KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

URINARY TRACT INFECTION BACTERIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN AMONG PREGNANT WOMEN ATTENDING ANTENANTAL CLINIC AT SELECTED HOSPITALS IN ILORIN

BY

RASHEEDAT, TOYOSI BABA

19/57MMB/00012

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M.Sc. THESIS SUBMITTED

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.SC) IN MICROBIOLOGY

DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY,
MICROBIOLOGY UNIT, FACULTY OF PURE AND APPLIED SCIENCES,
KWARA STATE UNIVERSITY, MALETE, NIGERIA.

APRIL, 2022.

DECLARATION PAGE

I hereby declare that this thesis titled (Urinary Tract Infection Bacterial Profile and Antibiotic Susceptibility Pattern among Pregnant Women attending ante natal clinic at selected Hospitals in Ilorin) is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

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NAME **SIGNATURE/DATE**

APPROVAL PAGE

This is to certify that this thesis by BABA RASHEEDAT TOYOSI has been read and approved as meeting the requirements of the Department of Microbiology for the award of the Degree of Masters (M.Sc) in Microbiology.

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DEDICATION

This work is dedicated to Almighty God who has been making my affairs easy and successful.

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ABSTRACT

Urinary tract infection in pregnancy is associated with significant morbidity for both the mother and the baby. The aim of this study was to determine the urinary bacterial profile and antibiotic susceptibility pattern among pregnant women attending Civil Service Clinic, Sobi Specialist Hospital and Okelele Primary Health Center in Ilorin, Nigeria. A total of 300 pregnant women with and without symptoms of urinary tract infection were enrolled as a study subject from July 2021 to October 2021. Organisms were identified from mid-stream clean catch urine samples and antibiotic susceptibility was performed using bacteriological standard tests. Molecular analysis was also carried out on some isolates for determination of *Mec*A gene. Out of 300 pregnant women, 49(16.3 %) were symptomatic and the rest 251(83.7 %) were asymptomatic. Bacteriological screening of urine samples revealed growth of bacteria in 28 (63.6 %) and 16 (36.4 %) for symptomatic and asymptomatic pregnant women respectively with overall prevalence of 14.5 %. The most common isolates detected were *Escherichia coli* (38.6 %) followed by Coagulase Negative *Staphylococcus*(CoNS) (22.7 %), *Klebsiella pneumoniae* (18.2 %), *Staphylococcus aureus* (13.6 %), *Enterococcus* sp (4.6 %) and *Pseudomonas* sp (2.3 %). Majority of Gram-negative isolates showed resistance rate of 19(73.1 %) to ampicillin and 17(65.4 %) to amoxicillin-clavulanic acid. Rates of resistance of Gram-negatives isolates against ceftriaxone, cefotaxime, cefuroxime range from 8(30.8 %) – 12(46.2 %). However, all Gram-negative bacteria isolates showed a relatively low level of resistance against nitrofurantoin 4(15.4 %) and ceftazidime 7(26.9 %). Both Gram positive and Gram negative bacteria showed high sensitivity against nitrofurantoin with a rate of 100 % and 69.2 % respectively. However, majority of Gram-positive isolates showed a high level of resistance to penicillin 16 (94.1 %). Forty- four (44 %) of CoNS isolated were cefoxitin resistance and all were *Mec*A gene positive. The isolation of bacterial pathogens both from symptomatic and asymptomatic pregnant women that are resistant to the commonly prescribed drugs in this study call for an early screening of all pregnant women to urinary tract infection.

CHAPTER ONE

1.0 INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection (Al Mijalli, 2017). In contrast to men, women are more susceptible to UTI, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Bale *et al*., 2021; Wong *et al*., 2015).

Urinary tract infection in pregnancy is associated with significant morbidity for both mother and baby. The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy (Mona *et al*., 2020).

In women UTIs account for about 25 % of all infections thus being one of the most frequent clinical bacterial infections (Balakrishnan *et al*., 2016). During pregnancy there occur many anatomical and hormonal changes in women which make them susceptible to develop UTI. Around 20 % of the pregnant women are reported to have UTI and it is the most common cause for admission to obstetric wards (Emiru *et al*., 2013). Its occurrence usually starts in week 6 and becomes most frequent during weeks 22–24 of pregnancy. There are many factors responsible like dilatation of urethra, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and uretero-vesical reflux (Derese *et al*., 2016). In addition, up to 70 % of women during pregnancy have glucose in urine, which increases the chances of bacterial growth in the urine (Koffi *et al*., 2016). Untreated UTI in pregnant women may have serious consequences like intrauterine growth restriction,

preeclampsia, caesarean delivery and preterm deliveries (Gopalakrishnan *et al*., 2017). Further untreated asymptomatic bacteriuria (ASB) is a significant risk of acute pyelonephritis in later pregnancy (Jayachandran *et al*., 2016).

Urinary tract infection is considered one of the most common medical complications during pregnancy (Gopalakrishnan *et al*., 2017). During pregnancy a number of risk factors of UTI are reported depending on the social, biological and geographical settings (Onuoha *et al*., 2014). The commonest cause of UTI among pregnant women has been found to be *Escherichia coli* because of its multidrug resistant strains Since ASB and obvious UTI has a close association, screening and treatment of pregnant women with ASB may also help to reduce adverse outcome for the child such as pre-term labour and low birth weight (Uddin *et al*., 2016). However, antibiotic resistance of urinary tract pathogens has been increasing worldwide, especially to the commonly used antimicrobials and pattern of antibiotic resistance in a wide variety of pathogenic organisms may vary over short periods and depend on site of isolation and different environmental conditions (Willy *et al*., 2015).

Urinary tract infection can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria (Gopalakrishnan *et al*., 2017). Asymptomatic bacteriuria (ASB) is a condition characterized by presence of bacteria in two consecutive clear-voided urine specimens both yielding positive cultures ($\geq 10^5$cfu/ml) of the same uropathogen, in a patient without classical symptoms (Uddin *et al*., 2016). *Escherichia coli* is the major etiologic agent causing UTI, which accounts for up to 90 % of cases. *Proteus mirabilis, Klebsiella* specie, *Pseudomonas aeruginosa* and *Enterobacte*r species are less frequent offenders (Kekelwa *et al*., 2021). Less commonly, *Enterococci*, *Gardnerella vaginalis* and *Ureaplasma urealyticum* are also known agents in UTIs.

Gram-positive organisms are even less common in which Group B *Streptococcus*, S*taphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* are recognized organisms (Jayachandran *et al*., 2016).

Current management of UTI is usually empirical, without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTI is increasing and is a major health problem in the treatment of UTI (Ifeanyichukwu *et al*., 2013).There is growing concern regarding antimicrobial resistance worldwide, particularly to *E. coli* which is the dominant causative agent of UTI in pregnant women (Kekelwa *et al*., 2021).

Urinary tract infections in pregnancy may lead to unfavorable pregnancy outcomes and complications like preterm delivery, low birth weight, pre-eclamptic toxaemia and anemia, so it must always be screened and treated timely (Ali *et al*., 2019).

In the female human subject, the urinary tract has an important relationship with the reproductive organs because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state the enlarging uterus affects all the tissues of the urinary tract at various times (Belete *et al*., 2020). Urinary Tract Infection (UTI) has become the most common hospital-acquired infection, accounting for as many as 35 % of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Rosana *et al*., 2016). Urinary tract infection accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria remained the most frequent cause of UTI, although the distribution of pathogens that cause UTI is changing (Yeva *et al*., 2020).Urinary tract infection (UTI) is the most common medical complications of pregnancy together with anaemia and hypertention and it occurs approximately in 5- 10 % of all pregnancies (Kekelwa *et*

al., 2020). It is also a common health problem among pregnant women (Ahmed *et al*., 2016). Proper investigation and prompt treatment are needed to prevent serious life threatening condition and morbidity due to urinary tract infection that can occur in pregnant women (Muhammed *et al*., 2016). Recent report in Addis Ababa, Ethiopia indicated the prevalence of UTI in pregnant women was 11.6 % and Gram negative bacteria was the predominant isolates and showed multi drug resistance (Rosana *et al*., 2016). In most developing countries including Nigeria, screening for UTI in pregnancy is not considered as an essential part of antenatal care. Therefore, this study was designed to determine the Urinary tract infection bacterial profile and antibiotic susceptibility pattern of uropathogen among pregnant women attending antenatal clinic at selected Hospital in Ilorin, kwara State.

1.1 Statement of Research Problem

Urinary Tract Infection (UTI) is one of the most common infectious diseases, with approximately 150 million diagnosed cases each year worldwide (Vazudevan, 2014). Pregnant women have 4 times higher rate of developing UTI compared to non-pregnant women (Muhammed *et al*., 2019).UTI is among the most commonly studied health problems in pregnancy ranging 3 % to 35 % prevalence worldwide in which increased prevalence is predominantly seen among developing countries especially Sub-Saharan Africa, Middle East, and Asia (Gilbert, 2013).

The high incidence and risk of developing UTI in the course of pregnancy are related to abnormal anatomical and physiological changes that occur during this period (Tolulope *et al*., 2015). Furthermore, previous history of UTI, increased age, multiparity, sexual activity, history of catheterization, immunodeficiency and lower socioeconomic status are identified as factors likely to increase risk of UTI during pregnancy (Tadesse *et al*., 2017).

Bacteria are the most common agents causing UTI including *Escherichia coli, Proteus mirabilis, Klebsiella* species, *Pseudomonas aeruginosa, Enterobacte*r species, enterococci, *Citrobacter, Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus* species and others (Banda *et al*., 2020). Gram-negative bacteria are the major isolates causing UTI in which the predominant isolates are *Escherichia coli* accounting majority (80 – 90 %) of infection (Elzayat *et al*., 2017).

UTI in pregnancy may be symptomatic or asymptomatic bacteriuria (ASB) which occurs in 2–11 % of pregnancies worldwide and is a major predisposition to the development of acute pyelonephritis in 20–50 % of untreated ASB cases (Celen *et al*., 2012). Furthermore, untreated UTI in pregnancy (symptomatic or asymptomatic) is associated with a 50 % increase in the risk of maternal complications of pregnancy which raise extent of preterm labor, prematurity and low birth weight resulting in high prenatal morbidity and mortality (Chisanga *et al*., 2017). Early diagnosis and clinical management reduce the incidence of these complications. Nevertheless, in developing countries including Nigeria, urine culture screening is not routinely done as part of antenatal care and treatment is empirical which may lead to emergence and spread of antimicrobial-resistant strains which is a leading cause of treatment failure in UTI.

1.2 Justification of the Study

Urinary tract infection is a common health problem among pregnant women (Olufadi *et al*., 2019). This usually begins in week 6 and peaks during weeks 22 to 24 of pregnancy due to a number of factors including urethral dilatation, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux and up to 70 % of pregnant women develop glycosuria, which encourages bacterial growth in the urine (Sekikubo *et al*., 2017). UTI may manifest as asymptomatic

bacteriuria (ASB) or symptomatic bacteriuria (SB). The prevalence of asymptomatic UTI has been previously reported to be 2 % to 13 % in pregnant women compared with that of symptomatic UTI which accounts 1–18 % during pregnancy (Ekwedigne *et al*., 2018).). The highest incidence of urinary tract infection occurs in the child bearing age and this has been linked to sexual activity and aging (Ahmed *et al*., 2016). The predominant organisms that cause UTIs during pregnancy are *Escherichia coli* which account for 80 %–90 % of infection (Banda *et al*., 2020). Treatment of asymptomatic bacteriuria offers no benefit for most healthy elderly or adult non pregnant women (Ekwedigne *et al*., 2018). However, screening and treatment of asymptomatic bacteriuria should be performed in pregnant women (Jain *et al*., 2013). Treatment of UTI in pregnancy is of paramount importance for mother and child. Given the close association between ASB and obvious UTI, screening and treatment of ASB in pregnancy may also help to reduce adverse outcome for the child such as pre-term labour and low birth weight. However, Antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials (Muhammed *et al*., 2019) and pattern of antibiotic resistance in a wide variety of pathogenic organisms may vary over short periods and depend on site of isolation and different environmental conditions (Muhammed *et al*., 2019). In many hospitals in developing countries including Nigeria, routine urine culture test is not carried out for antenatal patients instead many clinicians choose the strip urinalysis method for assessing urine in pregnant women (Sunday *et al*., 2018). The true picture of such urine specimen cannot be fully assessed as the strip cannot quantify the extent of infection in such a patient as well as provide antimicrobial therapy which is usually seen in the case of culture test. Antibiotics are usually given empirically before the laboratory results of urine culture are available (Sunday *et al*., 2018). To ensure appropriate therapy, current knowledge of the organism that causes UTIs

and their antibiotic susceptibility pattern is mandatory. This study is therefore aimed to determine the Urinary Bacterial Profile and Antibiotics Susceptibility pattern among pregnant women attending antenatal clinic in three different Hospitals in Ilorin.

l. 3 Aim of the study

The aim of the study was to determine the Urinary Tract Infection Bacterial Profile and Antibiotics Susceptibility Pattern among pregnant women attending antenatal clinic at Civil Service Clinic, Sobi Specialist Hospital and Okelele Primary Health Center. Ilorin.

1.4 Objectives of the study

The objectives of this study were to

i. Isolate urinary bacterial pathogens present in pregnant women's urine (Both symptomatic and asymptomatic pregnant women)
ii. Characterize and identify significant cultured organisms in the urine
iii. Determine the prevalence organisms causing urinary tract infection in pregnancy.
iv. Determine the antibiotics susceptibility patterns of the organisms isolated
v. Determine the resistant genes in significant isolates

CHAPTER TWO

2.0 LITERATURE REVIEW

A urinary tract infection (UTI) is an infection from microbes. These are organisms that are too small to be seen without a microscope (Judith et al., 2018). Most UTIs are caused by bacteria, but some are caused by fungi and in rare cases by viruses. UTIs are among the most common infections in humans (Edae *et al*., 2020). A UTI can happen anywhere in the urinary tract (Chisanga *et al*., 2017).The urinary tract is made up of the kidneys, ureters, bladder, and urethra. Most UTIs only involve the urethra and bladder, in the lower tract. However, UTIs can involve the ureters and kidneys, in the upper tract. Although upper tract UTIs are rarer than lower tract UTIs, they're also usually more severe (Chisanga *et al*., 2017).

In women UTIs account for about 25 % of all infections thus being one of the most frequent clinical bacterial infections (Derbie *et al*., 2017). During pregnancy there occur many anatomical and hormonal changes in women which make them susceptible to develop UTI (Ali *et al*., 2019). Around 20 % of the pregnant women are reported to have UTI and it is the most common cause for admission to obstetric wards (Belete *et al*., 2020). Its occurrence usually starts in week 6 and becomes most frequent during weeks 22–24 of pregnancy. There are many factors responsible like dilatation of urethra, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and uretero-vesical reflux (Foxman *et al*., 2014) In addition, up to 70% of women during pregnancy have glucose in urine, which increases the chances of bacterial growth in the urine (Demilie *et al*., 2012). Untreated UTI in pregnant women may have serious consequences like intrauterine growth restriction, preeclampsia, caesarean delivery and preterm deliveries (Bale *et al*., 2021). Further untreated asymptomatic bacteriuria (ASB) is a significant risk of acute pyelonephritis in later pregnancy

(Flores-Meireles *et al*., 2015). Urinary tract infection is considered one of the most common medical complications during pregnancy. During pregnancy a number of risk factors of UTI are reported depending on the social, biological and geographical settings (Derese *et al*., 2016). The commonest cause of UTI among pregnant women has been found to be *E. coli* because of its multidrug resistant strains (Al- Mijalli, 2017) Since ASB and obvious UTI has a close association, screening and treatment of pregnant women with ASB may also help to reduce adverse outcome for the child such as preterm labour and low birth weight (Muhammed *et al*., 2016). However, antibiotic resistance of urinary tract pathogens has been increasing worldwide, especially to the commonly used antimicrobials and pattern of antibiotic resistance in a wide variety of pathogenic organisms may vary over short periods and depend on site of isolation and different environmental conditions (Al-Naqshbandi *et al*., 2019).

2.1 Physiology of the Urinary System

Urine is formed by nephrons present inside the kidneys. The production of urine is the body's way of eliminating excess water, waste products and salt. After its formation in the nephrons, the urine flows through several structures in the kidney. From the kidney, the urine flows into the ureters downward into the bladder via peristaltic movement (Koffi *et al*., 2020). Contraction of the bladder through micturition (also known as urination), causes the lower end of the ureter to constrict to prevent reflux of the urine upwards. The bladder holds the urine until urination occurs which empties the urine through the urethra (Koffi *et al*., 2020). In a female, this lies above the vaginal opening. In a male, the urethra opening lies at the end of the penis. The internal urethral sphincter is at the intersection of the urethra and bladder. The external urethral sphincter is at the base of the urethra and the conscious nervous system directs its control. The bladder and internal urethra sphincter are innervated by the autonomic nervous system (Huether

and McCance, 2019). When the bladder contracts and empties urine, the sacral levels (S2-S4) of the spinal cord are at work through the parasympathetic fibers of the autonomic nervous system. When the bladder needs to retain urine, the urethral sphincter is excited by parts of the thoracic and lumbar spinal cord (T11-L2) through sympathetic fibers. An individual sense that the bladder is full by stretch receptors in bladder tissue and sends an impulse to the sacral part of the spinal cord. After the accumulation of roughly 300 mL of urine, the bladder contracts and the internal urethral sphincter muscle relaxes, and an individual feels the need to void (Huether and McCance, 2019).

In normal healthy individuals, there are several mechanisms that attempt to prevent bacteria from invading the bladder or progressing up through the upper urinary tracts. These mechanisms usually work together to prevent infection and they include:

- The process of urinating washes most bacteria out of the urethra
- In females: Mucus secreting cells in the urethra help trap bacteria so it can't move upward
- In males: the length of the urethra and the prostate and associated glands create secretions to shield bacteria from invading
- Several factors work to create a bactericidal effect: high osmolality and low PH of the urea, uromodulin presence (a protein synthesized in the kidneys), and the epithelial cells of the urinary tract.
- When the bladder contracts, the ureterovesical junction (functional one-way valve where the ureters lead into the bladder) closes, thus preventing urine from ascending upwards into the upper urinary tract
- In the distal urethra, the urethral sphincter prevents the upward movement of bacteria

If bacteria were to successfully invade, the immune system recruits toll-like receptors (TLR4) which recognize the pathogen and further recruits neutrophils and macrophages to induce phagocytosis. The ability of the pathogen to produce infection is influenced by the virulence of the specific pathogen and individual's specific immune response. If the immune system does not respond quick enough, the pathogen may be able to excessively multiply and inundate the individual's defense mechanism, causing a UTI (Huether and McCance, 2019).

2.2 Pathogenesis of urinary tract infection

During pregnancy, urinary tract changes predispose women to infection (Bradly *et al*., 2019). Ureteral dilation is seen due to compression of the ureters from the gravid uterus. Hormonal effects of progesterone also may cause smooth muscle relaxation leading to dilation and urinary stasis, and vesicoureteral reflux increases. The organisms which cause UTI in pregnancy are the same uropathogens seen in non-pregnant individuals (Kibret *et al*., 2014). As in non-pregnant patients, these uropathogens have proteins found on the cell-surface which enhance bacterial adhesion leading to increased virulence. Urinary catheterization, frequently performed during labor, may introduce bacteria leading to UTI. In the postpartum period, changes in bladder sensitivity and bladder over distention may predispose to UTI (Marami *et al*., 2019).

Pregnancy is a state of relative immunocompromise. This immunocompromise may be another cause for the increased frequency of UTIs seen in pregnancy (Marami *et al*., 2019).

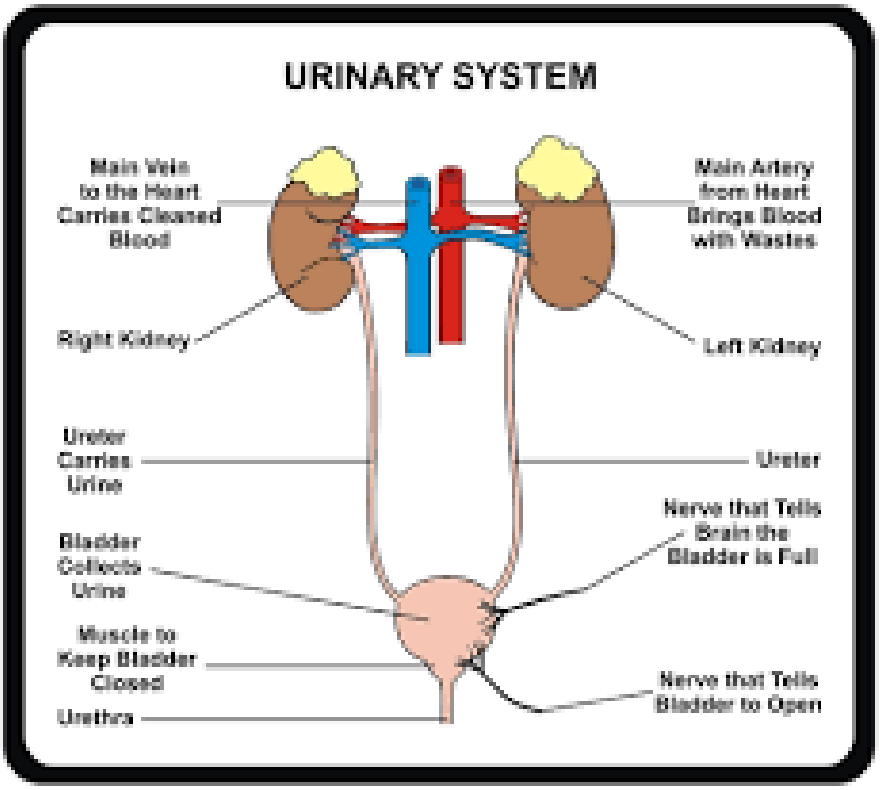

Figure 1. The Urinary System (Charity, 2016)

2.3 Epidemiology of Urinary Tract Infection

The most significant factor predisposing women to UTI in pregnancy is asymptomatic bacteriuria (ASB). ASB is defined as more than 100,000 organisms/mL on a clean catch urinalysis obtained from an asymptomatic patient (Onuoha *et al*., 2014). If asymptomatic bacteriuria is untreated in pregnancy, the rate of subsequent UTI is approximately 25 % (Kibret *et al.,* 2014). Due to both to the high rate and potential seriousness of pyelonephritis, it is recommended that all pregnant women be screened for ASB at the first prenatal visit. This is most often done with a clean catch urine culture. Treatment of ASB decreases the rate of clinical infection to 3 to 4 % (Kibret *et al.,* 2014).

The rate of asymptomatic bacteriuria in non-pregnant women is 5 - 6 % which compares similarly to estimated rates in pregnancy of 2 to 7 %. ASB is seen more frequently in parous women and women of low socioeconomic status. Women who are carriers for sickle cell trait also have a higher incidence of ASB (Muhammed *et al*., 2016).

UTIs are a common cause of serious infection in pregnant women. In one study, 3.5 % of antepartum admissions were due to UTI (Labi *et al*., 2015). Pyelonephritis is the most common cause of septic shock in pregnant women. Risk factors for UTIs in pregnancy include low socioeconomic status, young age, and nulliparity (Labi *et al*., 2015). As with ASB some patients may be predisposed to infection and may report a history of having had ASB, cystitis or pyelonephritis in the past. Pyelonephritis is more often right-sided however may be bilateral in up to 25 % of cases (Mei fong *et al.,* 2019).

2.4 Pathophysiology of Urinary Tract Infection

Organisms causing UTI in pregnancy are the same uropathogens which commonly cause UTI in non-pregnant patients. *Escherichia coli* is the most common organism isolated (Ahmed *et al*., 2016). An 18-year retrospective analysis found *Escherichia coli* to be the causative agent in 82.5 % of cases of pyelonephritis in pregnant patients (Wing *et al*., 2014). Other bacteria which may be seen include *Klebsiella pneumoniae, Staphylococcus, Streptococcus, Proteus,* and *Enterococcus* species (Wing *et al*., 2014).

2.5 Types of Urinary Tract Infection.

Urinary tract infections are caused by bacterium that invade the urinary epithelium cells causing irritation and inflammation of these cells. The infection can start in the urethra and can progress its way up to the bladder, ureters, or kidney (Gilber *et al*., 2013). Infection of the urethra or bladder is known as a lower urinary tract infection while infection of the ureters, renal pelvis or kidney tissue constitutes as an upper urinary tract infection (Derese *et al*., 2016). Women tend to be more prone to urinary tract infections due to their anatomy. Their urethra is shorter than a man's urethra and thus bacteria can reach the bladder more easily. In addition, a women's urethral opening is located closer to the anus making it easier for bacteria to migrate from the anus to the uretha (Mirella *et al*., 2016).

2.5.1 Acute Cystitis.

The most common site of a UTI, acute cystitis, is inflammation of the bladder. The urine is contaminated by bacteria that make its way up to the bladder. Most commonly a UTI occurs through the reversed movement of bacteria or pathogens (most commonly ***Escherichia coli***) from the gut (where it usually resides) up to the urethra then to the bladder (Huether and McCance, 2019). The migration of these particular bacteria from the perianal area to the urethra

opening may be due to poor wiping after a bowel movement, sexual intercourse or holding urine as urinating helps flush the bacteria from the body. *Escherichia coli* has several mechanisms that makes it more virulent and resistant to the immune system. They produce toxins called cytotoxin necrotizing factor-1 and hemolysis and they are resistant to complement (Kekelwa *et al*., 2021). Other bacteria that can cause UTIs work together to create a biofilm that helps with efficient reproduction and resists the defense mechanisms of the host as well as the antibiotic treatment that may be prescribed. The *E. coli* bacteria have particular structural features such as type-1 fimbriae that attach to the uroepithelial cells and their flagella help to push them upstream. In some women, genetics make them more prone to infection by some strains of ***E.*** *coli* **(**Jayachandran *et al*., 2016) Other pathogens that may contribute to infection include *Staphylococcus saprophyticus***,** *Pseudomonas, Proteus*, and *Klebsiella***.** Fungi such as *Candida*, viruses, and parasites such as *Schistosoma haematobium* are also common infection sources **(**Jayachandran *et al*., 2016). The cystitis, or inflammation of the bladder, can cause the epithelial cells of the bladder to appear red, pus-like or exudate in appearance as made visible by a cystoscopy, a procedure where the insertion of a flexible tube is used to view the structures of the bladder (Huether and McCance, 2019).

The inflammation of the bladder causes the common UTI symptoms of low back pain, urgency, frequency, and painful urination, also known as dysuria. The inflammation also causes the stretch receptors on the surface of the bladder to cause an individual to feel like they have a full bladder even when they only urinate a small amount. Other symptoms include flank pain, hematuria (blood in urine), and cloudy urine. Older adults with UTIs may demonstrate confusion and may be asymptomatic with regards to urinary symptoms (Huether and McCance, 2019).

2.5.2 Interstitial Cystitis/Painful Bladder Syndrome

Interstitial Cystitis (IC) or also known as Painful Bladder Syndrome (PBS) creates a chronic pain related to the lower urinary tract, more specifically, the bladder. Individuals with this experience a pain or pressure sensation symptom for greater than 6 weeks but no infection can be identified (Ailes *et al*., 2012). While the cause of interstitial cystitis is not known, an autoimmune response triggers inflammation that increases the sensitivity of neurons in the mucosa of the bladder, making it more vulnerable to bacteria colonization. The inflammation and hardening of the wall of the bladder can also create hemorrhagic ulcers and a decrease in bladder capacity. The epithelial cells of the bladder also secrete ant proliferative factor (AFP) which block cell growth of the inner wall of the bladder and causes an increased bladder sensation (Foxman *et al*., 2014).

2.5.3 Acute Pyelonephritis

Pyelonephritis is an infection of one or both upper urinary tracts. Acute pyelonephritis is usually associated with the microorganisms *Escherichia coli, Proteus,* and *Pseudomonas*. Urinary obstruction and reflux of urine from the bladder are the most common risk factors, along with being a female. These certain microorganisms make the urine more alkaline by splitting urea into ammonia, and this increases the risk of stone formation. The infection is possibly spread along the ureters or via the bloodstream. This triggers the inflammatory process and can cause unnecessary fluid buildup, inflammation or purulent urine. Both of the kidneys are usually involved as well as the renal tubules, but this rarely causes renal failure. Healing occurs with deposition of scar tissue, fibrosis, and atrophy of affected tubules after the acute phase. These individuals experience the same symptoms as those with acute cystitis in addition to fever, chills, flank pain and costovertebral tenderness (Huether and McCance, 2019).

2.5.4 Chronic Pyelonephritis

Chronic pyelonephritis is recurrent infection of the kidney which leads to scarring. Various causes are idiopathic, chronic UTI's, renal stones, or recurrent episodes of acute pyelonephritis. Chronic UTI's prevent the elimination of bacteria and triggers the inflammatory process which leads to destruction or atrophy of the tubules, significant scarring and impaired urine concentrating ability. These all ultimately lead to chronic kidney failure (Mirella *et al*., 2016).

2.5.5 Symptoms of UTI

Symptoms of a UTI depend on what part of the urinary tract is infected.

Lower tract UTIs affects the urethra and bladder. Symptoms of a lower tract UTI include:

- Burning with urination
- Increased frequency of urination without passing much urine
- Increased urgency of urination
- Bloody urine
- Cloudy urine
- Urine that looks like cola or tea
- Urine that has a strong odor
- Pelvic pain in women
- Rectal pain in men (Balakrishnan *et al*., 2016).

Upper tract UTIs affects the kidneys. These can be potentially life threatening if bacteria move from the infected kidney into the blood. This condition, called urosepsis, can cause dangerously low blood pressure, shock, and death (Balakrishnan *et al*., 2016).

2.5.5.1 Symptoms of an upper tract UTI include:

- Pain and tenderness in the upper back and sides
- Chills
- Fever
- Nausea
- Vomiting (Balakrishnan *et al*., 2016).

2.5.5.2 Symptoms of UTI in men

Symptoms of an upper tract urinary infection in men are similar to those in women. Symptoms of a lower tract urinary infection in men sometimes includes rectal pain in addition to the common symptoms shared by both men and women (Marami *et al*., 2019).

2.5.5.3 Symptoms of UTI in women

Women with a lower tract urinary infection may experience pelvic pain. This is in addition to the other common symptoms. Symptoms of upper tract infections among both men and women are similar (Marami *et al*., 2019).

2.6 Risk Factors of UTI

Urinary tract infections remain among the most common medical complications during pregnancy. It is estimated that the prevalence of ASB varies between 2% and 10–13%, similar to non pregnant women (Uddin *et al.,* 2016). There is a scarcity of data concerning acute cystitis in pregnancy (Willy *et al*., 2015). The prevalence of acute pyelonephritis in most reports ranges from 0.5 to 2% of pregnancies (Mirella *et al*., 2016).

Many women acquired bacteriuria before pregnancy. A large retrospective analysis with logistic regression modeling, embracing 8037 women from North Carolina, revealed that the two strongest predictors of bacteriuria at prenatal care at prenatal care initiation were: UTI prior to prenatal care initiation (OR = 2.5, 95% CI: 0.6–9.8 for whites, and OR = 8.8, 95% CI: 3.8–20.3 for blacks) and a pre-pregnancy history of UTI (OR = 2.1, 95% CI: 1.4–3.2) (Derese *et al.,* 2016). In a second analysis, prior antenatal UTI was found to be the strongest predictor of pyelonephritis after 20 weeks' gestation (OR = 5.3, 95% CI: 2.6–11.0). Other suggested risk factors for UTI during pregnancy are lower socioeconomic status, sexual activity, older age, multiparity, anatomical urinary tract abnormalities, sickle cell disease and diabetes, although the significance of some of them (age, parity or sickle cell trait) remains a matter of controversy (Derese *et al.,* 2016).

2.6.1 Predisposing Factors for Men

Most UTI risk factors for men are the same as those for women. However, having an enlarged prostate is one risk factor for a UTI that's unique to men (Kibret *et al*., 2014)

2.6.2 Predisposing Factors for Women

There are additional risk factors for women. Some factors that were once believed to be a cause of UTIs in women have since been shown to not be as important, such as poor bathroom hygiene. Recent studies have failed to show that wiping from back to front after going to the bathroom leads to UTIs in women, like previously believed (Yeva *et al*., 2020). In some cases, certain lifestyle changes such as good hygiene may help lessen the risk of some of these factors (Yeva *et al*., 2020).

2.6.3 Shorter urethra

The length and location of the urethra in women increases the likelihood of UTIs. The urethra in women is very close to both the vagina and the anus. Bacteria that may naturally occur around both the vagina and anus can lead to infection in the urethra and the rest of the urinary tract. A woman's urethra is also shorter than a man's, and the bacteria have a shorter distance to travel to enter the bladder (Vasudevan *et al*., 2014).

2.6.4 Sexual intercourse

Pressure on the female urinary tract during sexual intercourse can move bacteria from around the anus into the bladder. Most women have bacteria in their urine after intercourse. However, the body can usually get rid of these bacteria within 24 hours. Bowel bacteria may have properties that allow them to stick to the bladder (Judith *et al*., 2018).

2.6.5 Spermicides

Spermicides may increase UTI risk. They can cause skin irritation in some women. This increases the risk of bacteria entering the bladder (Judith *et al*., 2018)

2.6.6 Condom use during sex

Non-lubricated latex condoms may increase friction and irritate the skin of women during sexual intercourse. This may increase the risk of UTI (Judith et al., 2018)

However, condoms are important for reducing the spread of sexually transmitted infections. To help prevent friction and skin irritation from condoms, be sure to use enough water-based lubricant, and use it often during intercourse (Tadesse *et al*., 2018).

2.6.7 Diaphragms

Diaphragms may put pressure on a woman's urethra. This can decrease bladder emptying (Tadesse *et al*., 2018).

2.6.8 Decrease in estrogen levels

After menopause, a decrease in the estrogen level changes the normal bacteria in the vagina. This can increase the risk of UTI (Tadesse *et al.,* 2018).

2.7 Consequence of urinary tract infection in pregnancy

2.7.1 Asymptomatic bacteriuria

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 (Ade ojo *et al*., 2013; Assegai *et al*., 2008). 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. 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 (Mirella *et al.,* 2016).

However, the authors stressed the poor methodological quality of the available studies, their different design, lack of sufficient information about the randomization methods, different definitions used, low statistical power and some substantial biases, urging caution in drawing conclusions. A good example of these problems is presented by the Cardiff Birth Survey. In a prospectively studied large cohort of 25 844 pregnancies, several demographic, social and medical factors (including bacteriuria) were significantly associated with preterm birth in the

initial univariable analyses. However, after adjustments for other medical factors, bacteriuria retained an association of only borderline significance, and after further adjustment for demographic and social factors, the relationship completely disappeared. The results of the second analysis of the same cohort, aimed to compare associations of studied factors with spontaneous vs. indicated preterm birth, are even more interesting (Emiru *et al*., 2013). Two separate multiple logistic regression analyses revealed that spontaneous and indicated preterm births have different overall profiles of risk factors, and only the last of them was associated with bacteriuria. The authors concluded that ASB, if it does not progress to symptomatic UTI, is not associated with preterm delivery (Emiru *et al*., 2013).

2.7.2 Symptomatic Urinary Tract Infection

About 15–20 % of women with pyelonephritis have bacteremia (Derbie *et al*., 2017). 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. 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 (Bradly *et al*., 2019).

A number of observational studies have demonstrated the relationship between maternal symptomatic UTI and the risk of premature delivery and lower birth weight. 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 (Bradly *et al*., 2019). 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. A rare but severe complication is the transmission of the infection onto the newborn baby. Very often the transmitted infection originates from a heavily colonized birth canal, usually with GBS (Belete *et al*., 2020).

2.8 Treatment and Management of UTI

ASB and acute cystitis are treated with antibiotic therapy. Antibiotic choice can be tailored based on organism sensitivities when available from urine culture results (WHO, 2016). One-day antibiotic courses are not recommended in pregnancy, although 3-day courses are effective (Foxman, 2014). Antibiotics commonly used include amoxicillin, ampicillin, cephalosporins, nitrofurantoin, and trimethoprim-sulfamethoxazole. Fluoroquinolones are not recommended as a first-line treatment in pregnancy due to conflicting studies regarding teratogenicity. Short courses are unlikely to be harmful to the foetus, and thus, it is reasonable to use this class of drugs with resistant or recurrent infections (Brandon *et al*., 2015).

Recently evidence has developed suggesting a link between the use of sulfa derivatives and nitrofurantoin and congenital disabilities when these medications are prescribed in the first trimester (Kekelwa *et al*., 2021).

These studies have had limitations; however, it is currently recommended to avoid the use of these medications in the first trimester when alternatives are available. Because the potential consequences of untreated UTI in pregnancy are significant, it is reasonable to use these medications when needed as the benefit strongly outweighs the risk of use (Kekelwa *et al*., 2021).

Additional cautions exist with respect to these 2 classes of antibiotics. Patients with G6P deficiency should not be prescribed sulfa derivatives or nitrofurantoin as these medications can

precipitate hemolysis. In the late third trimester, trimethoprim-sulfamethoxazole should be avoided due to the potential risk for development of kernicterus in the infant following delivery (Mei fong *et al*., 2019).

Pyelonephritis in pregnancy is a serious condition usually requiring hospitalization. Once an evaluation has been completed, treatment consists primarily of directed antibiotic therapy and IV fluids to maintain adequate urine output. Fever should be treated with a cooling blanket and acetaminophen as needed. Commonly, second or third generation cephalosporins are used for initial treatment. Ampicillin and gentamicin or other broad-spectrum antibiotics are alternatives. Patients should be monitored closely for the development of worsening sepsis (Mei fong *et al*., 2019).

2.9 Safety of Antimicrobial Treatment

Nearly all antimicrobials cross the placenta, and some of them may exert teratogenic effects. Commonly accepted antibiotics used in treating UTIs during pregnancy, regardless of its period, include derivatives of penicillin and cephalosporins, particularly those with low protein-binding ability (such as cephalexin) all of FDA pregnancy category B (Table I) (Brandon *et al*., 2015).

Table I

US Food and Drug Administration (FDA) categories of medications in pregnancy

Antibiotic	**FDA risk category**	**Antibiotic**	**FDA risk category**
Amoxicillin	B	Trimethoprim/sulfamethoxazol	C
Cephalosporins	B	Ciprofloxacin	C
Piperacillin/tazobactam	B	Levofloxacin	C
Daptomycin	B	Imipenem/cilastatin	C
Azithromycin	B	Linezolid	C
Erythromycin	B	Clarithromycin	C
Meropenem	B	Spiramycin	C
Clindamycin	B	Gentamycin	C
Nitrofurantoin	B	Amikacin	D
Vankomycin ***iv***.	B	Tobramycin	D
Metronidazol ***iv***.	B	Netilmycin	D
Trimethoprim	C	Tetracyclines	D

A – Well-controlled studies available in humans with no adverse effects observed in human pregnancies; B – No adverse effects in well-controlled studies of human pregnancies with adverse effects seen in animal pregnancies OR no adverse effects in animal pregnancies without well-controlled human pregnancy data available; C – Human data lacking with adverse pregnancy effects seen in animal studies OR no pregnancy data available in either animals or humans; D – Adverse effects demonstrated in human pregnancies; benefits of drug use may outweigh the associated risks.

Nitrofurantoin and trimethoprim/sulfamethoxazole should be avoided during the first trimester due to a possible risk of fetal defects, although the studies on that issue yield somewhat contradictory results (Brandon et al., 2015). In the large American population-based National Birth Defects Prevention Study, maternal use of sulfonamides and nitrofurantoin (1 month before pregnancy to the end of the first trimester) was associated with more serious defects than any other antibacterial classes (Brandon et al., 2015). However, this study has been criticized for several significant limitations including recall bias (women were asked about antibiotic use after pregnancy and it was not confirmed by medical records), inability to determine whether the birth defect was due to the antibiotic itself, the infection for which the antibiotic was prescribed, or other confounding factors (Brandon et al., 2015).

In a two years study, the Committee of Obstetrics Practice of the American College of Obstetricians and Gynecologists, summarizing the available data on the relationship between prenatal exposure to both antimicrobials and birth defects, concluded that: 1) “When selecting an antibiotic for a true infection during the first trimester of pregnancy (that is, during organogenesis), health care providers should consider and discuss with patients the benefits as well as the potential unknown risks of teratogenesis and maternal adverse reactions; 2)

"Prescribing sulfonamides or nitrofurantoin in the first trimester is still considered appropriate when no other suitable alternative antibiotics are available"; 3) "Pregnant women should not be denied appropriate treatment for infections because untreated infections can commonly lead to serious maternal and fetal complications (Elzayat *et al*., 2017).

In the second and third trimester, trimethoprim/sulfamethoxazole and nitrofurantoin are well tolerated and by some considered even first line agents, except in the last week before delivery when they may increase neonatal jaundice and predispose to kernicterus (Bradley *et al.,* 2019). The use of fluoroquinolones (FDA pregnancy category C) is essentially contraindicated throughout pregnancy, since fetal cartilage development disorders have been reported in experimental animals, although not in human studies (Bradley *et al*., 2019).

Gentamicin and other aminoglycosides are FDA pregnancy category D, because of their potential nephro- and neurotoxicity (eighth nerve damage) to the fetus (Brandon *et al*., 2015). Tetracyclines lead to discoloration of deciduous teeth if given after 5 months' gestation. Macrolides have been assigned to pregnancy category C by the FDA (Table 1).

Although these drugs are used in pregnancy relatively often, the data on their embryotoxicity and teratogenicity are limited. Earlier reports suggested an association between prenatal exposure to macrolides and congenital heart defects or pyloric stenosis, whereas the results of some recent studies are rather reassuring (Brandon *et al*., 2015)

The first prospective controlled multicenter study of exposure to clarithromycin in early pregnancy suggested that this agent does not increase the risk of fetal malformations above the expected 1–3% (Sekikubo *et al*., 2017). However, there was a two-fold higher rate (14% vs. 7%) of spontaneous abortions in the exposed vs. control group, and although it still remained within

the expected baseline rate, the possibility that it could be a result of undiagnosed fetal malformation cannot be excluded. No significant teratogenic effect of erythromycin was identified in a Hungarian case-control study (Sekikubo *et al*., 2017).

2.9.1 Complications of UTI

Patients with pyelonephritis are at risk for several significant complications. Sepsis may worsen resulting in hypotension, tachycardia, and decreased urine output. Under this situation, ICU admission may be required (Chisanga *et al.,* 2017).

Pulmonary complications are not uncommon, occurring in up to 10% of pregnant patients undergoing treatment for pyelonephritis (Mirella *et al.,* 2016). This is due to endotoxin-mediated alveolar damage and may manifest as pulmonary edema or acute respiratory distress syndrome (ARDS). Urine output and oxygen status should be monitored closely, and patients may require ICU admission for respiratory support (Derese *et al.,* 2016).

Endotoxin release may lead to anemia, this typically resolves spontaneously following treatment. This is the most common complication seen with pyelonephritis occurring in up to 25% of patients (Wing *et al.,* 2014).

Endotoxin release may also cause uterine contractions and patients should be monitored for preterm labor; patients should be treated for preterm labor when indicated. Caution should be exercised in use of tocolytic therapy as the risk of pulmonary edema is increased in the setting of UTI (Al-Naqshbandi *et al.,* 2019).

A small number of patients may experience persistent infection. In these cases, consideration should be given to urinary obstruction or renal abscess. Antibiotic choice should be re-evaluated and culture results reviewed (Derese *et al*., 2016).

2.9.2 Antibiotics resistance to UTI

The emergence and rapid dissemination of antibiotic resistance is a major problem for human health (Blair *et al*., 2015). The adaptive power of bacteria is manifested by their ability to appropriate new properties either by modifying their genome (mutations) or by acquiring genetic information via mobile genetic elements such as plasmids and transposable elements (Xia *et al*., 2012; Wang *et al*., 2014; Xu *et al*., 2019). Most bacterial species are able to integrate different determinants of resistance into their genome. Thus, the dissemination of resistance genes between bacteria has led to the emergence of bacteria resistance to several antibiotics in particular methicillin-resistant *Staphylococci* (MRS), the enterobacteria producing extended-spectrum beta-lactamases (ESBL) and vancomycin resistant *enterococci* (VRE) (Hagel *et al*., 2019). Among bacteria species resistant to antibiotics, enterobacteria producing ESBL represent a major global threat to public health (Naas *et al*., 2016). Often associated with urinary tract infections, they can also cause serious blood stream infections (Naas *et al*., 2016). *Staphylococcus aureus* is the leader in the family of *Staphylococci* because of its involvement in suppurative, localized or severe systemic pathologies in humans (Boukhatem *et al*., 2015). However, other species of *Staphylococci* like coagulase negative *Staphylococci* (CoNS) can cause a lot of damage to their hosts. This is the case, for example, with *Staphylococcus saprophyticus*, which is the second most common bacterium responsible for human urinary tract infection after *Escherichia coli* (Koudokpon *et al*., 2017). The frequency of oxacillin resistance in CoNS strains has increased substantially over recent decades (Joshua *et.,al* 2016) and CoNS

are typically resistant to multiple drug classes. Increasing resistance of CoNS to commonly used antibiotics like glycopeptides, aminoglycosides and macrolides has been reported in different countries around the world [May *et.,al* 2014)]. Knowledge of the antibiotic susceptibility patterns of microorganisms is very important as it reduces unnecessary expenses, reduces development of resistance to useful and life saving antibiotics and also minimizes many side effects. The susceptibility patterns tend to vary from one country to another and within a country as antibiotic prescribing patterns and strains vary. The pathogenesis of CoNS species comes from the production of an impressive repertoire of virulence factors [Joshua *et.,al* 2016)]. These are properties coded for by specific genes and they enable microorganisms to establish themselves on or within a host. Some of these virulent genes include the *ica* gene (intercellular adhesion - operon *ica*) involved in biofilm formation and the *atl* E gene, which encodes the vitronectin-binding cell surface protein involved in primary attachment. The *mec* A gene controls the synthesis of the additional penicillin-binding protein PBP2a and is responsible for the resistance of some species to methicillin (Joshua *et.,al* 2016). The *ica* gene is considered to be the main marker of virulence in CoNS. It has been found to be the most amplified gene in virulent strains of CoNS (May *et.,al* 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Population

Ilorin is the state capital of Kwara State in western Nigeria (The World Gazetteer, 2013). It had a population of 973,671, making it the 7th largest city by population in Nigeria (Federal Republic of Nigeria, 2016) with 16 local Government. Civil Service Clinic, Sobi Specialist Hospital and Okelele Primary Health Center are all under Ilorin East Local Government Area with its headquater at Oke Oyi town. It has an area of 486km^2 and a population of 204,310 at 2006 census (the world Gazetteer, 2013).

3.1.1 Study location

Three hundred samples were collected at three different hospitals in Ilorin, Kwara State.

These are Civil Service Clinic (150 samples), Sobi Specialist Hospital Alagbado (65 samples) and Okelele Primary Health Center (85 samples).

3.2 Sample Size

The study population comprises pregnant women attending antenatal clinic at the three hospitals between July and August, 2021. The sample size was calculated using the Cochran's formula; N = Z^2 P (1-P) /d ^{2}). where N = sample size, Z = standard normal value corresponding to 95% confidence interval set at 1.96, D = degree of error tolerance at 5%, P = proportion used for estimation, obtained from previous studies in the study environment (26% was chosen from prevalence range of 1.3% - 60% reported in Nigeria).

Z: standard normal value corresponding to 95% confidence interval (Z) = 1.96

P: prevalence of the disease in the previous studies =26% (0.26)

d: degree of error tolerance at 5% = 0.05

N= 1.96^2 X 0.26 (1-0.26)/ 0.05^2 = 295.5 round up to 300

3.3 Inclusion Criteria

Pregnant women who were not on any antibiotics treatment within two weeks preceding sample collection were included.

3.3.1 Exclusion Criteria

Pregnant women who were on antibiotics treatment within two weeks preceding sample collection were excluded.

3.4 Preparation of Culture Media

Cysteine Lactose Electrolyte Deficient Agar and Blood Agar were prepared according to manufacturer's instruction by weighing the required quantities in (grams) and dissolving in required volume of distilled water in (ml). The media were allowed to dissolve completely on hot plate and mixing gently in other to achieve homogenous solution. The media were then sterilized by autoclaving at 121^0C for 15 minutes, they were allowed to cool down to 55^0C, dispensed in petri dishes and allowed to solidify before use.

3.5 Sample Collection

Mid stream urine were collected into sterile universal bottle and properly labeled.

The urine samples were transported in an ice-pack from the antenatal clinic to the laboratory where the samples were processed.

3.6 Macroscopic Observation

The urine samples were observed macroscopically for their colours, consistency i.e. turbidity or clarity.

3.6.1 Microscopic Observation

Each of the urine samples was pour into a clean test tube and centrifuged at 3000rpm for 5minutes. The supernatant was decanted and the sediment was poured on a clean slide, covered with cover slip and observed microscopically using x40 0bjective lens.

3.7 Isolation of Microorganisms

Calibrated wire loop was sterilized in the flame and allowed to cool and this wire loop was used to pick 0.001ml of urine and inoculated on CLED Agar using zigzag method, the same was done on Blood agar and the cultured plates were incubated at 37^0C for 24hrs. This procedure was carried out on all the urine samples collected.

Colony counts were carried out on those plates that have growth and those that have colony count of 10^5 CFU/ml and above were taken as significant, the Isolates were subcultured on Blood Agar to obtain pure culture of the organisms (Cheesbrough, 2006).

3.7.1 Gram's staining

Gram staining was carried out according to Cheesbrough (2006); Alemu *et al*. (2012). A drop of normal saline was placed on a microscopic slide. A loopful of the organism was picked using a sterilized wire loop and then emulsified on the normal saline to obtain homogenous smear. The smear was air dried and then heat fixed by passing over flame. The slide was flooded with crystal violet for 60s and rinsed with water. Secondly, the slide was also flooded with Lugor's iodine for 60s and rinsed with water. The slide was decolorized with Acetone for 3 seconds and washed

with water. The slide was finally flooded with safranin for 60s and was washed with water. It was allowed to air dry. A drop of Immersion oil was added and the slide was viewed under the microscope using x100 objective. Gram positive bacteria stained purple while Gram negative bacteria stained pink (Cheesbrough, 2006).

3.7.2 Motility Test

Sulphideindole motility medium (SIM) is a semi-solid media and was prepared to see how bacteria swarm or move. A straight sterilized wire loop was used to pick a colony of the organism and inoculated by stabbing the medium in the test tube. The test tubes were incubated for 37ºC and examined for a diffuse zone of growth flared out from the line of inoculation. Hazy growths that spread throughout the medium rendering it slightly opaque indicates a positive motility while growth confined to the stab-line, with sharply defined margins leaving the surrounding clearly transparent indicates negative motility (Tille and Forbes, 2014).

3.8 Biochemical Tests

3.8.1. Catalase Test

Catalase test was carried out as described by (Cheesbrough, 2006). A drop of hydrogen peroxide (H_2O_2) was placed on a slide and the edge of cover slip was used to pick a colony of the organism and then emulsify. Positive and negative controls were setup along with the test. The presence of catalase enzyme was indicated by production of bubbles (oxygen) that shows positive result, while no bubbles indicated absence of catalase enzyme.

3.8.2 Coagulase Test (Slide coagulase)

Coagulase is an enzyme capable of coagulating certain blood plasma. This test is used to differentiate *Staphylococcus aureus* from other *Staphylococcus* species. A drop of normal saline

was placed on a slide and a colony of the organism was emulsified on it to obtain a homogenous suspension. A drop of blood plasma was then mixed with the suspension on the slide and it was observed for agglutination. Positive and negative controls were setup along with the test. Coagulase positive was indicated by clumping of colonies while coagulase negative was indicated by absence of clumping of colonies (Cheesbrough, 2006).

3.8.3 Tube Coagulase Test

Three small test tubes were labeled as Test, Positive Control and Negative Control Exactly 0.2ml of plasma was put into each tube and 0.8ml of the test culture was added to tube T while 0.8ml of known *staphylococcus aureus* was added to positive control tube. 0.8ml of sterile broth was added to negative control tube and the three tubes were incubated at 37^0c for 2hrs. Coagulase positive was indicated by presence of clot while negative was indicated by absence of clot.

3.8.4 Indole Test

Indole test is used to determine the ability of the organism to produce indole. Each isolate was inoculated into sterile peptone water enriched with 1 % tryptophan in the test tube and incubated at 37^0c for 24hrs. Kovac's reagent was added and gently shaked. A red violet colour at the top surface of the tube indicated a positive result, while absence of coloration indicated a negative result. Positive and negative controls were set along with the test (Cheesbrough, 2006).

3.8.5 Citrate Test

This test is used to test ability of an organism to utilize citrate as a sole source of carbon and energy. A colony of each isolates was inoculated into a Simmon citrate agar slant and was incubated. A positive citrate test was confirmed by the formation of blue colour, while the initial

green color denotes negative test. Positive and negative controls were set along with the test (Cheesbrough, 2006).

3.8.6 Oxidase Test

The oxidase test is used in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella and Pasteurella* specie all of which produce the enzyme cytochrome.

A colony of the organisms was picked with the edge of a cover slip and emulsified on the oxidase strip. The appearance of deep purple colour within 30s indicated a positive result, while absence of purple colour indicated a negative result. Positive and negative controls were set along with the test (Cheesbrough, 2006).

3.8.7 Bile Aesculin Test

This test is used in the identification of *Enteroccoci* specie. A colony of the organisms was picked with a sterile wire loop and inoculated on Bile aesculin agar slant and incubated at 37^0C for 24hrs.

Changing of the medium from cream to dark colour indicated positive while the negative remained cream colour. Positive and negative controls were prepared along with the test (cheesbrough, 2006).

3.8.8 Lactose Fermentation Test

This test was carried out to determine the ability of organisms to ferment sugars with production of acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1% lactose and 0.01 % phenol red. About 10ml of sugar broth was dispensed into each of the test tubes, Durham tube which would trap the gas if produced was inverted carefully. The test tubes were autoclaved and inoculated with a loopful of 24 hour old culture of the test organisms after

then incubated for 48hrs at 37ºC and observed for acid and gas production. Yellow coloration indicated acid production while gas production was indicated by displacements of the medium in the Durham tube (Cheesbrough, 2006).

3.8.9 Methyl Red (MR) Test

A test organism was inoculated in the glucose phosphate water medium and incubated at 37ºC for 48 hours. Few drop of methyl-red was added to the culture and positive result was indicated with red colour formation, while no change in colour showed negative result. Positive and negative control were prepared along with the tests (Cheesbrough, 2006).

3.8.10 Nitrate Reduction Test

This test is used to determine ability of an organism to reduce nitrate to nitrite. Each test organisms was inoculated into nitrate broth using sterilized wire loop and incubated at 37º C for 48 hours, Two drops of Nitrite reagent (sulfanilic acid and α-naphthylamine) was added and the change in color was observed. Red coloration indicated positive result, absence of red colour showed negative result. Positive and negative controls were prepared along with the test (cheesbrough, 2006).

3.9 Antibiotics Sensitivity Test

A loop full colony was picked with sterilized wire loop and inoculated into normal saline diluted to a standard concentration of approximately 1.5 x 10^8 colony forming units per ml (according to 0.5 % McFarland standard). About 0.1ml of the diluted isolate was dropped and properly spread on the Mueller Hinton agar with the aid of sterile swab stick. Six discs were placed 60 degrees apart on the planted lawn. After 24 hours of incubation at 37° C, the diameter of the inhibitory zone surrounding the discs was measured. The isolate sensitivity/resistance pattern was

examined using the reference standard by the Clinical Laboratory Standards Institute CLSI, (2017). Antibiotics discs used were amoxicillin-clavulanic acid (AMC) 30μg, amoxicillin (AML) 10µg, nitrofurantoin (F) 30µg, ceftazidime (CAZ) 30µg, ceftriaxone (CRO) 30µg, erythromycin (E) 5µg, ofloxacin (OF) 5 µg and cefuroxime (CXM) 30µg. They were tested against all isolates. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as control (CLSI 2017). Methicillin resistant Coagulase negative *Staphylococcus were* determined using cefoxitin discs on Mueller-Hinton agar and those that were cefoxitin resistant were molecularly screened for *MecA* gene.

3.9.1 DNA Extraction of *Staphylococcus* species (CoNS).

The DNA of the isolates was extracted by suspending 4-5 bacteria colonies in 200 µl of 1XTBE buffer in Eppendorf tubes appropriately labeled. The cells were boiled at 100^{o}c for 10 minutes in a water bath and were cooled rapidly on ice for 30 minutes. 3µl of Proteinase K was added to the lysed cells and the mixture was incubated for 15 minutes at 60^{o}C in a water bath. The enzyme was denatured by boiling at 100^{0}C for 10 minutes and was centrifuged at 13, 400 rpm for 3 minutes. The supernatant containing the DNA was transferred into a fresh sterile Eppendorf tube and stored at -20^{O}C until required for PCR.

3.9.2 PCR procedure for Gene Amplification

A 25µl reaction containing 4µl of master mix of 5X, 1µl forward, 1µl of reverse primer, 13µl of nuclease free water and 2µl of DNA lysate were used for PCR. Amplification was subjected to initial denaturation at 95^{O}C for 5min, followed by 35 cycles of denaturation at 94^{O}C for 1 min, annealing at NUC (56^{O}C), *Mec*A (55^{O}C), for 1 min respectively, extension at 72^{O}C for 1 min and final extension procedure was carried out at 72^{O}C for 10min.

3.9.3 Gel electrophoresis

At the completion of the amplification, PCR products were resolved on l.5 % agarose gel prepared by dissolving 1.5g of agarose powder in 100 ml of 1X Tris-borate-EDTA (TBE) buffer solution inside a clean conical flask. The 1.5 % agarose solution was heated in a microwave oven for 2 minutes and was observed for clarity which was an indication of complete dissolution. The mixture was then allowed to cool to about 50 ^{0}C after which 0.5 µl of ethidium bromide was then added. It was allowed to cool further and then poured into a tray sealed at both ends with support to form a mould with special combs placed in it to create wells. The comb was carefully removed after the gel had set and the plate was placed inside the electrophoresis tank which contained 1X TBE solution, 5µl of amplicon was mixed with 1µl of loading buffer and the mixture was loaded to the wells of the agarose gel. The power supply was adjusted to 100 volts for 25 minutes. For each run, a 100 base-pair molecular weight DNA standard (size marker) was used to determine the size of each PCR product with control ATCC 2913. The DNA bands were then visualized with a short wave ultraviolet trans-illuminator and photographed using gene gel bioimaging system. The PCR product was then analyzed (Table 3.1).

Table 3.1: primers used for the amplification of genes

Primer(s)	Sequence 5^{1}-3^{1}	Product size(bp)	Annealing temp.(^{o}c)
*Mec*A F	AAAATCGATGGTAAAGGTTGGC	533	55
*Mec*A R	AGTTCTGCAGTACCGGATTTGC		

3.9.4 Quality Control

Control strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were run with the Gram positive and Gram negative isolates (CLSI, 2017).

3.9.5 Ethical Issue

The ethical clearance was obtained from the ministry of health and informed consent was obtained from all study participants for participation.

3.9.6 Statistical Analysis

Simple statistics were used to determine the mean age and standard deviation as well as proportion and percentage of the values.

CHAPTER FOUR

4.0 RESULTS

In this study, a total of 300 pregnant women 49 (16.3 %) with symptoms and 251 (83.7 %) without symptoms of UTI were investigated during the study period. The age of the study participants ranged from 19 to 43 years, with a mean age of 26.7 ± 4.7 years. Majority of 175 (58.3 %) of the study participants were in the age group of 25 to 34 years. Among the study participants, 188 (62.6 %) were self employed (Table 4.1). Out of 300 urine samples collected for analysis 240 (80 %) were amber and clear, 24 (8%) were slightly turbid while 36 (12 %) were turbid (Table 4.2). 65(21.7 %) of the samples contained white blood cells and 98 (32.7 %) contained epithelial cells, only 2 (0.7 %) had red blood cells when viewed under the microscope (Table 4.3).

The colonial morphology in terms of colour, shape, edge, texture and elevation of isolates and biochemical test in terms of Citrate utilization, Nitrate reduction, Methyl red, Catalase, Coagulase, Indole, Lactose fermentation, Bile aesculin and oxidase as well as motility and Grams staining were shown on (Table 4.4).

Six (6) different bacteria were isolated from the urine samples. Forty- four (44) of infected pregnant women have significant growth ($>10^5$CFU/ml) of bacteria in the urine culture in which 28(63.6 %) of them were Asymptomatic while 16(36.4 %) were Symptomatic. The predominantly isolated bacteria were *Escherichia coli* 17(38.6 %) then coagulase negative *staphylococcus* 10(22.7 %), *Klebsiella pneumoniae* 8(18.2 %), *Staphylococcus aureus* 6(13.6 %), *Enterococcus* specie 2(4.6 %) and *Pseudomonas* specie 1(2.3%) (Table 4.5).

Table 4.1: Sociodemographic characteristics of the study participants (n= 300)

Variables	Occurrences	Percentage (%)
Age(year)		
15- 24	102	34
25- 34	175	58.3
35- 44	23	7.7
Educational level		
No formal education	17	5.7
Primary	32	10.7
Secondary	145	48.3
College and above	106	35.3
Occupation		
Housewives	23	7.7
Self employed	188	62.6
Student	14	4.7
Employed	75	25
Symptoms of UTI		
Yes (Symptomatic)	49	16.3
No (Asymptomatic)	251	83.7

KEY: UTI urinary tract infection

Table 4.2: Macroscopic Observation of Urine Samples

Macroscopic Observations	Number and Percentage of Samples (%)
Amber and clear	240 (80)
Amber and slightly turbid	24 (8)
Amber and turbid	36 (12)

Table 4.3: Microscopic Observation of Urine Samples

Microscopic Observations / HPF	Number and Percentage (%) of Positive Cases	Number and Percentage (%) of Negative Cases
WBC	65 (21.7)	235 (78.3)
RBC	2 (0.7)	298 (99.3)
Epithelial cells	98 (32.7)	202 (67.3)

Key: WBC White blood cell, RBC Red blood cell, HPF High power field

Table 4.4: Colonial Morphology, Conventional Biochemical Test, Motility and Gram Reaction of Bacterial Isolates on CLED Agar

Isolates	Colonial Morphology					Conventional Biochemical Test									Motility	Gram Staining	Probable Isolates
	Colour	Shape	Edge	Texture	Elevation	Ci	NR	MR	Ca	Co	I	LF	BA	O	Motility	Gram staining	Probable Isolates
N1	Opaque yellow	Circular	Entire	Dry	Raised	-	+	+	+	-	+	+	-	-	+	- Rod	*Escherichia coli*
N2	Yellow	Circular	Entire	Mucoid	Flat	+	-	-	+	-	-	+	-	-	-	- Rod	*Klebsiella pneumonia*
N3	Green	Circular	Entire	Mucoid	Low Convex	-	-	-	+	-	-	-	-	+	+	- Rod	*Pseudomonas sp*
N4	Deep Yellow	Circular	Entire	Moist	Convex	-	-	-	+	+	-	+	-	-	-	+ Cocci	*Staphylococcus aureus*
N5	Pale yellow	Circular	Entire	Moist	Convex	-	-	-	+	-	-	+	-	-	-	+ Cocci	*Staphylococcus* sp(CoNS)
N6	Yellow	Circular	Entire	Moist	Convex	-	-	-	-	-	-	+	+	-	-	+ Cocci	*Enterococcus sp*

Key: Ci = Citrate, NR = Nitrate Reduction, MR = Methyl Red, Ca = Catalase, Co = Coagulase, I = Indole, LF = Lactose Fermentation, BA = Bile aesculin, O = Oxidase, Sp = Specie, N = Number, CoNS = Coagulase negative *Staphylococcus*

Table 4.5: Bacterial Uropathogens Isolates

Bacterial Isolates	Asymptomatic n(251)	Symptomatic n(49)	Total n(300)
Gram Negative	17 (65.3)	9 (34.7)	26 (59.1)
Organisms			
E .coli	11(64.7)	6(35.3)	17(38.6)
K. pnemoniae	6(75.0)	2(25.0)	8(18.2)
Pseudomonas sp	0(0.0)	1(100.0)	1(2.3)
Gram Positive	11(61.1)	7(38.9)	18(40.9)
S. aureus	4(66.7)	3(33.3)	7(13.6)
CoNs	7(77.8)	2(22.2)	9(22.7)
Enterococcus sp	0(0.0)	2(100)	2(4.6)
Total	28(63.6)	16(36.4)	44(100)

Key: E= *Escherichia, S= Staphylococcus, CoNS*= Coagulase negative *Staphylococcus,* sp = specie, K=*Klebsiella*

The majority of isolated Gram-negative uropathogens showed resistance rate of 19 (73.1 %) to ampicillin and 17(65.4 %) to amoxicillin-clavulanic (augmentin) acid. Rates of resistance of Gram-negatives isolates against ceftriaxone, cefotaxime, cefuroxime range from 8(30.8 %) – 12(46.2 %). However, all Gram-negative bacteria isolates showed a relatively low level of resistance against nitrofurantoin 4(15.4 %) and ceftazidime 7(26.9 %) (Table 4.6).

Gram-positive uropathogens showed a high level of resistance for Penicillin 16 (94.1 %). The rates of resistance of Gram-positive against ceftriaxone, ampiclox, and augmentin range from 5(29.4%) – 8(47.1 %). On the other hand, all Gram-positive isolates showed full sensitivity (100.0%) to nitrofurantoin. Moreover, 13(76.5 %), 10(58.8 %) and 9 (52.9 %) of the gram-positive isolates were sensitive to clindamycin, augmentin, and ampiclox, respectively (Table 4.7). Out of nine (9) CoNS that were phenotypically screen for cefoxitin. 4 (44.4 %) were resistant and 5(55.6 %) were sensitive. Cefoxitin resistant CoNS were molecularly screened for the presence of *Mec*A gene and all were positive for *Mec*A gene (100 %) (Figure 2).

Table 4.6: Antibiotics Susceptibility Patterns of Gram Negative Isolates.

Isolates (n)		CXM	CRO	CAZ	AUG	NF	AMP	CTX
E. coli (17)	S	3 (17.6)	9 (52.9)	8 (47.1)	3 (17.6)	13 (76.5)	1 (5.9)	6 (35.3)
	I	4 (23.5)	2 (11.8)	4 (23.5)	2 (11.7)	1 (5.9)	1 (5.9)	4 (23.5)
	R	10 (58.8)	6 (35.3)	5 (29.4)	12 (70.6)	3 (17.6)	15 (88.2)	7 (41.2)
K.pnemoniae (8)	S	4 (50.0)	5 (62.5)	5 (62.5)	1 (12.5)	5 (62.5)	3 (37.5)	3 (37.5)
	I	3 (37.5)	2 (25.0)	1 (12.5)	3 (37.5)	3 (37.5)	2 (25.0)	2 (25.0)
	R	1 12.5)	1 (12.5)	2 (25.0)	4 (50.0)	0 (0.0)	3 (37.5)	3 (37.5)
Pseudo sp (1)	S	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	1 (100)	1 (100)	0 (0.0)	1 (100)	1 (100)	1 (100)	0 (0.0)
Total (26)	S	7 (26.9)	14 (53.8)	14 (53.8)	4 (15.4)	18 (69.2)	4 (15.4)	10 (38.5)
	I	7 (26.9)	4 (15.4)	5 (19.2)	5 (19.2)	4 (15.4)	5 (19.2)	6 (23.1)
	R	12 (46.2)	8 (30.8)	7 (26.9)	17 (65.4)	4 (15.4)	19 (73.1)	10 (38.5)

Key: CRO, Ceftriaxone; AUG, Augmentin; CTX, Cefotaxime; CAZ, Ceftazidime; AMP, Ampicillin; NF; Nitrofurantoin, CXM Cefuroxime;R, Resistant; I, Intermediate; S, Sensitive.

Table 4.7: Antibiotic Susceptibility Patterns of Gram Positive Isolates

Isolates(n)		PEN	CRO	ACX	AUG	CL	NF
S.aureus (7)	S	0(0.0)	1(16.7)	4(66.7)	4(66.7)	4 (66.7)	6(100.0)
	I	0(0.0)	0(0.0)	1(16.7)	0(0.0)	1(16.7)	0(0.0)
	R	6(100.0)	5 (83.3)	1(16.7)	2(33.3)	1(16.7)	0(0.0)
CoNS(9)	S	0 (0.0)	4(44.4)	3(33.3)	4(44.4)	7(77.8)	9(100.0)
	I	0 (0.0)	2(22.2)	3(33.3)	2(22.2)	1(11.1)	0(0.0)
	R	9 (100)	3(33.3)	3(33.4)	3(33.3)	1(11.1)	0(0.0)
Enterococcus(2)	S	1(50.0)	0(0.0)	2(100.0)	2(100)	2(100)	2(100)
	I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	R	1(50.0)	0(0.0)	1(0.0)	0(0.0)	0(0.0)	0(0.0)
Total (18)	S	1(5.9)	5(29.4)	9(52.9)	10(58.8)	13(76.5)	18(100)
	I	0(0.0)	2(11.8)	4 (23.6)	2(11.8)	2(11.8)	0(0.0)
	R	16(94.1)	8 (47.1)	5(29.4)	5 (29.4)	2 (11.8)	0(0.0)

Key: CoNS, Coagulase-negative staphylococci; CL, Clindamycin; PEN, Penicillin; NF, Nitrofurantoin; ; CRO, Ceftriaxone; ACX, Ampiclox AUG Augmentin; R, Resistant; I, Intermediate; S, Sensitive.

***Mec*A gene Gel electrophoresis**

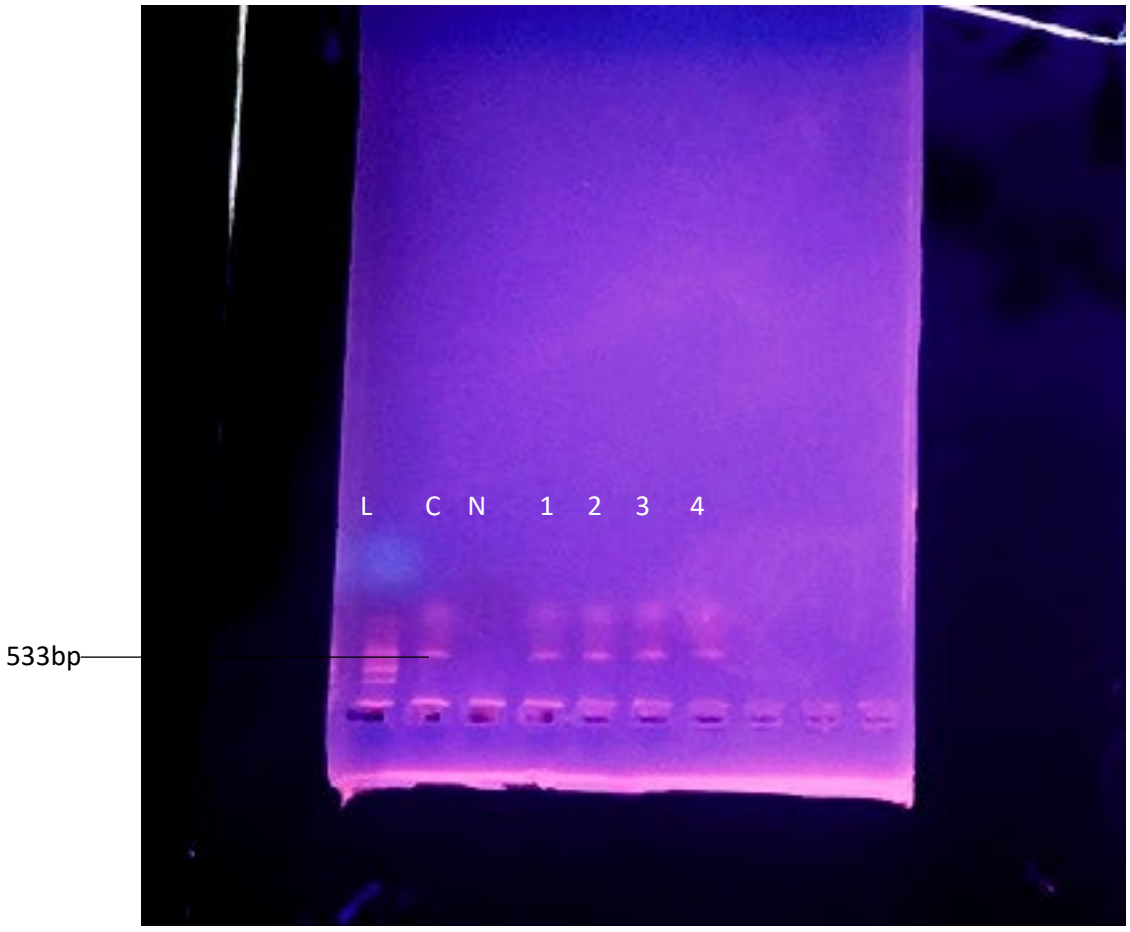

Figure 2: Gel Electrophoresis showing the PCR product of *Mec*A gene in CoNS (533bp).

CHAPTER FIVE

5.1 DISCUSSION

The incidence rate of UTI in this study was found to be 14.5 % which shows a lower prevalence rate compared to 27.9 % rate reported in a previous study in Ilorin (Olufadi *et al*., 2019), 43.3 % in Abuja (Yunusa *et al*., 2016), 26 % in Kaduna (Muhammed *et al*., 2014), 61.5 % in Aba (Ezeigbo *et al*., 2016) and 15.8 % in Kano (Muhammed *et al*., 2019). However, the value of this study is higher than 10.3 % reported in plateau (Oko *et al*., 2017) and 10.6 % in Enugu (Ojide *et al*., 2014). Prevalent rates of 6.1 % to 10.9 % have been reported in Iran (Emiru *et al*., 2013 and Ron Eremeko *et al*., 2020), while Obirikorang *et al*. (2012) reported 7.3 % prevalence in Ghana. This inconsistency in prevalence may be due to differences in the study participants particularly their socioeconomic status and study locations.

The most implicating organism causing urinary tract infections among the pregnant women in this study was *Escherichia coli* and it was responsible for 38.6 % of the cases of UTI. This was followed by Coagulase negative *staphylococcus* (22.7 %), *Klebsiella pneumoniae* (18.2 %), *Staphylococcus aureus* (13.6 %), *pseudomonas aeruginosa* (2.3 %). The pattern and frequency of occurrence of the bacterial isolates found in this study is similar to the previous studies. *Escherichia coli* is also the most common pathogen among patients with uncomplicated UTIs (Ezeigbo *et al*., 2016). This finding is similar to other reports which found that Gram negative bacteria, particularly *Escherichia coli* are the commonest pathogen isolated in patients with UTI (Flores- Mireles *et al*., 2015). In a similar study by Banda *et al*. (2020) the commonest isolates were also *Escherichia coli* follow by *Staphylococcus aureus.* Similar pattern was also reported by Kekelwa *et al*. (2021) but this was contrary to this study in which Coagulase negative *staphylococcus* was the next frequently isolated (22.7 %). The 18.2 % incidence rate reported for

klebsiella pnemoniae in this study reveals the fact that *Klebisella species* are achieving more prominence as aetiological agents of UTI than previously reported (Banda *et al*., 2020). According to Flores *et al*. (2015). *Staphylococcus aureus* is believed to cause cystis in mainly young sexually active females. It was also found to constitute a recognizable percentage in this study. This confirms that this organism may be achieving prominence as an aetiological agent of UTI in pregnant women. In this study, a total of 44 isolates were obtained from the 44 pregnant women with significant bacteriuria; only one bacterial specie was isolated from each subject, suggesting a mono-microbial nature of urinary tract infection in the study population.

In this study, the majority of isolated Gram-negative uropathogens showed high resistance rate against ampicillin and augmentin, moderate resistance against ceftriaxone, cefotaxime and cefuroxime. This is similar to the study of Olufadi *et al*. (2019) which found that isolates were highly resistant to augmentin and ceftriazone. However, all Gram negative bacteria isolates showed a relatively low level of resistance against nitrofurantoin and ceftazidime, that is, they were highly sensitive to nitrofurantoin. This is consistent with the study of Derese *et al*. (2016) where most of the isolates were sensitive to nitrofurantoin.

Gram-positive isolates showed full sensitivity (100.0 %) to nitrofurantoin. This finding is similar to the study of Bale *et al*. (2021) which reported that *Staphylococcus aureus* was 100% sensitive to nitrofurantoin. This is also similar to the study of Balakrishnan *et al*. (2015) which reported that 97 % of Gram positive organisms were sensitive to nitrofurantoin. Gram positive isolates were averagely sensitive to clindamycin, augmentin, and ampiclox. Gram-positive uropathogens showed a high level of resistance for penicillin. This is similar to the study of Kekelwa *et al*. (2021). Indiscriminate use of antibiotics, irrational administration of antibiotics and use of

antibiotics for poultry survival and poor administration of drugs may be responsible for this increasing antibiotic resistance (Yunusa *et al*., 2016).

Coagulase negative *staphylococcus was* found to be the most prominent Gram positive organism in the study 9(22.7 %) and these were phenotypically screen for Cefoxitin. Four 4(44.4 %) 0ut of nine (9) were resistant to Cefoxitin and 5(55.6 %) were sensitive to cefoxitin. This is contrary to the study of Garcia *et al*. (2019) in which 4 out of 12 (33.3 %) were sensitive and 8 out 12 (66.7 %) of CoNS were resistant to Cefoxitin. In this study, all the Cefoxitin resistant CoNS were molecularly screen and they were *Mec*A gene positive (100 %). This is similar to the study of Manon *et al*. (2020) in which 93 % Cefoxitin resistant CoNS were *Mec*A gene positive. This is contrary to the findings of Garcia *et al*. (2019) in which 75% of Cefoxitin resistant CoNS were *Mec*A gene positive. The variation could be as a result of differences in the species and strains of CoNS used in the study.

5.2 CONCLUSION

The isolation of bacterial pathogens from both symptomatic and asymptomatic pregnant women that are resistant to the commonly prescribed drug and the presence of *mec*A genes in the predominant isolates calls for early screening of all pregnant women to urinary tract infection. Early diagnosis and treatment of urinary tract infection during pregnancy can ensure the safety of the mother and fetus and also prevent complications during delivery.

5.3 RECOMMENDATION

- Urine culture screening should be included as part of the routine antenatal screening and empirical treatment should be discouraged in other to reduce the complications of UTI and spread of antimicrobial resistant strain.
- Further research work should be carried out on other genes that are responsible for antibiotic resistance to UTI pathogens.

5.4 CONTRIBUTION TO KNOWLEDGE

This study confirmed that screening of *Staphylococcus* species with Cefoxitin is enough to determine the resistance genes phenotypically because it is associated with *mec*A genotype, that is why all the Cefoxitin resistant Coagulase negative *Staphylococci* are *mec*A positive and Cefoxitin sensitive Coagulase negative *Staphylococci* are *mec*A negative.

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APPENDIX I

INFORMED CONSENT AND QUESTIONNAIRE ON URINARY TRACT INFECTION BACTERIAL PROFILE AND ANTIBIOTICS SUSCEPTIBILITY PATTERNS AMONG PREGNANT WOMEN.

Dear respondent,

This research is undertaken to study the "**Urinary Tract Infection Bacterial Profile and Antibiotics Susceptibility Patterns among Pregnant Women''**

It will involve collection of information that will help during the course of this research. It is very important to answer the questions correctly, and ensure your answers are valid to the best of your ability.

Your name is not needed on the questionnaire. Your participation in this study is voluntary. Your honest answers are very important, please be rest assured that the answers provided will be treated with absolute confidentiality.

Thank you for your time.

INSTRUCTION: Tick ( ) in the box corresponding to the appropriate response

CONSENT: I agree to participate in this study ( )

I decline to participate in this study ( )

APPENDIX II

QUESTIONNAIRE

Please kindly answer the questionnaire provided by ticking the appropriate space provided for the questions. Your answers and any other information provided will be treated with utmost confidentiality.

1. Initials……

2. Identification number……..

3. Age; 15 - 24 ( ) 25 - 34 ( ) 35 - 44 ( )

4. Religion: Christian ( ) Islam ( ) Traditional ( )

5. Tribe: Yoruba ( ) Hausa ( ) Igbo ( ) others (pleases specify) ( )

6. Marital status: Single ( ) Married ( ) Widowed ( ) Divorced ( )

7. Working status: Civil servant ( ) Self employed ( ) Student ( ) Housewife ( )

8. Educational level: Primary ( ) Secondary ( ) College and above ( ) No formal education ( )

8. Last menstrual period (LMP)……..

9. Do you have any of the following symptoms?

I.	Abnormal vaginal discharge	Yes ( )	No ( )
II.	Vaginal itching	Yes ( )	No ( )
III.	Burning or painful urination	Yes ( )	No ( )
IV.	Pelvic or lower back pain	Yes ( )	No ( )
V.	Frequent urination	Yes ( )	No ( )
VI.	Fever	Yes ( )	No ( )
VII.	Are you on any Antibiotics	Yes ( )	No ( )

APPENDIX III

MINISTRY OF HEALTH
KWARA STATE GOVERNMENT

MOH/KS/EU/777/466

15th February, 2021.

BABA RASHEEDAT TOYOSI

Department of Bioscience and Biotechnology (Microbiology),
College of Pure and Applied Science,
Malete, Kwara State.

APPROVAL TO CARRY OUT A RESEARCH TITLED: **"Urinary Bacterial Profile and Antibiotic Susceptibility Pattern Among Pregnant women Attending Antenatal Clinic in Selected Hospitals in Ilorin."**

Sequel to your request and the interest of the State Ministry of Health in Health-related research activities to improve the health of the citizens. I am directed to forward to you the approval of the Ministry of Health to carry out the dissertation as itemized in your protocol. This approval dates from 15th February, 2021 to 15th February, 2022.

2. You are mandated to acknowledge the State Ministry of Health by your presentations/publications and deposition of the final copy of the research findings/publications.

3. Best wishes in your research project.

Mr. ABDULMUTALIB A.A
For: Honourable Commissioner

CMD/Officer in charge.

.....

.....

Above for your information and necessary action, please.

P.M.B 1386, Fate Road, Ilorin, Kwara State
Telegram: GOV. ILORIN

www.kwarastate.gov.ng