spin column. 200 µl of buffer AL was added to the sample, and mixed thoroughly by vortexing. This was further be accompanied by the addition of 200 µl of absolute ethanol (96 - 100%) and mixed again by vortexing to yield homogenous solution. This mixture was pipetted into DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 min. The flow-through and collection tube was then discarded.

The DNeasy Mini spin column was then transferred into a new 2 ml collection tube and 500 µl buffer AW1 was added and centrifuged again at 8000 rpm for 1min before the flow-through was also discarded. For the third time, DNeasy Mini spin column was placed in 2 ml collection tube and 500 µl buffer AW2 was added and centrifuged at 14000 rpm for 3 min to dry the DNeasy

membrane in other to remove the residual ethanol that might interfere with subsequent reactions. The flow-through and collection tube was removed and discarded carefully so that the column does not come into contact with the flow-through, resulting in carryover of ethanol. The used DNeasy Mini spin column was now placed in a clean 2 ml micro-centrifuge tube and 200 µl buffer AE was introduced onto the DNeasy membrane to elute, and increase the final DNA concentration and yield in the eluate. The column was then incubated at room temperature for 1 min before being centrifuged at 8000 rpm for 1 min. The elution of the membrane was then repeated twice to ensure increased overall DNA yield in the same microcentrifuge tube used in step 7, but care was taken to prevent dilution of the first eluent.

3.14.3 Agarose gel electrophoresis

To ascertain that genomic DNA of the bacterial isolates was actually extracted, the eluent were subjected to agarose gel electrophoresis, prepared as stated below:

1% agarose gel was used to resolve the genomic DNA. The agarose gel was prepared by combining l.0 g of agarose in ten times concentration (10X) of Tris acetate ethylene diamine tetra acetate (2 ml 10X TAE) buffer and 98 ml distilled water in a 250 ml beaker flask and heating in a microwave for 2 minutes until the agarose is dissolved. 20 µl ethidium bromide (5.0 mg/ml) was added to the dissolved agarose solution as dye and mixed. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs inserted at the red bands to ensure easy view of the well, while filling the genomic DNA. It's then allowed to set for 30 minutes.The casting combs were carefully removed after the gel had completely solidified, one time concentration (1X) TAE electrophoresis buffer was then added to the reservoir until the buffer covers the agarose gel, 5 µl of gel tracking dye (bromo phenol blue) was added to 15 µl of each sample with gentle mixing, 20 µl of the sample was then loaded onto the wells of the gel,

the mini horizontal electrophoresis gel set up was covered and the electrodes connected running from cathode (-) to anode (+). Electrophoresis was carried out at 100 mV for 45 minutes to allow easy separation of sample based on molecular weight. At the completion of the electrophoresis, the gel was removed from the buffer (Tris) and the gel viewed under a trans-illuminator UV light of wavelength 302 nm. The band pattern of the DNA fragments was then photographed with a Polaroid camera and documented using an electrophoresis gel documentation system. Electrophoresis is employed to identify the number of plasmid copies present in different isolates. However, standard DNA molecular weight marker (1 kb) was used to estimate the genomic DNA size.

3.14.4 Detection of specific virulence genes in multi-drug resistant (MDR) *S. aureus* isolates and *E. coli* isolates

Specific primers were used to amplify sequences of the *fim*, *paphly*, *cnf*, and *aer* operons in the multidrug resistant ESBL *E. coli* isolates. The MDR *S.aureus* isolates were similarly screened for the presence of *mecA, pvl, ica A, ica D* operons. These were *E. coli* and *S. aureus* isolates with MARI ranging between 0.83 - 1.00 (i.e., isolates resistant to 10, 11 or all of the 12 antibiotics tested).

Using optimized and standard polymerase chain reaction (PCR) protocols as described by the DNeasy Blood and Tissue Handbook (2006), amplification of the various virulence DNA fragments were carried out using Dream Taq^{TM} DNA polymerase. Dream Taq^{TM} PCR master mix (2X) was vortexed and centrifuged for 30 seconds at 8000 rpm after thawing. The thin-walled PCR tube was then placed on an ice pack and the following components were added for each isolate for single reaction: (a) Dream Taq^{TM} PCR master mix. (b) Forward primers (c)

reverse primers (d) template DNA, Taq buffer, dNTP (e) the nuclease-free water. The samples were vortexed gently and spun down. The primers to be used for PCR are as contained in the Tables 3.1, 3.2, 3.3, 3.4, and 3.5.

3.14.5 Detection of resistance gene using their respective primer (PCR)

Amplification of resistant DNA fragments was carried out using Dream Taq™ DNA polymerase, optimized for all standard PCR applications as described by DNeasy Blood and Tissue Handbook (2006). Dream Taq™ PCR master mix (2X) was vortexed and centrifuged for 30 seconds at 8000rpm after thawing. The thin-walled PCR tube was then placed on an ice pack and the following components was added for each isolate for single reaction: (a) Dream Taq™ PCR master mix. (b) Forward primers (c) reverse primers (d) template DNA, Taq buffer, dNTP (e) the nuclease – free water. The samples were vortexed gently and spin down.

3.14.6 16srRNA sequencing of bacterial isolates

PCR products of multi-drug resistant isolates that coded for specific virulence genes were sequenced at *Inqaba* Biotechnical Industries (Pty) Ltd, South Africa, using an ABI PRISM 377 automated DNA sequencer. Current GenBank sequence databases (BLAST suite of programs) were used to evaluate the result of the sequenced genomic DNA. (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*).

3.14.7 Phylogenetic grouping of MDR ESBL *E. coli* isolates

Phylogenetic group of origin of the MDR ESBL *E. coli* isolates was determined using a three-locus PCR-based method as described by Clermont *et al*., (2000). This is a rapid technique for determining the phylogenetic groups of *E. coli* strains based on PCR detection of the *chuA* and

yjaA genes and DNA fragment TSPE4.C2. Phylogenetic grouping interpretative criteria according to Clermont *et al*., (2000); Gordon *et al*., (2008) and Ejrnaes *et al*., (2011) is as follows:

- *ChuA* + *Tsp*-/+*YjaA* or+ *IbeA*= Group B2
- If no *ChuA* = Groups B1 or A
- Virulent Extra-intestinal strains of Uropathogenic *E. coli* are mostly = Group B2.

3.14.8 Phylogenetic tree analysis

Molecular phylogenetic analysis of the sequenced bacterial isolateswas conducted by the maximum likelihood method (MLM) based on the Tamura - Nei model using the Molecular Evolutionary Genetics Analysis Version 7.0 (MEGA 7®)software. (Kumar *et al*., 2016).

CHAPTER FOUR

4.0: RESULTS

4.1 Prevalence of significant bacteriuria among pregnant women with reference to clinical symptoms

A total of 650 urine samples were analyzed. Using significant bacteriuria level as 10^5 cfu/ml, the prevalence of significant bacteriuria with respect to the clinical symptoms of urinary tract infections was ascertained from the information derived from the questionnaires administered. Bacteriuria was observed in 246 (37.8%); out of which 149(60.6%) were asymptomatic, while 97(39.4%) presented with clinical symptoms (Table 4.1).

4.2 Morphology and biochemical reactions of bacterial isolates from urine samples

The results of the morphological and biochemical reactions of the bacterial isolates from the urine samples of pregnant women attending antenatal clinics in Ibadan are shown in Appendix III.A total of246 isolates were obtained, ranging from *Staphylococcus* spp. 119 (48.37%) the most prevalent, followed by *Escherichia* spp. 52(21.1%) and *Acinetobacter* spp. 1(0.40%) the least.

4.3 Dipstick leucocyte esterase test

The resulting sensitivity, specificity, positive predictive value and negative predictive values as shown in Table 4.2 were ideal.

4.4 Diversity of bacterial urinary isolates from pregnant women in Ibadan metropolis

A wide diversity of bacterial isolates was observed among the pregnant women (Table 4.3). This includes the typical and atypical bacterial uropathogens.

Table 4.1 Prevalence of significant bacteriuria among pregnant women with reference to clinical symptoms

Bacteriuria type	Number of patients	Percentage Prevalence (%)
Symptomatic	97	39.4
Asymptomatic	149	60.6
Total	**246**	**100.0**

Table 4.2 Comparison of Dipstick Leucocytes esterase Test (LET) with culture for 250 urine samples (last batch)

Leucocyte	**Culture Negative**	**Culture Positive**	**Total**
Negative	64	13	77
Positive	11	162	173
Total	**75**	**175**	**250**

Sensitivity = 162/175 x 100 = 92.6 %

Specificity = 64/75 x 100 = 85.3 %

Positive predictive value = 162/173 x 100 = 93.6 %

Negative predictive value = 64/77 x 100 = 83.1 %

Table 4.3 Diversity of bacterial urinary isolates from pregnant women in Ibadan metropolis

Organism	Number	Percentage prevalence (%) (n=246)
Staphylococcus spp	119	48.37
Escherichia spp	52	21.13
Proteus spp	17	6.91
Streptococcus spp	17	6.91
Providencia spp	14	5.69
Enterobacter spp	9	3.65
Klebsiella spp	4	1.61
Morganella spp	4	1.61
Pseudomonas spp	3	1.21
Serratia spp	3	1.21
Alcaligenes feacalis	2	0.81
Salmonella spp	1	0.40
Acinetobacter spp	1	0.40

4.5Prevalence of Gram negative urinary isolates from pregnant women in Ibadan metropolis

The prevalence of Gram negative organisms isolated from the urine samples of pregnant women attending antenatal clinics is shown in Table 4.4. *E. coli* 48(19.5%) was the most prevalent, followed by *Proteus mirabilis* 13(5.28%) and *Providencia stuartii* 12(4.87%).

4.6Prevalence of Gram positive uropathogens from pregnant women attending antenatal clinics.

The prevalence of Gram positive isolates from pregnant women attending antenatal clinics in Ibadan metropolis is shown in Table 4.5. *S. epidermidis* 51 (21.95%) was the most prevalent followed by *S. aureus* 27(10.97%), *Streptococcus* spp. 17(6.09%) and *Staphyloccocus saprophyticus* 12(4.87%).

4.7 Prevalence of bacteriuria with respect to gestational age (trimester).

The number of urine samples colonized by bacteria with respect to the gestational age of the women is shown in Table 4.6. Bacteriuria was most prevalent among women in their third trimester 131(53.2%), followed by those in the second trimester 69(28.0%). The least bacteriuria level was observed in women in their first trimester 46(25.9%).

4.8 Prevalence of bacteriuria with respect to parity

Bacteriuria prevalence with respect to the women's parity was determined and shown in Table 4.7. Multipara women were the most 88(35.8%), followed by the primipara 67(27.2%). The least

Table 4.4 Prevalence of Gram negative urinary isolates from pregnant women in Ibadan metropolis

Organism	Number of isolates	Percentage (prevalence) (n=246)
Escherichia coli	48	19.5
Proteus mirabilis	13	5.28
Providencia stuartii	12	4.87
Enterobacter agglomerans	8	3.25
Proteus vulgaris	4	1.62
Escherichia fergusonii	4	1.62
Morganella morganii	4	1.62
Klebsiella pneumoniae	3	1.21
Pseudomonas aeruginosa	3	1.21
Serratia marcescens	3	1.21
Alcaligenes feacalis	2	0.81
Enterobacter cloacae	1	0.40
Klebsiella oxytoca	1	0.40
Providencia alcalifaciens	1	0.40
Providencia rettgeri	1	0.40
Salmonella enterica subsp enterica	1	0.40
Acinetobacter quilouiae	1	0.40
Total	**110**	**44.62**

*Isolates not identified to specie level with MB1130A Microbact GNB 12E, Microbact Staph 12S and MB0266A Microbact Oxidase Strips. Confirmation was by 16srRNA sequencing and NCBI BLAST.

Table 4.5 Prevalence of Gram positive uropathogens from pregnant women attending antenatal clinics.

Organism	Number of isolates	Percentage (prevalence) (n=246)
Staphylococcus epidermidis	51	21.95
Staphylococcus aureus	27	10.97
Streptococcus spp	17	6.09
Staphyloccocus saprophyticus	12	4.87
Staphylococcus haemolyticus	6	2.34
Staphylococcus capitis subsp.ureolyticus	4	1.62
Staphylococcus simulans	4	1.62
Staphylococcus lugdenensis	3	1.21
Staphylococcus warneri	2	0.81
Staphylococcus lentus	2	0.81
Staphylococcus aureus subsp. Anaerobius	1	0.40
Staphylococcus caprae	1	0.40
Staphylococcus chromogenes	1	0.40
Staphylococcus cohnii subsp. Urealyticum	1	0.40
Staphylococcus cohnii subsp. Cohnii	1	0.40
Staphylococcus intermidius	1	0.40
Staphylococcus xylosus	1	0.40
Staphylococcus equorium	1	0.40
Total	**136**	**55.38**

*Isolates not identified to specie level with MB1130A Microbact 12E, Microbact Staph 12S, and MB0266A Microbact oxidase strips

Table 4.6 Prevalence of bacteriuria with respect to gestational age (trimester)

Trimester	Number of patients	Number colonized	Percentage colonized (%)
First	177	46	25.9
Second	212	69	28.0
Third	261	131	53.2
Total	**650**	**246**	**37.84**

There is significant difference in percentage colonized with respect to gestational age, using $\chi 2$, at $P<0.05$

Table 4.7 Prevalence of bacteriuria with respect to parity

Parity	Number colonized	Percentage colonized (%)
Grandmultipara	49	20.0
Multipara	88	35.8
Primigravida	42	17.0
Primipara	67	27.2
Total	**246**	**100.0**

bacteriuria was observed in the primigravida 42(17.0%) women.

4.9 Prevalence of bacteriuria with respect to age group of pregnant women attending antenatal clinics.

As shown in Table 4.8, the highest prevalence of bacteriuria 101(44.2%) was observed in pregnant women aged 25-30 years. This was followed by those aged 30-35 years 69(35.0%), then women aged 35-40 years 42(39.6%). The least bacteriuria was seen in pregnant women aged 15-20 years 13(27.6%).

4.10 Prevalence of bacteriuria with respect to occupational status

From the result obtained, the house-wives (stay-at-home women) had the highest bacteriuria 87(35.3%). This was followed by the working-class women 83(33.7%) and then those that are either self-employed or are business women 76(30.8%). This is presented in Table 4.11.

4.11 Prevalence of bacteriuria with respect to educational level

The educational level of the women was one of the socio-economic factors (Table 4.10) seen to affect the bacteriuria level observed. Women with no formal type of education had the highest bacteriuria level 79(32.1%), while the least was observed in women with Postgraduate education 18(7.3%).

4.12 Phenotypic resistance pattern of uropathogens from pregnant women attending antenatal clinics.

Appendix III shows the phenotypic resistance profile of the isolates. The Multiple antibiotic resistance index (MARI) of the isolates were determined; and this varied from 0.08 to 1.0.

Twelve various antibiotics were used belonging to different class. Percentage resistance of the isolates also varied from 8.3% to 100%. Majority of the isolates (69.5%) were observed to be multi-drug resistant.

4.13 Summary of antimicrobial resistance profile of uropathogens from pregnant women

In table 4.14, the summary of the phenotypic resistance profile of the urinary isolates from pregnant women in Ibadan metropolis to commonly prescribed antibiotics is shown.

4.14 Extended spectrum beta-lactamase detection in uropathogenic *E. coli* isolates

Figure 4.1 shows the prevalence of ESBL production among the 48 *E. coli* isolates from the pregnant women; 35(72.9%) of them were ESBL producers, while 13(27.1%) were non-ESBL producers.

4.15 Methicillin resistant *Staphylococcus aureus* (MRSA) among pregnant women in Ibadan metropolis

The incidence of methicillin resistance among *S. aureus* isolates from the pregnant women indicates that out of the 28 *S. aureus* isolates [*S. aureus* 27; *S. aureus* subsp *anaerobius* 1], 16(57.0%) were MRSA while 12 were Methicillin sensitive *S. aureus* (MSSA). (Fig.4.2).

Table 4.8 Prevalence of bacteriuria with respect to age group of pregnant women attending antenatal clinics.

Age group (years)	Number of patients	Number colonized	Percentage colonized (%)
15-20	47	13	27.6
20-25	72	21	29.1
25-30	228	101	44.2
30-35	197	69	35.0
35-40	106	42	39.6
Total	**650**	**246**	**37.8**

There is significant difference in percentage colonized with respect to age groups using $\chi 2$at $P<0.05$

Table 4.9 Prevalence of bacteriuria with respect to occupational status

Occupational status	Number of women	Percentage (%)
House wives	87	35.3
Self employed/business	76	30.8
Working class	83	33.7
Total	**246**	**100.0**

There is significant difference in percentage colonized with respect to age groups using $\chi 2$at $P<0.05$

Table 4.10 Prevalence of bacteriuria with respect to educational level

Educational level	Number of women	Percentage (%)
No formal education	79	32.1
Primary	62	25.2
Secondary	51	20.7
Tertiary	36	14.6
Post Graduate	18	7.3
Total	**246**	**100.0**

Table 4.11 Resistance profile of bacterial urinary isolates to commonly prescribed antibiotics

Antibiotics (µg)	Gram positive (n)	Percentage resistance	Gram negative (n)	Percentage resistance	Total Percentage Resistance
Imipenem (10)	22	10.4	27	12.9	23.3
Ofloxacin (5)	31	14.8	19	09.0	23.8
Amoxicillin/clavulanic acid (20/10)	48	22.9	44	20.9	43.8
Nitrofurantoin (300)	23	10.9	20	0.95	11.9
Amoxicillin (10)	77	36.7	69	32.9	69.6
Gentamicin (10)	21	10.0	25	11.9	21.9
Ceftriaxone (30)	47	22.4	53	25.2	47.6
Cefuroxime (30)	41	19.5	63	30.0	49.5
Tetracycline (30)	26	12.4	66	31.4	43.8
Chloramphenicol (30)	59	28.1	51	24.3	52.4
Cotrimoxazole (1.25/23.75)	62	29.5	78	37.1	66.6
*Fosfomycin (200)	-	-	100	47.6	47.6

*Fosfomycin is used for testing and reporting *Escherichia coli* UTI isolates only. It is the only antibiotic not commonly prescribed in Ibadan metropolis, Nigeria.

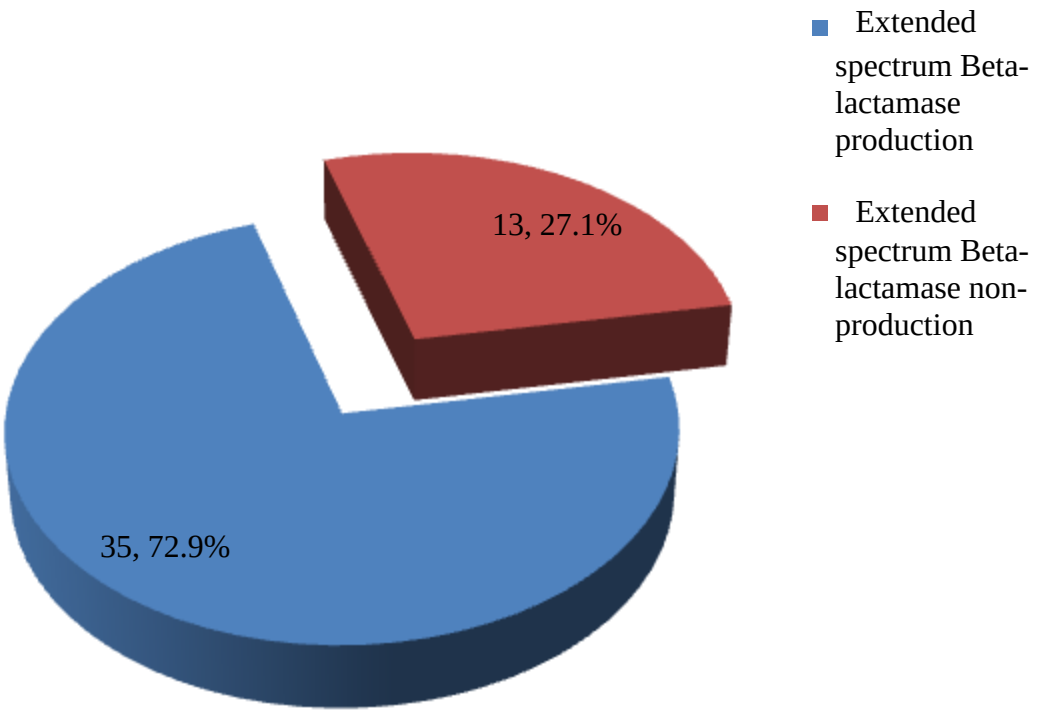


Figure 4.1: Detection of extended spectrum beta-lactamase production in uropathogenic *E. coli* (UPEC)

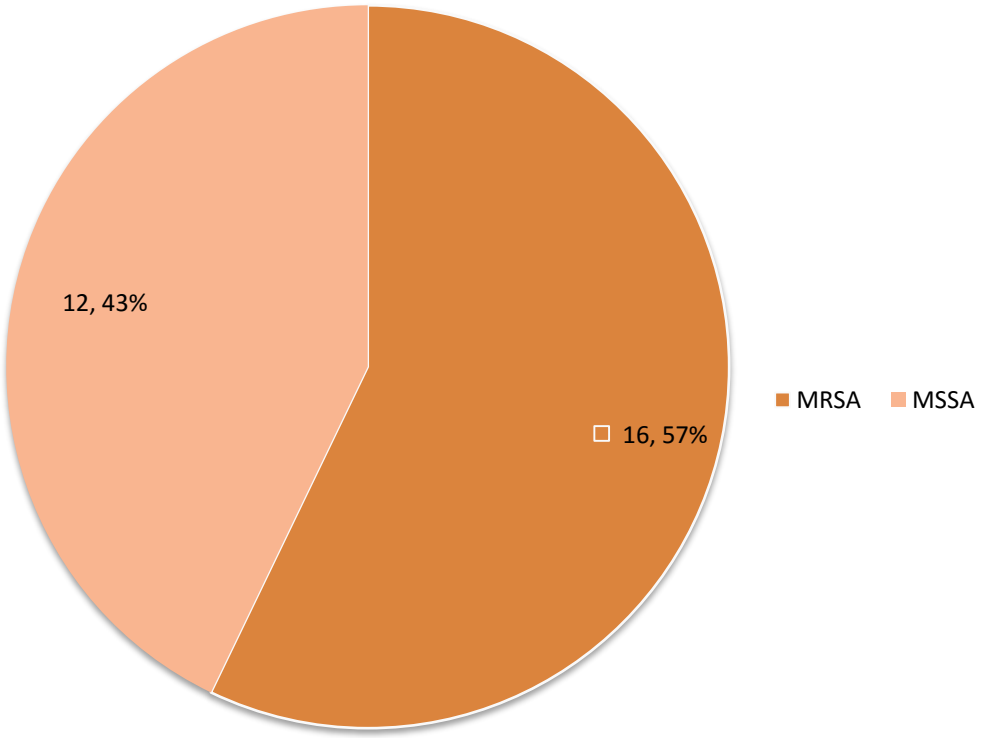


Figure 4.2: Incidence of Methicillin resistance among *S. aureus* isolates from pregnant women attending antenatal clinics

4.16 Molecular characterization of multi-drug resistant uropathogens from pregnant women

4.16.1 Genomic DNA Extraction

Amplification of the genomic DNA of 10 MDR Methicillin resistant *S. aureus* and 10 MDR ESBL producing *E. coli* isolated from pregnant women attending antenatal clinics in Ibadan metropolis is shown below. The purity (DNA conc *n*g/µl) was determined using a Nano drop machine (Appendix V). An absorbance 260/280 value of 1.8 is considered ideal for DNA. Lower absorbance values indicate the presence of protein, phenol or other contaminants. This factor influenced the degree of brightness of the DNA bands on electrophoresis gel when illuminated under UV light (Appendix IX).

4.16.2 Amplification of 16S rRNA in MDR *S. aureus* and MDR ESBL positive *E. coli* isolates purified for sequencing

The 16S rRNA amplification of 22 bacteria: 10 *S. aureus* isolates from the urine samples of pregnant women and a control *S. aureus* strain ATCC 25923; 10 *E. coli* isolates and also an *E. coli* control strain ATCC 35218 is shown in Plate 4.1.

4.16.3 Amplification of *mecA* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

M*ecA* gene that codes for Methicillin resistance was present in 2(20%) of the MDR *S. aureus* isolates (Plate 4.2), i.e. isolates S6 and S40 at 162 bp.

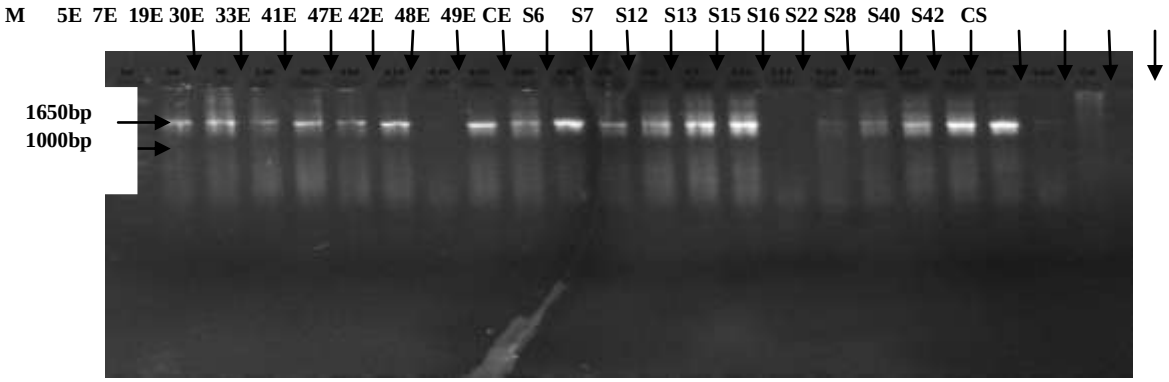


Plate 4.1 Amplification of 16S rRNA MDR *S. aureus* and MDR ESBL positive *E. coli*isolates purified for sequencing

Lane M= 1kb ladder
Lane 5E=*E. coli* 5E
Lane 7E=*E. coli* 7E
Lane 19E= *E. coli* 19E
Lane 30E=*E. coli* 30E
Lane 33E=*E. coli* 33E
Lane 41E= *E. coli* 41E
Lane 47E=*E. coli* 47E
Lane 42E=*E. coli* 42E
Lane 48E= *E. coli* 48E
Lane 49E= *E. coli* 49E
Lane CE=Control *E. coli* ATCC 35218
Lane S6= *S. aureus* S6
Lane S7= *S. aureus* S7
Lane S12= *S. aureus* S12
Lane S13=*S. aureus* S13
Lane S15=*S. aureus* S15
Lane S16= *S. aureus* S16
Lane S22= *S. aureus* S22
Lane S28= *S. aureus* S28
Lane S40= *S. aureus* S40
Lane S42= *S. aureus* S42
Lane CS=Control *S. aureus* ATCC 25923

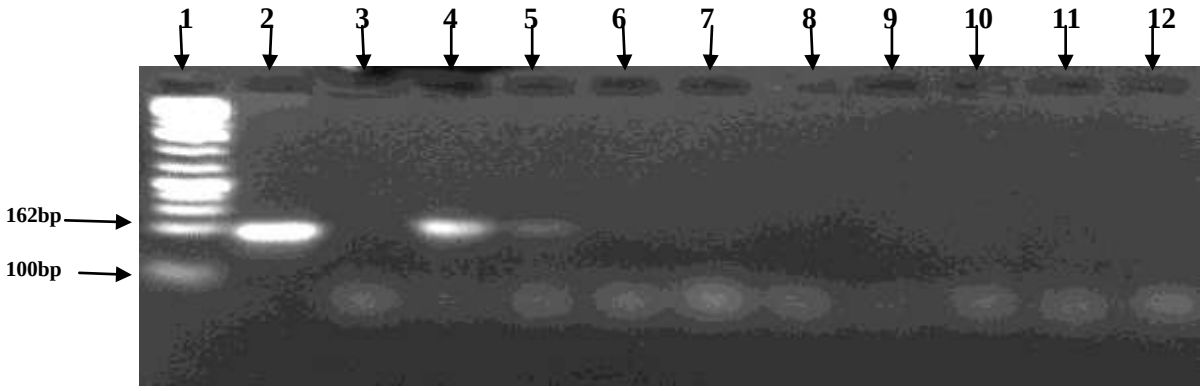


Plate 4.2 Amplification of *mecA* gene in MDR *S. aureus* isolates from pregnant womenattending antenatal clinics (*mecA* gene=162 bp Milheric *et al.*, 2007)

Lane1: 100 bp DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

4.16.4 Amplification of *pvl* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

The panton valentine leukocidin gene *pvl* (433 bp;Lina *et al*., 1999**)** evaluated in this study was absent in all the MDR *S. aureus.* Panton valentine leukocidin is a toxin responsible for pore-formation and is epidemiologically linked to some deep-seated infections and primary skin and soft tissue infections.

4.16.5: Amplification of *Amp C* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

The result in Plate 4.3 shows the amplification of *Amp C* gene in 10(100%) of MDR *S. aureus* isolates at 271 bp.

4.16.6 Amplification of *IcaD* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

Intracellular adhesion factor, *icaD* gene (198 bp) that codes for biofilm formation was observed in 8(80%) of the MDR *S. aureus* (Plate 4.4). *Staphylococcal* biofilm formation is mainly mediated by the expression of the (intracellular adhesion) *icaADBC* operon.

4.16.7 Amplification of *IcaA* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

Another intracellular adhesion factor, *icaA* (188 bp)gene that codes for biofilm formation was amplified in 5(50%) of the MDR *S. aureus* (Plate 4.5).

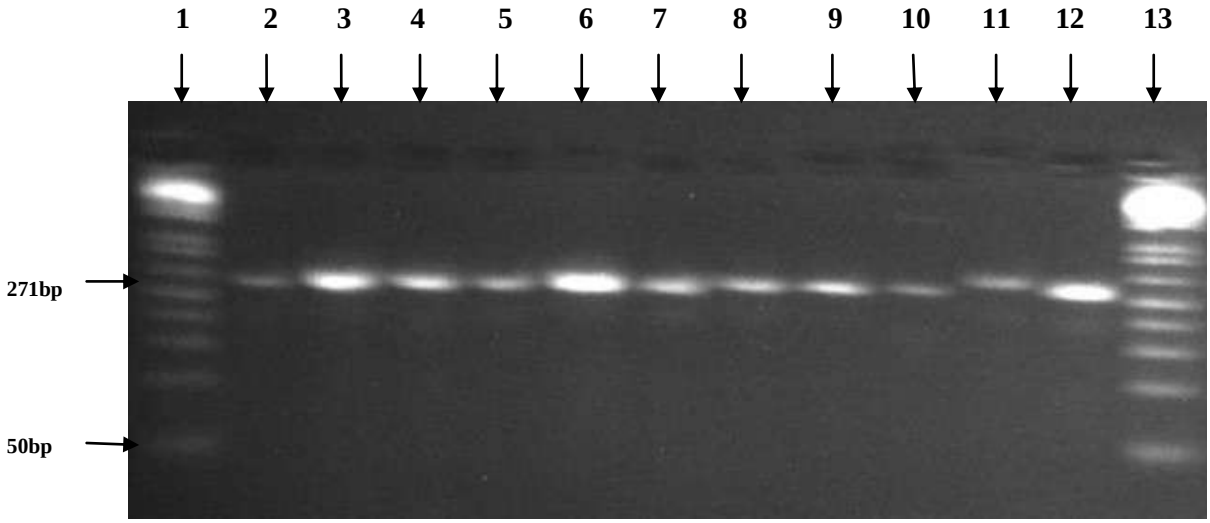


Plate 4.3 Amplification of *Amp C* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics (*Amp C* gene= 271 bp; Caroff *et al*., 2000)

Lane1: 100 bp DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S11
Lane 12: S16 = *S. aureus* S16
Lane 13: DNA marker

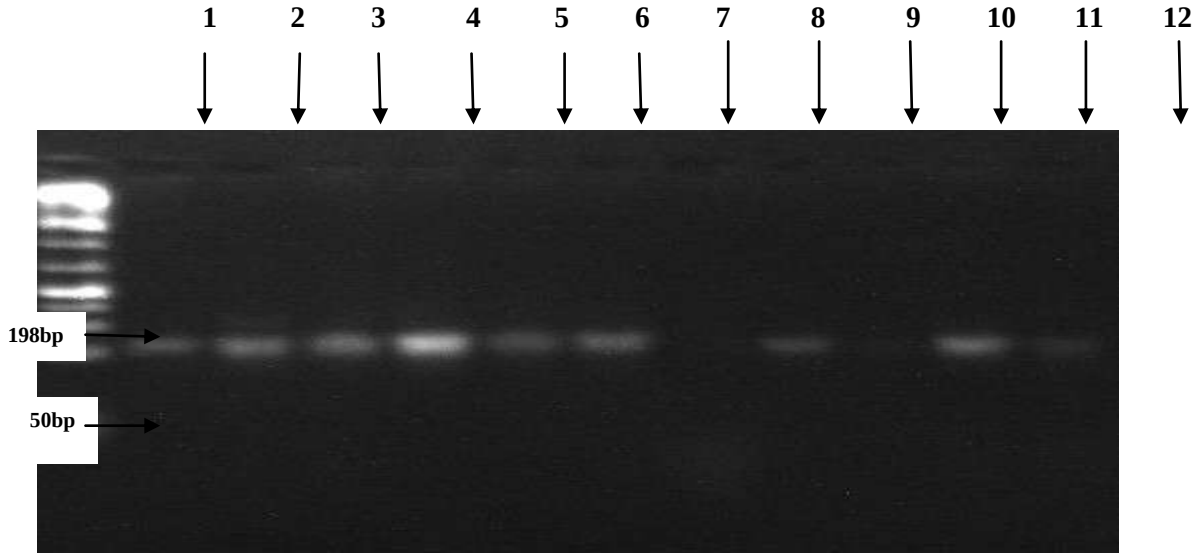


Plate 4.4 Amplification of *IcaD* genes in MDR *S. aureus* from pregnant women attending antenatal clinics (*icaD* gene= 198 bp; Cramton *et al*., 1999)

Lane1: 100 bp DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

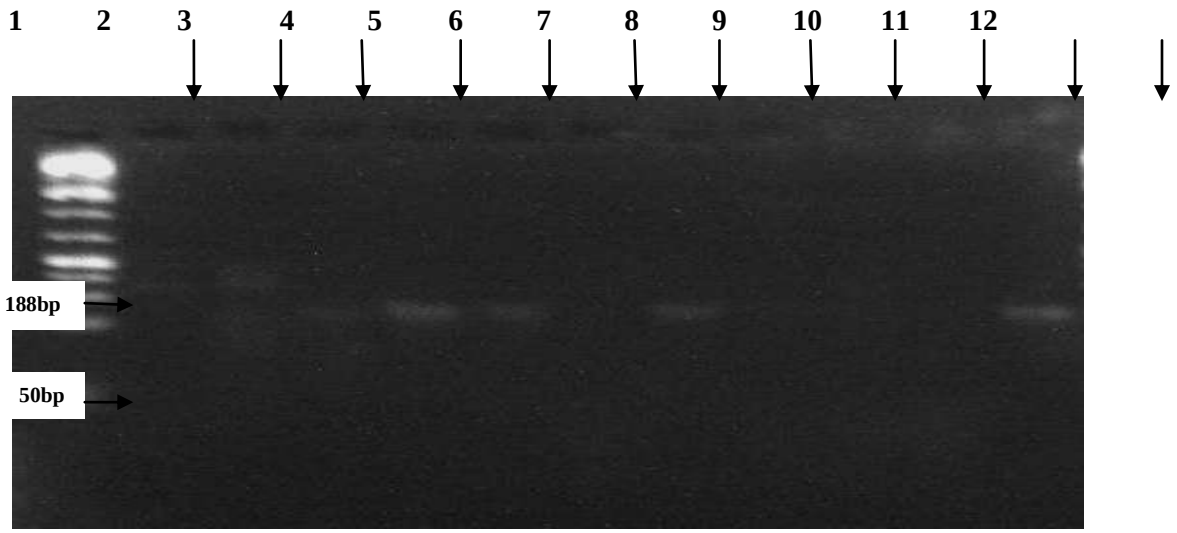


Plate 4.5 Amplification of *IcaA* genes in MDR *S. aureus* from pregnant women attending antenatal clinics (*IcaA* gene= 188 bp Cramton *et al*, 1999)

Lane1: 100 bp DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

4.16.8 Amplification of *Int 1* plasmid Integronin MDR *S. aureus* isolates from pregnant women attending antenatal clinics

Plasmid integron *int1* (160 bp) was observed in 10(100%) of the MDR *S. aureus* isolates (Plate 4.6). Most integrons from clinical isolates belong to class 1.

4.16.9 Amplification of *Aac(6')-lb*gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

The fluoroquinolone resistance gene *aac(6')-lbAac(6')-lb*(482bp) evaluated in this study was absent in all the MDR *S. aureus.*

4.16.10 Amplification of *IS256* in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

IS 256 insertion sequence was amplified in 7(70.0%) of the MDR *S. aureus* (Plate 4.7). *IS256* at 812 bp have been reported to be involved in phase variation of biofilm formation in *Staphylococci* especially *S. epidermidis*.

4.16.11 Amplification of *qepA* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

The quinolone efflux pump gene *qepA*,596 bpevaluated in this study was absent in all the MDR *S. aureus.*

4.16.12 Amplification of *upaBc* gene in MDR *S. aureus* isolates from pregnant women attending antenatal clinics

The result in Plate 4.8 shows that *Upa Bc* genes, that codes for auto transporter protein (a non-fimbrial adhesin) associated with aggregation, cell adherence, adhesion to extracellular

membrane proteins, and biofilm formation at 250 bp was found in 10(100%) of the MDR *S. aureus* from pregnant women attending antenatal clinics in Ibadan metropolis.

4.16.13 Amplification of *bla* $_{CTX-M}$ gene in MDR ESBL *E. coli* isolates from pregnant women attending antenatal clinics

Bla $_{CTX-M}$ gene at 550 bp that codes for ESBL production were present in 10(100%) of the MDR *E. coli* isolates, this is shown in Plate 4.9.

4.16.14 Amplification of *bla-*$_{OXA}$gene in MDR *E. coli* isolates from pregnant women attending antenatal clinics

This result shows that*bla-*$_{OXA}$gene (619 bp) that codes for ESBL production was observed in 10(100%) of the MDR *E. coli* isolates as seen in Plate 4.10.

4.16.15 Amplification of *bla-*$_{SHV}$gene in MDR *E. coli* isolates from pregnant women attending antenatal clinics

Bla-$_{SHV}$gene (753 bp) that codes for ESBL production was amplified in 7(70%) of the MDR *E. coli* isolates. This is shown in Plate 4.11.

4.16.16 Amplification of *bla-*$_{TEM}$gene in MDR *E. coli* isolates from pregnant women attending antenatal clinics

Bla-$_{TEM}$gene (822 bp) that codes for ESBL production was observed in 9(90%) of the MDR *E. coli* isolates (Plate 4.12).

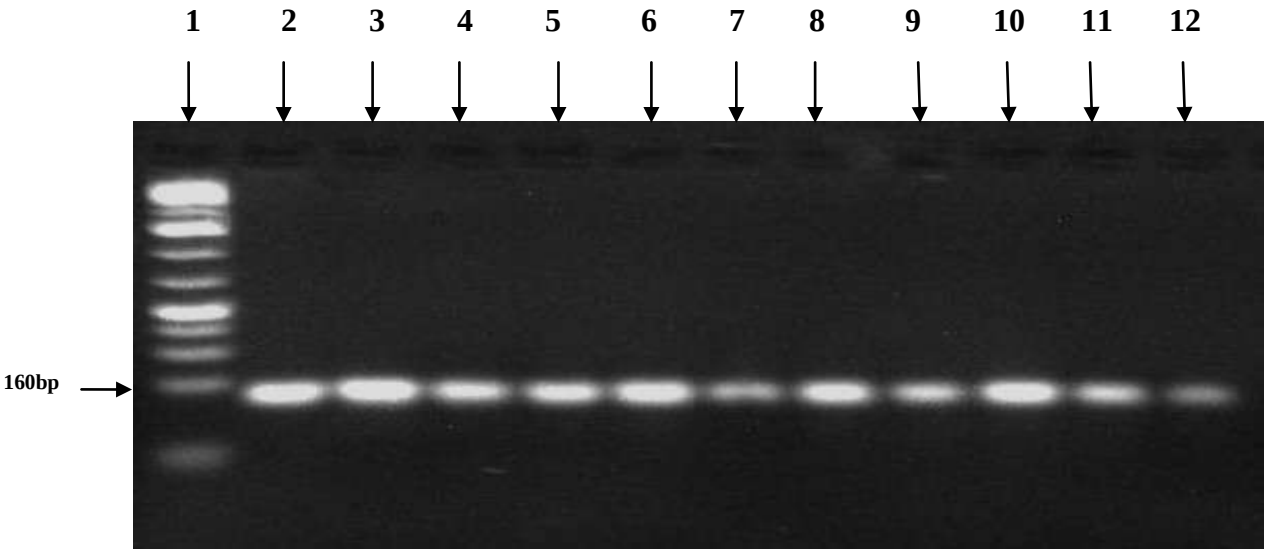


Plate 4.6 Amplification of *Int 1* plasmid integrons in MDR *S. aureus* isolated from pregnant women attending antenatal clinics(*Int1* gene=160 bp; Dillion *et al*., 2005)

Lane1: DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

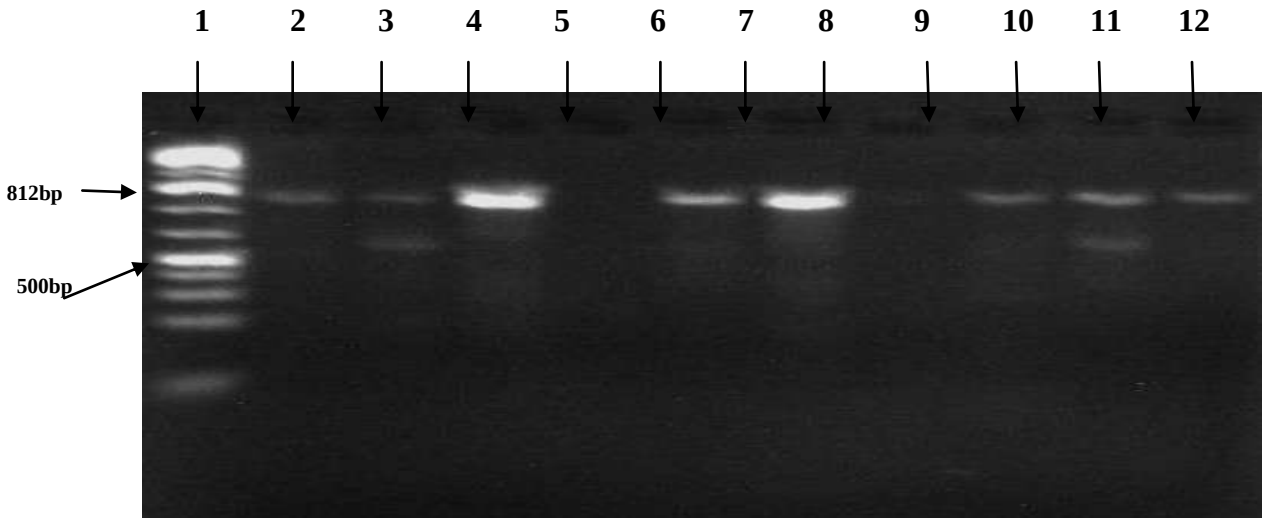


Plate 4.7 Amplification of *IS256* in MDR *S. aureus* isolates from pregnant women attending antenatal clinics (*IS256* = 812 bp Kozitskaya *et al*., 2004)

Lane1: DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

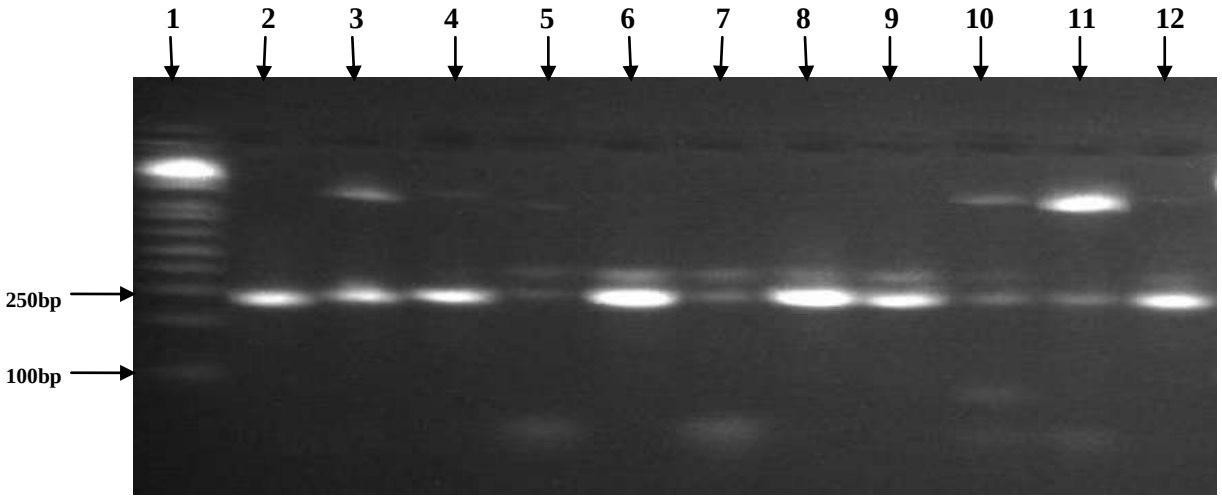


Plate 4.8 Amplification of *upaBc* genes in MDR *S. aureus* isolates from pregnant women attending antenatal clinics (*UpaBc* gene=250 bp, Allsopp *et al.*, 2011)

Lane1: DNA marker
Lane 2: Control *S. aureus* ATCC 25923
Lane 3: S42 = *S. aureus* S42
Lane 4: S6 = *S. aureus* S6
Lane5: S40 = *S. aureus* S40
Lane 6:S22 = *S. aureus* S22
Lane 7: S28 = *S. aureus* S28
Lane 8: S13 = *S. aureus* S13
Lane 9: S12 = *S. aureus* S12
Lane 10: S15 = *S. aureus* S15
Lane 11: S7 = *S. aureus* S17
Lane 12: S16 = *S. aureus* S17

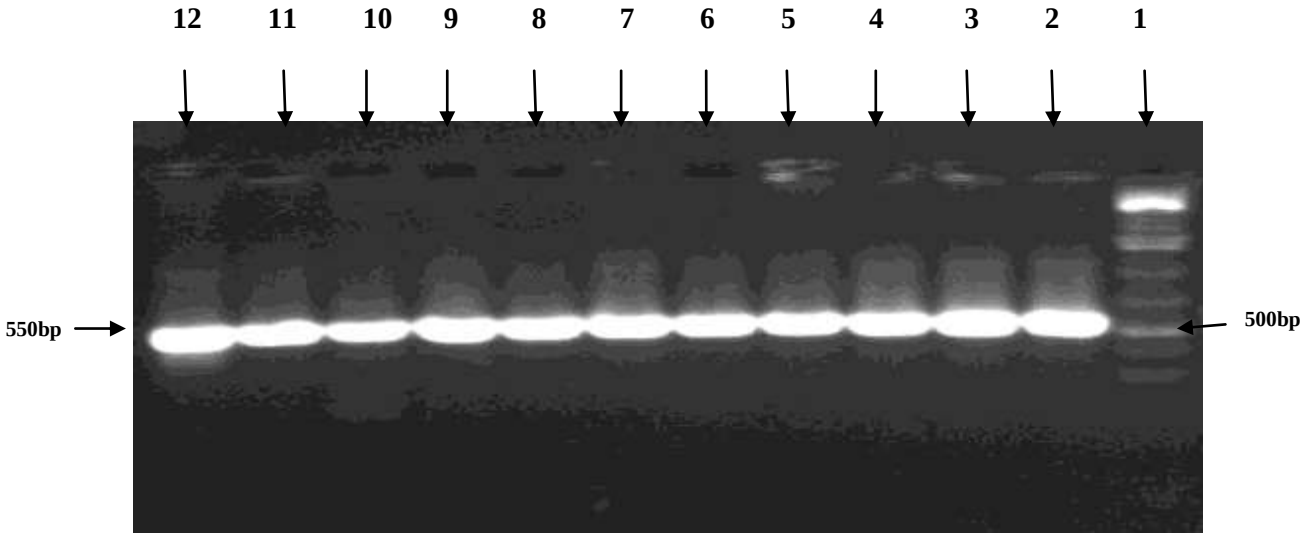


Plate 4.9 Amplification of *bla* $_{CTX-M}$ genes in MDR *E. coli* isolates from pregnant women attending antenatal clinics (Paterson *et al.*, 2003; Hujer *et al.*, 2006 = 550 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

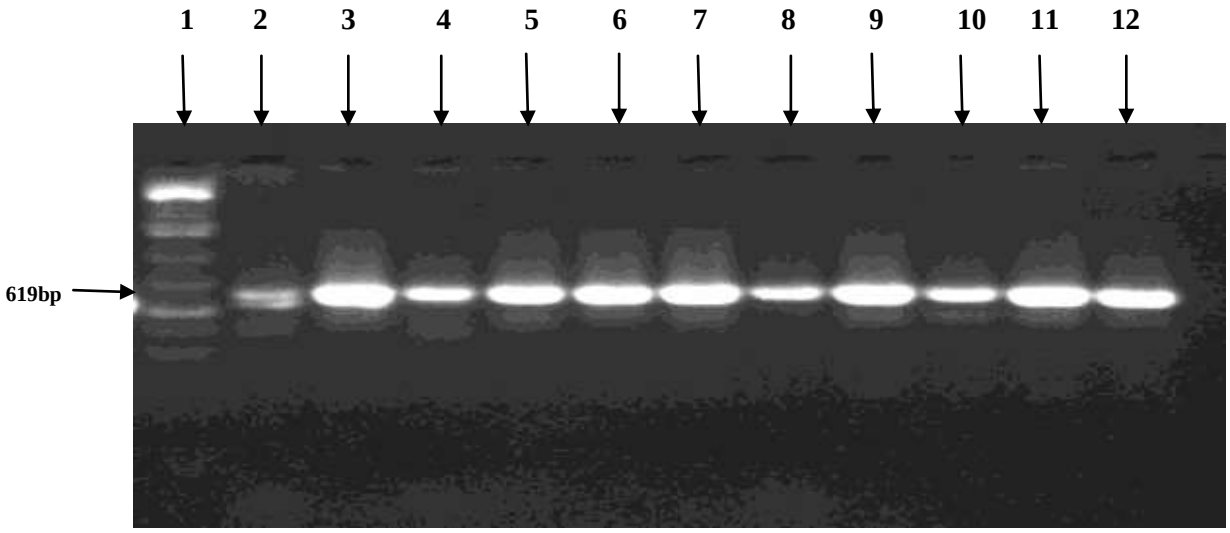


Plate 4.10 Amplification of *bla-*$_{OXA}$ genes in MDR *E. coli* isolates from pregnant women attending antenatal clinics (Karami *et al.*, 2008 = 619 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

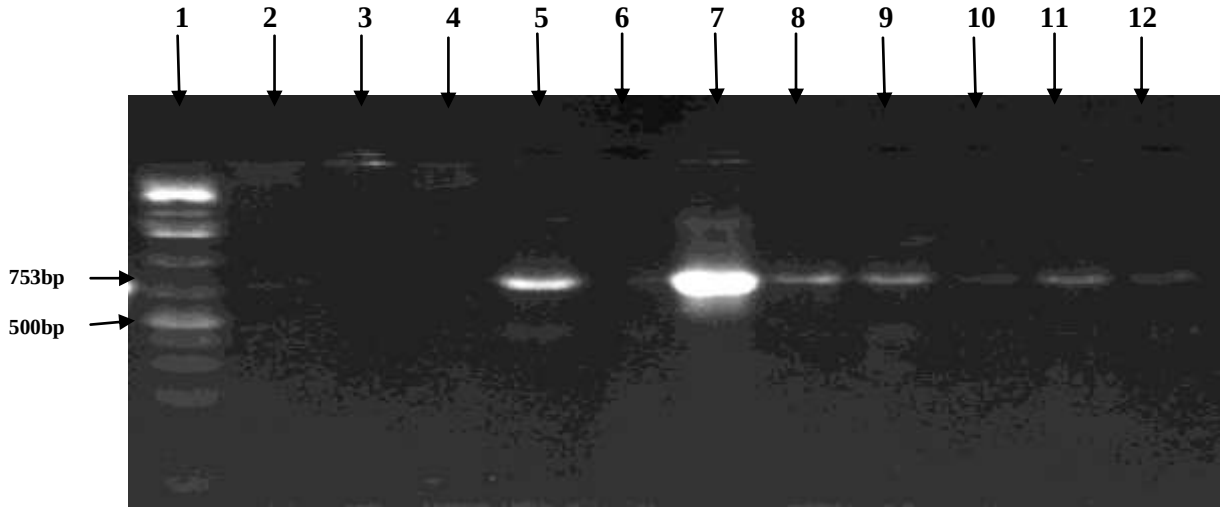


Plate 4.11 Amplification of *bla-$_{SHV}$* gene in MDR *E. coli* isolates from pregnant women attending antenatal clinics (Paterson *et al.*, 2003; Hujer *et al.*, 2006 =753 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

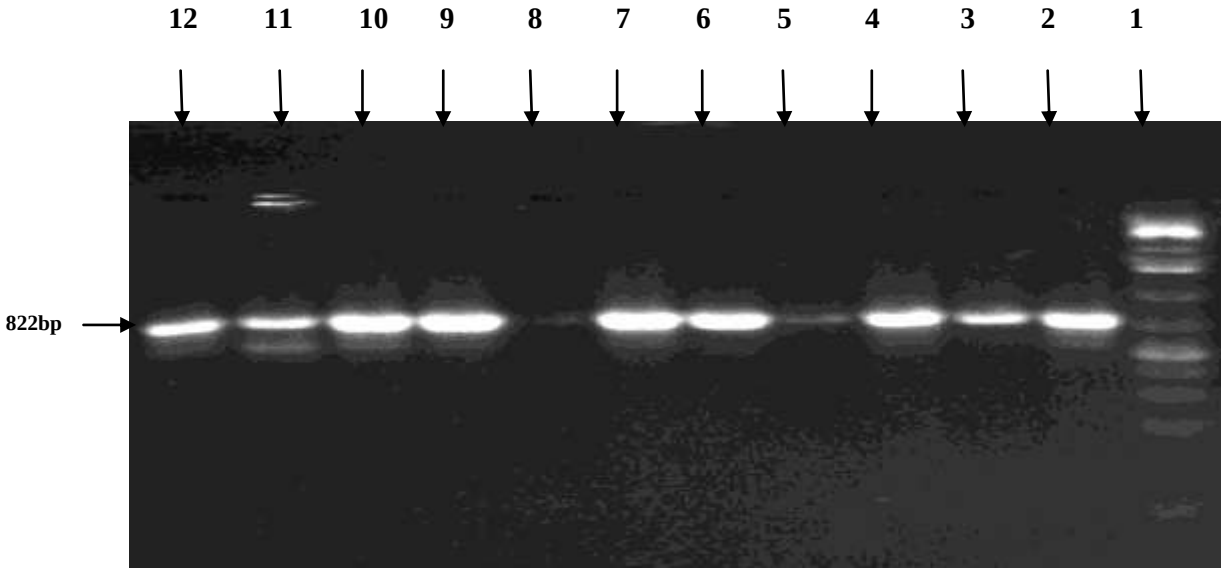


Plate 4.12 Amplification of *bla-$_{TEM}$* genes in MDR *E. coli* isolates from pregnant women attending antenatal clinics (Paterson *et al.*, 2003; Hujer *et al.*, 2006 = 822 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

4.16.17 Amplification of *fim H* gene in MDR ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

Fim H gene that codes for organelle tip-located adhesins responsible for adherence to the urinary tract epithelium were present in 10(100%) of the MDR ESBL producing *E. coli* isolates (Plate 4.13).

4.16.18 Amplification of *pap1* gene in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics

Pap1 gene that also codes for organelle tip-located adhesins responsible for adherence to the urinary tract epithelium were present in 10(100%) of the MDR ESBL producing *E. coli* isolates (Plate 4.14).

4.16.19 Amplification of *hly* gene in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics

The hemolysin gene *hly* evaluated in this study was absent in all the MDR ESBL producing *E. coli* isolates. Hemolysins are cytolytic protein toxins which are responsible for erythrocyte lysis.

4.16.20 Amplification of *cnf* gene in MDR ESBL *E. coli* isolates from pregnant women attending antenatal clinics

The *cnf* gene (693 bp) that codes for cytotoxic necrotizing factor (a hemolysin) which is responsible for promoting bacterial attachment to, invasion of uroepithelial cells and induction of inflammatory response were present in 5(50%) of the MDR ESBL producing *E. coli* isolates (Plate 4.15).

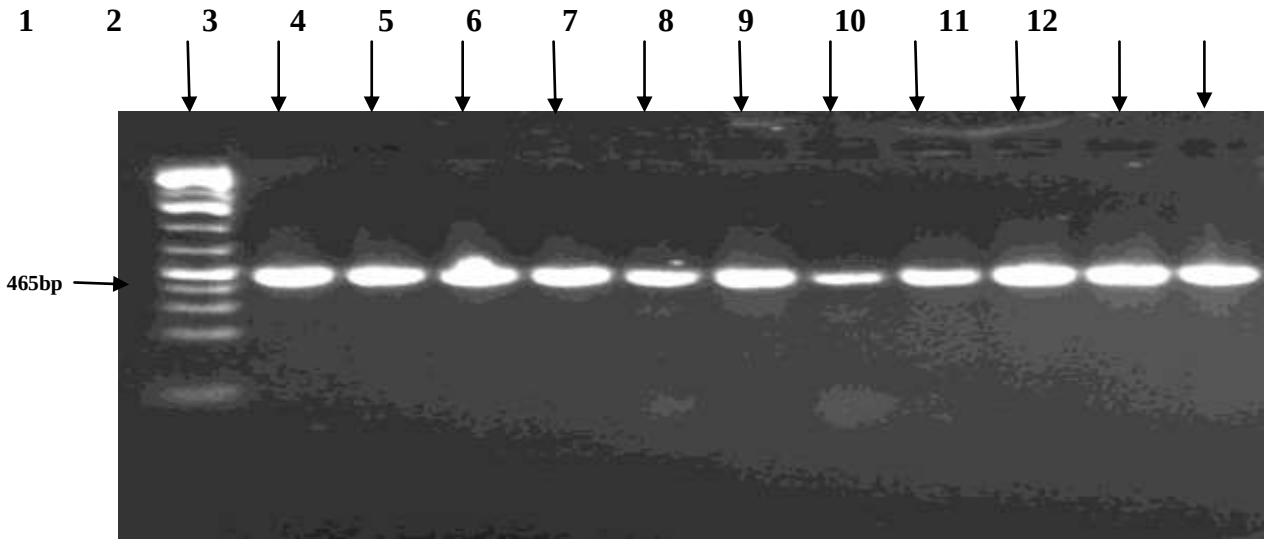


Plate 4.13 Amplification of *fim H* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (Farmer, 1999 = 465 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

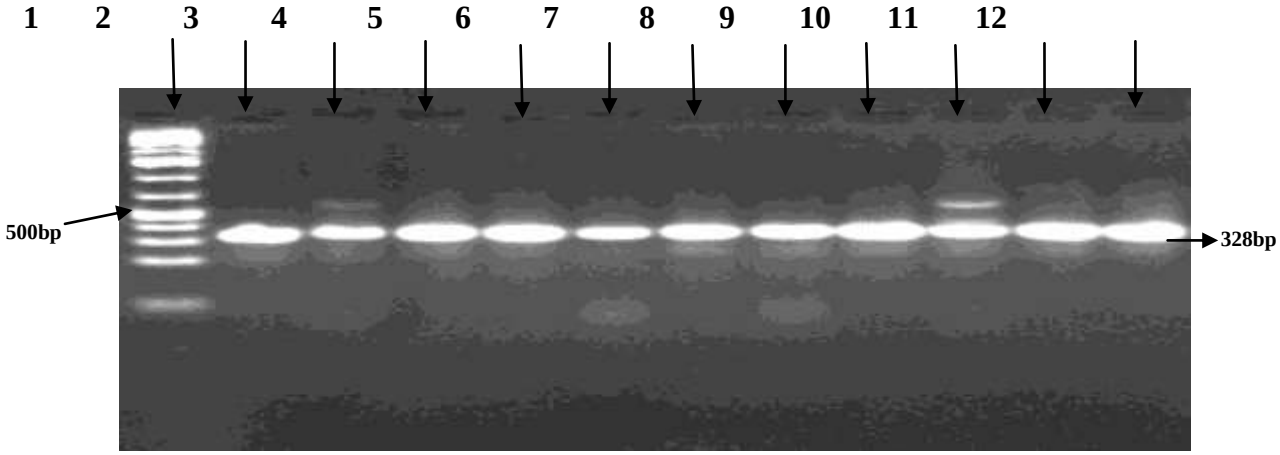


Plate 4.14 Amplification of *pap1* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (Soto *et al.*, 2011= 328 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

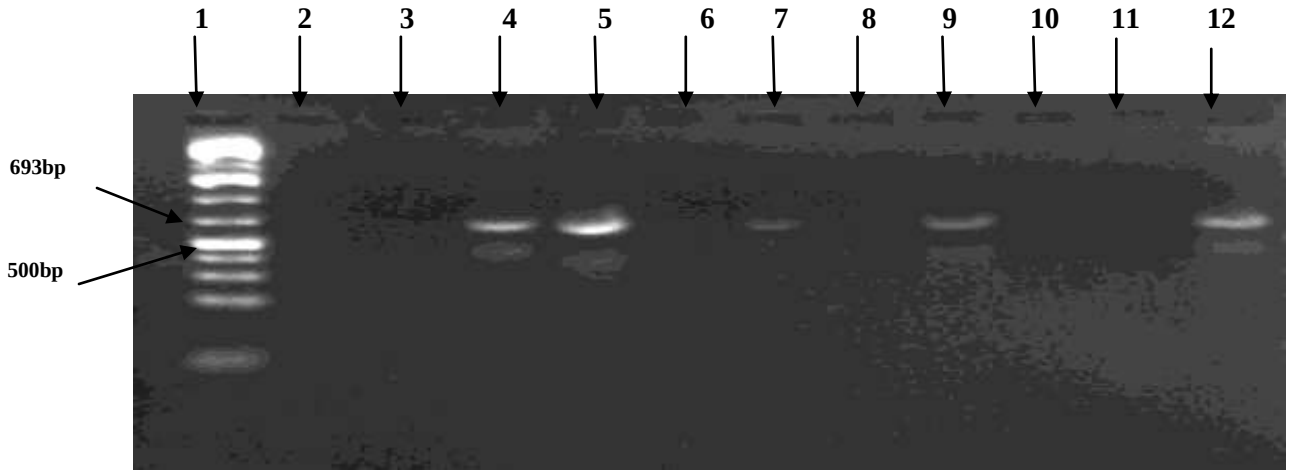


Plate 4.15 Amplification of *cnf* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (Licznar *et al*., 2003= 693 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

4.16.21 Amplification of aerobactin gene *aer* in MDR ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

The aerobactin gene *aer*responsible for iron acquisition evaluated in this study was absent in all the MDR ESBL producing *E. coli* isolates.

4.16.22 Amplification of *upaBc* gene in MDR ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

Amplification of *upaBc* gene at 250 bp was observed in 10(100%) of the MDR ESBL producing *E. coli* isolates (Plate 4.16). *Upa Bc* gene codes for autotransporter protein a non-fimbrial adhesion which is also responsible for biofilm formation.

4.16.23 Amplification of *qepA* gene in *MDR* ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

The *qepA* gene that codes for quinolone exflux pumpresponsible for promoting bacterial attachment to, invasion of uroepithelial cells and induction of inflammatory response were present in 5(50%) of the MDR ESBL producing *E. coli* isolates (Plate 4.17).

4.16.24 Amplification of *IS256* in MDR ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

Insertion sequence *IS 256* was amplified in 6 (60.0%) of the MDR ESBL producing *E. coli* isolates (Plate 4.18).

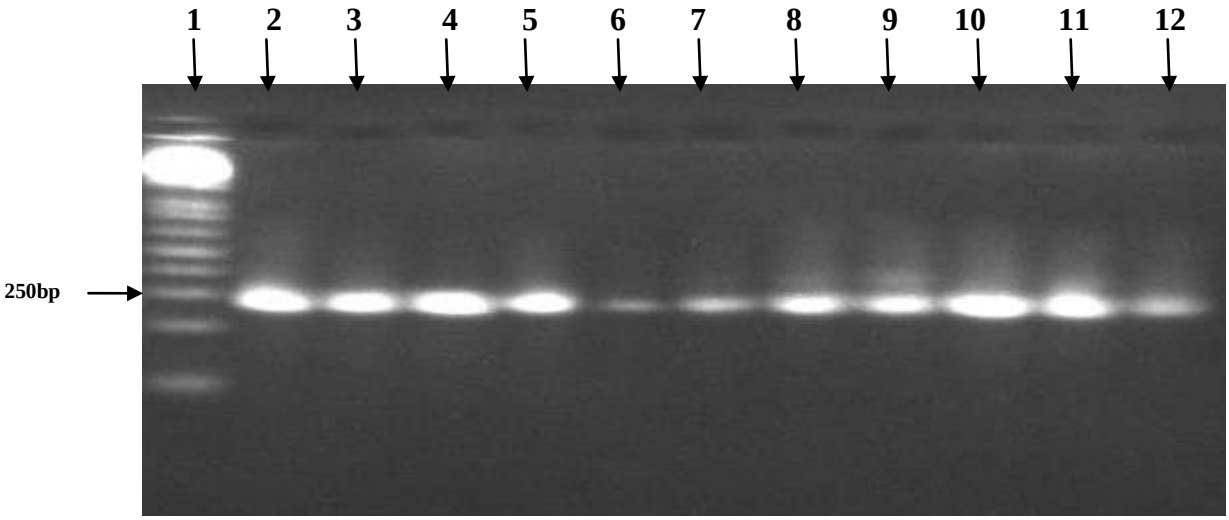


Plate 4.16 Amplification of *upaBc* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (*upa Bc* gene=250 bp; Allsopp *et al.*, 2011)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

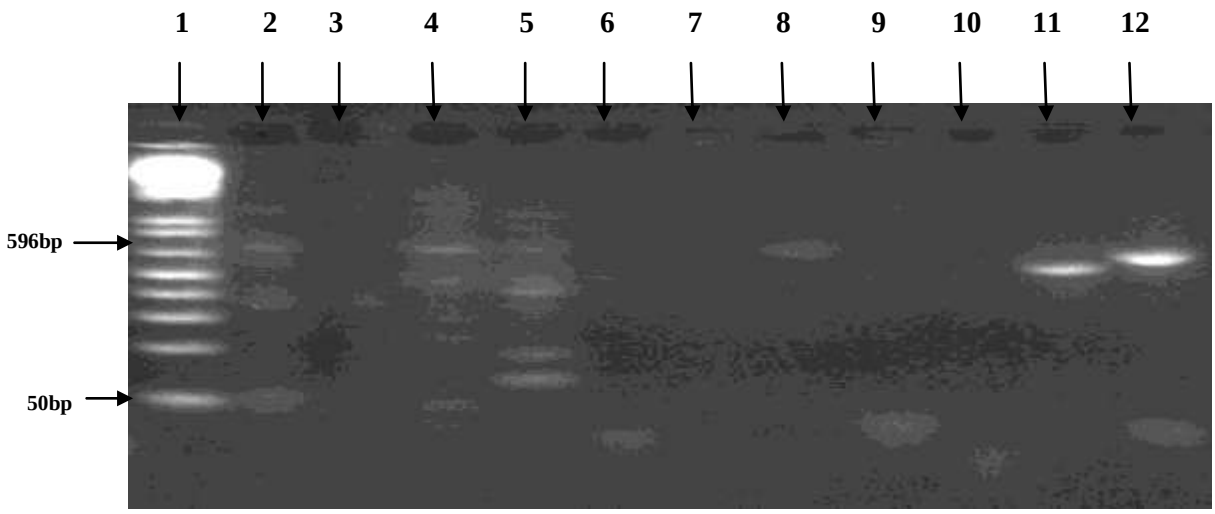


Plate 4.17 Amplification of *qepA* genes inMDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (Kim *et al*, 2009= 596 bp)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

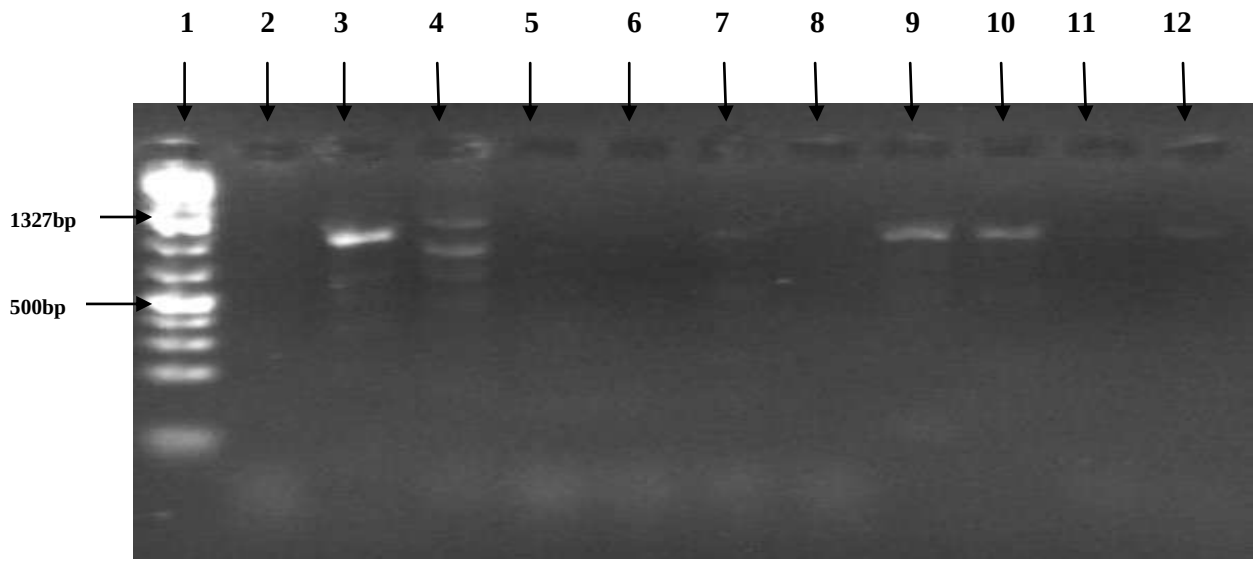


Plate 4.18 Amplification of *IS256* in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinic (*IS256*=1327 bp; Kozitskaya *et al*., 2004)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

4.16.25 Amplification of *AmpC* gene in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics

Amplification of *AmpC* gene 271 bp, was observed in 8(80%) of the MDR ESBL producing *E. coli* isolated from pregnant women attending antenatal clinics in Ibadan metropolis (Plate 4.19). *AmpC* confers resistance to the cephalosporins.

4.16.26 Amplification of *aac-Ib-6* gene in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics

The *aac-Ib-6* gene which confers resistance to the fluoroquinolones evaluated in this study was absent in all the MDR ESBL producing *E. coli* isolates.

4.16.27 Amplification of *Int 1* in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics

Int 1 integron was expressed in 9 (90.0%) of the MDR ESBL positive *E. coli* isolates from pregnant women in Ibadan metropolis (Plate 4.20). Class 1 and class 2 integrons are the most frequently found integrons in resistant bacteria.

4.17 NCBI Blasting of some MDR isolates from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.

The various level of identity that the nucleotide sequence of some of the MDR isolates expressed when compared with other strain sequence on NCBI data base is shown in Appendices X to XVI.

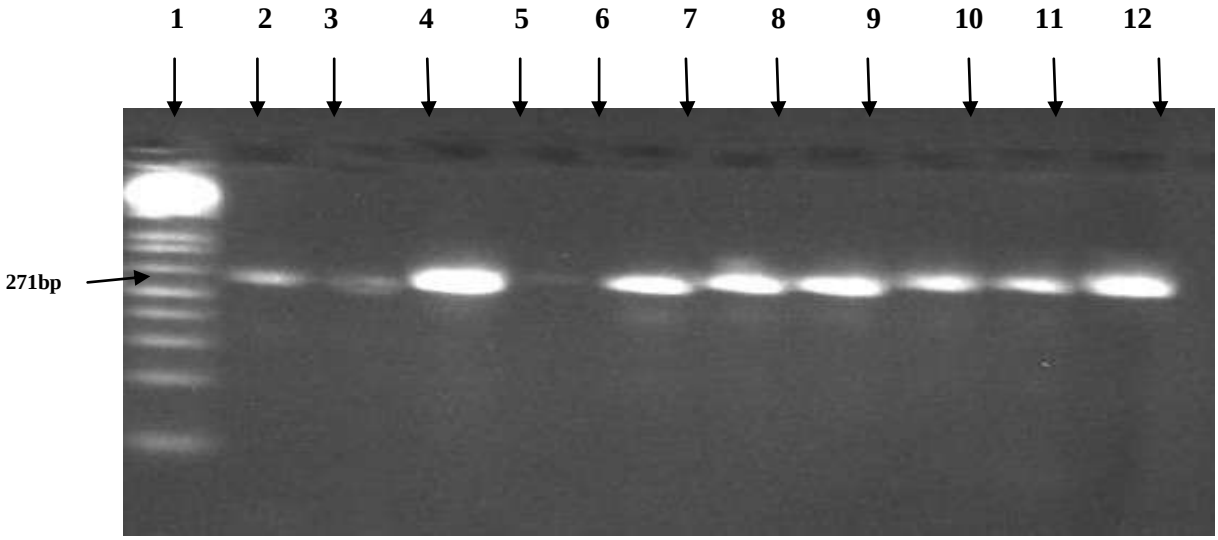


Plate 4.19 Amplification of *AmpC* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (*AmpC* gene=271 bp; Caroff *et al*., 2000)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

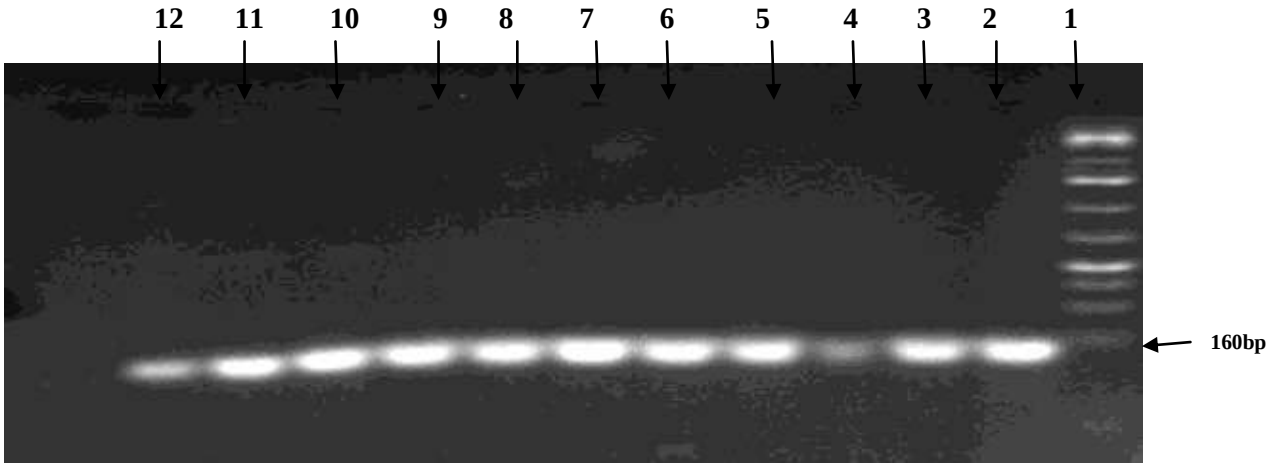


Plate 4.20 Amplification of *Int 1* integron in ESBL producing *E. coli* isolates from pregnant women attending antenatal (*Int1* gene = 160 bp; Dillion *et al.*, 2005)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

4.18 Phylogenetic study of the sequenced DNA of bacterial isolates from pregnant women in Ibadan metropolis

The evolutionary history of the isolates was inferred by the Tamura-Nei method. The analysis involved 23 nucleotide sequences, and all positions containing gaps and data were eliminated. There were a total of 132 positions in the final dataset. Evolutionary analyses were conducted using MEGA 7®.

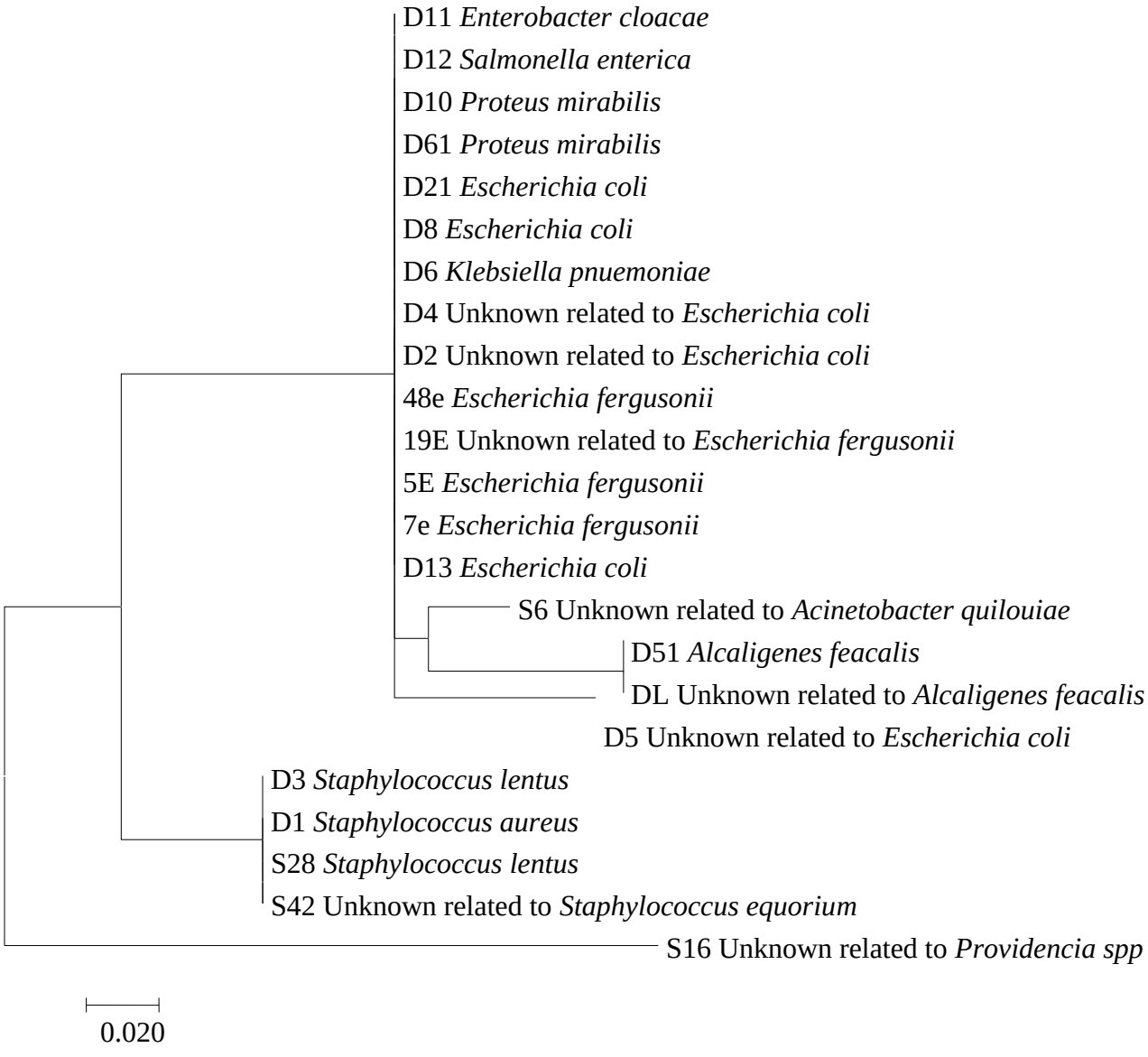


Fig 4.37 Molecular phylogenetic analysis by maximum likelihood method (MLM) based on the Tamura-Nei model

4.19 Phylogenetic grouping of MDR ESBL producing *E. coli* using PCR

ChuA, *Tsp*, and *YjaA* genes are used for the Phylogenetic classification of *E. coli* strains into A, BI, B2, and D. Phylogenetic grouping interpretative criteria described by Clermont *et al*., 2000; Gordon *et al*., 2008a; and Ejrnaes *et al*., 2011 was used.

4.19.1 Amplification of *Tsp* genes in MDR ESBL *E. coli* isolates from pregnant women attending antenatal clinics

Tsp gene (an anonymous DNA fragment) was amplified in 10 (100%) of the MDR ESBL producing *E. coli* isolates (Plate 4.21).

4.19.2 Amplification of *ChuA* genes in MDR ESBL *E. coli* isolates from pregnant women attending antenatal clinics

Amplification of *ChuA* gene was observed in 7(70%) of the MDR ESBL producing *E. coli* isolates. (Plate 4.22). *ChuA*is a virulence gene that encodes a hem transporter protein in *E. coli* O157: H7.

4.19.3 Amplification of *YjaA* genes in MDR ESBLproducing *E. coli* isolates from pregnant women attending antenatal clinics

YjaA gene, a virulence gene initially identified in the genome of *E. coli* K-12 was amplified in 9(90.0%) of the MDR ESBL producing *E. coli* (Plate 4.23).

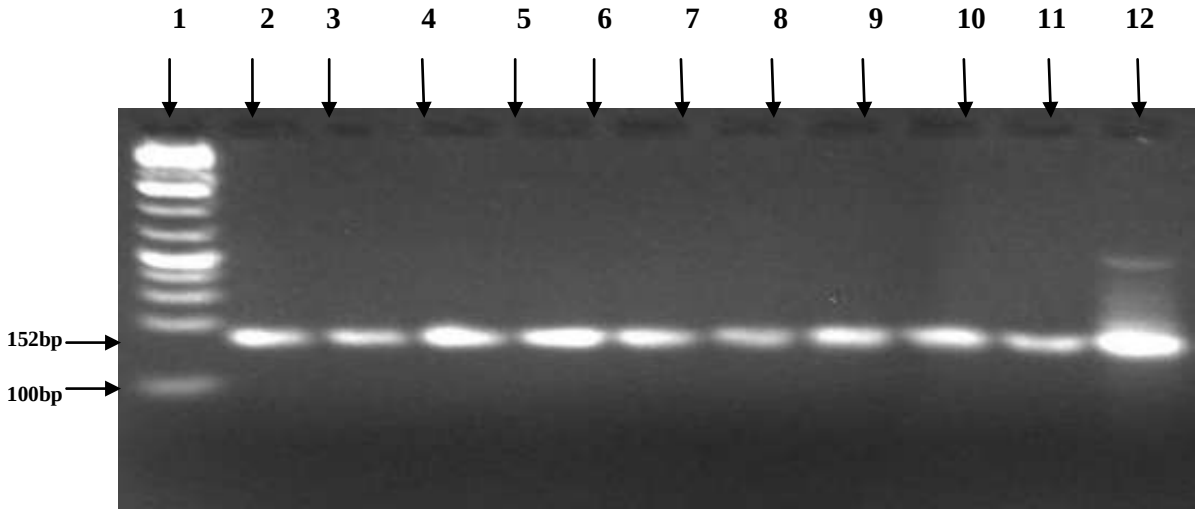


Plate 4.21 Amplification of *Tsp*genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (*Tsp* gene=152 bp; Clermont *et al.*, 2000)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

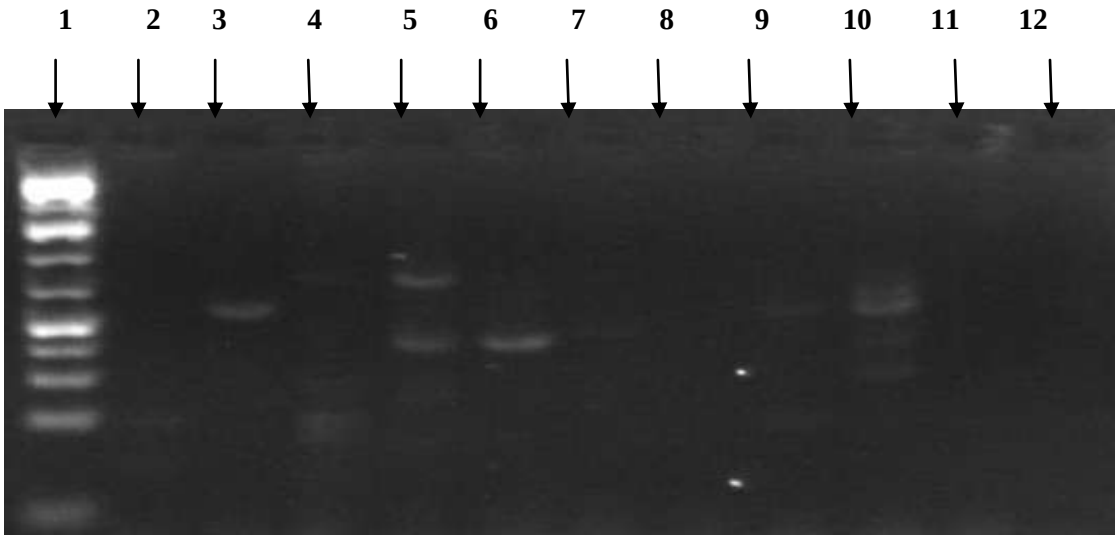


Plate 4.22 Amplification of *ChuA* gene in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (*ChuA* gene=279 bp; Clermont *et al.*, 2000)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

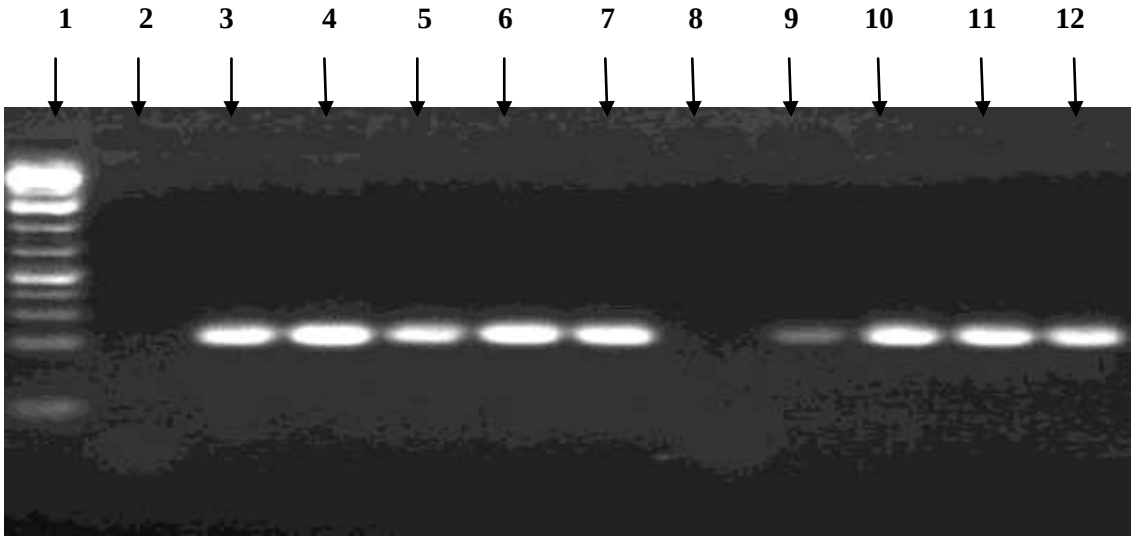


Plate 4.23 Amplification of *YjaA* genes in MDR ESBL producing *E. coli* isolates from pregnant women attending antenatal clinics (*YjaA* gene=211 bp; Clermont *et al*., 2000)

Lane1: 100 bp DNA marker
Lane 2: Control *E. coli* ATCC 35218
Lane 3: E5 = *E. coli* E5
Lane 4: E7 = *E. coli* E7
Lane5: E19 = *E. coli* E19
Lane 6: E30 = *E. coli* E30
Lane 7: E48 = *E. coli* E48
Lane 8: E47 = *E. coli* E47
Lane 9: E42 = *E .coli* E42
Lane 10: E41 = *E. coli* E41
Lane 11: E49 = *E. coli* E49
Lane 12: E33 = *E. coli* E33

4.19.4 Phylogentic grouping Interpretative criteria

From the PCR results of the amplification of the 3 markers (i.e., *ChuA, Tsp* and *YjaA* genes), 7(70.0%) of the MDR ESBL producing *E. coli* isolates are of group B2 i.e., they are of the virulent extra-intestinal strains. The interpretative criteria described by Clermont *et al*., 2000; Gordon *et al*., 2008; Ejrnaes *et al*., 2011 is as follows:

- *ChuA* + *Tsp*-/+*YjaA* or + *IbeA*= Group B2
- If no *ChuA* = Groups B1 or A
- Virulent extra-intestinal strains of uropathogenic *E. coli* are mostly = Group B2.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

Pregnant women were observed to harbor diverse micro-organisms in their urine, some of which are pathogenic, possessing virulent traits. The prevalence of significant bacteriuria among pregnant women in Ibadan metropolis was 246(37.8%), out of which 97(14.9%) were symptomatic and 149(22.9%) were asymptomatic. This is in agreement with studies conducted in Ibadan where Ogbolu *et al*., (2006) and Kehinde *et al*., (2011) reported 21.0% and 28.8% prevalence respectively. However, it is at variance with 45.3%, 47.5%, 56.5% and 58.4% found by Imade *et al*., (2010) in Benin city, Okonko *et al*., 2009 in Ibadan, Alex *et al*., (2012) in Ghana and Bolaji *et al*., (2013) in Zaria. Lower prevalence of 7.8%, 9.5%, 11.5 %, 13.3%, 15.2%, 16.0%, and 18.8% has been reported by Mokube *et al*., (2013) in Cameroon, Obirikorang *et al*., (2012) in Ghana, Dash *et al*., (2013) in India, Andabati *et al*., (2010) in Uganda, Durowaiye *et al*., (2015) in Kano, Ezeh *et al*., (2016) in Zaria, and Tadesse *et al*., (2014) in Ethiopia 18.8%.

Asymptomatic bacteriuria (ASB) prevalence is seen to vary across geographical regions within and outside the country. The high ASB observed in this study may be due to inadequate/poor personal hygiene practice by the women as well as other socio-economic factors. The resulting sensitivity (92.6%), specificity (85.3%), positive predictive value (93.6%) and negative predictive values (83.1%) of the dipstick leucocyte esterase test was ideal. This agrees with the findings of Priyanka *et al.,* (2015) where the test confirmed an actual infection (bacteriuria).

The diversity of urinary isolates as seen in this study suggests a changing bacterial colonization pattern of the urinary tract in pregnancy. *Staphylococcus epidermidis* 51(21.9%) was the most prevalent uropathogen, followed by *Escherichia coli* 48(19.5%).

The highest prevalence of bacteriuria with respect to gestational age was observed in women in their third trimester 131(53.2%), this agrees with the findings of Lindsay, (2003), Ogbolu *et al*., (2006) and Imade *et al*., (2010) that asymptomatic bacteriuria increases with increase in gestational period. There is usually an increased hormonal activity with increase in gestational age, and this aids bacterial growth. With respect to age group, bacteriuria was highest 101 (44.2%) among women aged 25-30 years, this also agrees with the report of Alex *et al*., (2012) where bacteriuria in pregnancy was highest among women aged 15-32 years. Women in this age group are more sexually active.

Other socio-economic factors affecting bacteriuria in pregnancy were occupational and educational level; bacteriuria was highest in women considered as housewives 87(35.3%), than those of the working class; and in women with no formal education 79(32.1%) than those with one form of education or the other. These could be as a result of their constant exposure to bacteria from regular house chores like sweeping and cleaning of dirty surfaces, toilets, as well as lack of enlightenment. The working class women are more educated, and enlightened about precautionary measures (which includes personal hygiene, etc) to take to avoid being infected. This is in consonance with the report of Durowaiye *et al*., (2015) from a study done in Kano, Nigeria.

The highest prevalence of bacteriuria with respect to parity 88(35.8%) were observed among the multipara women. This agrees with the findings of Ogbolu *et al*., (2006) in Ibadan where

multiparous women had the highest bacteriuria.Obirikorang *et al*., (2012) also reported bacteriuria to be highest among multiparous women in Ghana. All these socio-economic factors were statistically significant at P<0.05.

Antimicrobial resistance, including multiple antibiotic resistance was common among the bacterial isolates from the women. From their phenotypic resistance profile, MARI varied from 0.08 to 1.0. The percentage resistance of the isolates to twelve antibiotics (belonging to various classes) ranged from 10.0-36.7% and 0.9-47.6% for the Gram positive and Gram negative organisms respectively. Of the 52 *E. coli* isolates, 38(73.1%) were ESBL producers, while 14(26.9%) were non-ESBL producers (Fig. 4.1), which agrees with the following reports: 76.4% (Durowaiye and Adewuyi, 2017), 73.9% (Al-Mayehie, 2013), 68.5% (Faisal *et al.,* 2011), and 50.9% (Venkatadri *et al*., 2014). Out of the 28 *S. aureus* isolates, 16(57.0%) were Methicillin resistant *S. aureus* (MRSA), while 12 were Methicillin sensitive *S. aureus* (MSSA).

Molecular characterization of ESBL genes among 10 MDR *E. coli* isolates showed that all had *bla* $_{\text{CTX-M}}$ gene which concurs with the findings of Hertz *et al*., (2016) where it was reported that 98.0% of the *E. coli* isolates carried *bla*$_{\text{CTX-M}}$ gene; and 60.0% of the MDR *E. coli* isolates had 4 different ESBLs (*bla* $_{\text{CTX-M,}}$ *bla* $_{\text{TEM}}$ *bla* $_{\text{OXA,}}$ and *bla* $_{\text{SHV}}$). Jungmin *et al.,* (2005) and Shahi *et al.,* (2013), also reported the possibility of isolating more than one ESBL genes such as *bla* $_{\text{OXA-30}}$, *bla* $_{\text{TEM}}$, *bla* $_{\text{DHA-1}}$, and *bla* $_{\text{SHV-12}}$ in a single isolate of *E. coli* with *bla* $_{\text{CTX-M}}$. *Bla*$_{\text{TEM}}$ was also found in majority 9(90.0%) of the *E. coli* isolates. All the MDR *E. coli* isolates from pregnant women in Ibadan metropolis harboured *bla* $_{\text{OXA}}$, while *bla* $_{\text{SHV}}$ was present in 7(70.0%). These findings are in accordance with the report of Lei *et al.,* (2013) who reported that *bla*$_{\text{CTX-M}}$ is the most widely distributed gene encoding extended-spectrum β-lactamases in humans globally. Further molecular evaluation of virulence traits in these *E. coli* strains revealed the amplification

of *ampC* beta-lactamases in 8(80.0%). This high level of *ampC* beta-lactamase gene producers among ESBL producing *E. coli* observed in this study is in agreement with the report of Peter-Getzlaff *et al.,* (2011), who found 69.2% *ampC* producers among *Enterobacteriaceae.*

Virulence factor genes further investigated in the 10 MDR ESBL *E. coli* isolates from pregnant women in Ibadan metropolis revealed that *fim H* and *pap* genes that code for organelle tip-located adhesin responsible for adherence to the urinary tract epithelium were present in 10(100.0%) of the isolates respectively. This is in agreement with the report of Karimian *et al*., (2012) where *fimH* gene (79.7%) was the most prevalent virulence factor genes (VFG) among uropathogenic (UPEC) strains, and *pap* gene in 50.4 % in UTI patients in Iran. Santo *et al*., (2006) in Brazil reported 32.0% prevalence of pap genes in *E. coli* strains, which is lower than what was obtained here.

The *Hly* gene which codes for hemolysin production was completely absent in all the *E. coli* isolates, this is at variance with the report of Karimain *et al*., (2012) where 50.4% of the *E. coli* isolates produced hemolysin. Cytotoxic necrotizing factor (*cnf*) genes which is also responsible for hemolysin production was observed in 5(50.0%) in this study, this concurs with the findings of Karimian *et al*., (2012) where 50.4 % were observed.

Aer operon which codes for iron chelating agents was expressed by none of the *E. coli* isolates from pregnant women in this study. This is not in agreement with the report of Mladin *et al*., (2009) where *aer* genes was amplified in *E. coli* strains of UTI patients with neurogenic bladder.

The *upa Bc* gene which codes for an autotransporter protein; a non-fimbrial adhesin which is also responsible for biofilm formation was expressed by all the MDR ESBL *E. coli* molecularly characterized. *QepA* operon that codes for quinolone exflux pump was amplified in 5(50.0%) of

MDR ESBL producing *E. coli* isolates from pregnant women, while the *aac-Ib-6* gene which also confers resistance to the fluoroquinolones were absent.

The mobile genetic elements observed in the MDR ESBL producing *E. coli* isolates from pregnant women in Ibadan metropolis include *IS256* 6(60.0%). Majority 9(90.0%) expressed *Int 1* intergron. This aligns with the report of Sabate and Prats, 2002: class 1 and class 2 integrons are the most frequently found integrons in resistant bacteria. Idrees *et al.,* (2011) have established a strong association between the presence of integrons and antimicrobial resistance by studies on Uropathogenic *E. coli* (UPEC) in various regions of Asia, Europe and USA. The presence of these plasmid transferrable genes in our environment confers the possibility of fast dissemination of MDR genes (Sabate and Prats, 2002). Findings from this study indicate that UPEC strains with various virulence factors are a major aetiological agent for UTI in pregnant women either symptomatic or asymptomatic.

Molecular characterization of 10 MDR *S. aureus* isolates from pregnant women in Ibadan metropolis shows that *mecA* gene that codes for the production of the altered penicillin-binding protein (PBP2a) responsible for classical methicillin resistance was only amplified in 2(20%) of the MDR *S. aureus*, this is contrary to the findings of Ahmed, (2014) where *mecA* gene was present in all the MRSA strains from UTI patients. The MRSA phenotype observed in majority of the isolates was not the classical *mecA*-mediated resistance, but could be as a result of other mechanism as reported by Olayinka *et al*., (2009); as *ampC* gene was amplified in all of the MDR *S. aureus* isolates. *AmpC*β-lactamases are clinically important cephalosporinases encoded on the chromosomes of many of Enterobacteriaceae, where they mediate resistance to cephalosporins β-lactams [Jacoby, (2009); Mulvey and Simor, (2009)]. This further explains the high resistance observed in this study to the cephalosporins (cefuroxime and ceftriaxone).

Pvl gene which encodes a toxin responsible for pore-formation was completely absent in all the MDR *S. aureus* isolates from pregnant women, this agrees with the findings of Baba-Moussa *et al.*, (2008) where *pvl* was absent in all the UTI *S. aureus* isolates from a hospital in France. Other studies have also reported that no strains of *S. aureus* producing *pvl* have ever been isolated from urine (Prevost *et al*., 1995; Dufour *et al*., 2002; Van der Mee-Marquet *et al*., 2004). However, this is at variance with the report of Baba-Moussa *et al.*, (2008) who found 14(2.5%) of isolates among UTI in-patients in a hospital in Cotonou, Benin, carried *pvl*.

IcaD and *icaA* genes were expressed by 8(80%) and 5(50%) of the MDR *S. aureus* isolates, respectively. This concurs with the findings of (Kozitskaya *et al*., 2004) who observed that Staphylococcal biofilm formation is mainly mediated by the expression of the icaADBC operon. *IS256* has been reported to be involved in phase variation of biofilm formation in Staphylococci especially *S. epidermidis* (Cho *et al*., 2002; Ziebuhr *et al.*, 1999). Majority 7(70%) of the MDR *S. aureus* isolates from pregnant women in Ibadan metropolis were observed to harbour *IS256*. *Int 1* plasmid integron was observed in all the isolates. Most integrons from clinical isolates belong to class 1 (Martinez-Freijo *et al*., 1999). Both *aac(6')-lb* and *qep* efflux pumpgenes that codes for resistance to the fluoroquinolones were absent in the isolates; the low resistance to ofloxacin observed in this study might be due to other mechanisms that have been described for plasmid-mediated resistance to the fluoroquinolones e.g the *qnr* and *oqxAB* exflux pump genes which confer decreased susceptibility (Périchon *et al.*, 2007; Yamane *et al*., 2007; Wong *et al*., 2015).

UpaBc gene which codes for an auto transporter protein frequently associated with aggregation, cell adherence, adhesion to extracellular membrane proteins, and biofilm formation was amplified in all the MDR *S. aureus* isolates. This study also reveals the expression of various virulence genes the MDR *S. aureus* isolates from pregnant women in Ibadan metropolis. The

phylogenetic study reveals close evolutionary history between the *Escherichia* isolates, the *Staphylococci* isolates repectively.

The implication(s) of carriage of these resistance and virulence genes enumerated above by these isolates in urine samples of pregnant women was seen in their reduced susceptibility profile to the commonly prescribed antibiotics in the environment, and the presentation of clinical symptoms of UTI.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION

6.1 Summary

1. The prevalence of significant bacteriuria among pregnant women in Ibadan metropolis was 246(37.8%) out of which 97(14.9%) were symptomatic and 149(22.9%) were asymptomatic.

2. The specificity, sensitivity, positive predictive value and negative predictive values of the dipstick leucocyte esterase test was 92.6%, 85.3%, 93.6% and 83.1% respectively.

3. Of great maternal health concern is the huge diversity of urinary isolates from pregnant women in Ibadan metropolis which include some uncommon bacteria like *Acinetobacter quilouiae*, *Alcaligenes feacalis, Escherichia fergusonii*, *Serratiamarcescens*. Different *Staphylococcus* spp like *Staphylococcus equorium*, *Staphylococcus chromogenes, Staphylococcus lentus* etc.

4. Several socio-economic factors observed to influence bacteriuria in pregnancy includes: age of women, gestational age, parity, occupational status and their level of education.

5. The phenotypic resistance pattern recorded in this study is as follows: Nitrofurantoin(11.9%), Gentamicin(21.9%), Imipenem(23.3%), Ofloxacin(23.8%), Tetracycline(43.8%), Ceftriaxone(47.6%), Chloramphenicol(52.4%), Cotrimoxazole(66.6%). Amoxicillin and Amoxicillin/clavulanic acid are oral antibiotics most commonly prescribed in antenatal clinics; they exhibited 69.6% and 43.8% resistance pattern respectively. Fosfomycin, an antibiotic not

commonly prescribed in Ibadan metropolis, and used for testing and reporting *Escherichia coli* UTI isolates only showed 47.6% resistance.

6. Multidrug resistance was common (69.5%) among the urinary isolates in this study. The Multiple antibiotic resistance index (MARI) of the isolates varied from 0.08 to 1.0. Twelve antibiotics belonging to different classes were used.

7. Phenotypically, the prevalence of ESBL production among the 48 *E. coli* isolates from the pregnant women revealed that 35(72.9%) of them were ESBL producers, while 13(27.1%) were non-ESBL producers.

8. Phenotypically, out of the 28 *S. aureus* (both *S. aureus* and *S. aureus* subsp anaerobius) isolates, 16(57.0%) were MRSA while 12 were Methicillin Sensitive *S. aureus* (MSSA).

9. Molecular characterization of 10 MDR *S. aureus* isolates (by virulence factor gene amplification, antibiotic resistance gene and mobile genetic elements amplification) showed that *mecA* gene was present in 2(20.0%), *ampC* gene in 10(100.0%), *icaD* gene in 8(80.0%), *icaA* gene in 5(50.0%), *upaBc* gene in 10(100.0%), *Int 1* gene in 10(100.0%), *IS256* gene in 7(70.0%), while the following genes were absent *pvl, hly, aer,* and *qepA.*

10. Molecular characterization of 10 MDR ESBL producing *E. coli* isolates (by virulence factor genes amplification, antibiotic resistance and mobile genetic elements amplification) showed that $bla_{\text{CTX-M}}$, $bla_{\text{TEM,}}$ and bla_{OXA} genes were present in all the isolates, while bla_{SHV} was only present in 7(70.0%). Majority 6(60.0%) of the MDR ESBL producing *E. coli* had 4 different ESBLs ($bla_{\text{CTX-M,}}$ bla_{TEM} $bla_{\text{OXA,}}$ and bla_{SHV}).

11. Other virulence factor, antibiotic resistance genes and mobile genetic elements amplified in the MDR ESBL producing *E. coli* isolates were *fimH, pap* and *upaBc* genes in 10(100.0%), *cnf*

and *qepA* genes in 5(50.0%), *ampC* gene in 8(80.0%), *aac-Ib* gene in 1(10.0%), *Int 1* gene in 10(100.0%) and *IS256* gene in 8(80.0%).

12. Phylogenetic classification of the MDR ESBL producing *E. coli* isolates through PCR (amplification of the *chuA*, *Tsp* and *YjaA* genes) revealed that 7(70.0%) of them are of Group B2, i.e., the virulent extra-intestinal strains.

13. NCBI blast of the nucleotide sequences of the various isolates revealed close identity level to those in the Genbank data base respectively.

14. The molecular phylogenetic analysis by maximum likelihood method (MLM) based on the Tamura-Nei modelalsoreveals that these isolates are of closely related phylogeny.

6.2 Conclusion(s)

It can be concluded from this study that multidrug resistant bacterial uropathogens with virulence genes have emerged in pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria (alternate hypothesis). Majority of the women were seen to be asymptomatic (and might be seen as not requiring any form of therapy) which could in turn lead to eventual adverse maternal and neonatal outcomes. Several socio-economic factors were seen to also influence bacteriuria. The selection of appropriate and safe antibiotic for UTI therapy in pregnancy might become more difficult.

6.3 Recommendation(s)

1. There is need for early, proper and adequate diagnosis of both symptomatic and asymptomatic bacteriuria in pregnancy in order to prevent adverse maternal and neonatal outcomes. Multiparous women should be encouraged to report early at antenatal clinics.

2. Asymptomatic bacteriuria should be treated in pregnancy in order to prevent complications such as pyelonephritis, premature labour, still birth, hypertension, preeclampsia and septicemia.

3. Personal hygiene and healthy habits should be promoted at antenatal clinics; as some bacteria that were once considered as normal skin flora are now observed to be pathogenic.

4. Rational antibiotic use and awareness should be encouraged at antenatal clinics and communities at large. This would help curb the emergence and transmission of multidrug resistant uropathogens.

5. Continous local antibiotic surveillance studies like this should be greatly encouraged in this special population because of their reduced immunity and hormonal changes which make them more prone to infections.

6. The various socioeconomic factors influencing urinary tract infection(s) in pregnancy should be looked into both nationally and locally.

6.4 Contribution to knowledge

1. A changing bacterial colonization pattern of the urinary tract in pregnancy was also established in this study with uncommon microbes like *Staphylococcus lentus* (associated with infections in animals), *Staphylococcus equorium* (associated with fermented food).

2. This is the first time of detecting this variety of virulence genes in antenatal patients in South-west Nigeria to the best of our knowledge.

3. *MecA* gene was detected from two of the phenotypic MRSA isolates.

4. Sequencing analysis of the nucleotide of one of the MRSA isolates, revealed 100% identity with *S. aureus* strain ANT 05 with sequence ID (accession) number HM803171.1 from GenBank database.

5. Sequencing analysis of the nucleotide of one of the MDR ESBL producing *E. coli* isolates, revealed 99% identity with *E. coli* strain PEC 078 with sequence ID (accession) number CP004009.1 from GenBank database.

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APPENDICES

APPENDIX I: Research Questionnaire

Serial Number…….…

AHMADU BELLO UNIVERSITY ZARIA
FACULTY OF PHARMACEUTICAL SCIENCES
DEPARTMENT OF PHARMACEUTICS & PHARMACEUTICAL MICROBIOLOGY

RESEARCH TITLE: Molecular characterization of multi-drug resistant bacterial uropathogens from pregnant women attending antenatal clinics in Ibadan metropolis, Southwest, Nigeria.

INTRODUCTION: This study among pregnant women attending antenatal clinics seek information for research purposes only and all information supplied in this document shall be used strictly for research. Feel free to supply information as best describes your situations and your information shall remain confidential.

Thank you.

Mojirayo T. Durowaiye (Principal Investigator)

CONSENT STATEMENT

If you agree to participate in this study, kindly read this consent statement and append your signature: without expectation of compensation or other remuneration, now or in the future. I hereby give my consent to participate in this research for educational purposes. This consent is given in perpetuity and does not require prior approval by me.

Sign…… Date……

DEMOGRAPHIC INFORMATION

1. Age (a)20-24 (b)25-29 (c)30-34 (d)35-39 (e)40-44 (f)45-49 (g)50-54 (h)55-60
2. Nationality (a) Nigerian…… (b) Others……
3. Religion (a) Christianity…… (b) Muslim……. (c) Others……..
4. Occupational Status (a) Employed…..… (b) Self employed/Business……. (c) Not employed…..... (d) Housewife……
5. Educational Qualification (a) Primary…. (b)Secondary….. (c)Tertiary Institution…….....

(d) Postgraduate…….

CLINICAL SYMPTOMS OF URINARY TRACT INFECTION (UTI)

6. Do you experience any of these symptoms of urinary tract infection?

a) Burning sensation while urinating Yes……No……
b) Lower abdominal pain Yes……No……
c) Fever Yes……No……
d) Purulent vaginal discharge Yes……No……
e) Increased urgency of urination Yes…….... No……
f) Increased frequency of urination Yes……No……

GRAVIDITY/GESTATIONAL AGE

5. How old is your pregnancy?

(a) 1-3months (b) 4-6months (c) 7-9months (d) above 9months

6. How many children do you have?

7. Is this your 1st……2nd……3rd ……4th …….5th ……6th ……7th ……8th ……9th ……10th ……Pregnancy?

ANTIBIOTIC DRUG USE IN PREGNANCY

8. Are you on any antibiotic medication presently? a) Yes……. b) No…….

9. Have you taken any antibiotic medication since you became pregnant?

a) Yes…….. b) No……..

10. Who prescribed the antibiotic for you? a) A Medical Doctor…….. b) A Healthcare professional……...…. c) Self prescription……. d) A friend……

11. Did you start, take and complete the antibiotic medication the right way it was

prescribed? a) Yes…….b) No…….

12. If your response to (11) above is no, tick the reason why?

a) I forgot to complete my antibiotic medication……..b) I experienced an unfavorable side effect……c) The drug prescribed was expensive, I could not afford it……

d) Was afraid to take antibiotic despite the fact Doctor said it was safe……

APPENDIX II: Ethical Approval

TELEGRAMS..... TELEPHONE.....

MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. .....

All communications should be addressed to the Honorable Commissioner quoting

Our Ref. No. AD 13/ 479/385

15th April, 2013

The Principal Investigator,
Department of Pharmaceutics and Pharmaceutical Microbiology,
Ahmadu Bello University,
Zaria.

Attention: Durowaiye Mojirayo

Ethical Approval for the Implementation of your Research Proposal in Oyo State

This acknowledges the receipt of the corrected version of your Research Proposal titled: "Molecular characterization and Antibiotics Sensitivity of Urine Isolates from Pregnant women attending Antenatal Clinics in Ibadan, Nigeria."

2. The committee has noted your compliance with all the ethical concerns raised in the initial review of the proposal. In the light of this, I am pleased to convey to you the approval of committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the committee will monitor closely and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of the findings as this will help in policy making in the health sector.

4. Wishing you all the best.

OYO STATE RESEARCH ETHICAL REVIEW COMMITTEE
Signature & Date

Sola Akande (Dr)
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethical Review Committee

APPENDIX III: **Biochemical characterization and identification of bacterial urinary isolates**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
1	001	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
2	002	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
3	005	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
4	007	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
5	009	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
6	014	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
7	016	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
8	023	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
9	024	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
10	026	-	-	-	-	-	+	-	-	-	-	+	+	-	*Proteus vulgaris*
11	028	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
12	031	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
13	036	-	-	-	-	-	+	-	-	-	-	+	+	-	*Proteus vulgaris*
14	040	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
15	041	-	-	-	+	-	-	-	-	+	-	-	+	-	*Providencia rettgeri*
16	042	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
17	043	-	-	-	+	-	-	-	-	+	-	+	+	-	*Morganella morganii*
18	047	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
19	051	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
20	053	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
21	054	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
22	057	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
23	061	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
24	064	-	-	-	+	-	-	-	-	-	+	-	-	-	*Klebsiella pneumonia*
25	066	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
26	069	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
27	072	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
28	077	-	-	-	+	-	-	-	-	+	-	+	+	-	*Morganella morganii*
29	079	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia fergusonii*
30	081	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
31	083	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
32	085	-	-	-	+	-	-	-	-	-	+	+	-	-	*Serratia marcescens*
33	086	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
34	090	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
35	093	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
36	094	-	+	-	-	-	-	+	-	-	-	-	-	-	*P. aeruginosa*
37	098	-	-	-	-	-	+	-	-	-	-	+	+	-	*Proteus vulgaris*
38	102	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
39	107	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
40	109	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary isolates**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
41	110	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
42	114	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
43	116	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. xylosus*
44	119	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
45	120	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
46	124	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococccus spp*
47	126	-	-	-	+	-	-	-	-	-	+	-	-	-	*Klebsiella oxytoca*
48	129	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
49	130	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
50	134	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
51	137	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus subsp. Anaerobius*
52	139	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
53	140	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. warneri*
54	141	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
55	147	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
56	149	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
57	150	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
58	151	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
59	154	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. simulans*
60	158	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
61	161	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
62	164	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
63	166	-	-	-	+	-	-	-	-	-	+	-	-	-	*Klebsiella pneumonia*
64	171	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
65	172	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
66	176	-	-	-	+	-	-	-	-	-	+	+	-	-	*Salmonella sp*
67	181	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
68	185	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
69	187	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
70	191	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
71	193	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
72	196	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
73	199	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*
74	201	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
75	203	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
76	207	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
77	210	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
78	212	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
79	217	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
80	218	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
81	221	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary isolates**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
82	229	-	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
83	231	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. lentus*
84	234	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia fergusonii*
85	237	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
86	238	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
87	240	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
88	246	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
89	251	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
90	253	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
91	255	-	-	-	+	-	-	-	-	+	-	+	+	-	*Morganella morganii*
92	257	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
93	259	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
94	260	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*
95	263	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
96	267	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
97	270	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
98	271	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. simulans*
99	279	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
100	282	-	-	-	+	-	-	-	-	-	+	+	-	-	*Serratia marcescens*
101	287	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
102	290	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
103	292	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
104	295	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
105	297	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
106	310	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
107	313	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
108	316	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
109	318	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
110	320	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
111	321	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
112	325	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
113	327	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
114	329	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. warneri*
115	331	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
116	334	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
117	335	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
118	337	-	-	-	-	-	+	-	-	-	-	+	+	-	*Proteus vulgaris*
119	339	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptoccocus spp*
120	341	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
121	344	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
122	346	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary isolates**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
123	347	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. simulans*
124	349	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
125	351	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
126	353	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
127	355	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia fergusonii*
128	357	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
129	360	-	-	-	+	-	-	-	-	-	+	+	-	-	*Serratia marcescens*
130	363	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
131	367	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
132	369	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. cohnii subsp. cohnii*
133	370	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
134	373	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
135	375	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
136	378	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
137	381	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
138	384	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
139	387	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
140	391	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
141	392	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*
142	394	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
143	397	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
144	401	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
145	403	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
146	406	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. capitis subsp.ureolyticus*
147	437	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
148	439	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
149	440	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
150	441	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. lugdenensis*
151	443	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
152	446	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
153	448	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
154	451	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
155	452	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
156	455	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
157	456	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
158	459	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. lentus*
159	461	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*
160	465	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
161	467	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
162	468	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
163	471	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia fergusoni*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary isolates**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
164	473	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
165	476	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
166	477	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
167	479	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
168	480	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
169	483	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
170	485	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
171	486	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
172	489	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. intermidius*
173	491	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
174	493	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
175	495	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
176	496	-	-	-	+	-	-	-	-	+	+	-	-	-	*P. alcalifaciens*
177	498	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
178	500	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
179	503	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
180	505	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
181	507	-	-	-	+	-	-	-	-	-	+	-	-	-	*Klebsiella pneumonia*
182	509	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
183	511	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
184	513	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
185	516	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
186	519	-	-	-	+	-	-	-	-	+	+	-	-	-	*Providencia stuartii*
187	520	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
188	522	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
189	524	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. simulans*
190	527	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
191	529	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. lugdenensis*
192	533	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. saprophyticus*
193	537	+	N	-	-	-	-	-	+	-	-	-	-	+	*Staphylococcus caprae*
194	541	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
195	544	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
196	546	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
197	548	-	+	-	+	-	-	+	-	+	+	-	-	-	*P. aeruginosa*
198	550	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
199	551	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. lugdenensis*
200	552	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
201	555	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
202	557	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
203	559	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. capitis subsp.ureolyticus*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
204	561	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. haemolyticus*
205	563	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
206	565	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
207	566	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
208	567	+	N	-	-	-	-	+	-	-	-	-	-	-	*P. aeruginosa*
209	569	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
210	571	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. capitis subsp.ureolyticus*
211	574	-	-	-	+	-	-	-	-	+	-	+	+	-	*Morganella morganii*
212	575	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
213	578	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
214	582	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. cohnii subsp. urealyticum*
215	584	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
216	587	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
217	590	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
218	591	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
219	593	+	N	-	-	-	-	-	+	-	-	-	-	+	*Streptococcus spp*
220	596	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
221	599	+	N	-	-	-	-	-	+	-	-	-	-	+	*Streptococcus spp*
222	600	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
223	602	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
224	604	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
225	608	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
226	606	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
227	610	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
228	612	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
229	614	-	-	-	-	-	-	-	-	+	-	-	-	-	*Salmonella enterica subsp enteric*
230	616	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
231	621	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
232	623	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
233	625	-	-	-	-	-	+	-	-	-	-	-	+	-	*Proteus mirabilis*
234	627	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. capitis subsp.ureolyticus*
235	628	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. equorium*
236	630	-	-	-	+	-	-	-	-	-	+	-	+	-	*Enterobacter cloacae*
237	632	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. chromogens*
238	634	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
239	635	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
240	637	-	-	-	+	-	+	-	-	-	+	-	+	-	*E. agglomerans*
241	639	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*
242	641	+	-	-	+	-	-	-	-	-	-	-	-	+	*Streptococcus spp*
243	644	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*

APPENDIX III (continued): **Biochemical characterization and identification of bacterial urinary**

S/N	Isolate code	cat	oxi	coa	Differential media					MR	VP	IP	CT	GS	Probable Identity Microbact 12E/12S
					MAC	EMB	UA	CET	MSA						
244	647	+	-	+	-	-	-	-	+	-	-	-	+	+	*S. aureus*
245	648	+	N	-	-	-	-	-	+	-	-	-	-	+	*S. epidermidis*
246	649	-	-	-	+	+	-	-	-	+	-	+	-	-	*Escherichia coli*

Key:
MAC: maconkey agar, EMB: eosin methylene blue agar, UA: urea agar, CET: cetrimide agar, MSA: mannitol salt agar, Cat: catalase, Coa:coagulase, Oxi:oxidase, MR: methyl red, VP: voges proskeur, IP: Indole production, CT: citrate utilization, GS:gram staining, N: not done.
Microbact 12E: Gram negative bacillus, Microbact 12S: *Staphylococcus* spp

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

Inqaba Biotechnical Industries (Pty) Ltd
P.O. Box 14356, Hatfield 0028, South Africa
Tel: 012 343 5829
Fax: 012 343 0287
E-mail: info@inqaba.com

inqaba biotec

SYNTHESIS REPORT

08 Sep 2015

Client Detail:

Mojirayo Durowaiye
University of Ibadan
Department of Pharmaceutical Microbiology
Faculty of Pharmacy
Ibadan
Nigeria

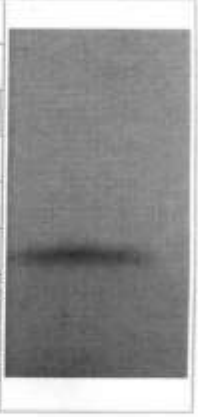
Name:	blaTEMF		**Barcode:** S43BE	**Length:** 17 bases	
Sequence:	AAACGCTGGTGAAAGTA				
OD	4.9476	**MW min \ max**	5267\5267	**5' Mod**	None
nmoles	23.87	**GC % min \ max**	41.18\41.18	**3' Mod**	None
Tm min \ max	52.37\52.37			**Purification**	Standard
For a 100 µM stock solution add 238.67 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	blaTEMR		**Barcode:** S43BF	**Length:** 14 bases	
Sequence:	AGCGATCTGTCTAT				
OD	5.0996	**MW min \ max**	4253.9\4253.9	**5' Mod**	None
nmoles	34.64	**GC % min \ max**	42.86\42.86	**3' Mod**	None
Tm min \ max	46.76\46.76			**Purification**	Standard
For a 100 µM stock solution add 346.44 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	blaSHVF			Barcode: S43C0	Length: 20 bases
Sequence:	ATGCGTTATATTCGCCTGTG				
OD	7.7197	MW min \ max	6113.8\6113.8	5' Mod	None
nmoles	37.79	GC % min \ max	45\45	3' Mod	None
Tm min \ max	58.35\58.35			Purification	Standard
For a 100 µM stock solution add 377.86 µl water or buffer					PAGE QC Image >>
Comments					



Name:	blaSHVR			Barcode: S43C1	Length: 20 bases
Sequence:	TGCTTTGTTATTCGGGCCAA				
OD	5.6734	MW min \ max	6113.8\6113.8	5' Mod	None
nmoles	27.77	GC % min \ max	45\45	3' Mod	None
Tm min \ max	58.35\58.35			Purification	Standard
For a 100 µM stock solution add 277.7 µl water or buffer					PAGE QC Image >>
Comments					



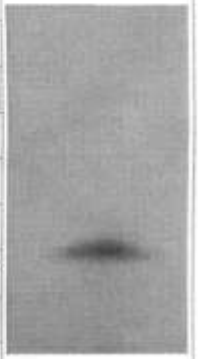
Name:	blaCTX-M F			Barcode: S43C2	Length: 17 bases
Sequence:	CGCTTTGCGATGTGCAG				
OD	3.9501	MW min \ max	5217.2\5217.2	5' Mod	None
nmoles	22.68	GC % min \ max	58.82\58.82	3' Mod	None
Tm min \ max	59.61\59.61			Purification	Standard
For a 100 µM stock solution add 226.76 µl water or buffer					PAGE QC Image >>
Comments					



Name:	blaCTX-M R		**Barcode:** S43C3	**Length:** 17 bases	
Sequence:	ACCGCGATATCGTTGGT				
OD	3.8684	**MW min \ max**	5201.2\5201.2	**5' Mod**	None
nmoles	21.74	**GC % min \ max**	52.94\52.94	**3' Mod**	None
Tm min \ max	57.19\57.19			**Purification**	Standard
For a 100 µM stock solution add 217.45 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	blaOXAF		**Barcode:** S43C4	**Length:** 22 bases	
Sequence:	ATATCTCTACTGTTGCATCTCC				
OD	8.094	**MW min \ max**	6611.1\6611.1	**5' Mod**	None
nmoles	37.59	**GC % min \ max**	40.91\40.91	**3' Mod**	None
Tm min \ max	58.95\58.95			**Purification**	Standard
For a 100 µM stock solution add 375.94 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



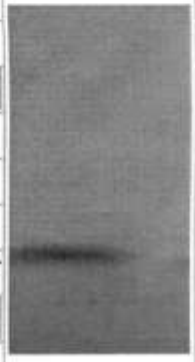
Name:	blaOXAR		**Barcode:** S43C5	**Length:** 18 bases	
Sequence:	AAACCCTTCAAACCATCC				
OD	7.657	**MW min \ max**	5356.1\5356.1	**5' Mod**	None
nmoles	39.76	**GC % min \ max**	44.44\44.44	**3' Mod**	None
Tm min \ max	55.34\55.34			**Purification**	Standard
For a 100 µM stock solution add 397.56 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	IS256F		**Barcode:** S43C6	**Length:** 23 bases	
Sequence:	TGAAAAGCGAAGAGATTCAAAGC				
OD	6.5417	**MW min \ max**	7137.6\7137.6	**5' Mod**	None
nmoles	22.72	**GC % min \ max**	39.13\39.13	**3' Mod**	None
Tm min \ max	59.2\59.2			**Purification**	Standard
For a 100 µM stock solution add 227.22 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	IS256R		**Barcode:** S43C7	**Length:** 21 bases	
Sequence:	ATGTAGGTCCATAAGAACGGC				
OD	9.8249	**MW min \ max**	6478.5\6478.5	**5' Mod**	None
nmoles	40.53	**GC % min \ max**	47.62\47.62	**3' Mod**	None
Tm min \ max	60.61\60.61			**Purification**	Standard
For a 100 µM stock solution add 405.32 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



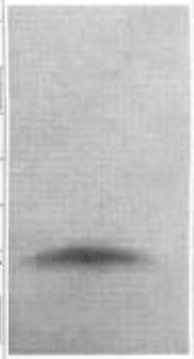
Name:	Class 1		**Barcode:** C43C8	**Length:** 17 bases	
Sequence:	CSGGCATCCAAGCAGCA				
OD	1.1305	**MW min \ max**	5148.8\5188.8	**5' Mod**	None
nmoles	6.08	**GC % min \ max**	64.71\64.71	**3' Mod**	None
Tm min \ max	62.02\62.02			**Purification**	Cartridge
For a 100 µM stock solution add 60.81 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Integron			**Barcode:** C43C9	**Length:** 19 bases
Sequence:	CSAAGCAGACTTGACCTGA				
OD	2.5232	**MW min \ max**	5781.2\5821.2	**5' Mod**	None
nmoles	11.92	**GC % min \ max**	52.63\52.63	**3' Mod**	None
Tm min \ max	60.16\60.16			**Purification**	Cartridge
For a 100 µM stock solution add 119.24 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Int 1-F			**Barcode:** S43CA	**Length:** 20 bases
Sequence:	CAGTGGACATAAGCCTGTTC				
OD	10.2125	**MW min \ max**	6116.5\6116.5	**5' Mod**	None
nmoles	47.28	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 472.8 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Int 1-R			**Barcode:** S43CB	**Length:** 18 bases
Sequence:	CCCGAGGCATAGACTGTA				
OD	6.1009	**MW min \ max**	5508.1\5508.1	**5' Mod**	None
nmoles	30.75	**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 307.51 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	fimH-f		**Barcode:** S43FA	**Length:** 26 bases	
Sequence:	AACAGCGATGATTTCCAGTTTGTGTG				
OD	7.4328	**MW min \ max**	8015.5\8015.5	**5' Mod**	None
nmoles	26.29	**GC % min \ max**	42.31\42.31	**3' Mod**	None
Tm min \ max	63.02\63.02			**Purification**	Standard
For a 100 µM stock solution add 262.92 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



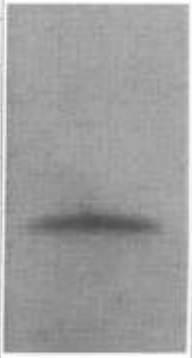
Name:	fimH-r		**Barcode:** S43FC	**Length:** 26 bases	
Sequence:	ATTGCGTACCAGCATTAGCAATGTCC				
OD	9.8705	**MW min \ max**	7929.3\7929.3	**5' Mod**	None
nmoles	35.38	**GC % min \ max**	46.15\46.15	**3' Mod**	None
Tm min \ max	64.59\64.59			**Purification**	Standard
For a 100 µM stock solution add 353.78 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Pap1		**Barcode:** S43FE	**Length:** 25 bases	
Sequence:	GACGGCTGTACTGCAGGGTGTGGCG				
OD	5.7437	**MW min \ max**	7794\7794	**5' Mod**	None
nmoles	21.5	**GC % min \ max**	68\68	**3' Mod**	None
Tm min \ max	72.78\72.78			**Purification**	Standard
For a 100 µM stock solution add 215.04 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	pap2		**Barcode:** S4400	**Length:** 25 bases	
Sequence:	ATATCCTTTCTGCAGGGATGCAATA				
OD	15.3463	**MW min \ max**	7655.3\7655.3	**5' Mod**	None
nmoles	56.17	**GC % min \ max**	40\40	**3' Mod**	None
Tm min \ max	61.3\61.3			**Purification**	Standard
For a 100 µM stock solution add 561.72 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	hly s		**Barcode:** S4402	**Length:** 21 bases	
Sequence:	AGATTCTTGGGCATGTATCCT				
OD	8.7704	**MW min \ max**	6426.9\6426.9	**5' Mod**	None
nmoles	39.92	**GC % min \ max**	42.86\42.86	**3' Mod**	None
Tm min \ max	58.66\58.66			**Purification**	Standard
For a 100 µM stock solution add 399.2 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	hly as		**Barcode:** S4404	**Length:** 21 bases	
Sequence:	TTGCTTTGCAGACTGTAGTGT				
OD	9.5703	**MW min \ max**	6458\6458	**5' Mod**	None
nmoles	44	**GC % min \ max**	42.86\42.86	**3' Mod**	None
Tm min \ max	58.66\58.66			**Purification**	Standard
For a 100 µM stock solution add 440.01 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	ChuA.1		**Barcode:** S43CC	**Length:** 20 bases	
Sequence:	GACGAACCAACGGTCAGGAT				
OD	7.0851	**MW min \ max**	6159.2\6159.2	**5' Mod**	None
nmoles	30.53	**GC % min \ max**	55\55	**3' Mod**	None
Tm min \ max	62.45\62.45			**Purification**	Standard
For a 100 µM stock solution add 305.26 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	ChuA.2		**Barcode:** S43CD	**Length:** 20 bases	
Sequence:	TGCCGCCAGTACCAAAGACA				
OD	8.2118	**MW min \ max**	6079.2\6079.2	**5' Mod**	None
nmoles	36.77	**GC % min \ max**	55\55	**3' Mod**	None
Tm min \ max	62.45\62.45			**Purification**	Standard
For a 100 µM stock solution add 367.75 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



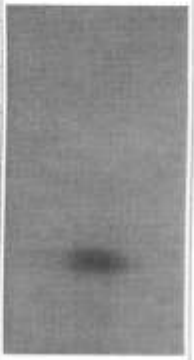
Name:	YjaA.1		**Barcode:** S43CE	**Length:** 20 bases	
Sequence:	TGAAGTGTCAGGAGACGCTG				
OD	7.9344	**MW min \ max**	6221.4\6221.4	**5' Mod**	None
nmoles	34.85	**GC % min \ max**	55\55	**3' Mod**	None
Tm min \ max	62.45\62.45			**Purification**	Standard
For a 100 µM stock solution add 348.46 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	YjaA.2		**Barcode:** S43CF	**Length:** 21 bases	
Sequence:	ATGGAGAATGCGTTCCTCAAC				
OD	6.5854	**MW min \ max**	6429.6\6429.6	**5' Mod**	None
nmoles	28.46	**GC % min \ max**	47.62\47.62	**3' Mod**	None
Tm min \ max	60.61\60.61			**Purification**	Standard
For a 100 µM stock solution add 284.59 µl water or buffer				**PAGE QC Image >>**	
Comments					



Name:	TspE4C2.1		**Barcode:** S43D0	**Length:** 20 bases	
Sequence:	GAGTAATGTCGGGGCATTCA				
OD	7.7444	**MW min \ max**	6196.5\6196.5	**5' Mod**	None
nmoles	34.45	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 344.5 µl water or buffer				**PAGE QC Image >>**	
Comments					



Name:	TspE4C2.2		**Barcode:** S43D1	**Length:** 20 bases	
Sequence:	CGCGCCAACAAAGTATTACG				
OD	5.4188	**MW min \ max**	6094.3\6094.3	**5' Mod**	None
nmoles	24.1	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 241.05 µl water or buffer				**PAGE QC Image >>**	
Comments					



Name:	aac-F		Barcode: S43D2	Length: 23 bases	
Sequence:	TTGCGATGCTCTATGAGTGGCTA				
OD	13.4596	MW min \ max	7085.1\7085.1	5' Mod	None
nmoles	55.37	GC % min \ max	47.83\47.83	3' Mod	None
Tm min \ max	62.77\62.77			Purification	Standard
For a 100 µM stock solution add 553.67 µl water or buffer				PAGE QC Image >>	
Comments					



Name:	aac-R		Barcode: S43D3	Length: 20 bases	
Sequence:	CTCGAATGCCTGGCGTGTTT				
OD	9.4943	MW min \ max	6114.7\6114.7	5' Mod	None
nmoles	47.69	GC % min \ max	55\55	3' Mod	None
Tm min \ max	62.45\62.45			Purification	Standard
For a 100 µM stock solution add 476.86 µl water or buffer				PAGE QC Image >>	
Comments					



Name:	qepA-F		Barcode: S43D4	Length: 20 bases	
Sequence:	AACTGCTTGAGCCCGTAGAT				
OD	8.8046	MW min \ max	6116.5\6116.5	5' Mod	None
nmoles	40.76	GC % min \ max	50\50	3' Mod	None
Tm min \ max	60.4\60.4			Purification	Standard
For a 100 µM stock solution add 407.62 µl water or buffer				PAGE QC Image >>	
Comments					



Name:	qepA-R		**Barcode:** S43D5	**Length:** 20 bases	
Sequence:	GTCTACGCCATGGACCTCAC				
OD	9.9313	**MW min \ max**	6037.4\6037.4	**5' Mod**	None
nmoles	49.16	**GC % min \ max**	60\60	**3' Mod**	None
Tm min \ max	64.5\64.5			**Purification**	Standard
For a 100 µM stock solution add 491.65 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Upa Bc0426.sF		**Barcode:** S43D6	**Length:** 20 bases	
Sequence:	GGAAAGGCAAAGTTTCAGGG				
OD	6.992	**MW min \ max**	6254.3\6254.3	**5' Mod**	None
nmoles	28.84	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 288.45 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Upa Bc0426.sR		**Barcode:** S43D7	**Length:** 20 bases	
Sequence:	GGTGGTATGTTTCTGTTTAC				
OD	7.1744	**MW min \ max**	6160\6160	**5' Mod**	None
nmoles	35.24	**GC % min \ max**	40\40	**3' Mod**	None
Tm min \ max	56.3\56.3			**Purification**	Standard
For a 100 µM stock solution add 352.38 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Upa Cc0478.sF		**Barcode:** S43D8	**Length:** 17 bases	
Sequence:	GGTGGTTGGGTAGATGC				
OD	6.0078	**MW min \ max**	5337.2\5337.2	**5' Mod**	None
nmoles	32.06	**GC % min \ max**	58.82\58.82	**3' Mod**	None
Tm min \ max	59.61\59.61			**Purification**	Standard
For a 100 µM stock solution add 320.59 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Upa Cc0478.sR		**Barcode:** S43DB	**Length:** 20 bases	
Sequence:	GTTCACATCCAGTACACCAC				
OD	8.664	**MW min \ max**	6005.4\6005.4	**5' Mod**	None
nmoles	41.38	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 413.75 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	ampCAB1		**Barcode:** S43E0	**Length:** 18 bases	
Sequence:	GATCGTTCTGCCGCTGTG				
OD	4.6056	**MW min \ max**	5497.4\5497.4	**5' Mod**	None
nmoles	26.33	**GC % min \ max**	61.11\61.11	**3' Mod**	None
Tm min \ max	62.18\62.18			**Purification**	Standard
For a 100 µM stock solution add 263.33 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



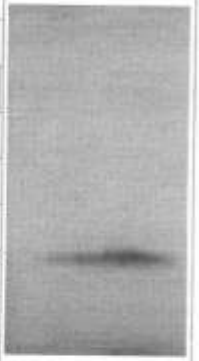
Name:	ampC2		**Barcode:** S43E2	**Length:** 20 bases	
Sequence:	GGGCAGCAAATGTGGAGCAA				
OD	7.7178	**MW min \ max**	6239.2\6239.2	**5' Mod**	None
nmoles	32.04	**GC % min \ max**	55\55	**3' Mod**	None
Tm min \ max	62.45\62.45			**Purification**	Standard
For a 100 µM stock solution add 320.37 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



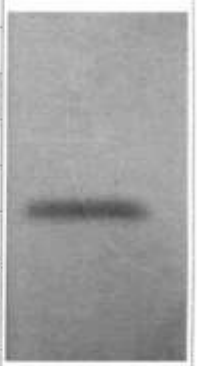
Name:	mecA-P4		**Barcode:** S43E4	**Length:** 22 bases	
Sequence:	TCCAGATTACAACTTCACCAGG				
OD	8.4569	**MW min \ max**	6662.7\6662.7	**5' Mod**	None
nmoles	35.53	**GC % min \ max**	45.45\45.45	**3' Mod**	None
Tm min \ max	60.81\60.81			**Purification**	Standard
For a 100 µM stock solution add 355.33 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	mecA-P7		**Barcode:** S43E6	**Length:** 20 bases	
Sequence:	CCACTTCATATCTTGTAACG				
OD	5.7228	**MW min \ max**	6026.7\6026.7	**5' Mod**	None
nmoles	27.81	**GC % min \ max**	40\40	**3' Mod**	None
Tm min \ max	56.3\56.3			**Purification**	Standard
For a 100 µM stock solution add 278.08 **µl water or buffer**				**PAGE QC Image >>**	
Comments					



Name:	Luk-pvl-F			Barcode: C43EC	Length: 31 bases
Sequence:	ATCATTAGGTAAAATGTCTGGACATGATCCA				
OD	5.8007	MW min \ max	9541\9541	5' Mod	None
nmoles	16.33	GC % min \ max	35.48\35.48	3' Mod	None
Tm min \ max	63.32\63.32			Purification	Cartridge
For a 100 µM stock solution add 163.26 µl water or buffer					PAGE QC Image >>
Comments					



Name:	Luk-pvl-R			Barcode: S43EF	Length: 27 bases
Sequence:	GCATCAAGTGTATTGGATAGCAAAAGC				
OD	10.6742	MW min \ max	8355.3\8355.3	5' Mod	None
nmoles	33.58	GC % min \ max	40.74\40.74	3' Mod	None
Tm min \ max	63.09\63.09			Purification	Standard
For a 100 µM stock solution add 335.77 µl water or buffer					PAGE QC Image >>
Comments					



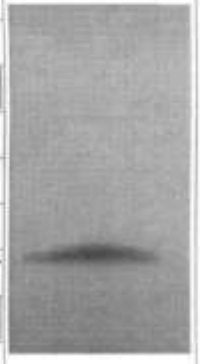
Name:	icaA-F			Barcode: S43F1	Length: 20 bases
Sequence:	TCTCTTGCAGGAGCAATCAA				
OD	8.9034	MW min \ max	6100.5\6100.5	5' Mod	None
nmoles	40.53	GC % min \ max	45\45	3' Mod	None
Tm min \ max	58.35\58.35			Purification	Standard
For a 100 µM stock solution add 405.25 µl water or buffer					PAGE QC Image >>
Comments					



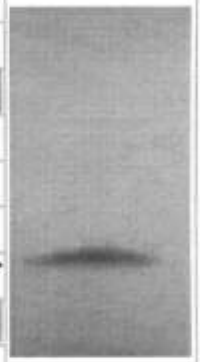
Name:	icaA-R			**Barcode:** S43F3	**Length:** 20 bases
Sequence:	TCAGGCACTAACATCCAGCA				
OD	6.4885	**MW min \ max**	6054.3\6054.3	**5' Mod**	None
nmoles	29.44	**GC % min \ max**	50\50	**3' Mod**	None
Tm min \ max	60.4\60.4			**Purification**	Standard
For a 100 µM stock solution add 294.4 **µl water or buffer**					**PAGE QC Image >>**
Comments					



Name:	icaD-F			**Barcode:** S43F5	**Length:** 20 bases
Sequence:	ATGGTCAAGCCCAGACAGAG				
OD	8.4721	**MW min \ max**	6159.2\6159.2	**5' Mod**	None
nmoles	36.5	**GC % min \ max**	55\55	**3' Mod**	None
Tm min \ max	62.45\62.45			**Purification**	Standard
For a 100 µM stock solution add 365.02 **µl water or buffer**					**PAGE QC Image >>**
Comments					



Name:	icaD-R			**Barcode:** S43F7	**Length:** 23 bases
Sequence:	CGTGTTTTCAACATTTAATGCAA				
OD	10.7825	**MW min \ max**	7012.2\7012.2	**5' Mod**	None
nmoles	42.91	**GC % min \ max**	30.43\30.43	**3' Mod**	None
Tm min \ max	55.64\55.64			**Purification**	Standard
For a 100 µM stock solution add 429.07 **µl water or buffer**					**PAGE QC Image >>**
Comments					



Name:	aer as			**Barcode:** S4408	**Length:** 21 bases	
Sequence:	ACCCGTCTGCAAATCATGGAT					
OD	8.3239	**MW min \ max**	6389.6\6389.6	**5' Mod**	None	
nmoles	36.67	**GC % min \ max**	47.62\47.62	**3' Mod**	None	
Tm min \ max	60.61\60.61			**Purification**	Standard	
For a 100 µM stock solution add 366.69 **µl water or buffer**					**PAGE QC Image >>**	
Comments						

RECOMMENDATIONS FOR HANDLING AND STORAGE OF OLIGOS

- Lyophilized oligo pellets might become displaced from the bottom of the tube during shipment. Briefly centrifuge each tube before opening to prevent the loss of the pellet.
- Prepare stock solution of oligos (e.g. 100 uM = 100 pmole per ul) preferably with a sterile buffered solution such as TE (10 mM Tris, pH 7.5 to 8.0, 1 mM EDTA). If sterile distilled water is used, make sure that the pH is above 7.0 since acidic solutions favours oligo depurination and subsequent loss of activity.
- Working solutions might be diluted from the stock solution with sterile, nuclease-free water to prevent inhibition of enzymatic reactions (e.g. PCR) by EDTA.
- Store the oligos as concentrated stock solution or lyophilized at -20° C.
- Avoid frequent freeze-thaw cycles by dividing the stock solution into smaller aliquots for long term storage and to prevent accidental contamination.
- Dye-modified oligos are light sensitive and should always be stored in the dark.
- Re-suspend modified oligos preferably in a slightly basic solution (i.e., TE at pH 8.0). However, Cy dye modified oligos are best kept at pH 7.0 at -20° C.
- Preferably store the modified oligos as dried aliquots at -20° C.

APPENDIX V: Quantification of extracted Genomic DNA Using Nanodrop

	Isolate Code	DNA Conc *n*g/µl	260/280	260/230
1.	41E	22.5	1.55	0.04
2.	48E	20.8	1.58	0.04
3.	7E	27.1	1.85	0.06
4.	47E	18.1	1.13	0.03
5.	33E	32.6	1.37	0.06
6.	49E	34.1	1.47	0.05
7.	30E	18.2	1.02	0.03
8.	42E	23.7	1.43	0.03
9.	5E	14.0	1.61	0.04
10.	19E	29.6	1.53	0.04
11.	S6	18.8	1.52	0.03
12.	S13	26.8	1.26	0.04
13.	S16	20.7	1.53	0.03
14.	S12	14.3	1.93	0.08
15.	S22	26.3	1.66	0.05
16.	S7	20.6	1.69	0.10
17.	S42	14.6	1.91	0.06
18.	S15	16.0	1.77	0.18
19.	S40	16.0	1.70	0.06
20.	S28	08.1	1.89	0.03

APPENDIX VI: Mc FARLAND STANDARD

1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water.

1% w/v solution of barium chloride was prepared by distilling 0.5g of dehydrated barium chloride in 50ml of distilled water.

0.05ml of barium chloride and 9.95ml of sulphuric acid was measured to make approximately a cell density of 1.5 x 10^8cfu/ml (Cheesbrough, 2002).

APPENDIX VII: CLSI 2012 guideline for screening and phenotypic confirmatory test for ESBL in *E. coli*

Antibiotic	Zone (mm)
Cefpodoxime	≤ 17
Ceftriaxone	≤25

Appendix VIII: Phenotypic resistance profile of uropathogens from pregnant women attending antenatal clinics.

Isolate code	Phenotypic resistance pattern	MARI	% Resistance	Resistance category
001	FOS, CEF	0.16	16.6	NMDR
002	CEF	0.08	8.3	NMDR
005	AML,SXT	0.16	16.6	NMDR
007	FOS	0.08	8.3	NMDR
009	FOS	0.08	8.3	NMDR
023	AML	0.08	8.3	NMDR
024	AML, FOS, CEF, CHL	0.33	33.3	MDR
028	FOS	0.08	8.3	NMDR
036	FOS	0.08	8.3	NMDR
040	OFL, AML	0.16	16.6	NMDR
053	CHL	0.08	8.3	NMDR
064	TET	0.08	8.3	NMDR
069	CEF	0.08	8.3	NMDR
081	OFL, AMC, NFT	0.25	25.0	MDR
083	AMC, NFT, AML, SXT, CFT, FOS, TET	0.58	58.3	MDR
086	IMI, AMC, AML, SXT, CFT, FOS, CEF, CHL, TET	0.75	75.0	MDR
090	IMI, SXT, GEN, CFT, CEF, TET	0.50	50.0	MDR
093	AMC, AML, SXT, CFT, FOS, CEF, CHL, TET	0.66	66.6	MDR
094	IMI, AMC, AML, FOS, CEF	0.41	41.6	MDR
098	AMC, GEN, CEF, CHL	0.33	33.3	MDR
102	IMI, AMC, AML, SXT, FOS, CEF	0.50	50.0	MDR
107	IMI, AMC, AML, CFT, FOS, CEF, TET	0.58	58.3	MDR
109	AMC, AML, SXT, CFT, CEF	0.41	41.6	MDR
110	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
114	OFL, SXT, GEN, CFT, CEF, CHL	0.50	50.0	MDR
116	SXT, GEN, CFT, CHL, TET	0.41	41.6	MDR
119	IMI. SXT, CEF, CHL	0.33	33.3	MDR
120	IMI, AMC, AML, SXT, CEF	0.41	41.6	MDR
124	AMC, AML, SXT, CEF, CHL, TET	0.50	50.0	MDR
126	IMI, CFT, CEF, TET	0.33	33.3	MDR
129	OFL, GEN, CHL	0.25	25.0	MDR
130	IMI, AMC, AML, SXT, FOS, CEF	0.50	50.0	MDR
134	IMI, OFL, AMC, AML, SXT, FOS, TET	0.58	58.3	MDR
137	IMI, OFL, AML, SXT, CFT, FOS, CHL, TET	0.66	66.6	MDR
139	OFL, AMC, AML, SXT, GEN, CFT, CEF, CHL	0.66	66.6	MDR
140	IMI, OFL, AMC, AML, SXT, CFT, CEF, CHL, TET	0.75	75.0	MDR
141	SXT, FOS, CEF, TET	0.33	33.3	MDR
147	SXT, CFT, CHL, TET	0.33	33.3	MDR
149	CHL	0.08	8.3	

Appendix VIII (continued): Phenotypic resistance profile of uropathogens from pregnant

Isolate code	Phenotypic resistance pattern	MARI	% Resistance	Resistance category
150	AML, SXT, CFT, CEF	0.33	33.3	MDR
154	OFL, AMC, AML, CHL, TET	0.41	41.6	MDR
158	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
161	SXT, FOS, CHL	0.25	25.0	MDR
166	IMI, AMC, NFT, AML, CFT, FOS, TET	0.58	58.3	MDR
171	AMC, AML, SXT, FOS, CHL	0.41	41.6	MDR
172	IMI, OFL, AMC, AML, CFT	0.41	41.6	MDR
176	AML, CEF, CHL	0.25	25.0	MDR
181	AMC, AML, SXT, FOS, CHL, TET	0.50	50.0	MDR
185	AMC, AML, SXT, CEF, CHL, TET	0.50	50.0	MDR
191	AMC, AML, SXT, CFT, CEF, CHL, TET	0.58	58.3	MDR
193	AML, SXT, GEN, CFT, CEF, CHL, TET	0.58	58.3	MDR
196	AMC, NFT, AML, SXT, CEF, CHL, TET	0.58	58.3	MDR
199	NFT, AML, SXT, CHL	0.33	33.3	MDR
201	AML, SXT, CFT, CEF, CHL	0.41	41.6	MDR
203	AML, SXT, CHL, TET	0.33	33.3	MDR
207	SXT, GEN, CFT, CEF, CHL	0.41	41.6	MDR
210	AMC, AML, GEN, CHL, TET	0.41	41.6	MDR
212	OFL, AMC, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
217	AML, GEN, CFT, CEF	0.33	33.3	NMDR
218	SXT, FOS	0.16	16.6	NMDR
221	AML	0.08	8.33	NMDR
229	CEF, TET	0.16	16.6	MDR
231	AMC, AML, SXT, CFT, CEF, CHL, TET	0.58	58.3	NMDR
234	SXT, FOS	0.16	16.6	MDR
237	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
238	AMC, AML, SXT, GEN, CFT, CEF, CHL	0.58	58.3	MDR
240	AMC, SXT, CEF	0.25	25.0	MDR
246	AMC, SXT, CEF	0.25	25.0	MDR
251	AMC, SXT, CFT, CEF	0.33	33.3	MDR
253	AML, SXT, CFT, CEF	0.33	33.3	MDR
255	GEN, CFT	0.16	16.6	NMDR
257	CFT	0.08	8.33	NMDR
259	AMC, AML, SXT, CFT, CEF	0.41	41.6	MDR
260	NFT, AML	0.16	16.6	NMDR
263	CFT, FOS, CEF	0.25	25.0	MDR
267	AML, SXT, FOS, CEF, TET	0.41	41.6	MDR
270	AMC, NFT, AML, CEF	0.33	33.3	MDR
271	AMC, FOS, CHL	0.25	25.0	MDR
279	AML, SXT, CEF	0.25	25.0	MDR

Appendix VIII (continued): Phenotypic resistance profile of uropathogens from pregnant

Isolate code	Phenotypic resistance pattern	MARI	% Resistance	Resistance category
282	AML, SXT, CFT, CEF	0.33	33.3	MDR
287	AML, SXT, CFT, CEF, CHL	0.41	41.6	MDR
290	NFT, AML, CEF, TET	0.33	33.3	MDR
292	CFT, CHL	0.16	16.6	NMDR
295	IMI, AMC, AML, SXT, GEN, CFT,CEF, CHL, TET	0.75	75.0	MDR
297	IMI, AMC, AML, SXT, GEN, CFT, FOS, CEF	0.66	66.6	MDR
310	IMI, OFL, NFT, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
313	SXT, FOS, CHL, TET	0.33	33.3	MDR
316	NFT, AML, SXT, GEN, FOS, CHL, TET	0.58	58.3	MDR
318	NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL	0.66	66.6	MDR
320	AML, CFT, FOS	0.25	25.0	MDR
321	AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.66	66.6	MDR
325	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
327	AMC, AML, CFT, CEF, CHL, TET	0.50	50.0	MDR
329	IMI, OFL, AMC, NFT, AML, SXT,CFT,CEF,CHL	0.75	75.0	MDR
331	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CHL, TET	0.91	91.6	MDR
334	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
335	OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
337	AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL	0.75	75.0	MDR
339	IMI, OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL, TET	0.91	91.0	MDR
341	OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.91	91.0	MDR
344	IMI, AMC, NFT, AML, SXT, CFT, CEF, CHL, TET	0.75	75.0	MDR
346	IMI, AMC, AML, SXT, GEN, CEF, CHL	0.58	58.3	MDR
347	IMI, AMC, AML, SXT, CFT, CEF, CHL	0.58	58.3	MDR
349	OFL, AMC, AML, SXT, GEN, CEF	0.50	50.0	MDR
351	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
353	IMI, OFL, AMC, AML, SXT, GEN, CFT, CEF, CHL, TET	0.83	83.3	MDR
355	OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF	0.66	66.6	MDR
360	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
363	OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, TET	0.75	75.0	MDR
367	OFL, AML, SXT, CFT, FOS, CEF, CHL	0.58	58.3	MDR
369	OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
370	IMI, OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL	0.83	83.3	MDR
373	AML, SXT, CFT, FOS, CEF, CHL, TET	0.58	58.3	MDR
375	IMI, AMC, NFT, AML, SXT, GEN, FOS, CEF, CHL, TET	0.83	83.3	MDR

Appendix VIII (continued): Phenotypic resistance profile of uropathogens from pregnant women attending antenatal clinics

Isolate code	Phenotypic resistance pattern	MARI	% Resistance	Resistance category
378	IMI, OFL, AMC, NFT, AML, SXT	0.50	50.0	MDR
381	IMI, OFL, AMC, AML, SXT, FOS, TET	0.58	58.3	MDR
384	OFL, AMC, NFT, AML, CFT, FOS, CEF, CHL, TET	0.75	75.0	MDR
387	IMI, OFL, AML, SXT, CFT, FOS, CHL, TET	0.66	66.6	MDR
391	OFL, AMC, AML, SXT, GEN, CFT, CEF, CHL	0.66	66.6	MDR
392	IMI, OFL, AMC, AML, SXT, GEN, CFT, CEF, CHL, TET	0.83	83.3	MDR
394	SXT, CFT, CHL, TET	0.33	33.3	MDR
397	AML, SXT, CFT, CEF, CHL, TET	0.50	50.0	MDR
401	CHL	0.08	8.33	NMDR
403	AML, SXT, CFT, CEF	0.33	33.3	MDR
406	OFL	0.08	8.33	NMDR
437	OFL, AMC, AML, CHL, TET	0.41	41.6	MDR
439	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
440	SXT, FOS, CEF, CHL	0.33	33.3	MDR
443	IMI, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
446	AMC, AML, SXT, FOS, TET	0.41	41.6	MDR
448	IMI, OFL, AMC, AML, CFT, CEF	0.50	50.0	MDR
451	AML, CEF, CHL	0.25	25.0	MDR
452	AMC, AML, SXT, FOS, CHL, TET	0.50	50.0	MDR
455	AMC, AML, SXT, CHL	0.33	33.3	MDR
459	AMC, AML, SXT, CFT, CEF, CHL, TET	0.58	58.3	MDR
461	IMI, AMC, AML, SXT, CFT, FOS, CEF, TET	0.66	66.6	MDR
465	SXT, CFT, FOS	0.25	25.0	MDR
467	AMC, AML, SXT, CFT, FOS, CEF, CHL, TET	0.66	66.6	MDR
468	IMI, AMC, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
471	AMC, AML, SXT, CFT, FOS, CEF, CHL	0.58	58.3	MDR
473	OFL, AMC, AML, SXT, CHL, TET	0.50	50.0	MDR
476	AMC, AML, FOS, CHL	0.33	33.3	MDR
477	AMC, AML, SXT, CFT, CEF, CHL, TET	0.58	58.3	MDR
479	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
480	OFL, SXT, GEN, CFT, CEF, TET	0.50	50.0	MDR
483	SXT, GEN, CFT, CEF, CHL, TET	0.50	50.0	MDR
485	SXT, CFT, FOS, CEF, CHL	0.41	41.6	MDR
486	IMI, AMC, AML, SXT, CHL	0.41	41.6	MDR
489	AMC, AML, CFT, CEF, CHL, TET	0.50	50.0	MDR
491	CHL	0.08	8.33	NMDR
495	FOS	0.08	8.33	NMDR
498	TET	0.08	8.33	NMDR
500	AML, SXT, CFT, FOS, CEF	0.41	41.6	MDR

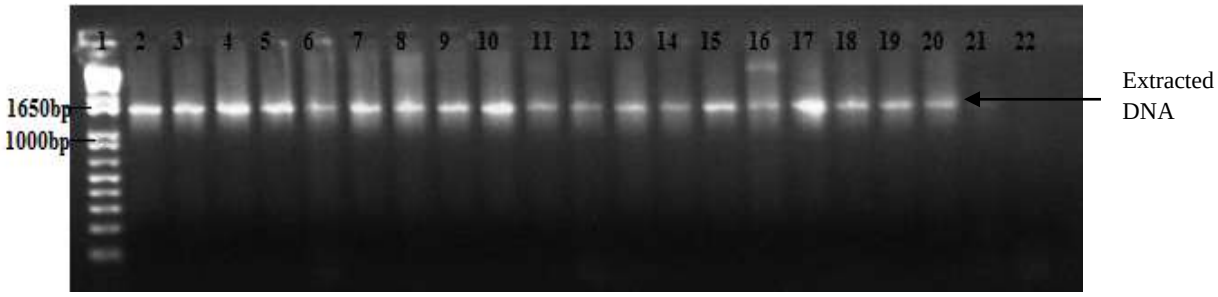
Appendix VIII (continued): Phenotypic resistance profile of uropathogens from pregnant

Isolate code	Phenotypic resistance pattern	MARI	% Resistance	Resistance category
507	OFL, AMC, NFT, AML, SXT, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
509	IMI, FOS, TET	0.25	25.0	MDR
511	OFL, GEN, FOS, CEF, CHL	0.41	41.6	MDR
513	IMI, AMC, AML, SXT, FOS, CEF, TET	0.58	58.3	MDR
516	FOS	0.08	8.33	NMDR
520	AML, SXT, FOS, CHL, TET	0.41	41.6	MDR
522	FOS	0.08	8.33	NMDR
524	AMC, AML, SXT, FOS	0.33	33.3	MDR
529	OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.91	91.6	MDR
541	AML, SXT, FOS	0.25	25.0	MDR
546	FOS	0.08	8.33	NMDR
550	AMC, AML, SXT, CFT, FOS, CEF	0.50	50.0	MDR
552	SXT, CEF	0.16	16.6	NMDR
557	AMC, AML, GEN	0.25	25.0	MDR
561	AMC, AML, SXT, FOS, CHL, TET	0.50	50.0	MDR
563	AML, SXT, FOS	0.25	25.0	MDR
565	NFT, AML, SXT, FOS	0.33	33.3	MDR
566	FOS	0.08	8.33	NMDR
567	AML, SXT, FOS	0.25	25.0	MDR
569	AML, SXT, GEN, CFT, CEF, CHL, TET	0.58	58.3	MDR
571	AMC, NFT, AML, SXT, CEF, CHL, TET	0.58	58.3	MDR
574	NFT, AML, SXT, GEN, CHL, TET	0.50	50.0	MDR
575	AML, SXT, CFT, CEF, CHL	0.41	41.6	MDR
578	AML, SXT, CHL, TET	0.33	33.3	MDR
582	SXT, GEN, CFT, CEF, CHL	0.41	41.6	MDR
584	AMC, AML, GEN, CHL, TET	0.41	41.6	MDR
587	OFL, AMC, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	0.83	83.3	MDR
590	AML, GEN, CFT, CEF	0.33	33.3	MDR
591	SXT, FOS	0.16	16.6	NMDR
593	AML	0.08	8.33	NMDR
596	CEF, TET	0.16	16.6	NMDR
599	AMC, AML, SXT, CFT, CEF, CHL, TET	0.58	58.3	MDR
600	SXT, FOS	0.16	16.6	NMDR
602	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
604	AMC, AML, SXT, GEN, CFT, CEF, CHL	0.58	58.3	MDR
606	AMC, SXT, CEF	0.25	25.0	MDR
608	AMC, NFT, AML, SXT, FOS	0.41	41.6	MDR
610	AML, SXT, FOS	0.25	25.0	MDR
612	AML, SXT, CFT, CEF	0.33	33.3	MDR
614	GEN, CFT	0.16	16.6	NMDR

Appendix VIII (continued): Phenotypic resistance profile of uropathogens from pregnant women attending antenatal clinic

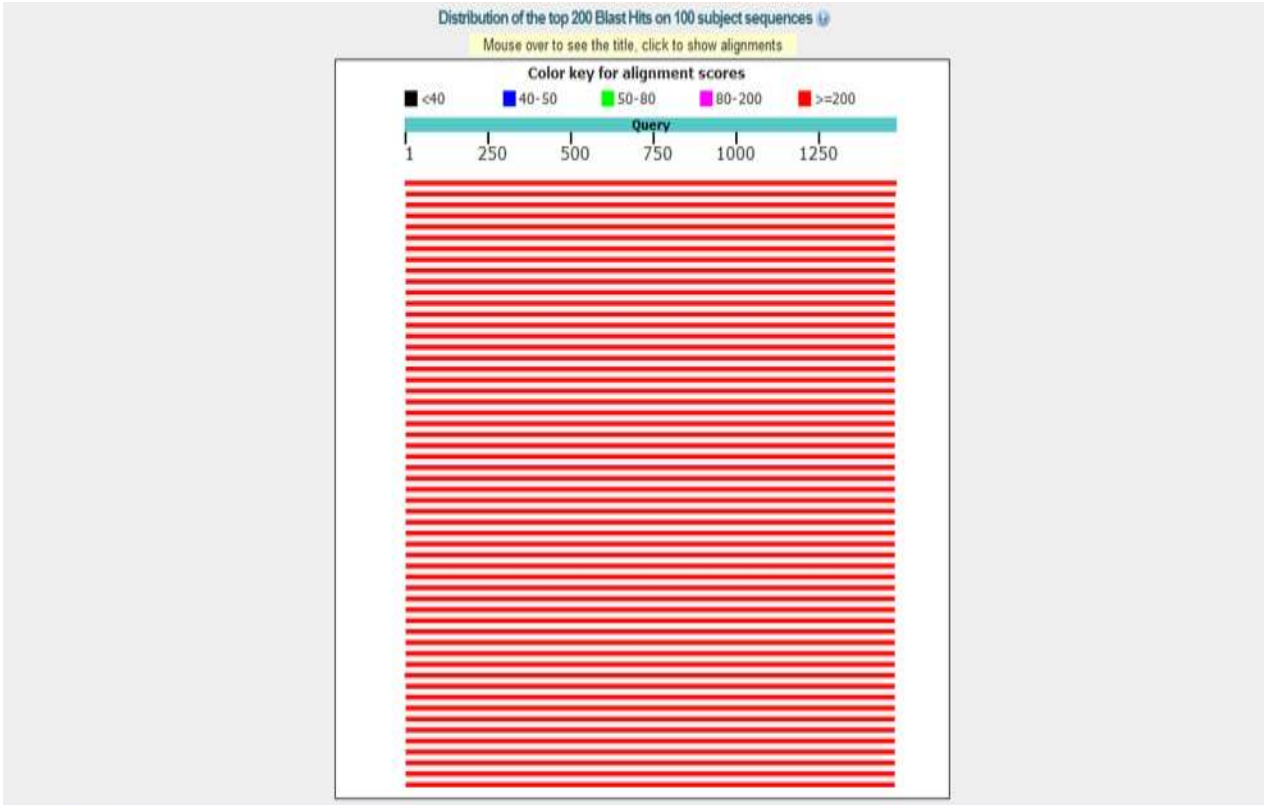
	Phenotypic Resistance Pattern	MARI	% Resistance	Resistance category
616	CFT	0.08	8.33	NMDR
621	AMC, NFT, AML, SXT, CFT, CEF, CHL	0.58	58.3	MDR
623	NFT, AML, SXT, FOS	0.33	33.3	MDR
625	NFT, CFT, FOS, CEF, CHL	0.41	41.6	MDR
628	AMC, AML, CEF	0.25	25.0	MDR
630	AMC, NFT, AML	0.25	25.0	MDR
632	AML, CEF	0.16	16.6	NMDR
634	AML, SXT, CFT, CEF, TET	0.41	41.6	MDR
635	AML, SXT, CFT, FOS, CHL, TET	0.50	50.0	MDR
637	AML, GEN, CFT, CEF, CHL	0.41	41.6	MDR
639	CFT, FOS, CEF, TET	0.33	33.3	MDR
641	IMI, AMC, AML, SXT, FOS	0.41	41.6	MDR
644	AMC, AML, SXT, FOS	0.33	33.3	MDR
647	IMI, OFL, AMC, NFT, AML, SXT, GEN, CFT, FOS, CEF, CHL, TET	1.00	100.0	MDR
648	AMC, AML, SXT, FOS, CHL, TET	0.50	50.0	MDR
649	AML, SXT, FOS	0.25	25.0	MDR

APPENDIX IX: Plate showing extracted genomic DNA on electrophoretic gel



Lane 1= 1kb ladder
Lane 2=*E. coli* 5E
Lane 3=*E. coli* 7E
Lane 4= *E. coli* 19E
Lane 5=*E. coli* 30E
Lane 6=*E. coli* 33E
Lane 7= *E. coli* 41E
Lane 8=*E. coli* 47E
Lane 9=*E.coli* 42E
Lane 10=*E.coli* 48E
Lane 11=*E.coli* 49E
Lane 12= *S. aureus* S6
Lane 13= *S. aureus* S7
Lane 14= *S. aureus* S12
Lane 15=*S. aureus* S13
Lane 16=*S. aureus* S15
Lane 17= *S. aureus* S16
Lane 18= *S. aureus* S22
Lane 19=*S. aureus* S28
Lane 20=*S. aureus* S40
Lane 21= *S. aureus* S42

APPENDIX X: NCBI Blasting showing identity level of MDR *S. aureus* from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



Descriptions

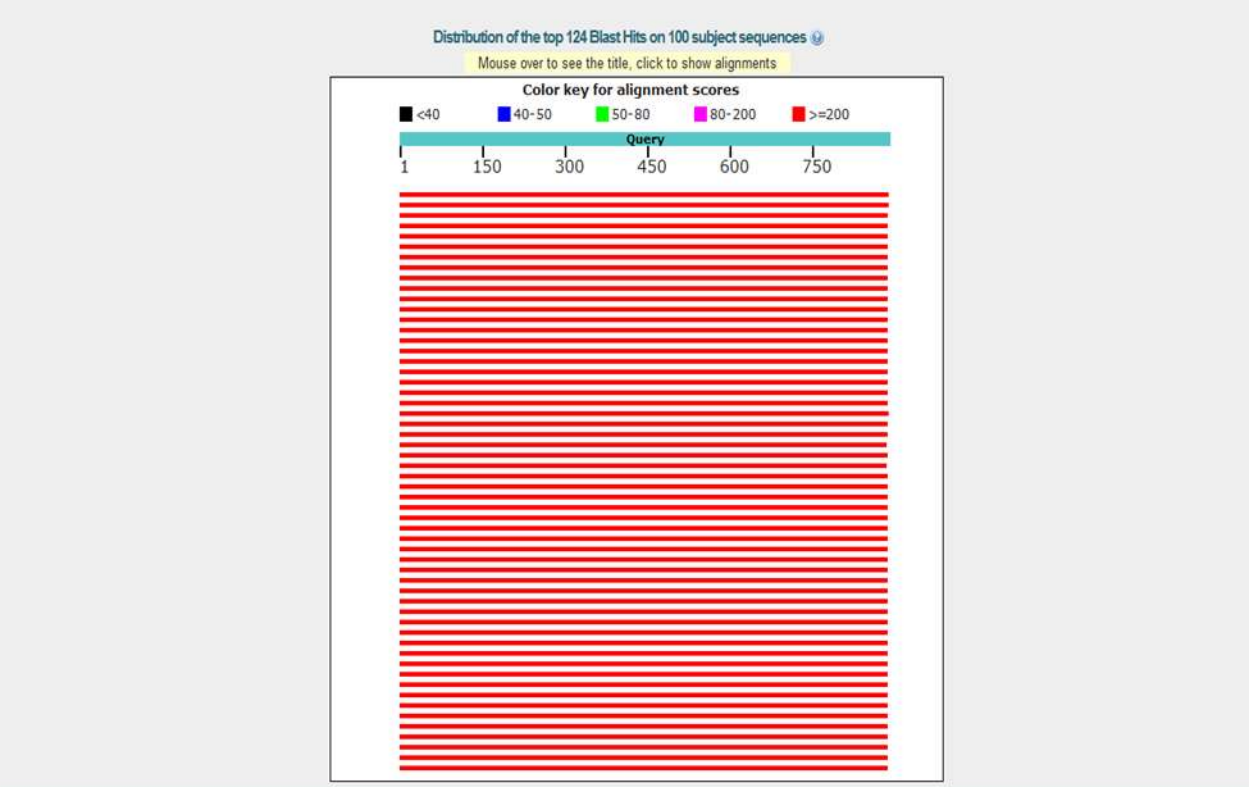
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus aureus strain ANT 05 16S ribosomal RNA gene, partial sequence	2736	2736	100%	0.0	100%	HM803171.1
Staphylococcus aureus strain FQXIII 16S ribosomal RNA gene, partial sequence	2695	2695	99%	0.0	99%	MF144454.1
Staphylococcus aureus strain MKV2011 16S ribosomal RNA gene, partial sequence	2695	2695	99%	0.0	99%	HQ658162.1
Staphylococcus aureus O11 chromosome, complete genome	2689	10731	99%	0.0	99%	CP024649.1
Staphylococcus aureus strain CFSAN007851 chromosome, complete genome	2689	16087	99%	0.0	99%	CP017680.1

APPENDIX XI: NCBI Blasting of MDR *Staphylococcus equorium* from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



Descriptions

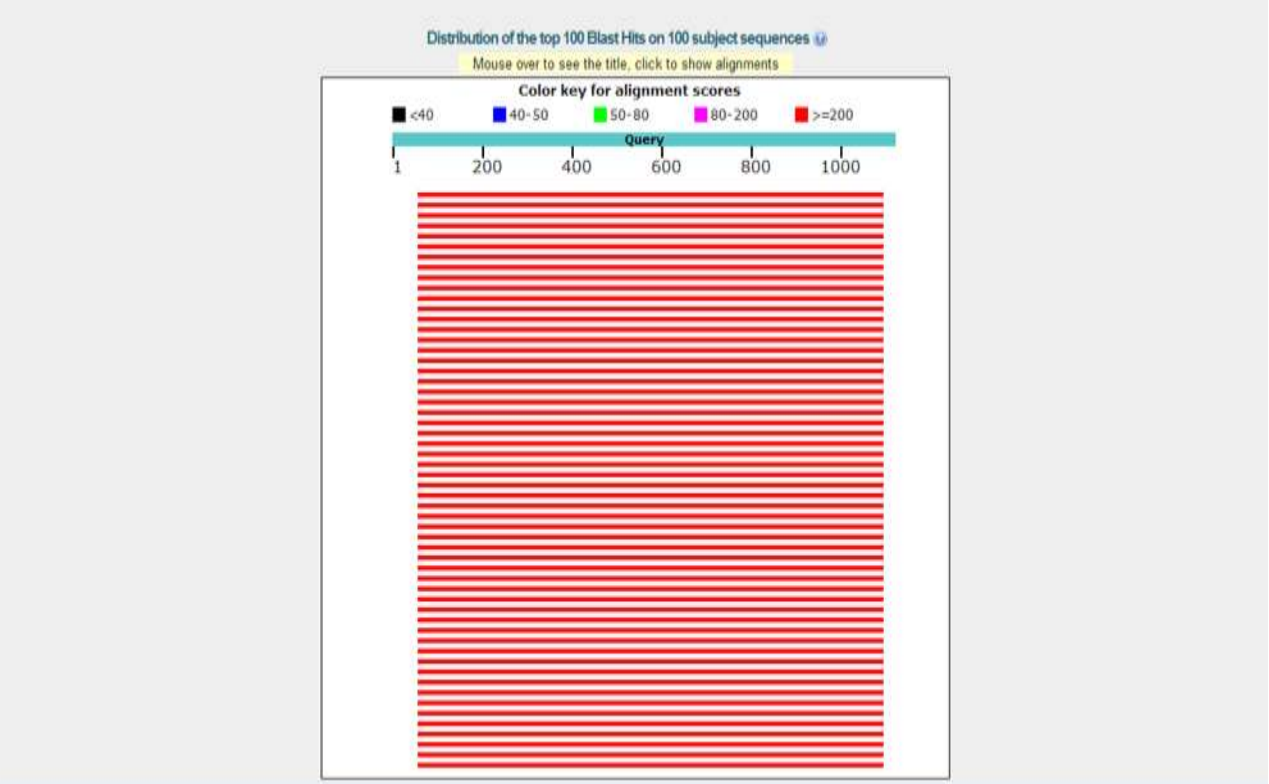
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus equorum strain B2S7 16S ribosomal RNA gene, partial sequence	1240	1240	99%	0.0	89%	EU221367.1
Staphylococcus equorum strain B2S6 16S ribosomal RNA gene, partial sequence	1240	1240	99%	0.0	89%	EU221366.1
Uncultured bacterium clone Wall_B_2 16S ribosomal RNA gene, partial sequence	1238	1238	99%	0.0	89%	MF092670.1
Uncultured bacterium clone Wall_A_81 16S ribosomal RNA gene, partial sequence	1238	1238	99%	0.0	89%	MF092626.1

APPENDIX XII: NCBI Blasting of MDR *Staphylococcus lentus* from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



escriptions

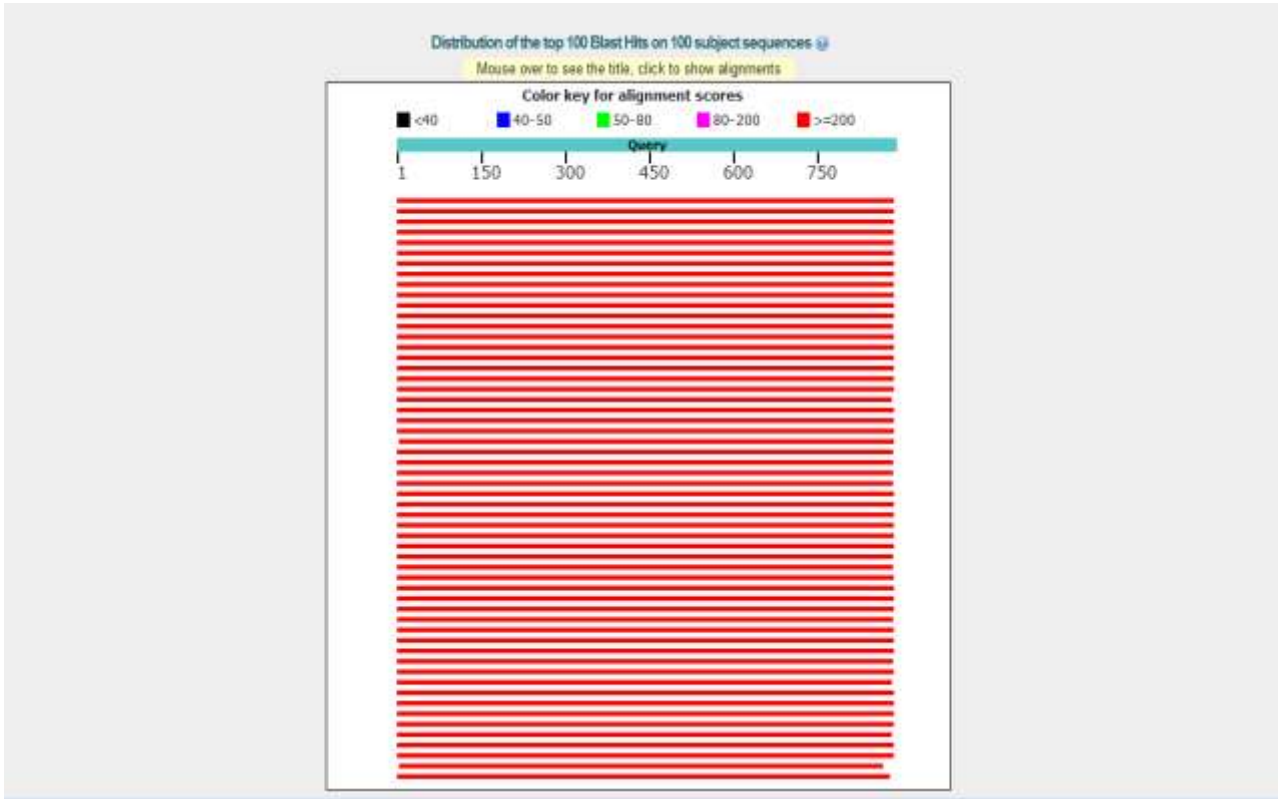
Sequences producing significant alignments:

Select: All None Selected 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus sp. b10 partial 16S rRNA gene, isolate b10	1740	1740	92%	0.0	97%	LK985359.1
Staphylococcus lentus strain 4v 16S ribosomal RNA gene, partial sequence	1738	1738	92%	0.0	97%	KX950710.1
Staphylococcus sp. strain 82584 16S ribosomal RNA gene, partial sequence	1738	1738	92%	0.0	97%	KX525724.1
Staphylococcus lentus strain Y8 16S ribosomal RNA gene, partial sequence	1738	1738	92%	0.0	97%	KU740177.1
Staphylococcus lentus strain SZ1 16S ribosomal RNA gene, partial sequence	1738	1738	92%	0.0	97%	KT947109.1

APPENDIX XIII: NCBI Blasting of MDR *Staphylococcus epidermidis* from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



escriptions

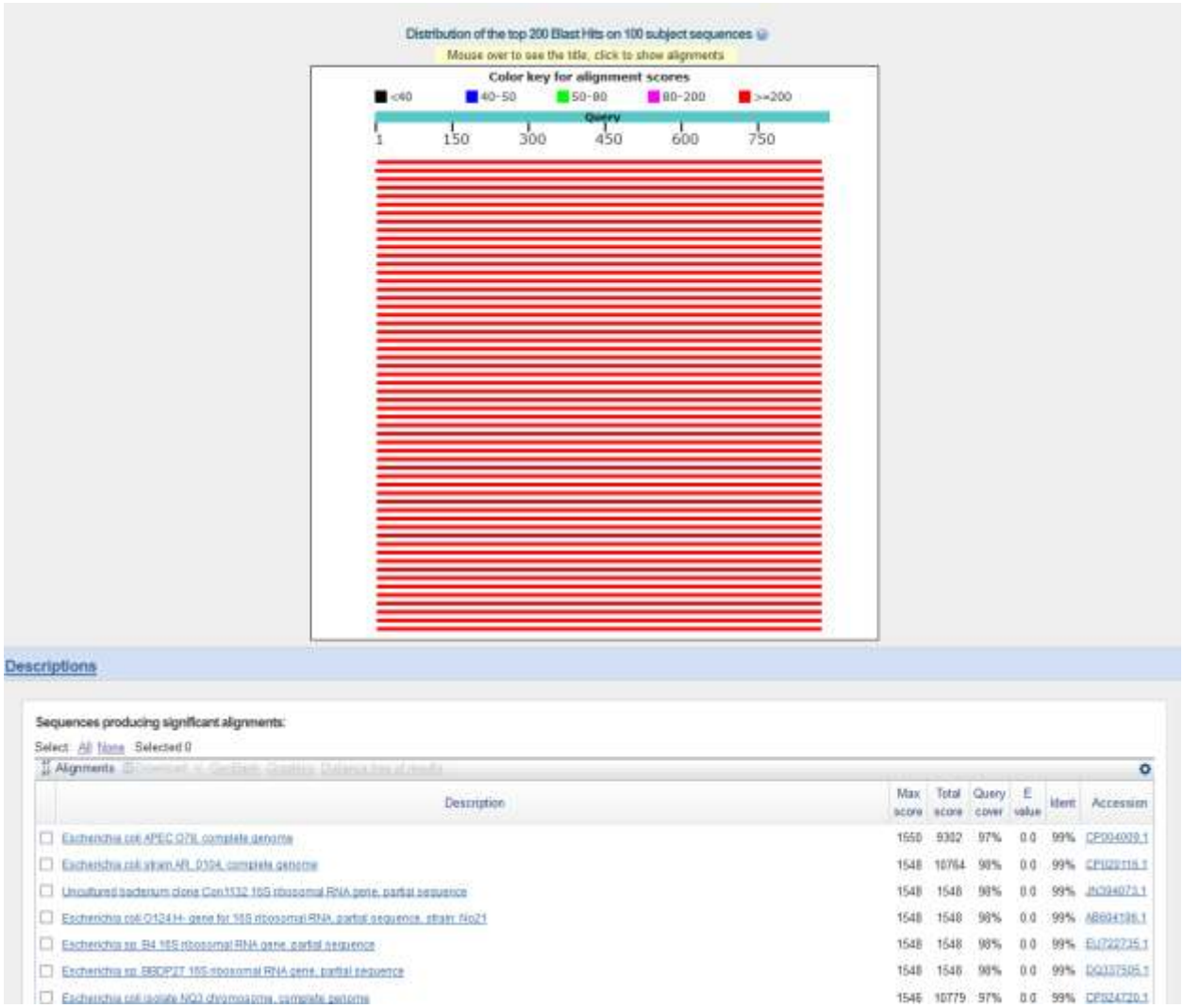
Sequences producing significant alignments:

Select: All None Selected 0

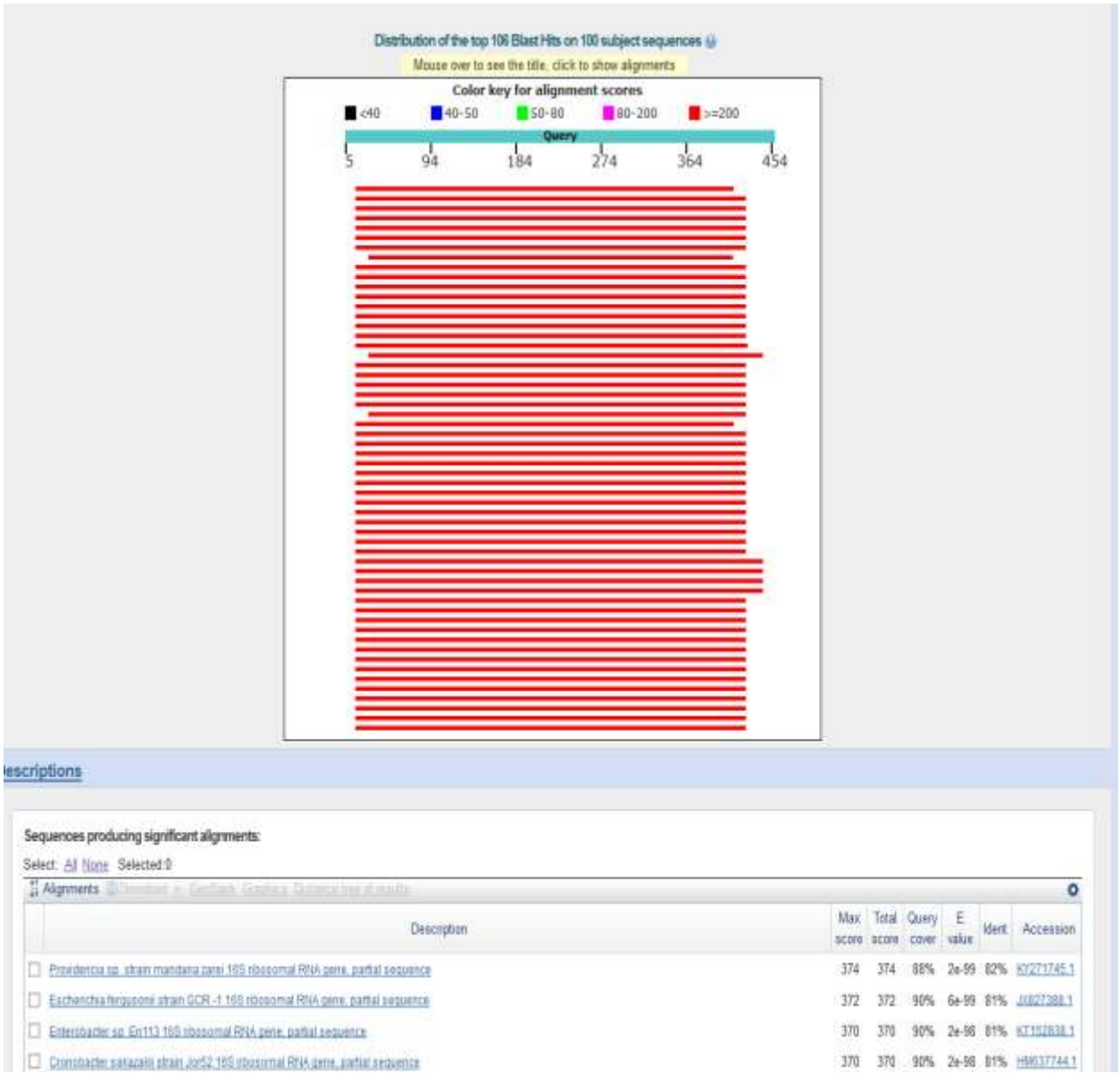
Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus sp. SC1 16S ribosomal RNA gene, partial sequence	1600	1600	99%	0.0	99%	KC951997.1
Uncultured bacterium partial 16S rRNA gene, clone PIST-ADA11	1600	1600	99%	0.0	99%	MW882532.2
Uncultured bacterium clone aab37a07 16S ribosomal RNA gene, partial sequence	1594	1594	99%	0.0	99%	DQ816712.1
Uncultured bacterium clone aaa84g01 16S ribosomal RNA gene, partial sequence	1594	1594	99%	0.0	99%	DQ816205.1
Uncultured bacterium clone aaa53f09 16S ribosomal RNA gene, partial sequence	1594	1594	99%	0.0	99%	DQ816140.1

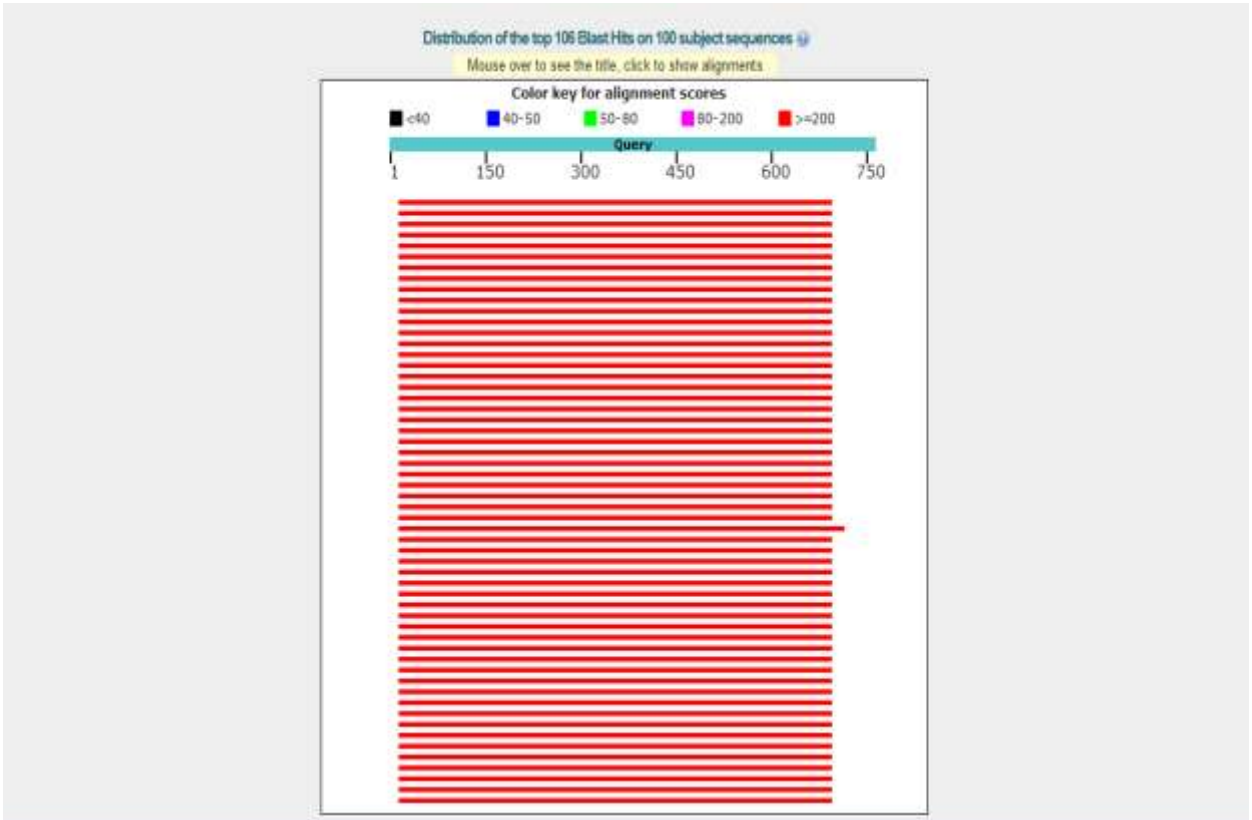
APPENDIX XIV: NCBI Blasting of MDR *ESBL* producing *E. coli* isolates from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



APPENDIX XV: NCBI Blasting of MDR *ESBL* producing *Providencia* isolates from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



APPENDIX XVI: NCBI Blasting of MDR *ESBL* producing *Acinetobacter* isolates from pregnant women attending antenatal clinics in Ibadan metropolis, Nigeria.



escriptions

Sequences producing significant alignments:

Select: All None Selected 0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Acinetobacter sp. strain 18_H2_V4 16S ribosomal RNA gene, partial sequence	946	946	89%	0.0	89%	MF872586.1
Acinetobacter guillouiae strain AAU PG4 16S ribosomal RNA gene, partial sequence	946	946	89%	0.0	89%	KX258087.1
Acinetobacter lwoffii strain L4 16S ribosomal RNA gene, partial sequence	946	946	89%	0.0	89%	KT767825.1
Acinetobacter guillouiae strain C43 16S ribosomal RNA gene, partial sequence	946	946	89%	0.0	89%	KT767748.1

List of papers presented, published, accepted or in press from this research thesis

1. **Paper Published**

   Durowaiye M.T., Onaolapo J,A., Oyi A.R. (2019). Diversity and antibiotic resistance pattern of bacteria associated with urinart tract infection in pregnant women in Ibadan metropolis South-west Nigeria**.***Nigerian Journal of Pharmaceutical Sciences.*Vol. 18, No 2, pp 8-15.

2. **Paper presented**

   Authors: Mojirayo T. Durowaiye, Josiah A. Onaolapo, Rukayat A. Oyi, Iruka N. Okeke

   Paper Title: Diversity and antibiotic resistance pattern of uropathogens from pregnant women in Ibadan metropolis Southwestern Nigeria.

   Presentation type: Poster presentation at the 2nd UNESCO MERCK Africa Research Summit, Addis-Ababa, Ethiopia, 28th and 29th November, 2016.

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DIVERSITY AND ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ASSOCIATED WITH URINARY TRACT INFECTION IN PREGNANT WOMEN IN IBADAN METROPOLIS SOUTH-WEST NIGERIA

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ABSTRACT

Women are more predisposed to urinary tract infection (UTI) during pregnancy. The emergence of multidrug resistant uropathogens has led to adverse obstetric and neonatal outcomes. This study is aimed to determining the prevalence of bacterial infecting agents, establishing their antibiogram and identifying socio-economic factors that influence UTI in pregnancy. Clean catch mid-stream urine samples from 650 women on their first visit to antenatal clinics were analyzed. Demographic information was obtained using a structured questionnaire. Dip-stick leucocyte esterase test was performed and isolates were identified using standard biochemical tests and further confirmed with Microbact® identification kits (Oxoid). Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility profile and interpreted as specified by the Clinical and Laboratory Standard Institute. Significant bacteriuria was observed in 246(37.8%) antenatal patients. *Staphylococcus epidermidis* 51(21.9%) was the most prevalent uropathogen, followed by *Escherichia coli* 48(19.5%). In all, bacterial species recovered include *Staphylococcus spp 119(48.3%),Escherichia spp* 52(21.1%), *Streptococcus spp* 17(6.9%), *Proteus spp 17 (6.9%) Providencia spp 14(5.6%), Enterobacter spp 9(3.6%), Morganella morganii* 4(1.6%), *Klebsiella spp 4(1.6%), Pseudomonas aeruginosa* 3(1.2%), *Serratia spp* 3(1.2%), *Alcaligenes spp* 2(0.8%), *and Acinetobacter spp* 1(0.4%). The highest prevalence of bacteriuria with respect to gestational age, women's age group, occupational status and educational level were 131(53.2%) third trimester, 101(44.2%) 25-30 years, 87(35.3%) housewives, and women with no formal education 79(32.1%) respectively. They were statistically significant at $P<0.05$. A wide range of bacterial etiologic agents are implicated and majority of these uropathogens are resistant to commonly prescribed antibiotics that are safe in pregnancy. Risk factors for bacteriuria include gestational age, age group, occupational status and educational level.

Keywords: Antibiotic resistance, pregnant women, urinary tract infection, socio-economic factors

INTRODUCTION

Urinary tract infectons (UTIs) are one of the most common medical complications of pregnancy (Mittal and Wing, 2005). Urinary tract infection often characterized by significant bacteriuria (asymptomatic or symptomatic) is one of the commone 224 infectious diseases of women worldwide including developing countries (Onyango *et al*., 2018). 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). Pregnant women are predisposed to UTI due to the

physiologic and hormonal changes that occur during pregnancy (Imade *et al*., 2010). In developing countries, especially in low resource settings to prevent or reduce maternal and fetal morbidities, antibiotics are usually given empirically before the urine culture reports are available (Nithyalakshmi *et al*., 2014). Poor socioeconomic status is reported to be a major risk factor with indigent patients having a five-fold increased risk (Johnson and Wolf, 2013). The emergence of multidrug resistant uropathogens with virulent traits has led to adverse obstetric and neonatal outcomes (Ramos *et al*., 2012).

This prospective experimental study, based in southwestern Nigeria, aimed at determining the prevalence of bacterial infecting agents in UTI during pregnancy, establishing their antimicrobial susceptibility profiles, and identifying socio-economic factors that promote UTI in pregnancy.

MATERIALS AND METHODS

Ethical Clearance

Ethical clearance (AD/13/376) was obtained from the Oyo State Ministry of Health (Appendix I) and permissions from relevant authorities of the various health facilities.

Study Design

A prospective experimental study involving 650 pregnant women on their first visit to the antenatal clinic was carried out over a period of 9 months.

Sample size/Study population

This was determined using the Leslie Kisch's formular: n=Z2pq/d2

Where Z: normal deviate=1.96; P: proportion in population (50%) = 0.50; q=1-p=0.50; d =5% level of significance=0.05; n= 384 patients.

A total of 400 pregnant women attending antenatal clinics at these various health facilities were initially used in this study; dip stick leucocyte esterase test was carried out on an additional 250 pregnant women to confirm actual infection and not contamination with normal flora of the skin making a total of 650 pregnant women.

Study Sites

Three maternity health facilities in Ibadan metropolis: Adeoyo Maternity Teaching Hospital, Yemetu, Jericho Nursing Home, and Our Lady of Apostles Hospital, Oluyoro.

Specimen Collection and Analysis

Clean-catch mid-stream urine samples were randomly collected and processed adequately (Hooton *et al*., 2000) from volunteers who consented.

Dip-stick Leucocyte Esterase Test

Dip-stick leucocyte esterase test (Wilkins*et al.,* 1985) was done on the last 250 samples to confirm actual infection; comparison was made with the first batch of 450 samples on which the test was not done. Specificity and Sensitivity was determined.

Specimen Processing

Using sterile distilled water, 1:100 dilution of urine samples were made, and inoculated (spread plate) unto cystein lactose

electrolyte deficient (CLED) agar plates, which were allowed to dry, incubated aerobically at 37°C for 24 hours, and observed for formed colonies (Cheesbrough, 2002). Colonies formed were multiplied by the inoculum volume and dilution factor to obtain the bacterial count present in each undiluted urine sample. Bacterial counts of 10^5cfu/ml and above were considered as significant for bacteriuria (Cheesbrough, 2002).

Bacterial Identification

Few specks were picked from each formed bacterial colonies and then streaked on the surfaces of already prepared selective media i.e. Mac Conkey agar, Cetrimide agar, Mannitol salt agar, Eosin methylene blue agar, Urea agar. The streaked plates were incubated at 37 C for 24 hours. Characteristics and morphology of the resultant colonies were noted (Cowan, 1974; Collee and Miles, 1989). The species of the isolates were later confirmed using the commercial Microbact® identification kits: MB1130A Microbact™ GNB 12E, Microbact™ Staph 12S and MB0266A Microbact™ Oxidase Strips (Oxoid, UK).

Antibiotic Susceptibility Testing

The modified Kirby-Bauer disc diffusion (Bauer *et al.,* 1996) method was used to determine the antibiogram and interpreted as specified by the Clinical and Laboratory Standard Institute (CLSI, 2012). Discs (Oxoid, UK) of twelve antibiotics of different classes were used.

RESULTS

Significant bacteriuria was observed in 246(37.8%) antenatal patients as shown in Table I.

Table I: Prevalence of bacteriuria among pregnant women attending antenatal clinics

Total no of urine samples screened	Samples with significant growth	Samples with insignificant growth	% of bacteriuria prevalence
650	246	404	37.8

(Significant level of bacteriuria=10^5 cfu/ml)

Table 2 shows the comparison of Dipstick Leucocyte Esterase Test (LET) with culture for 250 urine samples (subset) which reflects actual infection and not just colonization by normal commensals. The test exhibited good sensitivity (92.6%), specificity (85.3%), positive predictive value 93.6%, and negative predictive value 83.1%.

As shown in Fig 1, *Staphylococcus epidermidis* 51(21.9%) was the most prevalent bactreium, followed by *Escherichia coli* 48(19.5%). Other bacterial species recovered include *Staphylococcus aureus* 27(10.9%), *Streptococcus spp* 17(6.0%), *Proteus mirabilis* 13(5.2%), *Providencia stuartii* 12(4.8%), *Staphyloccocus saprophyticus* 12(4.8%), *Enterobacter agglomerans* 8(3.2%), *Staphylococcus haemolyticus* 6(2.3%). *Morganella morganii* 4(1.6%), *Proteus vulgaris* 4(1.62%), *Escherichia fergusonii* 4(1.62%), *Staphylococcus capitis subsp.ureolyticus* 4(1.6%), *Staphylococcus simulans* 4(1.6%), *Klebsiella pneumoniae* 3(1.2%), *Pseudomonas aeruginosa* 3(1.2%), *Serratia marcescens* 3(1.2%), *and Staphylococcus lugdenensis* 3(1.2%),*Alcaligenes feacalis* 2(0.8%),*Staphylococcus lentus*

2(0.8%),*Staphylococcus aureus subsp. anaerobius* 1(0.4%), *Staphylococcus caprae* 1(0.4%),*Staphylococcus chromogenes* 1(0.4%), *Staphylococcus cohnii subsp. urealyticum* 1(0.4%), *Staphylococcus cohnii subsp. cohnii* 1(0.4%), *Staphylococcus intermidius* 1(0.4%), *Staphylococcus xylosus* 1(0.4%), *Staphylococcus equorium* 1(0.4%), *Enterobacter cloacae* 1(0.4%), *Klebsiella oxytoca* 1(0.4%), *Providencia alcalifaciens* 1(0.4%), *Providencia rettgeri* 1(0.4%), *Salmonella enterica subsp enterica*1(0.4%),*and Acinetobacter quilouiae* 1(0.4%).

Table II: Comparison of Dipstick Leucocyte Esterase test (LET) with Culture for 250 Urine Samples (subset)

Leucocyte Esterase Test	Culture Negative	Culture Positive	Total
Negative	64	13	77
Positive	11	162	173
Total	75	175	250

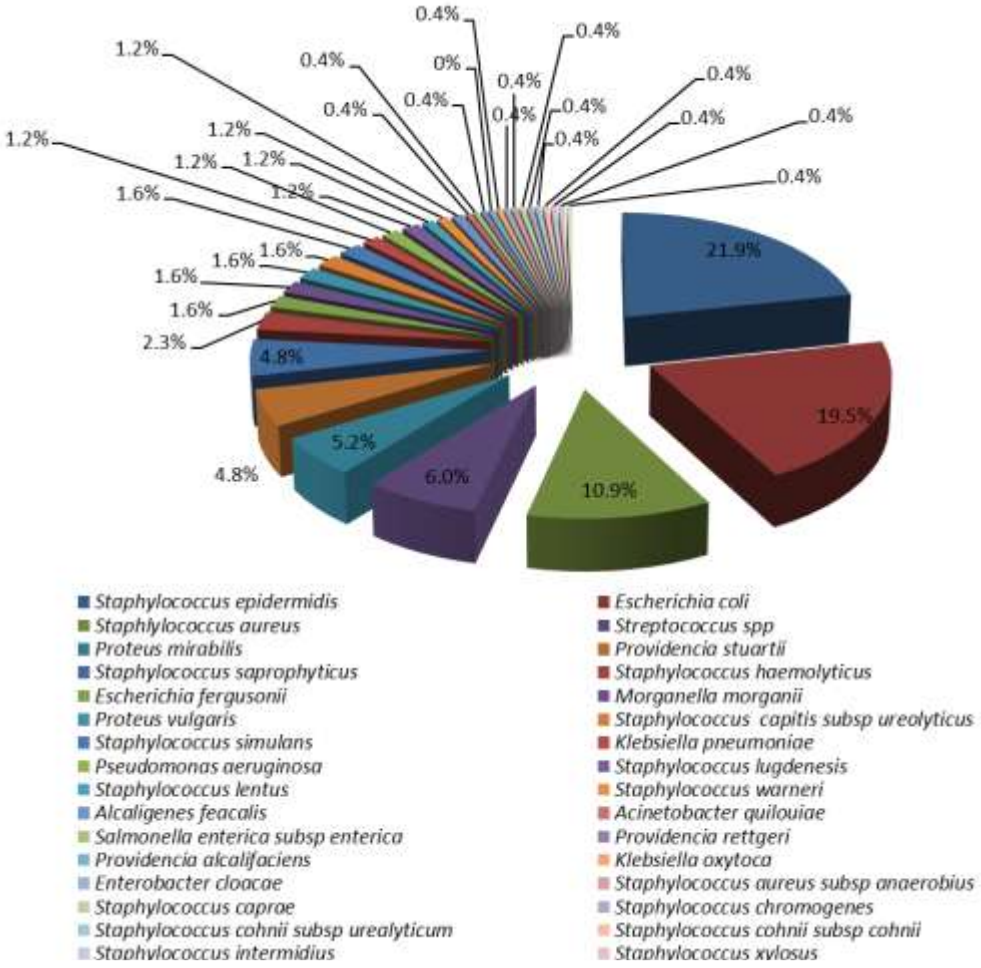


Figure I: Bacteria isolated from urine of antenatal patients in Ibadan metropolis

The highest prevalence of bacteriuria with respect to gestational age, women's age group, occupational status and educational level were 131(53.2%) third trimester, 101(44.2%) 25-30 years, 87(35.3%) housewives, and women with no formal education 79(32.1%) respectively and were statistically significant at P<0.05.



Figure II: Prevalence of bacteriuria with respect to gestational age

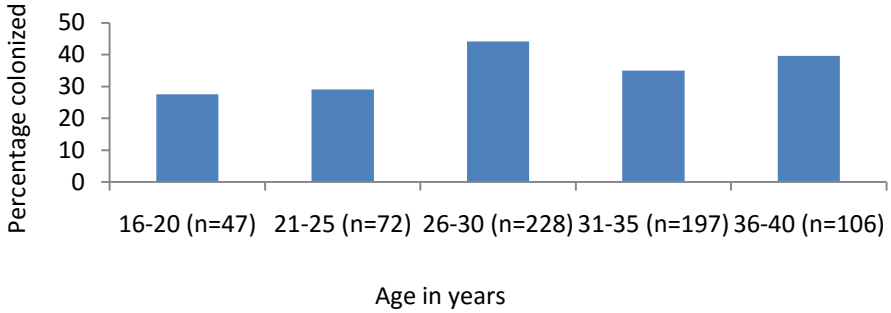


Figure III: Prevalence of bacteriuria with respect to age group

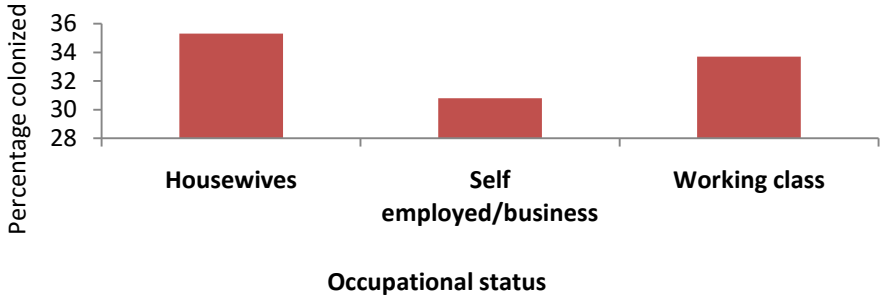


Figure IV: Prevalence of bacteriuria with respect to occupational status

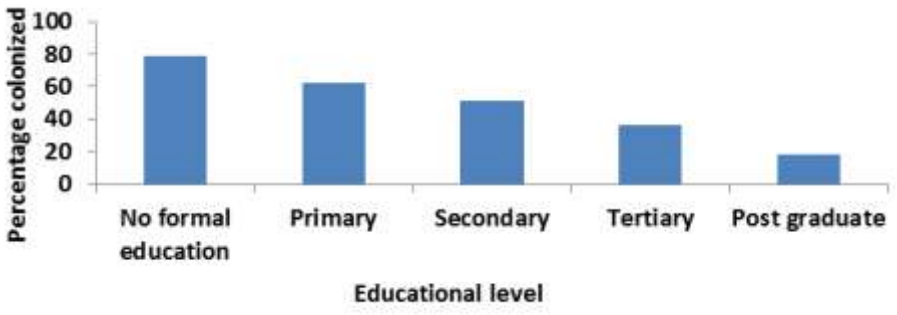


Figure V: Prevalence of Bacteriuria with Respect to Educational Status

Table III: Overall Antimicrobial Susceptibility Profile of Bacteria Isolated from Urine of Ante-natal Patients

Antibiotics (µg)	Gram positive	Percentage resistance	Gram negative	Percentage resistance
Imipenem (10)	22	10.4	27	12.9
Ofloxacin (5)	31	14.8	19	09.0
Amoxicillin/clavulanic acid (20/10)	48	22.9	44	20.9
Nitrofurantoin (300)	23	10.9	20	0.95
Amoxicillin (10)	77	36.7	69	32.9
Gentamicin (10)	21	10.0	25	11.9
Ceftriaxone (30)	47	22.4	53	25.2
Cefuroxime (30)	41	19.5	63	30.0
Tetracycline (30)	26	12.4	66	31.4
Chloramphenicol (30)	59	28.1	51	24.3
Cotrimoxazole(1.25/23.75)	62	29.5	78	37.1
Fosfomycin (200)	-	-	100	47.6

*Fosfomycin is used for testing and reporting *Escherichia coli* UTI isolates only

DISCUSSION

The significant bacteriuria was observed in this study 246(37.8%), is higher than 15.2 % observed in Kano, 28.8% in Ibadan, 23.9% in Sagamu and lower than 45.3% observed in Benin city (Durowaiye *et al*., 2015, Kehinde *et al*., 2011, and Imade *et al*., 2010). The higher prevalence may be attributed to the fact that majority of the women 87(35.3%) are housewives, and women with no formal education 79(32.1%). The findings from this study that *Staphylococcus epidermidis* now pathogenic from some studies was the most prevalent, followed by *E. coli*, a major faecal contaminant, is not at variance with the observations of several researchers where *Staphylococcus spp* followed by *E. coli* were the most prevalent uropathogens in antenatal patients even in other geographical areas, (Mukherjee *et al*., 2014; Mokube *et al*., 2013; Obirikorang *et al*.,2012; Imade *et al*., 2010; Perera *et al*., 2012).

The occurrence and apparent prevalence of these microorganisms in this special population, gives cause for concern as they are prone to several complications as well as foetal risks. *Staphylococcus epidermidis* a

normal skin flora may have been introduced to the urinary tract via improper cleansing after urinating and defecating. *Escherichia coli* is known to thrive well in the urinary stasis condition found in most pregnant women. Multiple antimicrobial resistance was common and several socio-economic factors are seen to influence bacteriuria in pregnancy.

Acknowledgement

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Diversity And Antibiotic Resistance Pattern Of Uropathogens From Pregnant Women In Ibadan Metropolis Southwestern Nigeria

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1. Introduction

Urinary tract infection (UTI) is the most frequent bacterial infection all around the world (Kudinha, *et al*., 2012). UTI often characterized by significant bacteriuria (asymptomatic or symptomatic) is one of the commonest infectious diseases of women worldwide including developing countries. Asymptomatic bacteriuria, occurring in 2–11% of pregnancies is a major predisposition associated with obstetric complications, such as preterm labour and low birth weight infants (Akobi *et al*., 2014). Pregnant women are predisposed to UTI due to the physiologic and hormonal changes that occur during pregnancy. The emergence of multidrug resistant uropathogens with virulent traits has led to adverse obstetric and neonatal outcomes (Ramos *et al*., 2012). This prospective experimental study, based in southwestern Nigeria aimed to determine the prevalence of bacterial infecting agents in UTI during pregnancy, to establish their antimicrobial susceptibility profiles, and to identify socio-economic factors that promote UTI.

2. Methods

- Clean catch mid-stream urine samples were randomly collected, processed and analyzed (Cheesbrough, 2002) from 650 women on their first visit to antenatal clinics of Adeoyo Maternity Teaching Hospital, Jericho Nursing Home and Our Lady of Apostles Hospital between January 2015 and January 2016. Demographic information was collected using a structured questionnaire.
- Dip-stick leucocyte esterase test (Wilkins *et al*., 1985) was done on the last 250 samples to confirm actual infection.
- Urinary isolates were identified using standard biochemical tests (Collee and Miles, 1989) and further confirmed with Microbact® identification kits (Oxoid)
- Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility profile and interpreted as specified by the clinical laboratory standard institute.

3. Results and Discussion

Table 1: Prevalence of bacteriuria among pregnant women attending antenatal clinics

Total no of urine samples screened	Samples with significant growth	Samples with insignificant growth	% of bacteriuria prevalence
650	246	404	37.8

Diversity of uropathogens (%)

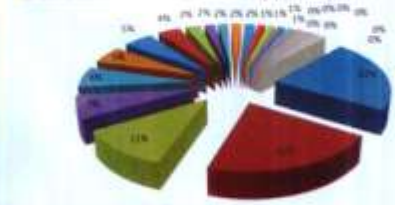


Figure 1: Uropathogens isolated from antenatal patients

Table 2: Prevalence of bacteriuria with respect to gestational age

Trimester	Number of patients	Number colonized	Percentage colonized (%)
First	177	46	25.9
Second	212	69	26.0
Third	261	131	53.0
Total	650	246	37.84

There is significant difference in percentage colonized with respect to gestational age using χ^2 at $P<0.05$

Table 3: Prevalence of bacteriuria with respect to women's age group

Age group (years)	Number of patients	Number colonized	Percentage colonized (%)
15-20	47	13	27.6
20-25	72	21	29.1
25-30	228	101	44.2
30-35	197	69	35.0
35-40	106	42	39.6
Total	650	246	37.8

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

Table 4: Prevalence of bacteriuria with respect to occupational status

Occupational status	Number of women	Percentage (%)
House wives	87	35.3
Self employed/business	76	30.8
Working class	83	33.7
Total	246	100.0

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

Table 5: Prevalence of bacteriuria with respect to educational level

Educational level	Number of women	Percentage (%)
No formal education	79	32.1
Primary	62	25.2
Secondary	51	20.7
Tertiary	36	14.6
Postgraduate	18	7.3
Total	246	100.0

There is significant difference in percentage colonized with respect to educational status using χ^2 at $P<0.05$

Table 6: Overall antimicrobial susceptibility profile of uropathogens from antenatal patients to commonly prescribed antibiotics

Antibiotics (µg)	Frequency	Percentage Sensitivity (%)	Percentage Resistance (%)
Imipenem (10)	49	76.7	23.3
Ofloxacin (5)	50	76.2	23.8
Amoxicillin/clavulanic acid (20/10)	92	56.2	43.8
Nitrofurantoin (300)	43	79.5	20.5
Amoxicillin (10)	146	30.5	69.5
Gentamicin (10)	46	78.1	21.9
Ceftriaxone (30)	100	52.4	47.6
Cefuroxime (30)	104	50.5	49.5
Tetracycline (30)	92	56.2	43.8
Chloramphenicol (30)	110	47.7	52.3
Cotrimoxazole(1.25/23.75)	140	33.4	66.6
Fosfomycin (200)	100	52.4	47.6

*Tetracycline is used for testing and reporting *Escherichia coli* (*E. coli*) isolates only

4. Conclusion

Bacteriuria (asymptomatic or symptomatic) in pregnancy is common in Ibadan, Nigeria. A wide range of bacterial etiologic agents are implicated and majority of these uropathogens are resistant to commonly prescribed antibiotics that are safe in pregnancy. Risk factors for bacteriuria include gestational age, age group, occupational status and educational level.

Ethical Approval

Ethical clearance was obtained from the Oyo State Ministry of Health and other relevant authorities.

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