

**ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF NOSOCOMIAL URINARY
TRACT PATHOGENS IN SURGICAL PATIENTS OF AHMADU BELLO UNIVERSITY
TEACHING HOSPITAL, SHIKA-ZARIA, NIGERIA**

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APPROVAL

This work meets the requirements for the partial fulfillment of the award of a Master of Science

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DECLARATION

I hereby declare that this thesis has been written by me. And, it has not been presented for the purpose of degree in any other University. Also, all references have being duly acknowledged.

Declarant.

DEDICATION

To the Glory of God.

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ABSTRACT

Urinary tract infections is one of the common infectious diseases diagnosed both in community and hospital settings. Due to rising antibiotic resistance among the uropathogens, it is important to have (local) community and hospital-based knowledge of the organisms implicated and their susceptibility pattern to choose correct treatment regime. This study was conceived with the objective to investigate the causative organisms, their antimicrobial susceptibility profile, and molecular characters of resistant isolates of UTI in the Surgical Wards of ABUTH Shika with a view to its effective management and control. This study was carried out in the Surgical Wards of ABUTH Shika between January and June 2014. A total of 182 urine samples of patients on admission who passed the inclusion criteria and consented to participate in the study were cultured aerobically. All isolates were identified by standard microbiological techniques and their antibiotic susceptibility was examined by disk diffusion method. Of the 182 samples examined, 107 (58.8%) showed significant growth. The positive culture of UTI was more among the females (67.9%) than the males (51.9%). The predominant age group was 21-30 while the age group with the least infection was 0-10.

In this study, *E.coli* was the predominant isolate constituting 48.6%, followed by *Staphylococcus* spp(21.5%), *Pseudomonas* spp(15.9%), *klebsiella* spp (9.3%), *Proteus* spp(2.8%) and *Candida* spp(1.9%) was the least encountered isolate. UTIs were associated with urinary catheters in 26 (63.4%) of the cases. Of the catheterized patients who showed positive UTI, those who had catheter for more than 3 days were found to be more likely to acquire nosocomial UTIs.

All the microorganisms were susceptible to amikacin, imipenem and gentamicin and resistant to amoxicillin. *E. coli* and *Staphylococcus* spp were observed to be very susceptible to amikacin, followed by imipenem and gentamicin.

Escherichiacoli was resistant to amoxicillin, nitrofurantoin, cotrimoxazole and ofloxacin while *Staphylococcus* spp showed resistance to amoxicillin and ofloxacin. *Pseudomonas* spp were sensitive to amikacin, imipenem and ciprofloxacin but displayed resistance to amoxicillin, ceftriaxone, cefuroxime, and cotrimoxazole. *Klebsiella* spp were susceptible to all antibiotics except amoxicillin. *Proteus* spp also displayed susceptibility to most of the tested antibiotics but showed resistance to amoxicillin, nitrofurantoin and ofloxacin.

Twelve (66.6%) of the 18 MAR isolates were found to be β -lactamase producers, implying that most of the bacterial species isolated developed resistance to the antibiotics as a result of β -lactamase production. Most of the isolates that produced β -lactamase were also found to harbor 1-2 plasmids. Comparison of plasmid sizes and numbers showed that some of the isolates had plasmid band of same number and sizes which indicates that they are likely of the same origin.

ABBREVIATIONS

ABUTH = Ahmadu Bello University Teaching Hospital

UTI = Urinary Tract Infection

NI = Nosocomial Infection

HAI = Hospital-Acquired Infection

CDC = Center for Disease Control and Prevention

WHO = World Health Organization

DNA = Deoxyribonucleic Acid

SSI = Surgical Site Infection

BSI = Bloodstream Infections

PBP = Penicillin Binding Protein

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CHAPTER ONE

1.0 INTRODUCTION

Nosocomial infection also called “hospital-acquired infection” is variously described as “an infection acquired in hospital by a patient who was admitted for a reason other than that infection” or as “an infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission” (WHO, 2002). This includes infections acquired in the hospital but appearing after discharge. Its source could be from other patients, health care workers, and/or hospital equipment and devices (Inyama *et al.*, 2011).

Nosocomial infection is a recognized public health problem world-wide with a prevalence rate of 3.0-20.7% and an incidence rate of 5-10% (WHO, 2002). It has become increasingly obvious that infections acquired in the hospital lead to increased morbidity and mortality and adding to economic burden. The cause of nosocomial infections might be endogenous or exogenous. Endogenous infections are caused by organisms present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel or medical devices (David and Famurewa, 2010; Samuel *et al.*, 2010).

Risk factors for hospital-associated infections are generally categorized into three areas:

- Iatrogenic risk factors: These includes invasive procedures (e.g., intubation, indwelling vascular lines, and urine catheterization), antibiotic use and prophylaxis.
- Organizational risk factors such as contaminated air-conditioning systems, contaminated water systems, staffing (e.g., nurse-to-patient ratio), and physical layout of the facility (e.g., open beds close together).

- Patient-related risk factors which include severity of illness, immunosuppression, and length of stay (Samuel *et al.*, 2010).

Hospital-acquired infections (HAI) are a major patient safety issue as a cause of preventable illness and death. As a result, the prevention and control of these infections have become a priority. Surveillance or monitoring of these infections has been recognized as key to the control of these infections (Ige *et al.*, 2011).

Nosocomial infection can affect any system of the human body; predisposition is dependent on the frequency of exposure of any system to microbial contamination via invasive medical procedures, presence of foreign body and susceptibility of the host. There are different types of nosocomial infections that can affect any system of the human body and the location of these infections usually depends on the nature of a patient's hospital procedure. The common ones include urinary tract infection (UTI), surgical site infection (SSI) respiratory tract infection (RTI) and bloodstream infections (BSI).

As the case for many other patient safety issues, health care associated infections create additional suffering and high cost for patients and their families. These infections prolong hospital stay, may lead to long term disability, and increase resistance to antimicrobials which represent a massive additional financial burden for health systems, generate high cost for patients and their families and cause unnecessary discomforts and/or even deaths. Such infections annually account for 37,000 attributed death in Europe and potentially many more that could be related, and they account for 99,000 death in USA (WHO, 2002). Though there has been decades of nosocomial infection surveillance and control world-wide, it still remains an important problem in hospitals. It is a major consequence of the failure/inadequacies of Infection Control Programme of a hospital (Inyama *et al.*, 2011).

It is well recognized that most hospitals in developing countries, especially Africa, have little or no effective infection control programme due to several factors such as lack of awareness of the problem, lack of personnel, poor water supply, erratic electricity supply, ineffective antibiotic policies leading to emergence of multiple-antibiotic resistant microbes, poor laboratory backup, poor funding and non-adherence to safe practices by health workers (Samuel *et al.*, 2010).

1.1 Statement of Research Problem

Hospital-acquired infections (HAIs) especially Urinary Tract Infections (UTIs), are largely preventable with implementation of effective control measures but have remained one of the leading cause of increased health care expenditures, morbidity and mortality in persons of all ages (Aschalew, 2011). Data from many hospitals and multi-hospital collaborative studies, has shown UTI to be one of the most common hospital-acquired infections and has been reported to be more serious because the causative bacteria are often resistant to antibiotic treatment and patients are often in poor general health (Iyad, 2008). Nosocomial urinary tract infections has been reported to account for more than 40% of the total hospital-acquired infections (WHO, 2002). Most of these infections result from direct introduction of urethral microorganisms at the time of catheterization and other instrumentation.

Worldwide, about 150 million people are diagnosed with UTI each year (Gupta *et al.*, 2001). In the United States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that UTIs account for over eight million cases annually and more than 1 million hospitalizations, with an overall annual cost in excess of \$1 billion (Gupta *et al.*, 2001). In most parts of sub-Saharan Africa as well as other developing parts of the world, UTIs are among the most common findings in everyday clinical practice showing high prevalence rates (Jombo *et al.*, 2011).

In Nigeria, increasing involvement of hospital-acquired over community-acquired uropathogens and their resistance to most antibiotics commonly used have been reported. Jombo *et al.* (2006) showed that the prevalence of nosocomial and community-acquired UTI in Jos (North Central, Nigeria) was 21.6% (12.3% nosocomial and 9.3% for Community). In Calabar (South Eastern, Nigeria), nosocomial incidence rate was 30.8% and in Kano (North western, Nigeria), it was 11.1% (Nwadioha *et al.*, 2010; Jombo *et al.*, 2011).

Few authors have reported significant high prevalence of UTI in the Surgical Wards than in other wards in hospital settings. For example, Afolabi *et al.* (2011) in their study reported that surgical ward contributed 38% of nosocomial infections while the ICU contributed the least accounting for 6.4%. In another study by Ige *et al.* (2011), it was reported that surgical and medical wards had the highest nosocomial infection incidence of 48.3% and 20.5% respectively. UTIs and surgical site infections were the most prevalent (43.9% and 30.7% respectively) with UTIs significantly higher in surgical and medical wards.

It has also been reported that antibiotics commonly used in treating UTIs are becoming less effective and species distribution and their susceptibility to antibiotics are changing all over the world (Saleh *et al.*, 2009). Although *E. coli*, *S. aureus* and *P. aeruginosa* remain important nosocomial pathogens responsible for urinary tract infection, there are reports of changing patterns in the prevalence of uropathogens;

For example, organisms such as Coagulase-negative *Staphylococci*, *Enterococci* and *Candida albican* are pathogens of increasing importance (Jarvis and Martone, 1992).

Furthermore, urinary pathogens especially from hospital patients have been known to include strains that are resistant to many of the commonly used antibiotics. According to the CDC, more than 70% of the bacteria isolated from hospital-associated infections are resistant to at least one

of the antibiotics most commonly used to treat them. As a result, continuous surveillance to monitor the prevalence of UTI and antimicrobial resistance among isolates from hospital patients is very necessary (Oladeinde *et al.*, 2011).

Effective management of nosocomial infections is often hampered due to lack of adequate facilities for proper microbial isolation as well as for their antimicrobial susceptibility testing. This often gives rise to urologic or other complications arising from untreated, and undetected as well as improperly treated UTIs (David and Famurewa, 2010; Dienye and Gbeneol, 2011).

Most of the published studies carried out on nosocomial UTI in the country are retrospective in nature and were done by reviewing records of urine microscopy, culture and sensitivity obtained from the microbiology and parasitology laboratories of the hospitals concerned (Sadiq *et al.*, 2006; Jombo *et al.*, 2006; Nwadioha *et al.*, 2010 and Jombo *et al.*, 2011;). The results obtained in these studies did not go beyond assessing the antimicrobial susceptibility patterns of the isolates implicated in UTI. Another gap that needs to be filled includes further analysis of resistant isolates by determining their molecular characteristics with a view to elucidating/tracing their origin of resistance.

Since the bacterial pathogens that predominate in a particular hospital ward often change in relation to newly admitted patients and altered therapy protocols, the knowledge of the bacterial agents that have been known and used in the past as problem in these wards might result in wrong selection of antibiotics. This study therefore, will assess the distribution of bacterial pathogens implicated in UTIs in surgical wards of ABUTH, Shika, their antimicrobial susceptibility patterns, resistance profiles and molecular characterization of the resistant isolates.

1.2 Justification of the Study

UTI is one of the most common complications affecting hospital patients, and in many cases are preventable (Jarvis and Martone, 1992). A high frequency of hospital-acquired UTI has been reported to be an evidence of inadequate or poor infection control practices by health care personnel, because one third of nosocomial infections are preventable (WHO, 2002).

A continuous review of the pattern of microbial isolates causing UTIs and their antimicrobial susceptibility patterns in clinical practice is essential because this would provide useful and up-to-date information about this common clinical disease, especially as concerns its correct and timely antimicrobial treatment; in order to forestall the irreversible damages that may follow thereafter (Ducel, 2002).

There is often regional variability of pathogens and their susceptibility patterns, and these are capable of changing over time. Thus, determining the aetiological agents and their antibiotic sensitivity patterns is needed to help in empirical treatment. UTI is generally treated empirically by general practitioners, for which they need to be aware of the locally prevalent strains and their sensitivity pattern. Over the last few decades, the resistance pattern of urinary isolates has been showing dramatic changes all over the world (Kadri *et al.*, 2004; Kemebradikumo *et al.*, 2012).

Evidence-based knowledge about the bacterial distribution implicated in UTIs in the hospital wards will be important for designing and implementing effective prevention and control measure to tackle nosocomial UTIs and other forms of hospital-acquired infections in the hospital. Moreover, the findings of the study will give an insight for health professionals in ABUTH, Shika and to enable them to take the utmost care for their patients by breaking the chains of transmission.

The study will also play a great role in describing antimicrobial susceptibility pattern of isolates and their resistance profiles to the common antibiotics used in the hospital and its environs.

Hence, the study finding will be important in setups where immediate culture and sensitivity tests are not available. A sound epidemiological knowledge of bacterial pathogens will also help in rationale selection of antibiotics for prophylaxis and empiric treatment options.

1.3 Objectives of the Study

1.3.1 Research aim

The aim of this study is to determine the incidence of nosocomial UTI in the surgical wards of Ahmadu Bello University Teaching Hospital Shika with a view for its effective management and control.

1.3.2 The specific objectives

- i. To isolate and characterize causative organisms of UTI from urine samples of surgical patients in ABUTH Shika using standard microbiological and biochemical methods.
- ii. To determine the antimicrobial susceptibility pattern of the isolates by the agar disc diffusion technique.
- iii. To carry out molecular characterization of the plasmid DNA in antibiotic resistant isolates.

1.4 Hypothesis

- **Null hypothesis (Ho)** – There is no incidence of nosocomial urinary tract infections in Surgical Wards of ABUTH, Shika.
- **Alternate hypothesis (Hi)** – There is significant incidence of nosocomial UTIs in surgical wards of ABUTH, Shika.

1.5 Limitation

- i. Only patients on admission in the male and female surgical wards of ABUTH, Zaria were included in the study. Thus, patients managed by invasive procedures in an out-patient or day-case basis that develop nosocomial UTI were excluded from the study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Urinary Tract Infections

Urinary tract infection (UTI) is considered one of the most common bacterial infections in humans in both community and hospital settings and affects both males and females of all ages. The cumulative incidence rate in children reaches 10%. In adults, almost half of all women experience at least one episode of UTI sometime in their lives (WHO, 2002).

The term urinary tract infection describes a variety of conditions relating to the parts of the urinary tract in which microorganisms are present in significant quantities. It is defined as the microbial invasion and subsequent multiplication on a part or the entire urinary tract.

The urinary tract consists of the urethra, prostate gland, urinary bladder, ureters, kidneys and seminal vesicles in males (Sadiq *et al.*, 2006; Alex *et al.*, 2012).

Urinary tract infections are mostly caused by retrograde ascent of bacteria of the faecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (Gupta *et al.*, 1999; Kolawole *et al.*, 2009).

Although urine is a fluid with a variety of molecules and salts some of which are waste products, it does not usually have bacteria as a normal component. Urine formed in the kidney is a sterile fluid that serves as a good culture medium for the proliferation of bacteria, therefore when a bacterium by any means gets into the bladder; it subsequently multiplies, and may cause UTI (Otajevwo, 2013).

2.1.1 Classification of UTI

Infections in the urinary system are often classified by the site of infection or the organ involved, presence or absence of symptoms, first or recurrent infection, and presence or absence of complicating factors.

I. Site of infection or the organ involved

This involves infection of the urethra (urethritis), the bladder (cystitis), or the kidney (pyelonephritis).

- a. Urethritis refers to an inflammation or infection of the urethra which is the passage way that connects the bladder with exterior of the body. It is usually caused by bacterial invasion mostly as a result of contamination from the anus especially in women because their urethra is shorter and is in close proximity to the vagina and anus.
- b. Cystitis is the inflammation of the bladder. It is caused by bacterial invasion of the bladder that often ascends from the urethra as a result of faecal contamination. Cystitis can often occur at the same time as urethritis. Sometimes cystitis and urethritis are referred collectively as a lower urinary tract infection.
- c. Pyelonephritis is an infection of the kidneys that is usually a result of an infection that has spread up the tract, as a consequence of bacteraemia or from an obstruction in the urinary tract. An obstruction in the urinary tract causes urine to back flow into the ureters and kidneys. UTIs that affect the kidney are referred to as an upper UTI. Usually, UTI is caused by bacteria that can also live in the digestive tract, in the vagina, or around the urethra, which is at the entrance to the urinary tract. Most often these bacteria enter the urethra and travel to the bladder and kidneys.

II. Presence or absence of symptoms

UTI can also be classified as symptomatic or asymptomatic.

- a. It is symptomatic when patient has a positive urine culture and is experiencing defined UTI signs or symptoms with no other recognized cause of the symptoms.
- b. When a person has no symptoms of UTI but significant numbers of bacteria ($\geq 10^5$ cfu/ml) have colonized the urinary tract, the condition is called asymptomatic UTI (also called asymptomatic bacteriuria). About 75% to 90% of patients with asymptomatic bacteriuria do not develop a systemic inflammatory response or other clinical manifestations suggesting infection. Asymptomatic cases of UTI are usually detected during routine urinary investigations. The condition is usually harmless in most people and rarely persists, although it does increase the risk for developing symptomatic UTIs.

III. Presence or absence of complicating factors/severity

UTIs are also often classified as complicated or uncomplicated and its clinical manifestation depend on the portion of the urinary tract involved, the aetiologic organisms, the severity of the infection, and the patient's ability to mount an immune response to it (Akinjogunla *et al.*, 2010).

- a. It is uncomplicated if it involves only the bladder and are not associated with the presence of foreign bodies or anatomic abnormalities. It is usually an infection in which there is no structural or neurological abnormality of the urinary tract that interferes with the normal flow of urine in the voiding mechanism of an otherwise healthy patient (Sadiq *et al.*, 2006).
- b. On the other hand if the infection involves the upper urinary tract, and the person has other disease such as diabetes mellitus, or is pregnant, or immunocompromised, it is considered complicated. A complicated UTI is mostly the result of a congenital abnormality or distortion of the tract, trauma, a stone, an indwelling catheter, an enlarged prostate, a neurological

deficit, or an infection of a normal tract in a patient with an underlying disease. Complicated UTIs may include pyelonephritis, urosepsis and the presence of foreign bodies or anatomic disorders (Sadiq *et al.*, 2006).

IV. First and recurrent infection

The initial UTI documented by a proper urine culture is the first infection. Recurrent infections can be further subdivided into unresolved bacteriuria, bacterial persistence, and reinfection (Steven *et al.*, 2006).

- a. Unresolved bacteriuria is most commonly caused by inadequate antimicrobial therapy. Sub-therapeutic levels of the antimicrobial agents may be as a result of noncompliance, malabsorption, suboptimal drug metabolism, and resistant uropathogens unresponsive to attempted therapy. In these cases, infection typically resolves after altering the therapy according to antimicrobial sensitivities determined by a proper urine culture.
- b. Bacterial persistence and reinfection occur after sterilization of the urine has been documented. In the case of bacterial persistence, the nidus of infection in the urinary tract is not eradicated. Characteristically, the same pathogen is documented on urine cultures during subsequent episodes of UTI despite negative cultures after treatment. The uropathogen frequently resides in a location that is shielded from antimicrobial therapy.

These protected sites are often anatomic abnormalities, including infected urinary calculi or foreign objects, such as urethral catheters which once infected may not be sterilized. Identification of the anatomic abnormality is essential because surgical intervention may be necessary to eradicate the source of infection.

c. In contrast to bacterial persistence, reinfection is characterized by different pathogens documented on proper urine cultures with each new UTI. Urinary tract infection most commonly occurs by periurethral colonization and by the faecal-perineal urethral route. Rarely, a fistula between the urinary tract and gastrointestinal tract serves as the source of reinfection.

In both males and females, UTI may be asymptomatic, acute, or chronic. Asymptomatic infection can be diagnosed by culture. Acute UTI is more frequently seen in females of all ages; these patients are usually treated on an outpatient basis and are rarely admitted to hospital. Chronic UTI in both males and females of all ages is usually associated with an underlying disease (e.g. pyelonephritis, prostatic disease, or congenital anomaly of the genitourinary tract) and these patients are most often hospitalized (WHO, 2002).

2.1.2 Epidemiology

According to data from numerous hospitals and multi-hospital collaborative studies, UTI is one of the most common infections and has been reported to be more serious because the bacteria that cause them are often resistant to antibiotic treatment and patients are often in poor general health (Iyad, 2008). UTIs are common with an estimated annual global incidence of at least 250 million cases, and are costly to both patients and healthcare funding systems (Ronald *et al.*, 2001). Worldwide, about 150 million people are diagnosed with UTI each year (Gupta *et al.*, 2001). In the United States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that UTIs account for over eight million cases annually and more than 1 million hospitalizations, with an overall annual cost in excess of \$1 billion (Gupta *et al.*, 2001).

In the paediatric population, boys are at greater risk before the age of three months but girls become at greater risk thereafter (Larcombe, 2012). Age group wise, women are at greater risk than that of men of developing a UTI. Differences in anatomy among other factors contribute to likelihood of the females being at a higher risk of UTI than males. The moist periurethral and vaginal areas promote the growth of uropathogens. The shorter urethral length also increases the chance for ascending infection into the urinary tract. As many as 40-50% of female report having at least one symptomatic UTI in their lifetime (Kunin, 1974). Young sexually active women are particularly prone to UTI because of sexual activity and pregnancy. Sexual activity increases the chances of bacterial contamination of female urethra because bacteria in the vaginal area are sometimes massaged into the urethra by the motion of the penis (Kolawole *et al.*, 2009). Also, in pregnancy, the various anatomical, physiological, and biochemical changes of pregnancy alongside the structural alterations caused by the gravid uterus in the pelvis places pregnant women at a high risk of developing UTI.

In older people generally, UTI is also the most common bacterial infection that is often asymptomatic. In the community, approximately 5-10% of older men and 10-20% of older women have asymptomatic bacteriuria. Asymptomatic bacteriuria affects between 4% and 7% of pregnant women (Andriole and Patterson, 1991). Older men become more susceptible to UTIs after 50 years of age, when they are more likely to develop prostate problems. In older women, biologic changes due to menopause them at particular risk for primary and recurring UTIs. With estrogen loss, the walls of the urinary tract thin out, weakening the mucous membrane and reducing its ability to resist bacteria (Ani and Mgbechi, 2008; Obiogbolu *et al.*, 2009).

Urinary tract infection is mainly caused by gram negative organisms that include *E.Coli* 60-70%, *Klebsiella* 10%, *Proteus* 5-10%, *Pseudomonas* 2-5%, gram-positive bacteria, group B

Streptococcus and *Staphylococcus* species (Patterson and Andriole, 1987). These organisms are mainly from the external genitalia, vagina, the genital tract, rectum and gastro-intestinal tract.

UTI occurs more frequently in developing countries among the low socio-economic populations. In the USA surveys estimated that there about 8 million cases of UTI annually with huge economic implications (Christensen, 2000). In most parts of sub-Saharan Africa as well as other developing parts of the world, UTIs are among the most common findings in everyday clinical practice showing high prevalence rates (Jombo *et al.*, 2011).

In Nigeria, reports from different parts of the country have shown varying high prevalence and incidence rates and high levels of multiple resistance of uropathogen isolates to quite a large number of antibiotics commonly used in treatment of UTI. Kolawale *et al.*, (2009) in Lafia, Nasarawa state (Northern Nigeria) showed an overall prevalence rate of 60%. The most common organism was *Escherichia coli*. The Gram negative bacteria showed susceptibility to Quinolones (ofloxacin, ciprofloxacin, pefloxacin) and Erythromycin, while the Gram positive isolates were susceptible to lincomycin, erythromycin and quinolones. Olaleinde *et al.* (2011) similarly reported 39.9% prevalence in Okada, Edo state (Western Nigeria). *Staphylococcus aureus* was the most predominant isolate causing UTI. The susceptibility profile showed that Ciprofloxacin was the most active antibacterial agent, while nalidixic acid, nitrofurantoin, sulphamethoxazole–trimetoprim, amoxicillin and amoxicillin–clavulanate were poorly active against the bacterial isolates. In a similar findings by Aniebo and Dike, (2009) in Rivers state (Eastern Nigeria) reported a prevalence rate of 51% amongst pregnant women in Port Harcourt and its environs was reported. *Escherichia coli* was the most common Gram-negative while *Staphylococcus* spp was the commonest Gram-positive organism isolated. Ciprofloxacin and nitrofurantoin were found to be the most effective antibiotics and tetracycline the least effective.

2.1.3 Causative Organisms

Many different microorganisms can cause UTIs, the most common pathogens are *Escherichia coli* and other *Enterobacteriaceae*, which accounts approximately 75% of the isolates. Less often is urinary tract infection caused by viruses, yeast and other intracellular organisms (Getenet and Wondewosen, 2011). In the United States, the predominant organisms implicated in nosocomial UTI were reported to be *Escherichia coli*, *Enterococci* and *Pseudomonas aeruginosa*. Among hospitals conducting intensive care unit (ICU) surveillance, the commonest pathogens were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative staphylococcus, *Candida* spp., *Enterobacter* spp and *Enterococci* (Jarvis and Martone, 1992).

In Nigeria the leading causes of acute and uncomplicated nosocomial UTIs in patients have been reported to be due to *Escherichia coli*, *Staphylococcus aureus*, *Proteus* spp, *Klebsiella* spp, *Pseudomonas aeruginosa*, and coagulase-negative staphylococci (Ehinmidu, 2003; Jombo *et al.*, 2006; Ojo and Anibijuwon, 2010,).

Escherichia coli is a member of the intestinal flora and normally does not cause any infection. It only becomes pathogenic when it reaches tissues outside the intestinal tract, particularly the urinary and biliary tract, lungs, peritoneum and meninges, causing inflammation at these sites. The urinary tract is the most common site of *E.coli* infection and more than 80% of all uncomplicated urinary tract infections are caused by *E.coli* infection. *Escherichia coli* bacteraemia is usually associated with urinary tract infection, especially in cases of urinary tract obstruction of any origin. *Escherichia coli* is the leading cause of both community acquired and nosocomial UTI.

Staphylococcus aureus is the most isolated Gram-positive uropathogen that is not very common. Some *Staphylococcus* spp. are members of the normal flora of the skin and mucous membranes

of humans. The organism may cause infections through tissue invasion and toxin production. *Staphylococcus aureus* bacteriuria can usually lead to subsequent invasive infection.

Pseudomonas aeruginosa is a Gram-negative non-Enterobacteriaceae. It is a frequent cause of nosocomial infections, UTI and bacteraemia. *Pseudomonas* infections can usually be very complicated. *Pseudomonas* UTIs are mostly hospital-acquired and are associated with catheterization. *Pseudomonas aeruginosa* is the third most common pathogen associated with hospital acquired catheter associated UTIs (Jarvis and Martone, 1992).

Proteus species are part of the Enterobacteriaceae family of Gram-negative bacilli. *Proteus mirabilis* causes 90% of *Proteus* infections. *Proteus mirabilis* is one of the most common causes of UTIs among individuals with long term indwelling catheters, complicated UTIs, and bacteraemia among the elderly.

Klebsiella species is another member of the family Enterobacteriaceae. They are opportunistic pathogens; the principal pathogenic reservoirs of infection are the gastro intestinal tract of patients and the hands of hospital personnel. *Klebsiella* accounts for 6 to 17% of all nosocomial UTIs and shows an even higher incidence in specific group of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus.

Candida species are less common uropathogens but are usually common in the hospital setting or among patients with predisposing diseases and structural abnormalities of the kidney. Candidiasis is a fungal infection of any of the candida species of which *C. albicans* is the most pathogenic species.

2.1.4 Pathogenesis

Bacterial Studies strongly support bacterial entry into the urinary tract by the faecal-perineal-urethral route with subsequent retrograde ascent into the bladder. Because of differences in

anatomy, females are at a higher risk of UTI than males. In females, the moist periurethral and vaginal areas promote the growth of uropathogens. The shorter urethral length also increases the chance for ascending infection into the urinary tract. Once the uropathogen reaches the bladder, it may ascend to the ureters and then to the kidneys (Kolawole *etal.*, 2009).

Additional pathways of infection include nosocomial infection through instrumentation, hematogenous seeding in the setting of systemic infection or a compromised immune system, and direct extension caused by the presence of fistulae from the bowel or vagina (Larcombe, 2012).

The urinary tract (i.e. kidney, ureter, bladder, and urethra) is a closed, normally sterile space lined with mucosa composed of epithelium. The main defense mechanism against UTI is constant antegrade flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra. This washout effect of the urinary flow usually clears the urinary tract of pathogens. The urine itself also has specific antimicrobial characteristics, including low urine pH, polymorph nuclear cells, e.t.c., which inhibits bacterial adherence to the bladder mucosal wall. UTI occurs when the introduction of pathogens into this space is associated with adherence to the mucosa of the urinary tract. If uropathogens are cleared inadequately by the washout effect of voiding, then microbial colonization potentially develops. Colonization may be followed by microbial multiplication and an associated inflammatory response.

2.1.5 Risk Factors

Risk factors for UTIs are factors that do not seem to be a direct cause of the disease, but seem to be associated in some way. Having a risk factor for UTIs increases the chances of getting a

condition but does not always lead to UTIs. Some factors that may contribute to urinary tract infections are:

a. Structure of the female urinary tract

Approximately, 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will have a UTI during their lifetime (Mansour *et al.*, 2009). In general, the higher risk in women is mostly due to the shortness of the urethra, which is 1.5 inches compared to 8 inches in men. Bacteria from faecal matter can be easily transferred to the vagina or the urethra (Okonko *et al.*, 2009).

b. Pregnancy

UTI in women is more prevalent during pregnancy, with a rate of 12-35%. It was reported that UTI is the most common complication of pregnancy and that it results in 5 times as many febrile episodes as viral infections (Jodi *et al.*, 1997). This is due to the various anatomical, physiological, and biochemical changes of pregnancy alongside the structural alterations caused by the gravid uterus in the pelvis. The increased progesterone levels lead to reduced ureteral, bladder, and urethral tone with dilatation and urine stasis. The increased glomerular filtration rate leads to increased urine volume, glycosuria and proteinuria that form good culture medium for bacteria; the gravid uterus compresses the ureter causing stasis and dilatation leading to infection in the kidneys (Larcombe, 2012).

c. Sexual intercourse

For many women, sexual intercourse seems to trigger an infection. Sexual activity increases the chances of bacterial contamination of female urethra because bacteria in the vaginal area are sometimes massaged into the urethra by the motion of the penis (Kolawole *et al.*, 2009).

d. Irregular urination

Infrequent visits to the toilet can cause a woman to be more susceptible to UTIs. The urine that stays in the bladder is more likely to grow bacteria and cause infections.

e. Birth control methods

Contraceptive use has been reported as a significant risk factor for acquiring UTI, with the barrier methods being more predisposing. Using barrier methods such as the diaphragm can lead to UTIs because diaphragms push against the urethra and make it harder to completely empty the bladder. Urine retention is more likely to grow bacteria and cause infections (Dienye and Gbeneol, 2011).

f. Insufficient water intake

This will cause less urination, which flushes out the urinary system. Bacteria that get into the bladder have more time to multiply and to take hold, causing an infection (Jombo *et al.*, 2006).

g. Inadequate personal hygiene

Bacteria from faecal matter or vaginal discharges can enter the female urethra because its opening is very close to the vagina and anus.

h. Catheters or tubes placed in the bladder

One of the most common sources of infection is catheters, or tubes, placed in the bladder. Urinary catheterization can cause UTI by introducing bacteria into the urinary tract. The risk for developing a UTI increases when long term catheterization is required (Jombo *et al.*, 2006).

i. Menopause

Studies indicate that between 20% and 25% of women over 65 years old have UTIs. In general, biologic changes due to menopause put older women at particular risk for primary and recurring UTIs. With estrogen loss, the walls of the urinary tract thin out, weakening the mucous membrane and reducing its ability to resist bacteria. Also, estrogen is essential to maintain the normal acidity of vaginal fluid (Ani and Mgbechi, 2008; Obiobolu *et al.*, 2009).

j. Age.

A man's risk for UTI increases with age. Men become more susceptible to UTIs after 50 years of age, when they are more likely to develop prostate problems.

k. Immunosuppressive medications

Medications that lower immunity can result to a decrease in general body resistance and increase risk for developing UTI. Diseases or health conditions that cause an increased risk of developing UTI e.g. anatomical problems, such as narrowing of the urethra or ureters, urine retention, vesicoureteral reflux (the abnormal flow of urine from the bladder back to the ureters), Kidney disorders, Kidney stones (which cause urinary tract obstruction that leads to infection), Spinal cord injuries, diabetes (causes changes to the immune system, damage to the kidneys and often results in sugar in the urine, that promotes the growth of bacteria). In men, an enlarged prostate may inhibit the flow of urine (Hackett, 2005).

2.1.6 Diagnosis

The diagnosis of UTI is suggested by the presentation of classical symptoms such as frequency and dysuria and by the presence of white blood cells and nitrates in the urine. Apart from the clinical manifestations of UTI, the diagnosis also depends on the portion of the urinary tract involved, the aetiologic organisms, the severity of the infection, and the patient's ability to mount an immune response to it (Akinjogunla *et al.*, 2010). However, acute and uncomplicated UTIs are usually established or confirmed via;

- a. Urinalysis:** Markers of infection in urinalysis can be used to provide additional evidence for a UTI. Certain bacteria, particularly Gram-negative bacteria, reduce nitrates to nitrites. Leukocyte esterase is produced by activated leukocytes. This chemical, however, depends on white blood cells, which may not always be present during a UTI. The presence of nitrites and leukocyte esterase serves as indirect evidence of a UTI, although it is not a replacement for urine culture (Jeff *et al.*, 2005).
- b. Urinemicroscopy:** Having collected the specimen, for routine examination, urine is subjected to a microscopic examination and culture. Urine microscopy reveals the presence of leukocytes, red blood cells, bacteria and "casts". These are proteinaceous deposits formed within the diseased kidney, and shed in the urine. They may be clear (hyaline casts) or may have leukocytes or red cells stuck to their surface. Urine sample containing squamous (skin-type) epithelial cells are considered contaminated (Fogazzi and Garigali. 2003).
- c. Urineculture:** This is deemed positive if it shows a bacterial colony count of greater than or equal to 10^5 cfu per ml of not more than two typical urinary tract organisms. Because urine culture typically requires at least 24 hours of incubation, urinalysis and urine microscopy are often used to guide initial empiric therapy (Graham and Galloway, 2001).

d. Antibioticsensitivity: This can also be tested with these cultures, making them useful in the selection of antibiotic treatment.

2.2. Nosocomial Infections

Based on the body systems involved, nosocomial infections are classified into thirteen major categories. These include:

- i. Urinary Tract Infections (UTI)
- ii. Surgical Site Infections (SSI)
- iii. Respiratory Tract Infections (RTI)
- iv. Blood Stream Infections (BSI)
- v. Bone and Joint infections (BJ)
- vi. Central Nervous System infections (CNS)
- vii. Cardio Vascular Infections (CVI)
- viii. Ear, Eye, Nose and Throat infections (EENT)
- ix. Gastro Intestinal infections (GI)
- x. Lower Respiratory Tract infections (LRT)
- xi. Reproductive Tract Infections (RTI)
- xii. Skin and Soft Tissue infections (SST) and
- xiii. Systemic Infections (SI).

The frequent sites of infection are urinary tract, surgical site, respiratory tract (pneumonia) and blood stream which together account for more than 80% of all hospital-acquired infections (Zeamanuel, 2007).

2.2.1 Nosocomial Urinary Tract Infections

Urinary tract infection (UTI) is a broad term used to describe both colonization of urine (bacteriuria), and invasion of structures in any part of the urinary tract. This has been reported to be the most common nosocomial infection constituting as much as 40% of all nosocomial infections (WHO, 2002). Infection may involve the kidneys (pyelonephritis), bladder (cystitis), prostate (prostatitis), and urethra (urethritis) or may affect the blood stream (bacteremia, septicemia). Once urine, or any part of the tract is infected, the entire system is at a risk of infection. Infection may be by bacteria, yeasts, mycoplasma, viruses or protozoa (Kaye, 1972; Kunin, 1974).

In this type of nosocomial infection, the nosocomial bacteria may be endogenous (i.e. the patient's flora-meatal, rectal, or vaginal colonization) in two thirds of cases, or exogenous (i.e. from other patients, hospital staff or equipment) with about 80% being associated with the use of an indwelling catheter (catheterization). The infection may be symptomatic UTI or asymptomatic bacteriuria. Symptomatic UTI has signs and symptoms of infection such as fever, urgency, frequency and dysuria. Infection in children less than one year may show additional symptoms like hypothermia, apnea, bradycardia and lethargy. In asymptomatic bacteriuria, urine culture is positive with 10^5 cfu per ml of urine with no more than two types of microorganisms. (Zeamanuel, 2007; Cheesbrough, 2000).

It is recognized that between 10% and 20% of patients who are hospitalized receive an indwelling urinary catheter especially in surgical procedures. Once catheter is in place, the risk

of bacteriuria is approximately 5% per day (Harry *et al.*, 1996). In long-term catheterization, bacteriuria is inevitable. Catheter-associated UTIs account for 40% of all nosocomial infections and are the most common source of Gram-negative bacteraemia in hospitalized patients (WHO, 2002).

Bacterial contamination of the hands of personnel, hand towels, fluids for bladder irrigation, bottles used for urine collection, bedpans, rectal thermometers, lubricants for catheter insertion, and also dust and air from the ward atmosphere have been implicated in hospital cross infections (Dutton and Ralston, 1957).

Ordinarily, the healthy urinary bladder is sterile, which means it does not have bacteria or other microorganisms in it. There may be bacteria in or around the urethra possibly because the urinary tract is in direct contact with the exterior, but normally these bacteria do not enter the bladder. During insertion, catheter can pick up bacteria from the urethra and allow them into the bladder, causing an infection to start. The most common sites of UTI are the urethra and the urinary bladder. From these sites, the infection may ascend into the ureters and subsequently involve the kidney.

UTIs are associated with less morbidity than other types of nosocomial infections, but can occasionally lead to bacteraemia and even death. It has been reported to be the second most common cause of bacteraemia in hospitalized patients (WHO, 2002). Infections are usually defined by microbiological criteria such as: a positive quantitative urine culture of $\geq 10^5$ cfu per ml and not more than two species of microorganisms. (CDC, 2006; Kolawole *et al.*, 2009).

2.2.2 Nosocomial Surgical Site Infections

Surgical site infection (SSI) is an infection that occurs at the incised site. It has been estimated to be the second most common type of hospital-acquired infection with incidence of 0.5 to 15%

depending on the type of operation and underlying patient status. Surgical procedures increase a patient's risk of acquiring an infection in the hospital. Surgery directly invades the patient's body, giving bacteria a way into normally sterile parts of the body. An infection can be acquired from contaminated surgical equipment or from healthcare workers. Following surgery, the surgical wound can become infected. Other wounds from trauma, burns, and ulcers may also become infected (WHO, 2002).

It can be classified as superficial incision, deep incision and organ/space surgical site infection.

a. The superficial incision involves the skin and subcutaneous tissue of the incision, and usually occurs within 30 days after the operative procedure.

b. Deep incision SSI involves deep soft tissues such as soft tissue of the chest, soft tissue of the leg, and facial and muscle layers. The infection is considered as hospital-acquired SSI if it occurs within 30 days of the operative procedure if no implant is left in place and the infection appears to be related to the operative procedure.

c. An organ/space SSI involves any part of the body, excluding the skin incision, fascia, or muscle layers that is opened or manipulated during the operative procedure (Zeamanuel, 2007).

The diagnosis of SSI is mainly clinical: purulent discharge around the wound or the insertion site of the drain, or spreading cellulitis from the wound. The infection is usually acquired during the operation itself; either exogenously (e.g. from the air, medical equipment, surgeons and other staff), endogenously from the flora on the skin or in the operative site or, rarely, from blood transfused at surgery. The main risk factor is the extent of contamination during the procedure, which is to a large part dependent on the length of the operation, and the patient's general condition (CDC, 2006).

2.2.3 Nosocomial Respiratory Tract Infections (Pneumonia)

Nosocomial pneumonia occurs in several different patient groups. The most important are patients on ventilators in intensive care units, where the rate of pneumonia is 3% per day (WHO, 2002). Bacteria and other microorganisms are easily brought into the throat by respiratory procedures commonly carried out in the hospital. The microorganisms come from contaminated equipment or the hands of health care workers. Some of these procedures are respiratory intubation, suctioning of material from the throat and mouth, and mechanical ventilation. The introduced microorganisms quickly colonize the throat area and form colony, but do not yet cause an infection. It is easy for a patient to inhale the microorganisms into the lungs where it cause infection (WHO, 2002; CDC, 2006).

The diagnosis of pneumonia may be based on clinical and radiological criteria which are readily available but non-specific: recent and progressive radiological opacities of the pulmonary parenchyma, purulent sputum, and recent onset of fever. Risk factors for infection include the type and duration of ventilation, the quality of respiratory care, severity of the patient's condition (organ failure), and previous use of antibiotics.

2.2.4 Nosocomial Bacteraemia

These infections represent a small proportion of nosocomial infections (approximately 5%) but case fatality rates are high — more than 50% for some microorganisms (WHO, 2002). Infection may occur at the skin entry site of the intravascular device, or in the subcutaneous path of an intravenous catheter.

Bacteria transmitted from the surroundings, contaminated equipment, or healthcare workers' hands can invade the site where the catheter is inserted. A local infection may develop in the skin around the catheter. The bacteria also can enter the blood through the vein and cause a

generalized infection. The main risk factors are the length of catheterization, level of asepsis at insertion, and continuing catheter care (CDC, 2006).

2.3 Epidemiology of Nosocomial Infections

Hospital-acquired infection has been estimated to be 5-10% in developed countries, and 10-30% in developing countries. The rate usually vary between countries, within the country, within the districts and sometimes even within the hospital itself, due to complex mix of the patients, aggressive treatment, and local practices (WHO, 2002).

In the United States, it is estimated that about 10% of hospital patients or more than 2 million hospitalized patients annually suffer from hospital infection with estimated annual death rate of 20,000, which may reach up to 88,000 deaths per year. The hospital-acquired infection follows basic epidemiologic patterns that can help to direct prevention and control measures. (Weinstein, 2005; Zeamanuel, 2007).

The pathogens that cause hospital infection have reservoirs, transmitted by predictable routes, and require a susceptible host. The reservoirs and sources of the infection could be the inanimate environment such as surgical instrument and the operative theatre, and the animate environment such as infected or colonized health care workers, patients and hospital visitors.

The possible mode of transmission for hospital-acquired infection are either cross-infection due to indirect spread of the pathogens via patients contact stay or autoinfection from an endogenous flora found in the patient.

Significant rates of nosocomial infection are reported from various studies conducted in different hospitals in different parts of the world. In the 1996 CDC report, the rate of catheter associated UTI was 34% followed by SSI (17%), BSI (14%) and Pneumonia (13%) (Weinstein, 1998). Similarly, in a study on the prevalence of hospital-acquired infection in 47 hospitals in 14

countries in four continents, the rates in the individual hospitals varied from 3% to 21% .The highest rate were seen in intensive care (13.3%), surgical (13.1%) and orthopaedic wards (11.2%). Children under the age of 1 year (infection prevalence of 13.5%) and adults over 64 years (prevalence of 12.0%) suffered more infection than others. In children, the commonest infections were of the lower respiratory tract, the skin and gastroenteritis. In the elderly, UTIs predominated (Mayon-White *et al.*, 1988).

In Morocco, the prevalence of HAI was reported as 17.8% (Jroundi *et al.*, 2007). The prevalence was higher in intensive care units (50%), and the most frequently infected sites were urinary tract (35%) and surgical wounds (32.5%). In India, Ginawi *et al.*(2014)reported a higher incidence rate of HAI (26.1%). In Ethiopia, an overall incidence of hospital acquired infections of 17.8% was reported (Melaku *et al.*, 2012) with 18.1% episodes of bacterial infections. Urinary tract and surgical site infections were detected in 48% and 45.6% of the cases, respectively.

In Nigeria, Ige *et al.* (2011) reported a prevalence of nosocomial infection of 2.6% over a 5 year period in Ibadan (South Western, Nigeria) with Surgical and Medical wards having the highest figures of 48.3%and20.5% respectively. UTI and SSI were the most prevalent (43.9% and 30.7% respectively) with UTIs significantly higher in surgical and medical wards, surgical site infections in obstetrics and gynaecology wards.

In another similar study covering the period of 2000-2009 by Afolabi *et al.* (2011) in Ile-Ife, (South Western, Nigeria), a prevalence 3.0% of HAI was reported. The highest prevalence of 9.0% was reported in the year 2006.The Intensive Care Unit (ICU) had the highest period prevalence of 14.7% followed by Orthopaedics ward (7.7%).Surgical ward contributed the highest number of cases with 38.3% followed by medicine (18.4%), Orthopaedics (13.9%),

Obstetrics and Gynaecology (13.6%), ICU (6.4%), paediatrics (5.0%), and neonatal contributed the least (4.3%).

2.4 Factors influencing the development of nosocomial infections

2.4.1 The microbial agent

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not necessarily result in the development of clinical disease. Other factors also influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient's own flora (endogenous infection). Some organisms may be acquired from an inanimate object or substances recently contaminated from another human source (environmental infection).

Before the introduction of basic hygienic practices and antibiotics into medical practice, most hospital infections were due to pathogens of external origin (foodborne and airborne diseases, gas gangrene, tetanus, etc.) or were caused by microorganisms not present in the normal flora of the patients (e.g. diphtheria, tuberculosis). Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients (*Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, Enterobacteriaceae) (WHO, 2002).

2.4.2 Patient susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, and diagnostic and therapeutic interventions.

The extremes of life (infancy and old age) are associated with an inadequate/decreased resistance to infection. Patients with chronic disease such as malignant tumours, leukaemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) also have an increased susceptibility to infections with opportunistic pathogens. The latter are infections with organism(s) that are normally innocuous, e.g. part of the normal bacterial flora in the human, but may become pathogenic when the body's immunological defenses are compromised. Immunosuppressive drugs or irradiation may also lower resistance to infection. Injuries to skin or mucous membranes bypasses natural defense mechanisms poses risk of infection (Borby *et al.*, 1998).

Malnutrition is also a risk factor. Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, intubation/ventilation and suction and surgical procedures increase the risk of infection. Contaminated objects or substances may be introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract.

2.4.3 Environmental factors

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for other patients and staff. Patients who become infected in the hospital are a further source of infection. Crowded conditions within the hospital, frequent transfers of patients from one unit to another, and concentration of patients

highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials used in one procedure which subsequently contact susceptible body sites of other patients. In addition, new infections associated with bacteria such as waterborne bacteria (atypical mycobacteria) and/or viruses and parasites continue to be identified (Haley *et al.*, 1985).

2.4.4 Bacterial resistance

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multiple antibiotic resistant strains of microbes; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The widespread use of antimicrobials for therapy or prophylaxis is the major stimulant of resistance.

Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. Multiple resistant *Klebsiella* and *Pseudomonasaeruginosa* are prevalent in many hospitals (Kollef *et al.*, 2000).

This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable

2.5 Impact of Nosocomial Infections

Hospital-acquired infections, though largely preventable, progress rapidly and the organisms are frequently resistant to antibiotics. According to available evidence, impact of hospital acquired

infections include prolonged hospital stay, long-term disability, increased resistance of microorganisms to antimicrobials, massive additional financial burden for health systems, high costs for patients and their family, unnecessary deaths and also reduces the chances of treatment for others (David and Famurewa, 2010).

The significance of nosocomial infection lies not only in its ability to substantially alter morbidity and mortality statistics, but also in its economic implications. The increased length of stay for infected patients is the greatest contributor to cost (WHO, 2002; Samuel *et al.*, 2010).

It has been estimated that between 5 and 10 percent of patients admitted to acute care hospital in developing nations acquire one or more infections, and the risks are increasing annually. This is by far more serious in low-resource countries that are unable either to prevent and control nor possess financial and technological skills to manage such situations (Afolabi *et al.*, 2011).

Some of the factors that have contributed to the rise in nosocomial infections include the followings;

- Increased use of modern medical methods.
- Patient populations (that are getting older or are infected with chronic diseases).
- Increasing proportions of patients with immune-compromised conditions or diseases (principally HIV patients).
- The misuse and abuse of antibiotics contribute largely to increased incidence of antimicrobial-resistant pathogens.
- Prolong stay of patients increases the likelihood of transmission of such nosocomial pathogens to other patients.

2.6 Control Measures and Management of Nosocomial Infections

Not all hospital-acquired infections are preventable. The very old, the very young, those undergoing invasive procedures and those with suppressed immune systems are particularly susceptible but a large percentage are preventable with implementation of effective control measures.

The Center for Disease Control and prevention (CDC) has estimated that 36% of nosocomial infections can be prevented if health care workers adhere to specific infection control guidelines when caring for patients (CDC, 2006).

In the developing countries where the burden is estimated to be highest, information on surveillance activities in the prevention and control of HAI is not often available. The developments in the surveillance and monitoring of HAI in these countries also lag behind those of more industrialized countries and the mandatory surveillance requirements as it obtains in some other countries have largely not being implemented. As a result of this relative lack of information, there has been no significant change to improve existing surveillance systems (Pittet *et al.*, 2008; Ige, 2011). In Nigeria, the inadequate knowledge of the risks of HAI and the measures of risk reduction have limited control activities. To prevent HAIs it is necessary to identify sources and modes of transmission of infection and to implement data driven prevention guidelines and practices (Ige, 2011).

The CDC's study on the Efficacy of Nosocomial Infection Control stated that hospitals can reduce infection control rates by approximately one third when the following four key infection control components are implemented:

- Existence of an effective hospital epidemiologist
- Presence of an infection control practitioner for every 250 beds
- Existence of an active surveillance mechanism

- Ongoing control efforts

In addition, the CDC (2006) also recommend the adoption of hand washing as an important measure for preventing the spread of nosocomial bacterial pathogens. Most guidelines recommend hand washing before and after contact with patients, before invasive procedure and after contact with contaminated inanimate objects.

The major overall advances in control of infectious diseases have been immunization and improved hygiene, particularly hand washing. It is imperative to uphold the activities that ensure adequate infection control practices where healthcare is provided. This may include but not limited to the followings;

- Provision of facilities and equipment that make it possible for the health care workers to maintain good infection control practices. Standards (policies and guidelines) for procedures or systems used within the healthcare setting and implementation of educational programmes for all personnel in the use of such standards should be available and should not be neglected.
- Establishment of surveillance systems that identify problem areas early enough.
- Putting in place a policy for the prudent use of antibiotics and work to ensure adherence to the policy. This is the most desirable as it has truly been established that unnecessary exposure of bacterial to antimicrobial agents results in selective pressure with ultimate emergence of resistant forms.
- Production of guidelines for cleaning, disinfection and decontamination and work to ensure adherence to those guidelines (Yvette, 2006).

In most parts of sub-Saharan Africa as well as other developing parts of the world, UTIs are among the most common findings in everyday clinical practice (Jombo *et al.*, 2011). Effective

management of these infections is often hampered by the lack of adequate facilities for proper microbial isolation as well as for their antimicrobial susceptibility testing.

This often gives rise to urologic or other complications arising from untreated, undetected as well as improperly treated UTIs (Sadiq *et al.*, 2006).

Management of UTI is often empirical without recourse to urine culture or susceptibility testing to guide therapy. Though, improved antimicrobial drug stewardship and intervention for resistance control is often cited in this part of the world, they are inadequately implemented (Okezie and Uchenna, 2011).

A continuous review of the pattern of microbial isolates causing UTIs and their antimicrobial susceptibility patterns in clinical practice is essential. This would provide useful and up-to-date information about this common clinical disease, especially as concerns its correct and timely antimicrobial treatment; in order to forestall the irreversible damages that may follow thereafter (Ozumba, 2005).

2.6.1 Prevention

a. Prophylaxis: Prophylactic therapy goal is to decrease chance for renal damage. A daily low dose prophylactic antibiotic reduces the rate of infection in patients suffering from recurrent infections. However, this does not alter the underlying propensity to develop infections once the prophylaxis is stopped.

b. Taking plenty of water: Water helps flush your urinary tract, so drinking plenty of plain water daily prevents bacteria from lodging and multiplying in the urinary tract.

c. Frequent urination: Don't hold urine when you need to urinate. Holding urine when you need to go can help any bacteria that may be present develop into a full-fledged urinary tract infection.

d. Personal hygiene: Taking showers regularly helps prevent bacteria from entering the urethra and causing a UTI. Washing the genital area both before and after sexual intercourse helps prevent transferring bacteria to the urethra or vaginal area, which can create a breeding ground for a UTI. Also, avoid wiping from back to front after a bowel movement. This is especially important to help prevent bacteria from the anus from entering the vagina or urethra.

2.7 Health and Cost Implication

UTI is one of the most common causes of hospitalization and referral to outpatient, having an estimated figure of 150 million per annum worldwide (Akinjogunla *et al.*, 2010)

UTIs are one of the leading causes of increased health care expenditures, morbidity and mortality in persons of all ages worldwide. Although it is associated with less morbidity than other infections, it can occasionally lead to bacteraemia and death. (Okonko *et al.*, 2009).

In the United States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that UTIs account for over eight million cases annually and more than 1 million hospitalizations, with an overall annual cost in excess of \$1 billion (WHO, 2002; Abdulhadi *et al.*, 2008).

Majority of UTIs are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia (Manikandan *et al.*, 2011).

UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and has also been reported to be the second most common cause of bacteraemia in hospitalized patients (Kolawole, 2009).

In this part of the world, effective management of UTI is often hampered by the lack of adequate facilities for proper microbial isolation as well as for their antimicrobial susceptibility testing.

This often gives rise to urologic or other complications arising from untreated, undetected as well as improperly treated UTIs (Sadiq *et al.*, 2006).

2.8 Antibiotic Management

Treatment of urinary tract infections (UTIs) is becoming difficult due to the increasing trend of antibiotics resistance and this may necessitate an up to date knowledge of resistance pattern (Jombo *et al.*, 2011). Adequate treatment and control of these conditions need a good knowledge of the bacteria species involved and their susceptibility to antimicrobial agents followed by prompt therapeutic intervention to prevent cases of asymptomatic UTI from becoming symptomatic with resultant damage. (Obiogbolu *et al.*, 2009)

Majority of the treatments is done completely empirically, the knowledge of the organisms, their epidemiological characteristics and their antibacterial susceptibility is therefore mandatory. Data obtained are essential to optimize the treatment and avoid the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failure. Prompt therapeutic intervention is also essential to prevent cases of asymptomatic UTI from becoming symptomatic with resultant damage (Sadiq *et al.*, 2006).

The flouroquinolones (ciprofloxacin, ofloxacin and pefloxacin), aminoglycosides (Gentamicin) and the cephalosporins have been reported in most studies in Nigeria to be the most sensitive antibacterial agent against majority of the uropathogens (Okezie and Uchenna, 2011; Sadiq *et al.*, 2006).

Earlier researches also revealed that the success of ciprofloxacin could be due to its broad spectrum activities, its bactericidal activity on organisms both in replicating and resting state and its ability to disrupt DNA functions leading to the death of the bacterium (Ojo and Anibijuwon, 2010).

Ciprofloxacin when compared to most antibiotics frequently used is relatively expensive. This probably had restricted its procurement and indiscriminate use by the various residents investigated, thereby reducing emergence of resistant bacterial strains and making the organisms susceptible to it (Ehinmidu, 2003;Inabo and Obanibi, 2006; Chedi *et al.*, 2009; Ojo and Anibijuwon, 2010; Oladeinde *et al.*, 2011).

2.8.1 Antibiotics used in treatment of UTI

Antimicrobial therapy has been the first and main medical intervention treatment choice against urinary tract infections and other infectious diseases caused by bacterial pathogens. Antimicrobial activity is due to the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell. The three main bacterial targets of antimicrobial agents are cell wall, protein, and nucleic acid biosynthesis. Various mechanisms neutralizing the action of antimicrobial agents have developed in bacteria. The most widespread antimicrobial resistance mechanisms are enzymatic drug inactivation, modification or replacement of the drug target, active drug efflux, and reduced drug uptake (Kollef *et al.*, 2000).

a. Quinolones

Nalidixic acid, the first (generation) quinolone, was discovered in 1962 and introduced for clinical use in the treatment of urinary tract infections in humans in 1967. Modification of the chemical structure with a fluorogroup improved and expanded the antibacterial efficacy of quinolones. Fluoroquinolones became available for use in the mid-1980s. Quinolones are purely synthetic broad-spectrum antimicrobials that exert their antibacterial effect by inhibiting certain bacterial topoisomerase enzymes. Topoisomerases are enzymes that unwind and wind DNA in

the DNA replication process. Examples of quinolones include ofloxacin, ciprofloxacin, norfloxacin, and levofloxacin. Ciprofloxacin is one of the most used fluoroquinolones, but ofloxacin and levofloxacin are also prescribed in general practice (Donadio *et al*, 2010).

Ciprofloxacin is a fluoroquinolone, a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division (Drlica and Zhao., 1997).

Resistance to quinolones is mainly due to two mechanisms: target alterations (mutations in the code of the topoisomerases) and decreased accumulation inside the bacteria due to impermeability of the membrane or due to increased efflux. Furthermore, plasmids conferring quinolone resistance have been described and were an important finding in the explanation of the rapid emergence of quinolone resistance. The strong association between resistance to quinolones and resistance to other agents also suggest the importance of plasmids in this process. Today, several plasmid-mediated quinolone resistance (PMQR) genes have been discovered (Cirz *et al*, 2005).

b. β -lactam/ β -lactamase Inhibitor combinations

B-lactam antimicrobials are among the most commonly prescribed drugs worldwide and include penicillins, narrow-and extended-spectrum cephalosporins, monobactams and carbapenems. This group of antimicrobials share a structural feature, β -lactam ring. B-lactam antimicrobials exert their effect by interfering with cell wall synthesis through binding to the penicillin binding proteins (PBPs) in the cytoplasmic membrane of the bacterium. Covalent binding to PBPs interferes with synthesis of the cell wall and ultimately leads to cell death. B-lactam

antimicrobials have an especially lethal effect on Gram-positive bacteria and less so for Gram-negative bacteria as their cell wall has an outer membrane (Poole, 2004).

Resistance to β -lactam antimicrobials arises through one or more of the following mechanisms:

(1) PBP modifications (mutations or the acquisition of supplementary foreign genes encoding new PBPs), (2) decreased permeability due to alterations in the porins, (3) the production of β -lactamases inactivating the antimicrobial.

To increase the utility of β -lactam antimicrobials, this antimicrobial was combined with β -lactam inhibitors. The β -lactam inhibitor is designed to overwhelm all β -lactamases and bind irreversibly to them, allowing the β -lactam antimicrobial to work. Clavulanic acid was the first β -lactam inhibitor introduced in clinical medicine in 1970, and later sulbactam and tazobactam were developed as synthetic compounds (Drawz and Bonomo 2010).

Until recent years, the standard treatment for a UTI was 10 days of amoxicillin, a penicillin antibiotic, but it is now ineffective against *E. coli* bacteria in up to 25% of cases. A combination of amoxicillin-clavulanate (Augmentin) is sometimes given for drug-resistant infections. Amoxicillin or Augmentin may be useful for UTIs caused by Gram-positive organisms, including *Enterococcus* species and *S. saprophyticus* (Poole, 2004).

Cephalosporins are also alternatives for infections that do not respond to standard treatments or for special populations. They are often classed as first, second, or third generation. Cephalosporins used for treatment of UTIs include cephalexin (Keflex), cefadroxil (Duricef) cefuroxime (Ceftin),loracarbef (Lorabid), and cefixime (Suprax), among others.

Mechanisms of resistance to the combination β -lactam/ β -lactam inhibitors in *E.coli* are through the production of β -lactamases not susceptible to the inhibitors or enzyme hyperproduction. Penicillin is a β -lactam antimicrobial agent. Co-amoxyclav contains the combination of

amoxicillin and potassium clavunate. This combination has an increased spectrum of action including Gram-negative bacteria (Pitout *et al.*,1997; Drawz and Bonomo 2010).

c. Aminoglycosides

Aminoglycosides (gentamicin, tobramycin, amikacin) are given by injection for very serious bacterial infections. They can be given only in combination with other antibiotics. Gentamicin is the most commonly used aminoglycoside for severe UTIs. Gentamicin is an aminoglycoside antibiotic composed of a mixture of related gentamicin components and fractions and is used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms (Moulds and Jeyasingham, 2010)

The antibacterial actions of Gentamicin involve two possibly synergistic effects. First, the positively charged aminoglycoside binds to negatively charged sites on the outer bacterial membrane, thereby disrupting membrane integrity. It is likely that the aminoglycoside-induced bacterial outer membrane degradation accounts for the rapid concentration dependent bactericidal effect of these compounds. Second, gentamicin binds to various sites on bacterial 30S ribosomal subunits, disrupting the initiation of protein synthesis and inducing errors in the translation of messenger RNA to peptides. They also bind to sites on bacterial 50S ribosomal subunits, although the significance of this binding is uncertain (Craig and Stitzel, 2004).

d. Carbapenems

Imipenem (N-formimidoyl thienamycin) is the first carbapenem antimicrobial agent developed for clinical use. It is a semisynthetic derivative of thienamycin which is produced by *Streptomyces* spp. Imipenem binds to PBP1 and PBP2 of gram negative and gram positive bacteria, causing cell elongation and lysis. It is stable toward most plasmid or cromosomally

mediated beta lactamases. Imipenem has the widest spectrum of antibacterial activity of the currently available antibiotics.

It has excellent invitro activity against aerobic gram positive species. More than 90% of *Enterobacteriaceae* are susceptible to imipenem, including those resistant to other beta lactams and aminoglycosides. Other members of this group are panipenem, meropenem etc (Marrie, 2002).

e. Tetracyclines

Tetracyclines act by binding to the 30S subunit of the ribosome at the A-site. During protein biosynthesis, the new t-RNA with the amino acid attempts to bind to A-site of the ribosome. However, since the A-site is blocked by the tetracycline, the aminoacyl-tRNA cannot bind to it. Thus without the sequential attachment of the tRNA at the A-site, protein biosynthesis cannot occur. By inhibiting protein biosynthesis tetracyclines cause cell death of the bacterial cell. Tetracycline are broad spectrum agents that inhibit a wide variety of aerobic and anaerobic gram positive and gram negative bacteria. Tetracycline are used to treat many different bacterial infections, such as urinary tract infections, acne, gonorrhoea, chlamydia, and others. Tetracycline are incompletely absorbed orally. They usually affected by meal as milk and cheese. Common members of this group include doxycycline, tetracycline, and minocycline. Doxycycline is commonly used for treatment of UTI (Poole, 2004).

Resistance to the tetracyclines results from changes in permeability of the microbial cell envelope. In susceptible cells, the drug is concentrated from the environment and does not readily leave the cell. In resistant cells, the drug is not actively transported into the cell or leaves it so rapidly that inhibitory concentrations are not maintained. This is often plasmid-controlled. Mammalian cells do not actively concentrate tetracyclines (Marrie, 2002).

f. Sulphonamides

Sulfonamides are synthetic bacteriostatic antibiotics that competitively inhibit conversion of *p*-aminobenzoic acid to dihydropteroate, which bacteria need for folate synthesis and ultimately purine and DNA synthesis. Humans do not synthesize folate but acquire it in their diet, so their DNA synthesis is less affected. Most sulfonamides are readily absorbed orally and are distributed throughout the body. They are metabolized mainly by the liver and excreted by the kidneys. Most sulfonamides are rapidly excreted and very soluble in urine so they are used to treat infections of the urinary tract. Sulfonamides are active against a broad spectrum of gram-positive and many gram-negative bacteria. Trimethoprim-sulfamethoxazole is a combination that synergistically interferes with folate metabolism and is used frequently in the treatment of uncomplicated UTI (Marrie, 2002).

Both chromosomal and R-factor-mediated resistance to sulfonamides have been attributed to altered forms of dihydropteroate synthetase (for which sulfonamides have a lowered affinity). Because sulfonamides act in a competitive fashion, overproduction of PABA can preclude inhibition of dihydropteroate synthetase.

g. Nitrofurans

Nitrofurans are synthetic antimicrobials used to treat UTI. Nitrofurans have a bacteriostatic effect mediated through the inhibition of enzyme synthesis and a bactericidal effect which causes lesions in the DNA for which the normal enzymatic repair is also inhibited by nitrofurans. Nitrofurans' bactericidal effect is activated by its rapid reduction inside the bacterial cell. Although the specific mode of action of nitrofurantoin is still not fully understood, studies of *E.coli* extracts have shown that strains resistant and susceptible to nitrofurans differ in their

ability to reduce the compounds, suggesting that nitrofurans need to be activated by reduction to exert their antimicrobial effect.

Nitrofurans became available in 1953 as nitrofurantoin, furazolidone and nitrofurazone. Nitrofurantoin is taken orally and prescribed for use in treatment of uncomplicated UTIs. Resistance to nitrofurans occurs by step-wise mutations where increased resistance is accompanied by a decrease in the activity of their reductive capacity (Donadio *et al.*, 2010).

2.9 Antibiotic/Drug Resistance

The use of antimicrobials combined with improvements in sanitation, housing, and nutrition, and the introduction of widespread immunization programmes, has caused a dramatic decline in the often fatal diseases that were previously untreatable. These gains are today seriously jeopardized by another development: the emergence and spread of microbes that are resistant to these, once described as “Wonder drugs” (W.H.O, 2002).

The emergence and spread of antimicrobial resistance is a complex problem involving antimicrobial agents, bacterial species, resistant genes and various mechanisms of resistance. Antimicrobial resistance is a relative term and in its clinical definition a strain is defined resistant when it survives antimicrobial therapy.

This resistance can be intrinsic, due to a structural or functional trait which diminishes the effect of a particular drug by all members of a bacterial species (tolerance). Acquired resistance, on the other hand, is a major threat to health because it is the source of the emergence and spread of resistance in normally susceptible bacterial populations and consequently may lead to therapeutic failure.

Bacterial resistance was present before antimicrobials were used. This intrinsic resistance is the innate ability of a bacterial species to resist the activity of a particular antimicrobial agent

through its inherent structural or functional characteristics. Acquired bacterial antimicrobial resistance is a result of a genetic change, which occurs in the presence or absence of the antimicrobial. This genetic change can be the result of a mutation or horizontal exchange of genetic material (transformation, transduction and conjugation). Whereas transformation and transduction are processes limited to closely related bacteria belonging to the same species or genus, conjugation is not restricted like this and is therefore likely to play a much larger role in the spread of antimicrobial resistance. Conjugation is a mechanism of horizontal genetic material transfer, most often with plasmids or transposons, due to which resistance can be passed on to other species.

These genetic events occur in the presence or absence of antimicrobials. Resistance of urinary pathogens to commonly prescribed antibiotics has been reported, and multi-drug resistant uropathogenic bacteria have been isolated from urine specimens in different parts of the world.

Antibiotic resistance is an alarming problem worldwide and it is spreading rapidly due to overuse, self-medication, and nontherapeutic use of antimicrobials. It is a serious medical problem because of very fast rise and spread of mutant strains that are insusceptible to medical treatment (Jombo *et al.*, 2011; Aluyi *et al.*, 2013).

Most of the prevalent pathogens of UTIs have been found to be resistant to most chemotherapeutic agents. Development of resistance to these antimicrobial agents in UTI cases will affect future treatment and management of the infection with these drugs if not controlled (Okonko *et al.*, 2009).

The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable

quality in circulation (Manikandan *et al.*, 2011). In Nigeria antibiotics are readily available as over the counter drugs and can be obtained easily without a prescription from medical personnel. Self-medication is a common practice among Nigerians especially where there is no ready access to a medical facility or when it is considered convenient (Okezie and Uchenna, 2011).

An additional issue with respect to UTIs is recurrent infections which are more likely to be associated with resistant organisms due to exposure to antimicrobial agents in the treatment of the previous episode. Culture and susceptibility test results and antimicrobial treatment of previous episodes of UTI may also be able to guide empiric therapy in subsequent episodes.

Higher rates of multiple resistances among hospital acquired bacterial isolates has also been reported and was attributed to established incessant exposure of bacteria to drugs in hospital settings (Jombo *et al.*, 2006; 2011).

Also, prescription of antibiotics without laboratory guidance as well as over the counter sales of antibiotics without prescription are rife has been reported in the Nigerian setting which has contributed to emergence of resistant strains to the commonly used antibiotics (Oladeinde *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Laboratory Media

The following laboratory media were prepared according to manufacturer's specification: Simmon Citrate, MacConkey, Saboraud Dextrose, Nutrient, Eosine Methylene Blue, and Muller Hinton agar which are products of Oxoid Ltd., England. Nutrient Broth and Peptone Water are products of Fluka Spain. Cetrinide, Urea and Triple sugar Iron agar are products of Biotech Laboratory Ltd., UK. Agarose gel is a product of Schwarz/Mann, England.

3.1.2 Antibiotic Discs

The following antibiotics were obtained from Oxoid Ltd., Basingstoke, UK:

Amikacin (10 µg), Gentamicin (10 µg), Ofloxacin (5 µg), Ciprofloxacin (5 µg), Amoxicillin (10 µg), Cotrimoxazole (25 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Nitrofurantoin(300 µg), and Imipenem(10 µg).

3.2 METHODS

3.2.1 Study Area

The study was conducted at Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria, Nigeria, a tertiary health care facility that serves as a referral Centre for specialized health services for Kaduna State, and some other states in the northern part of the Country.

The study was carried out in the Male and Female Surgical wards of the hospital. The study was limited to only inpatients in the Surgical Wards (Male and female) of ABUTH, Shika between the period of January to June, 2014.

3.2.2 Study population

All patients that were admitted to the Male and Female Surgical Wards of ABUTH, Shika that passed the inclusion criteria during the period of study were recruited.

3.2.3 Inclusion criteria

- Patients on admission for more than 24 hours in the Male and Female Surgical wards irrespective of initial diagnosis other than UTI.
- Patients with or without risk factors for UTI and
- Patients who consented to participate by signing the consent form.

3.2.4 Recruiting and consenting

The study involved recruitment of all patients admitted in the Male (A and B section) and Female (A section) Surgical Wards of ABUTH, Shika after applying the inclusion criteria.

The purpose of the study and other ethical concerns were explained to the patients. A written consent form (Appendix VI) was availed to and signed by the patient for accepting to participate.

3.2.5 Exclusion criteria

- Patients who or whose relatives declined to sign consent form.
- Those already diagnosed with UTI at the time of admission or were primarily admitted for the treatment of UTI.
- Patients initially enrolled in the study but were discharged before the completion of sample collection were removed from the list of participants.

3.2.6 Ethical clearance

Ethical clearance certificate (Appendix IV) was obtained from the Health Research Ethics Committee of the Hospital to carry out this study after presentation and defense of research proposal.

3.2.7 Sample collection

The clean catch mid-stream technique was employed to collect early morning urine samples. Following signed consent of the patient, urine sample was collected in a sterile container.

a) Female patients – Patients were instructed to properly position their thigh to spread the labia with one hand and cleanse the area with soaped swabs with the other hand, and then pass a small amount of urine into toilet, and finally urinate into the wide mouthed sterile container.

b) Male patients – Patients were instructed to wash hands first, and pass the clean catch midstream urine into the bottle.

c) Catheterized patients - Urine was collected through the draining portal of the urinary catheter using aseptic technique.

3.2.8 Sample transport

Approximately 20 ml of urine was collected aseptically in a sterile container. Each sample in the container was properly labeled with patients ID number. The specimens were then transferred to the laboratory for processing.

3.2.9 Sample Processing

Following collection, urine samples were inoculated onto Blood and MacConkey agar plates for isolation of bacteria and onto Sabouraud Dextrose Agar (SDA) for isolation of fungi. The

inoculated agar plates were incubated aerobically for 24-48 hours at 37°C for bacteria and 25°C for fungi.

3.2.10 Preliminary Identification

Presumptive identification of the bacteria in the urine samples was based on standard identification procedures of colony morphology, colonial characteristics on differential media and biochemical reactions of the organisms (Elmer *et al.*, 1997).

3.2.11 Cultural characterization

Cultural characterization of the isolates was based on their morphological and growth characteristics on differential media. Growth from samples with positive bacteriuria was subcultured unto selective media such as Mannitol Salt Agar, MacConkey Agar, Cetrimide Agar and Eosin Methylene Blue Agar, for preliminary detection of *Staphylococcus* spp, Enterobacteriaceae, *Pseudomonas* spp and *Escherichia coli* isolates respectively.

3.2.12 Gram staining

This was carried out by making smears of the bacterial isolates on slides. It was allowed to dry, heat-fixed and then covered with crystal violet solution for 30-60 seconds, and then washed with water. The smears were covered with Lugols iodine solution for 30-60 seconds, then drained and decolourized with acetone for 30-60 seconds. This was immediately washed with water, covered with neutral red stain for 1 minute, rewashed with water and air-dried. The slides were then viewed under the microscope (X100 magnification) to observe the shapes of the cells.

3.2.13 Biochemical Tests

Biochemical tests were performed on colonies from primary cultures for final identification of the bacterial isolates. Tests such as catalase, oxidase, urease, coagulase, sugar fermentation, Ejikman and IMViC (Indole, Methyl red, Voges-Proskauer, and Citrate) tests were carried out as detailed by Cheesbrough (2000).

- **Coagulase test**

This test is used to identify *S. aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin.

The slide agglutination test was adopted. One drop of normal saline (0.8% sodium chloride) was placed on a clean microscopic slide. One or two colonies of test organisms were taken and emulsified in the drop of saline to form a smooth milky suspension. A loop-full of human plasma was added to the bacterial suspension. Appearance of coarse clumping visible to the naked eye within 5-10 seconds was indicative of positive reaction.

- **Catalase test**

This test is useful in differentiating members of the genus *Streptococcus* spp which are non-catalase-producing bacteria from members of the genus *Staphylococcus* spp that produce the catalase enzyme. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

A small amount of the culture to be tested was picked up from an agar medium with inoculating wire loop. This was inserted into hydrogen peroxide (3%) solution held in a small, clean tube. The production of gas bubbles indicates a positive reaction.

- **Indole test**

This test was used to identify and differentiate members of the Enterobacteriaceae Family. Some bacteria split tryptophan into indole and pyruvic acid using the hydrolase called tryptophanase. Isolates were cultured on tryptone broth containing tryptophan. Indole production is detected with Kovac's reagent (Indole reagent) with the observation of red colouration. This is very important in differentiating *E. coli* (indole positive) from some closely related enteric bacteria. It also differentiates *Proteus mirabilis* (indole negative) from all other *Proteus* species (indole positive).

The test organism was suspended in a Bijou bottle containing 5 ml of sterile peptone water and incubated at 37°C for 48 hours.

Five drops of Kovac's reagent were added to the bijou bottle containing the test organism and shaken gently. A positive reaction was indicated by the development of a red colour in the surface layer within one minute. In the negative reaction, the indole reagent retained its yellow colour.

- **Methyl Red test**

The MR-VP medium was used for this test to differentiate the members of the Enterobacteriaceae Family. Many Gram-negative intestinal bacteria can be differentiated based on the products produced when they ferment the glucose in MR-VP medium. The large amounts of acids produced lower the pH of the medium. Methyl red (a pH indicator) turns red when added to the medium if the organism is a mixed acid fermenter which is indicative of a positive reaction. *Escherichia coli*, *Salmonella*, and *Proteus* ferment glucose to produce lactic, acetic, succinic, and formic acids with production of carbondioxide, hydrogen gas and ethanol.

About 2.5 ml of MR-VP broth was inoculated with the test organism and incubated at 37°C for 48 hours. After incubation, the culture was transferred to a small serological tube. To this small quantity, 2-3 drops of methyl red were added, and the colour change was noted.

- **Voges-Proskauer test**

Organisms that are negative in the methyl red test may be producing 2, 3 butanediol and ethanol instead of acids. These non-acid products do not lower the pH as much as acids do. *Enterobacter*, *Serratia* and some species of *Bacillus* produce these substances.

About 2.5ml of MR-VP broth tubes was inoculated with the test organism and incubated at 37°C for 48 hours. After incubation, 0.6ml of 5% α -naphthol was added and shaken followed by addition of 0.2 ml of 40% potassium hydroxide. The development of a red colour indicates a VP positive test and a no colour change indicates a VP negative test result.

- **Citrate test**

Simmon's citrate agar was used for this test. This was carried out to detect the ability of an organism to use citrate as its sole source of carbon.

The test organism was inoculated onto citrate agar slant in a Bijou bottle and incubated at 37°C for 24-48 hours. The development of a bright blue colour indicates a positive reaction. No change in colour indicates a negative citrate test.

- **Urease test**

The urea agar base was used. This was done to test for the urease enzyme activity to differentiate the enteric bacteria.

The agar slants was heavily inoculated with the test organism and incubated for 24hrs at 37°C. The production of a pink colouration indicates a positive test.

- **Triple Sugar Iron Agar Test**

This test identifies carbohydrate fermenters and H₂S producers among the Enterobacteriaceae. Slopes of medium in test tubes were prepared as recommended by the manufacturer. Sterile wire was first used to streak the slope with the organisms and then the bottom. The tubes were incubated at 37°C for 24 hours. Glucose fermenters were identified when tube bottom turns yellow; lactose/sucrose fermenter turns tube slant yellow; H₂S producers form black spot on the surface of the slope; no fermentation is indicated by no color changes at the bottom and the slant.

- **Eijkman Test**

This is the fermentation of lactose and gas production at 44.5°C. It is used for further confirmation of *E. coli*. The organism was inoculated into a sterile lactose broth containing inverted Durham tube and incubated at 44.5°C for 24 to 48 hours. The development of turbidity and gas production inside the Durham tube confirmed the presence of *E. coli*.

3.2.14 Preparation of Barium Sulphate Standard (McFarland 0.5)

One percent (v/v %) solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water. One percent (w/v%) solution of barium chloride was then prepared by dissolving 0.5 g of dihydrate barium chloride (BaCl₂.2H₂O) in 50 ml of distilled water. A 0.6 ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution and mixed. Small volume of the turbid solution was then transferred to a screw cap bottle of the same types as used for preparing the test and control inocula (Cheesbrough, 2000).

3.2.15 Inoculum Preparation

The inoculum was prepared by picking 3-5 discrete colonies of the test organism with a sterile wire loop. This was suspended in a sterile peptone water and incubated at 37°C for about two hours to allow organisms reach their log phase in growth. This was then diluted to match the turbidity standard (McFarland 0.5) which contains approximately 1.5×10^8 cfu/ml (McFarland, 1907).

3.2.16 Antibiotic Susceptibility Testing

Susceptibility testing was performed on isolates based on the agar disc diffusion technique developed by Bauer *et al.* (1966) on Muller-Hinton agar. Bacterial inocula were prepared by suspending the freshly isolated bacteria in 5 ml sterile nutrient broth and adjusted to 0.5 MacFarland standard. A sterile cotton swab was used to streak the surface of Mueller-Hinton agar (MHA) plates. After the agar surface has dried, the appropriate antibiotic discs were placed on it with a sterilized forceps at reasonable equidistance, on the seeded MHA. Inoculated plates were incubated at 37°C for 24 hours. On the next day, plates were read by taking measurement of zone of inhibition. The diameter of the zone of inhibition produced by each antibiotic disc was measured using metric ruler and recorded in millimeter. The result was interpreted as either susceptible 'S', intermediate 'I' or resistant 'R' to the antibiotic agent used, depending on the diameter of zone of inhibition produced as defined by EUCAST and CLSI (2014) standard zone size interpretive manual. The antibiotics used for antibiogram determination of the isolates in the presence of any potential growth were obtained from Oxoid limited, Basingstoke, UK in the following concentrations: amoxicillin (10 µg), amikacin (10 µg), gentamicin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cotrimoxazole (25 µg), nitrofurantoin (300 µg), and imipenem (10 µg).

3.2.17 Determination of Multiple Antibiotics Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was determined for each isolate as shown in the equation below. MAR index (MARI) is the number of antibiotic(s) to which the organism is resistant divided by the total number of antibiotics tested (Krumperman, 1983).

$$\text{MARI} = \frac{\text{Number of Antibiotic (s) to which isolate was resistant}}{\text{Total number of antibiotics tested}}$$

3.2.18 Test for the Presence of Plasmids in the isolates

The acridine orange method as described by Rasool *et al.* (2003) was employed in this investigation. The MICs value of acridine orange to eighteen selected highly resistant isolates was determined. Sub-MIC of acridine orange found to be 500ug/ml was used to cure the isolates of plasmid they might contain. Sterile nutrient broths was inoculated with standardized overnight inoculum of the test isolates and solutions of acridine such that the final concentration of acridine in the broth became 500ug/ml. These were then incubated at 37°C for 18-24 hrs. Thereafter, growths from the tubes was subcultured and the susceptibility of the eighteen resistant isolates after acridine treatment was redetermined using the agar diffusion method. The zones of diameter of inhibition of the test antibiotics against the acridine-treated isolates was then compared with the values before plasmid curing with acridine.

3.2.19 Test for β -Lactamase

The β -Lactamase identification sticks obtained from Oxoid limited was used to carry out this test. The tips of the sticks are impregnated with a solution of nitrocefin, phosphate buffer and dimethylsulphoxide. The stick end changes colour from yellow to red as the amide bond in the β -lactam ring is hydrolysed by a β -lactamase.

A well separated representative colony from an overnight growth was touched with the impregnated end of the stick and thereafter placed in the moisture condensate on the Petri dish

lid and allowed to stay for 5-10 minutes before reading results. A colour change from yellow to pink/red on the end of the stick was taken as positive for presence of β -lactamase enzyme.

3.2.20 Molecular characterization of some antibiotic-resistant isolates

This experiment was carried out at the Molecular Diagnostic Laboratory, Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.

a. Culture purification

Purity of the cultures to be used for the test was ascertained by sub-culturing the isolates on Nutrient agar plates where discrete colonies were picked.

b. Plasmid separation

A 24hr old culture of isolates was inoculated into 5ml of Luria-Bertani (LB) broth and incubated at 37°C for 18 hrs. The culture was spun at 10,000rpm for 5 minutes using centrifuge. The supernatant was discarded leaving pellets which was further vortexed in a Stuart vortex mixer for few seconds.

- i. A 100 μ l of buffer 1 (50 mM Tris-HCl, 10 mM EDTA, 100 μ g/mL RNase A, pH 8.0) was added to each tube to resuspend the cells. It was mixed properly by vortexing, making sure that the cell suspension was homogenous and no clumps were visible.
- ii. A 200 μ l of Buffer 2 (1% SDS, 0.2 M NaOH) was added to each tube. The caps were tightened and the solutions mixed rapidly by inverting them a few times. The tubes were then left to stand on ice for 5 minutes.
- iii. Thereafter, 150 μ l of ice-cold Buffer 3 (3.0 M potassium acetate, pH 5.2) was added to each tube. The caps were closed and the solutions mixed rapidly by inverting them a few times. The

tubes were again left to stand on ice for 5 minutes after which they were placed in a centrifuge and spun at 10000rpm for 2 minutes. The supernatants was then transferred into clean 1.5 mL tubes and precipitate discarded.

- iv. To each tube of supernatant an equal volume (400µl) of isopropanol was added. The caps were closed and content mixed vigorously. The tubes were left to stand at room temperature for 2 minutes, and then centrifuged at 10,000rpm for 5 minutes. The supernatant was carefully removed and discarded.
- v. The DNA pellet was then washed with 200µl of 95% ethanol and mixed by inversion several times. The tubes were again centrifuged at 10,000rpm for 2-3 minutes. The supernatant was then carefully removed and discarded. The tubes were left with the caps open under vacuum for 15-20 minutes to dry off the last traces of ethanol (Birnboim and Doly., 1979; Feliciello and Chinali., 1993).

c. Electrophoresis

Agarose gel (1%) was prepared by weighing out 1 g of agarose and dissolving in 100 ml of 1x TBE (Tris-Boric Acid-EDTA buffer) with the aid of heat. The hot gel solution was cooled to 45°C and then 5µl of 10 mg/mL stock ethidium bromide was added. The gel solution (100 mL) was poured into electrophoresis tray to which a comb was fixed to create holes on the solidified agarose gel.

The combs were removed after the gel has solidified. The tray with the solidified gel was placed in a TBA buffered tank and using the micropipette, the plasmid preparations from the different bacterial isolates (to which loading dye, 5µl was added) were loaded into the different wells. A

standard 100bp molecular DNA ladder was also loaded in the first well. The gel was allowed to run for 1hour at 75Volts.

After 1hour running the electrophoresis, the chamber was disconnected and gel containing the separated plasmids was visualized under a Trans-illuminator UV light at wavelength 302 nm. This was then photographed with a Polaroid camera and documented using BioRad gel electrophoresis documentation system. The DNA was captured and documented. After photographing, the distance of migration of each isolate were determined relative to the standard DNA ladder loaded in the first well (Gersten, 1996).

d. Determination of Molecular Weight of Plasmids of the Isolates

Using the distance of migration of the bands in each isolate, the isolate and matching the value of the marker in the standard with it, the plasmid sizes of the isolates were determined using the BioRad Image Lab software.

CHAPTER FOUR

4.0 RESULTS

4.1 INCIDENCE OF UTI

Out of the 182 urine samples examined during the period of the study, 107 were positive with significant growth ($\geq 10^5$ cfu/mL) giving a incidence rate of 58.8%. Samples from female patients had a higher prevalence of 67.9% compared with those from males (51.9%) as show on Table 4.1.

The patients who consented to participate in the study were diagnosed and admitted in the surgical wards for different surgical reasons which included hernia, typhoid perforations, wound burns, tumours/cancer, appendectomy, prosectomy, e.t.c. and were being managed by different medical invasive procedures as shown on Table 4.2

Of the forty one (41) subjects associated with urinary catheterization, 29 (70.7%) had catheter while 12 (29.3%) were post-catheterized before sample collection. Twenty six (63.4%) of the 41 patients that had catheter showed positive growth. Highest incidence of UTI was observed in patients with catheter after three days of catheterization (Table 4.3). The age groups of 31-40 and 41-50 recorded the highest incidence of catheter-associated UTI with the males predominating (Tables 4.4 and 4.5).

Incidence of UTI was observed to be highest among patients in the age bracket of 21-30 while the age group of 0-10 years had the least number with positive growth. Patients in the age bracket of 21-30 and 31-40 years had higher proportion of female with bacteriuria (Table 4.6).

Table 4.1: Prevalence of UTI among patients in the Surgical Wards of ABUTH, Shika

Gender	No. sample Screen	% incidence of bacteriuria
Male	104	51.9
Female	78	67.9
Total	182	58.8

Table 4.2: Relationship between Surgical invasive procedures and UTI among surgical patients in ABUTH

Invasive procedure	No. screened	No.with UTI (%)
Intubation	30	15 (50)
Urethral cystoscopy	9	3 (33.3)
Thoracotomy	31	17 (54.8)
Urethral catheterization	41	26 (63.4)
Arterial/Vascular lining	7	2 (28.5)
Endoscopy	13	6 (46.1)
Others	51	38 (74.5)
Total	182	107 (58.8)

Table 4.3 Relationship between duration of catheterization before sample collection and urine positive growth in surgical patients of ABUTH, Shika

Duration of catheterization Before sample collection (Days)	No. screened (n)	% with UTI
0-3	11	5 (45.5)
4-6	13	10 (76.9)
7 and above	5	2 (40.0)

Table 4.4: Relationship between Age and catheter associated UTI

Age group	No. associated with Catheterization	No. with UTI
0-10	3	1
11-20	1	0
21-30	1	1
31-40	11	7
41-50	10	7
51-60	7	4
61 and above	8	6
Total	41	26

Table 4.5: Relationship between gender and catheter associated UTI

Gender	No. associated with Catheterization	No. with UTI (%)
Male	27	18 (66.6)
Female	14	8 (57.1)
Total	41	26 (63.4)

Table 4.6: Incidence of UTI among patients in the surgical wards of ABUTH based on age distribution

Age group (years)	No. screened	No. with UTI (%)
0-10	8	5 (4.6)
11-20	24	13 (12.1)
21- 30	49	31 (28.9)
31-40	37	23 (21.4)
41-50	29	18 (16.8)
51-60	18	9 (8.4)
61 & above	17	8 (7.4)
Total	182	107 (58.8)

4.2 DISTRIBUTION OF ISOLATES IN THE URINE SAMPLES

Standard biochemical tests showed that organisms isolated were distributed across six (6) genera and a high proportion of the isolates belongs to the Enterobacteriaceae Family. *Staphylococcus* spp, a Gram-positive was isolated in about 21% of the cases while only 2 isolates of *Candida* spp were identified. Table 4.7 shows the distribution of microorganisms isolated from the urine of subjects with significant bacteriuria.

Table 4.8 shows the relationship between invasive medical procedures and the organisms isolated from patients with positive growth. *Escherichia coli* was isolated more in patients that were managed with urethral catheters while *Staphylococcus* spp was frequently isolated in patients that underwent thoracotomy as invasive medical procedure.

4.3 ANTIBIOTIC RESISTANCE PROFILES OF ISOLATES

Data presented on table 4.9 show the susceptibility profiles of the isolates to the antibiotics.

A high proportion of *E.coli* isolates were sensitive to the inhibitory effect of imipenem, ceftriaxone, amikacin and ciprofloxacin. Susceptibility to amoxicillin, nitrofurantoin, cotrimoxazole and ofloxacin was relatively low. On the other hand, *Staphylococcus* spp isolates were comparatively more sensitive to most of the antibiotics, with percentages higher than 50% in 8 of the 10 antibiotics. Only amoxicillin and ofloxacin displayed low effectiveness against the isolates.

Pseudomonas spp isolates were sensitive to amikacin, imipenem and ciprofloxacin but were generally resistant to amoxicillin, ceftriaxone, cefuroxime, and cotrimoxazole.

Klebsiella spp and *Proteus* spp were sensitive to the inhibitory effects of the test antibiotics. Only *Klebsiella* spp was resistant to amoxicillin while resistance to *Proteus* spp isolates included nitrofurantoin and ofloxacin.

4.4 DETERMINATION OF MAR INDEX

MAR indices presented on Table 4.10 show that most of the isolates are multiple antibiotic-resistant.

4.5 TEST FOR BETA-LACTAMASE

Twelve (66.6%) of the eighteen multiple antibiotic-resistant isolates as shown in Table 4.11 were found to be β -lactamase positive.

Table 4.7: Isolates obtained using standard biochemical tests

Isolated Organism	Occurrence n (%)
<i>Escherichiacoli</i>	52 (48.6)
<i>Klebsiellaspp</i>	10 (9.3)
<i>Proteusspp</i>	3 (2.8)
<i>Pseudomonasspp</i>	17 (15.9)
<i>Staphylococcispp</i>	23 (21.5)
<i>Candidaspp</i>	2 (1.9)
Total	100

Table 4.8: Relationship between Surgical invasive procedures and organisms isolated from urine of surgical patients in ABUTH

Invasive Procedures	<i>E.coli</i>	<i>Staphylococci</i> spp.	<i>Pseudomonas</i> spp.	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Candida</i> spp.	Total
Intubation	8	3	2	1	1	0	15
Urethral cystoscopy	2	1	0	0	0	0	3
Thoracotomy	8	7	1	1	1	0	18
Urethral catheterization	16	4	3	3	0	0	26

Arterial/Vascular lining	2	0	0	0	0	0	2
Endoscopy	3	2	1	0	0	0	6
Others	13	6	10	5	1	2	37

Table 4.9: Resistance profiles of nosocomial UTI Isolates to the test antibiotics

Antibiotics	% Resistance of Isolates					
	<i>E.coli</i> n=52	<i>Staphylococci</i> n=23	<i>Spp.</i> n=17	<i>Pseudomonas</i> n=10	<i>Klebsiella</i> n=3	<i>Proteus</i> n=3
Amoxicillin	80.7	78.2	82.3	70.0	100	
Ofloxacin	51.9	41.1	52.9	10.0	33.3	
Ciprofloxacin	40.3	30.4	23.5	20.0	33.3	
Gentamicin	23.0	8.6	35.2	10.0	00.0	
Amikacin	7.6	4.3	0.0		00.0	00.0
Cefuroxime	51.9	47.8	76.4		30.0	00.0
Ceftriaxone	42.3	34.7	47.0		20.0	00.0
Cotrimoxazole	61.5	39.1	64.7	20.0		33.3
Imipenem	1.9	00.0	00.0		00.0	00.0
Nitrofurantoin	80.7	43.7	58.8		30.0	0.00

Table 4.10: MAR indexes of nosocomial UTI isolates from surgical patients of ABUTH, Shika.

MARI	Isolates				
	<i>E.coli</i> n=52	<i>Staphylococci</i> spp n=23	<i>Pseudomonas</i> spp n=17	<i>Klebsiella</i> spp n=10	<i>Proteus</i> spp n=3
0.0	3	0	1	2	0
0.1	5	5	0	2	0
0.2	13	6	4	3	2
0.3	0	0	0	0	0
0.4	13	7	2	2	0
0.5	9	2	4	1	1
0.6	0	0	0	0	0
0.7	6	0	3	0	0
0.8	2	3	3	0	0
0.9	0	0	0	0	0
1.0	1	0	0	0	0

Table 4.11: β -Lactamase producing isolates from urine of Surgical patients of ABUTH, Shika.

Isolate	Organism	β -Lactamase
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No		
3	<i>Klebsiella Spp</i>	+
9	<i>Escherichia coli</i>	+
10	<i>Staphylococci spp</i>	-
17	<i>Staphylococci spp</i>	-
20	<i>Escherichia coli</i>	+
24	<i>Escherichia coli</i>	+
26	<i>Pseudomonas spp</i>	-
31	<i>Staphylococci spp</i>	+
33	<i>Pseudomonas spp</i>	+
35	<i>Escherichia coli</i>	+
37	<i>Proteus spp</i>	-
42	<i>Escherichia coli</i>	-
48	<i>Pseudomonas spp</i>	+
49	<i>Escherichia coli</i>	+
50	<i>Escherichia coli</i>	+
57	<i>Pseudomonas spp</i>	-
77	<i>Escherichia coli</i>	+
81	<i>Escherichia coli</i>	+

4.6 DETERMINATION OF NATURE OF RESISTANCE

In order to prove the involvement of plasmids R-factor in the multiple antibiotic-resistant (MAR) isolates, the cultures were treated with acridine orange. After acridine curing, most of the isolates irreversibly lost their antibiotic resistance and became susceptible to inhibition to same concentrations of the different antibiotics to which they were previously resistant (Table 4.11 and 4.12).

For example before plasmid curing, isolate No 81 (*Escherichia coli*) which was resistant to the 10 test antibiotics, was to 5 after curing the isolate of plasmids. Similar patterns were observed for the rest of the isolates.

Table 4.12 which shows the resistance of the test antibiotics before and after curing indicated that the proportion of reduction in the number of isolates that became susceptible varied among the test antibiotics.

For example, more than 50% of the MAR isolates that were resistant to ciprofloxacin, gentamycin, ceftriaxone and nitrofurantoin reverted to being susceptible. The only isolate initially resistant to imipenem and the five isolates also previously resistant to amikacin became susceptible.

Gel electrophoresis of plasmid DNA showed that 14 of the 18 isolates examined had plasmids with plasmid bands of 1 to 2 and plasmid sizes ranging from 73.2-708.4 kb (Plate 4.1 and 4.2). Some of the isolates have the same number of plasmid bands and sizes as shown in Table 4.12.

Table 4.13 shows the relationship of β -lactamase producing and plasmid carrying isolates. Twelve (66.6%) of the 18 multiple antibiotic-resistant isolates produce β -Lactamase enzymes. All the isolates which produced β -Lactamase enzymes also possessed plasmids, except isolates No. 31 (*Staphylococcus*spp) and 77 (*Escherichia coli*) as shown in Table 4.13.

Table 4.12: Resistant pattern of some Antibiotic Resistant bacteria species isolated from urine of surgical patients of ABUTH before and after curing

Isolate No	Organism	No of Antibiotics Resistant to		Increase in Susceptibility (%)
		Before curing	After curing	
3	<i>Klebsiella</i> spp	5	2	60.00
9	<i>Escherichia coli</i>	7	3	57.14
10	<i>Staphylococci</i> spp	7	4	42.85
17	<i>Staphylococci</i> spp	8	6	25.00
20	<i>Escherichia coli</i>	8	4	50.00
24	<i>Escherichia coli</i>	8	3	62.50
26	<i>Pseudomonas</i> spp	8	4	50.00
31	<i>Staphylococci</i> spp	8	5	37.50
33	<i>Pseudomonas</i> spp	6	4	33.33
35	<i>Escherichia coli</i>	7	4	42.85
37	<i>Proteus</i> spp	5	4	20.00
42	<i>Escherichia coli</i>	7	4	42.85

48	<i>Pseudomonas spp</i>	6	4	33.33
49	<i>Escherichia coli</i>	6	3	50.00
50	<i>Escherichia coli</i>	6	2	66.66
57	<i>Pseudomonas spp</i>	8	5	37.50
77	<i>Escherichia coli</i>	7	6	14.28
81	<i>Escherichiacoli</i>	10	5	50.00

Table 4.13: Comparison of resistance of some antibiotics to 18 Multiple Antibiotic Resistant bacteria species isolated from urine of surgical patients of ABUTH, Shika before and after plasmid curing

Antibiotic	No of Isolates Resistant out of 18		Reduction in resistance (%)
	Before	After	
Amoxicillin	18/18	14/18	22.22
Ofloxacin	17/18	14/18	17.65
Ciprofloxacin	16/18	6/18	62.50
Gentamicin	9/18	1/18	88.89
Amikacin	5/18	0/18	100.00
Cefuroxime	17/18	16/18	05.88
Ceftriaxone	14/18	4/18	71.43
Cotrimoxazole	15/18	11/18	26.67
Imipenem	1/18	0/18	100.00
Nitrofurantoin	14/18	4/18	71.43

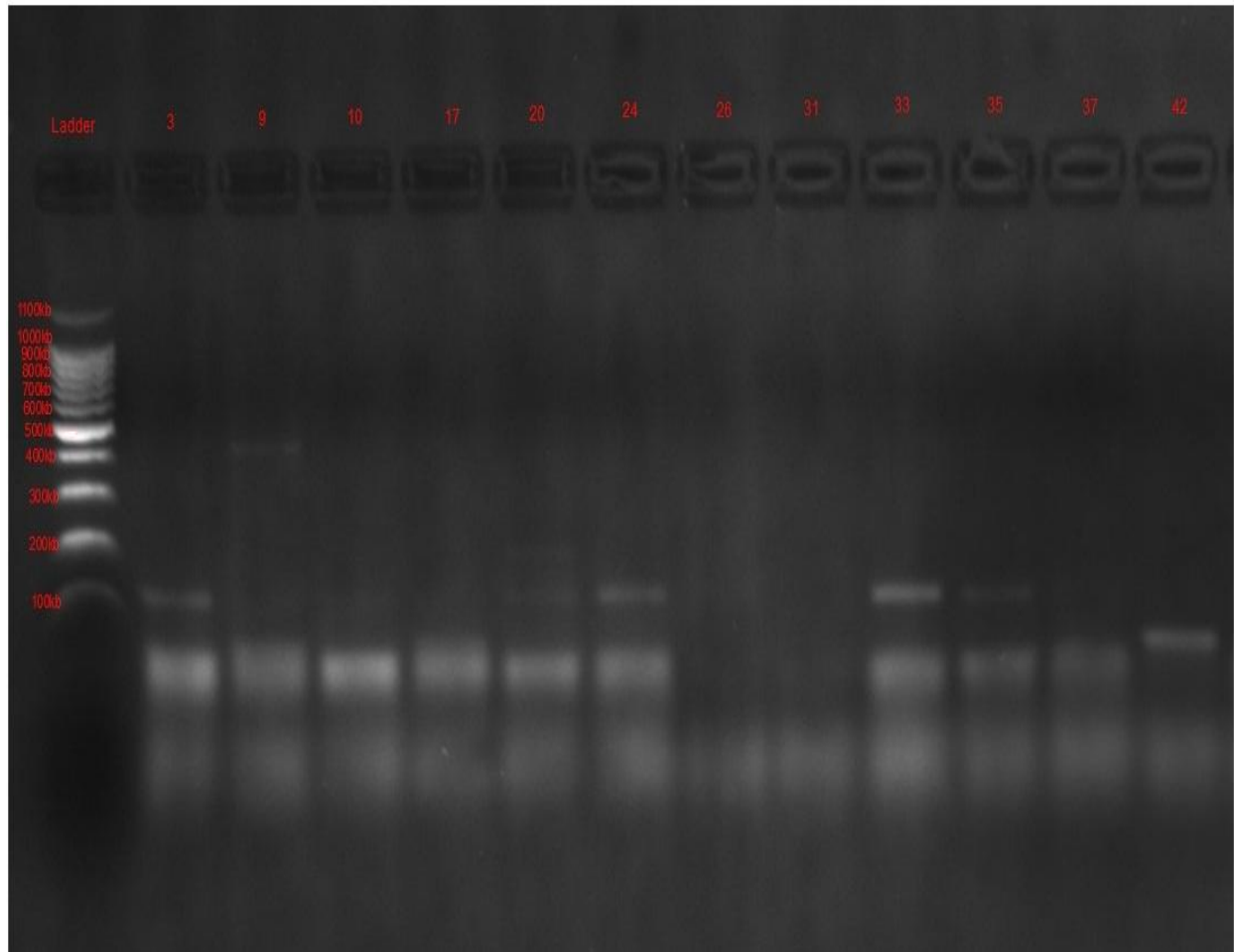


Plate a. Different plasmid bands exhibited by some of the antibiotic-resistant isolates

Key :

Lane 1 : Marker standard

Lane 2: Isolate 3. (*Klebsiella* spp)

Lane 3: Isolate 9. (*Escherichia coli*) Lane10: Isolate33.(*Pseudomonas* spp)

Lane 4: Isolate 10. (*Staphylococci* spp)

Lane 5: Isolate 17.(*Staphylococci* spp)

Lane 6: Isolate 20.(*Escherichia coli*)

Lane 7: Isolate 24.(*Escherichia coli*)

Lane 8: Isolate 26.(*Pseudomonas* spp)

Lane 9: Isolate 31.(*Staphylococci* spp)

Lane11: Isolate 35.(*Escherichia coli*)

Lane12: Isolate 37.(*Proteus* spp)

Lane13: Isolate 42.(*Escherichia coli*)

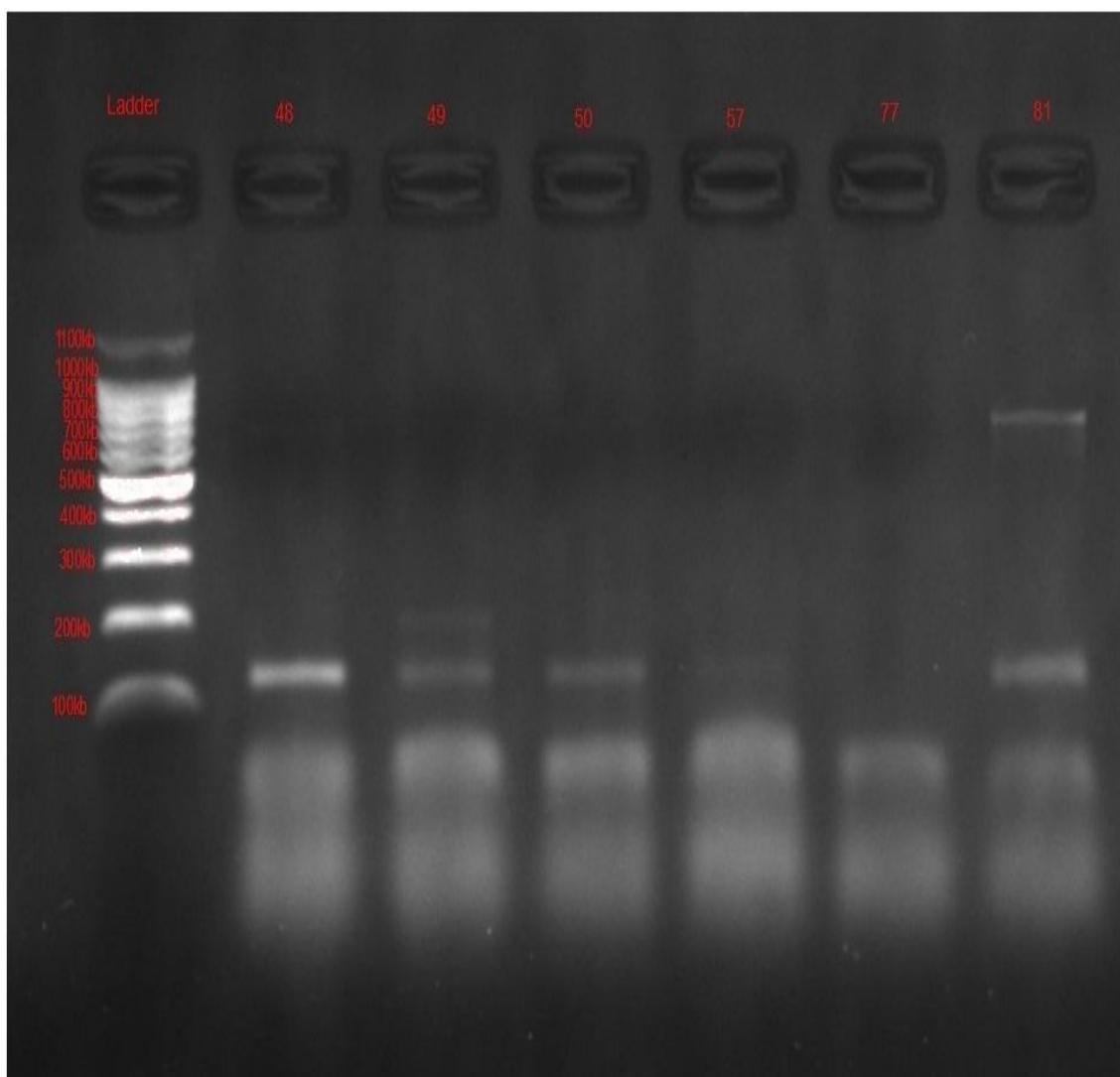


Plate b. Different plasmid bands exhibited by some of the antibiotic-resistant isolates

Key :

- Lane 14: Marker standard
- Lane 15: Isolate 48. (*Pseudomonas* spp)
- Lane 16: Isolate 49. (*Escherichia coli*)
- Lane 17: Isolate 50. (*Escherichia coli*)
- Lane 18: Isolate 57. (*Pseudomonas* spp)
- Lane 19: Isolate 77. (*Escherichia coli*)
- Lane 20: Isolate 81. (*Escherichia coli*)

Table 4.14: Relationship between β -Lactamase producing and Plasmid carrying isolates from urine of Surgical patients of ABUTH, Shika.

Isolate	Organism	β -Lactamase	Plasmids	Plasmid sizes
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No			Bands number	
3	<i>Klebsiella</i> spp	+	2	73.2, 87.2
9	<i>Escherichia coli</i>	+	2	73.2, 431.1
10	<i>Staphylococci</i> spp	-	1	73.2
17	<i>Staphylococci</i> spp	-	1	74.2
20	<i>Escherichia coli</i>	+	2	73.2, 95.7
24	<i>Escherichia coli</i>	+	2	74.2, 95.7
26	<i>Pseudomonas</i> spp	-	Nil	
31	<i>Staphylococci</i> spp	+	Nil	
33	<i>Pseudomonas</i> spp	+	2	73.2, 100
35	<i>Escherichia coli</i>	+	2	75.2, 100
37	<i>Proteus</i> spp	-	1	77.3
42	<i>Escherichia coli</i>	-	1	88.1
48	<i>Pseudomonas</i> spp	+	1	129.5
49	<i>Escherichia coli</i>	+	1	129.5
50	<i>Escherichia coli</i>	+	1	129.5
57	<i>Pseudomonas</i> spp	-	Nil	
77	<i>Escherichia coli</i>	+	Nil	
81	<i>Escherichiacoli</i>	+	2	131.8, 708.4

CHAPTER FIVE

5.0 DISCUSSION

Hospital acquired UTI is one of the common causes for prolonged hospital stay, increased resistance of microorganisms to antimicrobials, massive additional financial burden for patient's families and health systems among other things. Effective management of patients suffering from bacterial UTIs is dependent on the timely identification of the type of organisms that caused the disease and the selection of an effective antibiotic agent to the organism in question.

In this study, which was carried out to investigate the antimicrobial susceptibility of nosocomial UTI pathogens in patients admitted in the surgical wards of ABUTH, Shika, an overall prevalence of 58.8% was recorded which is comparatively higher than other reports in the country. Ige *et al.*(2011) in a retrospective survey of records over a 5 year period from the Infection Control Unit of the University College Hospital, Ibadan, Nigeria reported a prevalence of nosocomial UTIs to be 43.9% while Jombo *et al.*(2011) reported an prevalence rate of 30.8% in a study carried out at the University of Calabar Teaching Hospital (UCTH),Nigeria. Lower rates of 12.3% and 11.1% have also been reported by Jumbo *et al.*(2006) and Nwadioha *et al.*(2010) in different studies carried out in University of Jos Teaching Hospital (JUTH), Nigeria and Aminu Kano Teaching Hospital (AKTH) Nigeria respectively. The higher rate observed in this study as compared to other studies carried out in the country might have been either as a result of sample size variation or because their studies might have been based on retrospective survey. However, the percentage obtained in this study is virtually in line with other findings in Bangladesh and Italy by Shahnaz (2009) and Zotti *et al.*(2004) who reported prevalence rates of 50% and 52.7% respectively.

It is also in agreement with other studies in Ethiopia carried out by Zeamanuel (2007) and Melaku *et al.*(2012) that obtained incidence rates of 55.9% and 48% respectively which are closer to the finding in this study.

The indwelling urinary catheter as an invasive device has a significant association with hospital-acquired UTIs: it provides either a portal of entry for microorganisms or a place for colonization by microorganisms. In this study, 26 (63.4 %) of the isolates were associated with indwelling urinary catheters of which 17 were catheterized and 9 were post catheterized. It was also discovered that of the 74.1% of the patients managed with invasive procedures such as urethral catheterization, intubation, thoracotomy etc., urethral catheterization contributed the highest rate of UTI whereas *E.coli* was the predominant isolate. This finding could be due to the close proximity of the urethral catheter to the anal passage especially in females. Reasons advanced for this poor catheter management such as improper and delayed cleaning after a bowel action which allows bacteria to colonize the entry site of the catheter (Inyama *et al.*, 2011). Similar findings have also been reported by Mohammed and Shaikh (2012) and Inyama *et al.*(2011) in Sudan and Kenya respectively. Study by Dennis and Maki(2001) also found that catheter-associated bacteriuria developed in about 25% of patients who required urinary catheter. The finding in this study that patients with indwelling catheter for at least 3 days are more likely to develop nosocomial UTI is in agreement with reports of Inyama *et al.*(2011).

In this study, distribution according to sex reveals a predominance of the females (67.9%) with *E.coli* contributing 43.4% over the males (51.9%). Sexual activity has been reported to influence higher prevalence of UTI in females. This may not be unconnected with the fact that most of the infecting organisms are commensals of perianal and vaginal regions. Males are less prone to UTIs possibly because of their longer urethra and the presence of antimicrobial substances in

prostatic fluid (Adedeji and Abdulkadir, 2009; Farajnia *et al.*, 2009; Oluremi *et al.*, 2011). The higher rate in females reported in this study is in agreement with that of Inyama *et al.* (2011) who reported 62.5% in females as against 37.5% in males in a study carried out in the Intensive Care Unit of the National Hospital of Kenya. Refai and Ramy (2011) also reported higher figures in females (35.16% vs. 31.93%) at a Teaching hospital in Nineveh. Similarly, Gupta *et al.* (2001) recorded 51.23% for females while Shahnaz, (2009) reported 58% for females. Observation in this study also reveals a predominance of young (21-30) and middle aged (31-40) females over the males whereas in older age groups of 50 and above the males are more affected than the females which agrees with the findings of Shahnaz (2009) who reported same pattern. There are many factors which could lead to increase in the risk of infection among elderly persons such as: poor nutrition, immobility leading to poor hygiene, chronic illnesses, and increased stress on the body and strain on the body's defense mechanisms. Pathological factors such as prostatic hypertrophy and degenerative nerves which can cause urine stasis predisposes people to urinary tract infection (Dewit 2005). Chronic diseases lead to impairing of the normal defense mechanisms and serious illness exerts the immune system causing greater susceptibility to other pathogens.

It has been established that 22.5% of patients with chronic diseases such as diabetes mellitus, benign prostatic hyperplasia and cancer could contribute significantly to the development of nosocomial infections (WHO, 2002). The observed increase in prevalence of UTI in the male age group of 50 years and above might be caused by the higher incidences of urinary tract pathologies like prostate diseases. The elevated incidence in the female group in the age bracket 20-40 might be due to obstetric and gynaecological causes (Mukherjee *et al* 2005).

UTIs are common conditions worldwide and the pattern of uropathogens and their antimicrobial resistance usually vary between countries and within the country, and sometimes even within the hospital. The uropathogens identified in this study are similar to those of many other studies conducted in different countries in this region and internationally.

In this study, *E.coli* was the predominant isolate and *Candida* spp was the least encountered isolate. The pattern in this study is similar to a study carried out in same institution in 2006 (Sadiq *et al*, 2006).

Nwadioha *et al*,(2010) in a study at Aminu Kano Teaching Hospital (AKTH) Northwestern, Nigeria also reported *E.coli* as the predominant isolate across the community-acquired and hospital-acquired groups. Jumbo *et al*.(2011), also showed in a study carried out at University of Calabar Teaching Hospital (UCTH) Southeastern, Nigeria that the most common isolate to be *E. coli* with *C. albicans* as the least common. The same pattern was also reported by same author in a work carried out in Nguru, Yobe state (Jumbo *et al*, 2005). Similar findings have also been reported in USA (Davies *etal.*, 1989), and in Kenya (Mwamba, 2005; Inyama *etal.*, 2011).

However, some authors have reported different organisms as most prevalent isolates. Jombo *etal*.(2006) reported *Klebsiella* spp as the most prevalent in hospital-acquired organism in University of Jos Teaching Hospital (JUTH) North central, Nigeria while Mohammed and Shaik (2012) reported *P. aeruginosa* as the most prevalent in Sudan.

The observed differences in the distribution of the uropathogens might be due to different environmental conditions, host factors, and practices such as, complex mix of the patients, healthcare and education programmes, socioeconomic standards and local hygiene practices (WHO, 2002; Mansour *et al*, 2009).

Consistent with other studies, the Gram-negative bacteria have been commonly associated with hospital-acquired infections (Ige *et al.*, 2011). This findings are also consistent with that of Mehmet *et al.* (1999) in a study carried out in Turkish military hospital. Afolabi *et al.* (2011) in a study carried out at Obafemi Awolowo University Teaching Hospital (OAUTH), Ile-Ife, Southwest, Nigeria also obtained similar trend.

Nosocomial UTIs are the largest institutional reservoir of nosocomial antibiotic-resistant pathogens (Jarvis and Martone, 1992). In this study the antibiogram of urinary pathogens isolated revealed that all the isolates were very sensitive to amikacin, imipenem, and gentamicin while amoxicillin, ofloxacin, and cotrimoxazole displayed resistance.

This high level of susceptibility to these three antibiotics could be due to its restricted use in the clinical practice. The observed widespread resistance to amoxicillin, ofloxacin and cotrimoxazole could be attributed to exposure of the isolates to these agents, which may have enhanced resistance development. This finding agrees with results obtained in same institution by Sadiq *et al.* (2006) in a study that evaluated the level of drug utility in UTI patients in ABUTH Zaria. They established that gentamicin, ampicillin/cloxacillin, cotrimoxazole, amoxicillin/clavulanate and ciprofloxacin were the most commonly utilized antibiotics in the hospital. This is no wonder that some of the antibiotics listed among the most used have become resistant over the years. A previous study carried out in same environment also established that ciprofloxacin and gentamicin as the most effective drugs whereas penicillin, amikacin and amoxicillin were ineffective (Ehinmidu, 2003).

Shahnaz (2009) had also reported that imipenem and amikacin were the most effective agents in both community and hospital-acquired UTI. In another study, Mazed *et al.* (2008) reported that

among the Enterobacteriaceae and Gram-positive cocci causing UTI, imipenem was the most effective antibiotic with ampicillin and cotrimoxazole being virtually ineffective.

The finding in this study that cefuroxime, ofloxacin and ceftriaxone are not very effective against a high proportion of the isolates seems to contradict the observations by Jombo *et al.* (2005, 2006, 2011) and Nwadioha *et al.* (2010). The empirical use of commonly used antibiotics is majorly responsible for antibiotic-resistant phenomenon. Amoxicillin, ofloxacin and cotrimoxazole have and are still being among the commonly used antibiotics to treat UTI. Majority of the UTI patients in the study environment received fluoroquinolone, cephalosporins, aminoglycosides or a combination of these antibiotics as empirical therapy or as definitive treatment.

There are many factors that may have contributed to the decreased sensitivity of uropathogens to many of these antimicrobial agents. They range from the use of antimicrobial agents as prophylactic in the presence of bacteriuria in patients, antibiotic use in animal feeds and under dosing of antibiotics. Resistance could also occur in the community as a result of clustering and overcrowding, widespread use of broad-spectrum antibiotics, the sale of antibiotics over the counter, self-treatment with antibiotics, the inappropriate use of antibiotics and decreased funding for public health surveillance (Amyes, 2000). It is possible that the high resistance to amoxicillin, ofloxacin and cotrimoxazole observed in this study could be due to the widespread use of these antibiotics and its use for a long period of time in the community and hospital settings. It is well established that antibiotic therapy can select for antibiotic-resistant strains and R-plasmid-mediated antibiotic resistance can spread in a population subjected to heavy antibiotic therapy (Levy *et al.*, 1976).

Twelve (66.6%) of the 18 MAR isolates were found to be β -lactamase producers, implying that most of the bacterial species isolated developed resistance to the antibiotics as a result of β -

lactamase production. Most of the isolates that produce β -lactamase enzymes were also found to harbor plasmid. Most cases of multiple antibiotic-resistant strains have been demonstrated to be due to transferable, extrachromosomal circular DNA, plasmids (Harkness *et al.*, 1975; O'Brien *et al.*, 1980).

Plasmid curing experiment showed that the resistance to the antibiotics was in most cases plasmid-mediated

The plasmid profile studies showed plasmid band number ranging from 1-2 and plasmid sizes ranging from 73.2-708.4 kb in the MAR isolates.

Comparative analysis of data on β -lactamase production and presence of plasmid in some of the MAR isolates showed that all the isolates except isolate 31 (*Staphylococcus* spp) and 77 (*Escherichia coli*) that produced β -lactamase also had plasmid. These findings confirmed that most of the β -lactamases produced by some of the isolates were plasmid encoded and are most likely responsible for the resistance of the isolates to most of the antibiotics tested. Plasmid mediated resistance to β -lactam antibiotics is most often as a result of β -lactamases (Fred and Denis, 1988). The result also provides explanation for the antibiotic-resistance of the isolates, since majority of the isolates possessed 1-2 plasmid bands.

This finding is in agreement with the report by some researchers that high percentage of many commonly encountered Gram-positive and Gram-negative nosocomial pathogens have at least one and frequently multiple plasmids (Cynamon and Palmer, 1983; Chow *et al.*, 1985).

The result of this study also shows that plasmid-encoded antibiotic resistance encompasses most classes of antibiotics commonly employed at the forefront of clinical antibiotic therapy such as the penicillins, fluoroquinolones, sulphonamides, and cephalosporins.

The similarities in the plasmid sizes among the isolates indicates that they are likely of the same origin, most probably from other patients, hospital staff, hospital equipment and/or hospital environment.

Multiple factors are probably contributing to increased incidence of UTI and other hospital-acquired infections among surgical patients which is often due to the breached skin defenses resulting from invasive surgical procedures (which invades the patient's body giving bacteria a way into normally sterile parts of the body). Urethral catheter induced infection coupled with compromised host immunity promote hospital-acquired UTI. Also, patients who have undergone surgeries may or must be in many instances immobile, and the perineal care after elimination (urination or defaecation) is usually difficult, inadequate and unhygienic practices further exposes this area to more contamination (Refai and Ramy, 2011).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Due to the rising antibiotic resistance among uropathogens especially of the nosocomial origin, it is important to have hospital based knowledge of the organisms causing UTI and their sensitivity pattern to select correct treatment regimen. This study was carried out with the objective to determine the antimicrobial susceptibility profile of nosocomial UTI pathogens in the surgical wards of Ahmadu Bello University Teaching Hospital, Shika-Zaria during January to June 2014. A total of 182 urine samples of patients who consented to participate in the study were cultured aerobically. All the isolates were identified by standard microbiological and biochemical techniques and their antibiotic susceptibility was observed by disk diffusion method. A prevalence of 58.8% was recorded. The culture positivity of UTI among the females was more (67.9%) compared with the males (51.9%). The predominant age group with UTI was 21-30 and 31-40 years while the least age group was 0-10 years.

The causative microorganisms isolated were *E.coli* constituting 48.6%, followed by *Staphylococcus* spp(21.5%), *Pseudomonas* spp(15.9%), *klebsiella* spp (9.3%), *Proteus* spp(2.8%) and *Candida* spp(1.9%) was the least encountered isolate. UTIs were associated with urinary catheters in 63.4% of the cases. Of the 74.1% of the patients managed with invasive procedures, urethral catheterization contributed the highest of which *E.coli* was the predominant isolate. Patients with indwelling catheter for at least 3 days were observed to most likely develop nosocomial UTI.

Antibiotic susceptibility profiles of the bacteria isolates showed that most of the isolates were not susceptible to the inhibitory activities of amoxicillin, ofloxacin, cotrimoxazole and cefuroxime.

They were generally sensitive to amikacin, imipenem, gentamycin, nitrofurantoin and ciprofloxacin, and moderately sensitive to ceftriaxone. *E. coli* and *Staphylococcus* spp were observed to be very susceptible to amikacin, followed by imipenem and gentamycin. *Escherichia coli* was resistant to amoxicillin, nitrofurantoin, cotrimoxazole and ofloxacin while *Staphylococcus* spp showed resistance to amoxicillin and ofloxacin. *Pseudomonas* spp showed sensitivity to amikacin, imipenem and ciprofloxacin but displayed resistance to Amoxicillin, ceftriaxone, cefuroxime, and cotrimoxazole. *Klebsiella* spp was sensitive to all antibiotics except amoxicillin. *Proteus* spp also displayed susceptibility to most of the tested antibiotics but showed resistance to amoxicillin, nitrofurantoin and ofloxacin.

Twelve (66.6%) of the 18 MAR isolates were found to be β -lactamase producers, implying that most of the bacterial species isolated developed resistance to the antibiotics as a result of β -actamase production. Most of the isolates that produce β -lactamase enzymes were also found to harbor 1-2 plasmids. Comparison of plasmid size and number showed that some of the isolates have plasmid band of the same number and sizes which indicates that they are likely to be of the same origin

6.2 CONCLUSION

Hospital-acquired infections have increased worldwide, contributing considerably to morbidity and mortality of the hospitalized patients, prolonged hospital stay, adding significantly to the economic burden of underlying disease. Hospitalized patients acquire nosocomial infections due to important risk factors including advancing age, intravenous lines, indwelling urinary catheter and surgical wounds in the Intensive Care Unit (ICU), Surgical Wards, and/or Medical Specialties Wards (Mohamed and Shaik, 2012).

To prevent HAIs, it is necessary to identify sources and modes of transmission of infection and to implement data-driven prevention guidelines and practices. Determination of their prevalence and risk factors are keys to the prevention of hospital-acquired UTIs and other hospital-acquired infections. Efforts to limit HAI should be guided by local surveillance data if progress is to be made in improving the quality of patient care in Nigeria (Ige *et al*, 2011).

Generally, this study deduced the following conclusions:

1. The incidence of UTI in the surgical patients of ABUTH Shika is relatively high, much higher than the general incidence of UTI commonly reported.
2. The incidence of catheter-associated UTI was found to be 63.4%.
3. In this study, distribution according to sex reveals a predominance in the females (67.9%) than in the males (51.9%).
4. The predominant age groups with UTI was 21-30 and 31-40 years while the least age group was 0-10 years
5. Findings in this study also showed a predominance of the Gram-negative (76.6%) with the Enterobacteriaceae dominating. *Staphylococcus* spp (21.5%) was the only Gram positive and *Candida* spp (1.9%) the only yeast isolate in UTI.
6. Antibiotic susceptibility tests showed that the uropathogens isolated are not only resistant to the commonly used antibiotics, they are also multiple antibiotic-resistant.
7. Most of the multiple antibiotic-resistant isolates were beta lactamase producers, which are largely plasmid-mediated.

6.3 RECOMMENDATIONS

This study therefore, recommends the following:

1. There is need for periodic update/ refresher courses on infection control and prevention measures on UTIs at the Surgical Wards of ABUTH, Shika in particular and in Hospitals in general.
2. Amoxicillin, ofloxacin, and Cotrimoxazole should no longer be prescribed for treatment and management of UTI among surgical patients unless susceptibility tests proved otherwise. It is suggested that amikacin, imipenem and gentamicin could be employed as empirical treatment for UTI until causative agent and its susceptibility is defined. However, to decrease the chance of microorganisms to attain resistance, these antibiotics should be used cautiously.
3. Finally, periodic monitoring (surveillance) of the bacterial and susceptibility pattern of urinary pathogens among surgical patients in order to document such changes is highly recommended. The data obtained by monitoring may then serve as a basis for urgent empirical prescription until the culture results become available.
4. Infection control and prevention unit should be adequately empowered.

CONTRIBUTIOIN TO KNOWLEDGE

The findings in this study has provided an up-to date data on the antimicrobial susceptibility profile of nosocomial urinary tract pathogens in the surgical patients of Ahmadu Bello University Teaching Hospital, Shika-Zaria which can be used for designing effective infection control program in the hospital. The data obtained from this study can serve as a basis for urgent empirical prescription until the culture results become available because past knowledge of these pathogens might result in wrong selection of antibiotics.

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APPENDICES

Appendix I: Cultural and biochemical characterization of the isolates from urine samples of patients studied

Escherichia coli

Gram stain: Gram-negative bacilli

Colony morphology:

MacConkey agar: Produce smooth pink colony

Blood agar: Rounded colonies of 1-4 mm diameter with or without haemolysis.

Biochemical test:

On KIA agar: Yellow slope and butt with gas production

On EMB: Green metallic sheen

Indole-positive, Urease-negative, Citrate-Negative, Oxidase-Negative, Eijkman Positive, VP negative

Klebsiella spp

Gram stain: Gram-negative bacilli

Colony morphology:

MacConkey: Mucoid pink colonies

Blood agar: Large grey white mucoid colonies

Biochemical test:

On KIA agar: Yellow slope and butt with gas production

Indole-negative, slow urease producer, Citrate-Positive, Oxidase-Negative

Proteus spp

Gramstain: Gram-negative motile bacilli

Colony morphology:

MacConkey agar: Produce lactose fermenting colony

Blood agar: Fishy odor, swarming growth

Biochemical test:

On KIA agar: Red slope and yellow butt with gas and H₂S production, Indole-negative, urease producer, Citrate-Positive, Oxidase-Negative

Pseudomonas aeruginosa

Gram stain: Gram negative motile bacilli occur as single bacteria, in pairs and occasionally in short chain.

Colony morphology:

MacConkey agar: Produce non-lactose fermenting pale colony

Blood agar: Large flat, spreading colonies, often hemolytic, greenish blue colour pigment production, some produce mucoid colony.

Biochemical test:

On KIA- Red slope & butt without production of gas and H₂S

On Cetrimide: Growth with greenish pigmentation

Citrate-Positive, Oxidase-Positive

Staphylococcus aureus

Gram stain: Gram-positive cocci, appeared in grape-like clusters with some single or paired spherical arrangement.

Colony morphology:

MacConkey: Pink color colony

Blood agar: yellow to cream or white colony.

Biochemical test: Coagulase-positive, Catalase-positive

***Candida* spp**

Colony morphology:

On Sabouraud Dextrose Agar: Round white colonies

Under the microscope: Budded yeast cells seen in a wet preparation.

Appendix II: Resistant pattern of some Antibiotic Resistant bacteria species isolated from urine of surgical patients of ABUTH before and after curing

Isolate No	Isolates	Resistance Profile before curing	Resistance Profile after curing
3	<i>Klebsiella</i> spp	F-SXT-CXM-AML-CRO	F-CRO
9	<i>Escherichia coli</i>	OFX-CN-CXM-AK-AML-CIP-CRO	OFX-CXM-AML
10	<i>Staphylococci</i> spp	OFX-F-CN-SXT-CXM-AML-CIP	OFX-CXM-AML-CIP
17	<i>Staphylococci</i> spp	OFX-F-SXT-CXM-AK-AML-CIP-CRO	OFX-SXT-CXM-AML-CIP-CRO
20	<i>Escherichia coli</i>	OFX-F-SXT-CXM-AK-AML-CIP-CRO	OFX-SXT-CXM-AML
24	<i>Escherichia coli</i>	OFX-F-SXT-CXM-AK-AML-CIP-CRO	OFX-CXM-CIP
26	<i>Pseudomonas</i> spp	OFX-F-CN-SXT-CXM-AML-CIP-CRO	OFX-F-CXM-CIP
31	<i>Staphylococci</i> spp	OFX-F-CN-SXT-CXM-AML-CIP-CRO	OFX-SXT-AML-CIP-CRO
33	<i>Pseudomonas</i> spp	OFX-F-SXT-CXM-AML-CIP	OFX-SXT-CXM-AML
35	<i>Escherichia coli</i>	OFX-F-SXT-CXM-AML-CIP-CRO	OFX-SXT-CXM-AML
37	<i>Proteus</i> spp	OFX-F-SXT-AML-CIP	OFX-SXT-AML
42	<i>Escherichia coli</i>	OFX-CN-SXT-CXM-AML-CIP-CRO	OFX-SXT-CXM-AML
48	<i>Pseudomonas</i> spp	OFX-F-CN-SXT-CXM-AML	F-SXT-CXM-AML
49	<i>Escherichia coli</i>	OFX-SXT-CXM-AML-CIP-CRO	SXT-CXM-AML
50	<i>Escherichia coli</i>	OFX-F-CXM-AML-CIP-CRO	CXM-AML
57	<i>Pseudomonas</i> spp	OFX-F-CN-SXT-CXM-AML-CIP-CRO	OFX-F-SXT-CXM-AML
77	<i>Escherichia coli</i>	OFX-CN-SXT-CXM-AML-CIP-CRO	OFX-CN-SXT-CXM-CIP
81	<i>Escherichia coli</i>	OFX-F-CN-SXT-IPM-CXM-AK-AML-CIP-CRO	OFX-SXT-CXM-AML-CIP

Appendix III: Zone Diameter Interpretative Standard according to European Committee on Antimicrobial Susceptibility Testing, 2014.

S/No	Antibiotics		Zones of inhibition (mm)	
	Intermediate	Susceptible	Resistance	
1	Amoxicillin (10µg)		≥14	14
2	Ofloxacin (5µg)		19-21	≥22
3	Ciprofloxacin (5µg)		19-21	≥22
4	Gentamicin (10µg)		14-16	≥17
5	Amikacin (10µg)		13-15	≥16
6	Cefuroxime (30µg)			≥18
7	Ceftriaxone (30µg)		20-22	≥23
8	Cotrimoxazole (25µg)		13-15	≥16
9	Imipenem (10µg)		16-21	≥22
10	Nitrofurantoin (300µg)			≥11

Appendix IV: Zone Diameter Interpretative Standard according to Clinical and Laboratory

Standard Institute Guidelines (CLSI, 2014.)

S/No	Antibiotics	Zones of inhibition (mm)		
		Intermediate	Susceptible	Resistance
1	Amoxicillin (10µg)	14-16	≥17	≤13
2	Ofloxacin (5µg)	13-15	≥16	≤12
3	Ciprofloxacin (5µg)	16-20	≥21	≤15
4	Gentamicin (10µg)	13-14	≥15	≤12
5	Amikacin (10µg)	13-16	≥17	≤14
6	Cefuroxime (30µg)	15- 22	≥23	≤14
7	Ceftriaxone (30µg)	16-20	≥21	≤15
8	Cotrimoxazole (25µg)	11-15	≥16	≤10
9	Imipenem (10µg)	20-22	≥23	≤19
10	Nitrofurantoin (300µg)	15-16	≥17	≤14

Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates.

S/N	Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R
1	1	I	S	S	S	S	S	S	S	S	S	0
2	2	I	S	S	R	S	R	S	S	S	R	3
3	3	I	R	S	R	S	R	S	S	S	R	4
4	4	S	R	S	R	S	R	S	S	S	R	4
5	5	R	R	S	S	S	R	S	S	S	R	4
6	6	I	R	S	S	S	R	S	S	I	R	3
7	7	I	R	S	R	S	S	S	S	S	S	2
8	8	R	R	S	R	S	S	S	R	R	S	5
9	9	R	S	R	S	S	R	R	R	R	R	7
10	10	R	R	R	R	S	R	S	R	R	S	7
11	11	R	S	S	R	S	S	S	R	R	S	4
12	12	R	S	S	S	S	S	S	S	S	I	1
13	13	S	S	S	S	I	R	S	R	S	R	3
14	14	S	S	S	S	S	R	S	R	S	R	3
15	15	S	S	S	S	I	R	S	R	S	R	3

Appendix III: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates cont'd.

S/N	Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R
16	16	S	S	S	S	I	R	S	R	S	R	3
17	17	R	R	S	R	S	R	R	R	R	R	8
18	18	R	S	S	S	S	S	S	S	S	S	1

19	19	S	S	S	S	I	R	S	R	S	R	3
20	20	R	R	I	R	I	R	R	R	R	R	8
21	21	S	S	S	S	S	R	S	S	S	R	2
22	22	S	S	S	S	S	S	S	S	S	S	0
23	23	S	S	S	S	S	S	S	S	S	S	0
24	24	R	R	I	R	I	R	R	R	R	R	8
25	25	S	S	S	S	S	R	S	R	S	R	3
26	26	R	R	R	R	I	R	I	R	R	R	8
27	27	S	R	I	R	S	R	S	R	S	R	5
28	28	R	S	S	R	S	R	S	S	S	I	3
29	29	S	S	R	R	I	R	S	S	S	R	4
30	30	S	S	S	S	S	R	S	R	S	R	3

Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates cont'd.

S/NIsolateN0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R	
31	31	R	R	R	R	I	R	S	R	R	R	8
32	32	R	S	S	S	S	S	S	S	S	I	1
33	33	R	R	S	R	S	R	S	R	R	S	6
34	34	R	S	S	R	S	R	S	R	R	S	5
35	35	R	R	I	R	I	R	S	R	R	R	7
36	37	R	R	S	R	S	S	S	R	R	S	5
37	38	S	S	S	S	S	R	S	R	I	R	3
38	40	R	S	R	R	S	R	S	R	R	S	6
39	41	S	R	S	S	I	S	S	R	S	S	2

40	42	R	S	R	R	S	R	S	R	R	R	7
41	44	S	S	S	S	S	S	S	S	S	S	0
42	45	S	S	S	S	S	R	S	R	S	R	3
43	46	R	S	S	R	S	S	S	R	R	I	4
44	47	S	R	R	R	S	R	S	R	S	S	5
45	48	R	R	R	R	S	R	S	R	S	I	6

Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates cont'd.

S/N Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R	
46	49	R	S	S	R	I	R	S	R	R	R	6
47	50	R	R	I	I	I	R	S	R	R	R	6
48	51	I	R	R	R	S	R	S	R	S	I	5
49	52	S	S	S	S	I	R	S	R	S	R	2
50	53	S	S	S	S	S	R	S	R	S	R	3
51	54	I	S	S	S	S	R	S	R	S	S	2
52	55	S	R	S	R	S	R	S	R	S	I	4
53	56	S	S	R	S	S	R	S	S	S	R	3
54	57	R	R	R	R	S	R	S	R	R	R	8
55	58	S	S	S	S	S	S	S	R	S	S	1
56	59	R	R	S	R	S	S	S	R	S	S	4
57	60	S	R	S	S	S	S	S	R	S	R	3
58	61	R	S	S	R	S	S	S	R	R	S	4
59	62	R	S	S	S	S	S	S	S	S	S	1
60	63	R	S	S	R	S	S	S	R	R	S	4

Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates cont'd.

S/N	Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R
61	64	R	R	S	S	S	S	S	R	R	S	4
62	65	R	R	S	S	S	S	S	R	R	S	4
63	66	R	R	S	S	S	S	I	R	R	S	4
64	67	I	S	S	S	S	R	S	R	S	S	2
65	68	R	S	R	R	S	R	I	S	R	I	5
66	69	S	R	S	S	S	R	S	R	S	R	4
67	70	I	S	I	S	S	S	S	R	S	S	1
68	71	R	S	R	R	S	R	S	R	R	S	6
69	72	R	S	R	R	S	S	S	R	R	S	5
70	73	S	S	S	R	S	S	S	R	S	R	3
71	74	S	R	S	R	S	S	S	R	I	S	3
72	75	R	S	S	R	S	S	S	R	R	S	4
73	77	R	S	R	R	S	R	S	R	R	R	7
74	78	S	S	S	S	S	S	S	R	S	S	1
75	79	S	S	R	R	S	S	S	R	S	R	7

Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different

Bacterial Isolates cont'd.

S/N	Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R
76	80	S	S	S	S	S	S	S	S	S	S	0
77	81	R	R	R	R	R	R	R	R	R	R	10
78	83	S	S	S	S	S	R	S	R	S	R	3

79	84	R	S	S	S	S	S	S	R	S	S	2
80	85	R	R	S	S	S	R	S	R	I	S	4
81	86	R	R	S	S	S	S	S	R	R	S	4
82	87	R	S	S	R	I	S	S	R	R	S	4
83	88	S	S	S	R	S	R	S	R	S	R	4
84	89	R	S	R	R	S	S	S	R	R	S	5
85	90	S	S	S	I	S	S	S	R	S	S	1
86	91	S	S	S	R	S	R	S	R	S	R	4
87	92	S	S	S	S	S	S	S	R	S	S	1
88	93	S	S	I	R	S	S	I	R	S	S	2
89	94	R	S	R	R	S	R	S	R	R	S	6
90	95	I	S	I	R	S	S	S	R	S	S	2


Appendix V: Interpretation of Antibiotic Susceptibility Tests of the different Bacterial Isolates cont'd.

S/N Isolate N0	OFX	F	GN	SXT	IPM	CXM	AK	AML	CIP	CRO	Σ R	
91	96	R	S	S	R	S	S	S	R	S	S	3
92	97	I	S	S	S	S	S	S	R	S	S	1
93	98	S	S	S	R	S	S	S	R	S	S	2
94	99	R	S	I	R	S	S	S	R	I	S	3
95	100	R	S	S	R	S	S	S	R	S	S	3
96	101	I	R	S	S	S	S	S	R	S	S	2
97	102	R	S	S	R	S	S	S	R	S	S	3
98	103	I	S	S	R	S	S	S	R	S	S	2
99	104	R	S	S	R	S	R	S	R	S	S	4
100	105	S	S	S	S	S	S	S	S	S	S	0

101	106	S	S	S	R	S	S	S	R	S	S	2
102	107	S	R	S	S	I	S	S	R	S	S	2
103	108	S	S	R	S	S	R	S	R	R	S	4
104	109	S	S	S	S	S	S	S	R	S	S	1
105	110	S	R	S	S	S	S	S	R	S	S	2

Key: SXT= COTRIMOXAZOLE, CN= GENTAMICIN, CIP= CIPROFLOXACIN, AK= AMIKACIN, CXM= CEFUROXIME, OFX=OFLOXACIN, AML= AMOXICILLIN, CRO= CEFTRIAZONE, F= NITROFURANTOIN, IPM= IMIPENEM.

Appendix VI: Ethical clearance certificate to carryout study in ABUTH, Shika



HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuth@yahoo.com Website: www.abuth.org

Chairman of Board: Chief. Shualb Oyedokun Afolabi *FRII*
Chief Medical Director: Prof. Lawal Khalid, *MBBS, FMCS, FWACS, FRCS(ED) mni*
Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, *MBBS, FWACP, FICS*
Director of Administration: Barr. Ishak Bello, *LL.B, BL., LL.M, PGDM, AHAN, FCAI*

Our Ref: _____ ABUTH/HREC/TRG /36 _____ Date: _____ 7th Feb, 2014
Your Ref: _____

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

Re: "Incidence of nosocomial urinary tract infection in the surgical wards of Ahmadu Bello University Teaching Hospital Zaria."

ABUTH Ethics Committee assigned number: - ABUTH/HREC/ HO2//2013

Name of the principal Investigator: - Mr. Djauro Paul Mutah,

Address of the Principal Investigator: - Dept. of Pharmaceutical Microbiology
ABU, Zaria.

Date of receipt of valid application: - 31st January, 2014

Date of meeting when final determination on ethical approval was made: - 7th February, 2014

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and **given full approval by the ABUTH Ethics Committee.**

Please note: this approval dates from 7th February, 2014 – 7th February, 2015.

No participant recruitment into this research may be conducted outside these dates.


All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: <http://www.nhrec.net>.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.


Prof. A. I. Mamman
Chairman, ABUTH HREC

Appendix VII: Consent forms issued to recruited subjects.

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PROFORMA

Age of patient 40

Sex of patient Male Female

Initial diagnosis on admission Prostate cancer

.....

Any invasive procedure? Yes

Nature of invasive procedure urethral catheteri-
zation

Any antibiotic given? Yes No

What antibiotic given Flagyl, Ciprofloxacin

Any new diagnosis other than initial diagnosis? Yes No

2

6

CONSENT FORM

Title of Research Thesis

THE INCIDENCE OF NOSOCOMIAL URINARY TRACT INFECTION IN THE SURGICAL WARDS OF AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, SHIKA, NIGERIA

I have read the information provided above (it has been adequately explained to me). I have had the opportunity to ask questions about it and my participation and my questions have been answered to my satisfaction.

I voluntarily accept to participate /allow my ward or children to participate in this study and I understand that I have the right to withdraw from the study at any time without compromising the quality of care I deserve.

Yes No

[Signature]

14/03/14

Signature/thumb print of research respondent

Date

Printed name of research subjects legal guardian

[Signature]

14/03/14

Signature/thumb print of person obtaining consent

Paul Ajam

14/03/14

Printed name of person obtaining consent

Date

2 weeks
40 days

Address *Benue*

GSM/Telephone number _____

PROFORMA

30

Age of patient 35

Sex of patient Male Female

Initial diagnosis on admission Rapidly growing

umbilical swelling & appears on its own
with pus & stained discharge

Any invasive procedure?

Nature of invasive procedure.....

Any antibiotic given? Yes No

What antibiotic given..... Flagey, amoxicillin

Any new diagnosis other than initial diagnosis? Yes No

30

CONSENT FORM

Title of Research Thesis

THE INCIDENCE OF NOSOCOMIAL URINARY TRACT INFECTION IN THE SURGICAL WARDS OF AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, SHIKA, NIGERIA

I have read the information provided above (it has been adequately explained to me). I have had the opportunity to ask questions about it and my participation and my questions have been answered to my satisfaction.

I voluntarily accept to participate /allow my ward or children to participate in this study and I understand that I have the right to withdraw from the study at any time without compromising the quality of care I deserve.

Yes No

[Handwritten signature]

08/03/14

Signature/thumb print of research respondent

Date

Printed name of research subjects legal guardian

[Handwritten signature]

8/03/14

Date

Signature/thumb print of person obtaining consent

Paul Djamo

8/03/14

Date

Printed name of person obtaining consent

Date

Address *Kachia LGA*

GSM/Telephone number

PROFORMA

Age of patient 24

Sex of patient Male Female

Initial diagnosis on admission post-operative abdominal ulcer

Any invasive procedure? no

Nature of invasive procedure.....

Any antibiotic given? Yes No

What antibiotic given Penicillin, carbamazepine

Any new diagnosis other than initial diagnosis? Yes No

CONSENT FORM

Title of Research Thesis

THE INCIDENCE OF NOSOCOMIAL URINARY TRACT INFECTION IN
THE SURGICAL WARDS OF AHMADU BELLO UNIVERSITY
TEACHING HOSPITAL, SHIKA, NIGERIA

I have read the information provided above (it has been adequately explained to me). I have had the opportunity to ask questions about it and my participation and my questions have been answered to my satisfaction.

I voluntarily accept to participate /allow my ward or children to participate in this study and I understand that I have the right to withdraw from the study at any time without compromising the quality of care I deserve.

Yes No

Signature/thumb print of research respondent

Date

Printed name of research subjects legal guardian

Date

Signature/thumb print of person obtaining consent

Date

Printed name of person obtaining consent

Date

Address *kwai*

GSM/Telephone number *08106686417*