ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM URINE SAMPLES OFPATIENTS WITH URINARY TRACT INFECTIONS ATTENDING GENERAL HOSPITAL MINNA, NIGER STATE, NIGERIA

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DEPARTMENT OF MICROBIOLOGY

SCHOOL OF LIFE SCIENCES

FEDERAL UNIVERSITY OF TECHNOLOGY MINNA

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ABSTRACT

This study determined the antibacterial susceptibility pattern of bacterial isolates from urine samples of patients attending General Hospital, Minna, Niger State. Four hundred (400) urine samples were collected and cultured on Cysteine Lactose Electrolyte Deficient agar (CLED), MacConkey and Nutrient agar for isolation of bacteria. The isolated bacteria were identified using colonial, microscopic and biochemical tests. Antibacterial susceptibility profiles of the isolates were carried out using disc diffusion method.The bacteria isolates identified include: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus* aureus, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Salmonella enterica*, *Streptococcus pneumoniae*, *Enterobacter faecalis* and *Serratia marcescens. Escherichiacoli* 25(30.1 %) occurred more frequency than other bacterial isolates, followed by *Klebsiella pneumoniae* 20(24.0 %) and *Staphylococcus aureus* 16(19.3 %) *Enterobacter faecalis* 2(2.4 %) and *Serratia marcescens* 2(2.4 %) recorded theleast bacterial isolates. Female patients recorded more bacterial isolates than their male counterparts. Age groups 15-24 years revealed diverse bacteria, when compared to other age groups. Married patients recorded higher prevalence rate of bacterial infections 46(55.4 %), when compared to patients that were single 37(44.5 %). Similarly, patients with no formal education 35(42.1 %) were infected with diverse bacteria, when compared to those with formal education 11(13.2 %). *Salmonella enterica* 2(66.7 %) recorded the highest susceptibility to Nalidixic acid, Gentamicin and Sulfamethoxazole-trimepthoprim while, *Staphylococcus aureus* 16(62.5 %) and *Streptococcuspneumoniae*, 3(66.7 %) were susceptible to Levofloxacin, Chloramphenicol and Amoxil. Similarly, all the isolated bacteria were resistant to atleast three (3) antibiotic tested (MDRI=0.3). Multidrug resistance uropathogens exist in this study area. Therefore, regular surveillance and stewardship should be encouraged.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Urinary tract infection (UTI) is the microbial invasion of any of the tissues (kidneys, ureters, bladder, urethra and accessory structures) of the urinary tract (Otajevwo *et al*., 2013). Typical symptoms associated with UTI include painful urination (dysuria), the enhanced desire to void the bladder (urgency) and increased rate of urination (frequency) (Ogbukagu *et al*., 2016).

The evidence of UTI is confirmed by the presence of 10^5 cfu/mL in urine samples of a single strain of bacterium per milliliter in two consecutive midstream sample of urine. Urinary tract infections are caused by bacteria in the gastro intestinal tract that colonized the periurethral area (Hooton *et al*., 2013). Gram negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Proteus* species, *Enterobacter* species, and *Serratia* species are the major genera associated with urinary tract infections (Ogbukagu *et al*., 2016). Other bacterial pathogens less frequently isolated include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* (Ogbukagu *et al*., 2016).

UTI constitutes a major public health problem among male and female, particularly in women (Adabara *et al.*, 2012). The proximity in female urethra to the vagina orifice aids microbial contamination (Ebie *et al*., 2001). The risk factor of urinary tract infections (UTIs) is in many time found with pregnant women, partly due to the pressure of gravid uterus on the ureters causing stasis of urine flow and is also attributed to the immunological changes during normal pregnancy (Ramzan *et al*., 2004). Bacteriuria is one of the most common complications during pregnancy which may be

either symptomatic or asymptomatic. It occurs in 2–7 % of pregnant women in the first trimester (Thomas *et al*., 2018). Urinary tract infection is associated with increase maternal and perinatal morbidity namely spontaneous rupture of membrane, preterm labour and delivery, septicaemia, preterm baby and neonatal infection (Matuszkiewicz-Rowińska *et al*., 2015).

1.2 Statement of the Research Problem

Urinary tract infection is a public health challenge worldwide with fatal complications (WHO 2014). Many people are harboring the infection unknowingly due to reluctance of people to undergo routine screening, lack of awareness and knowledge on some remote mode of prevention and management of the infection such as personal hygiene, poor anal hygiene, indiscriminate use of antibiotics and improper medical diagnosis (Idris *et al*., 2014). The high number of urinary tract infection occur among all ages and women especially those who are sexually active or pregnant which put them at high risk of infection in Minna, Niger State and Nigeria at large (Adabara *et al.*, 2012).

Various tests have been used in the diagnosis of Urinary tract infection (UTI), but urine culture remains gold standard (Idris *et al*., 2014) because the sensitivity of bacteria to the antibiotics varies according to time in any geographical and regional location (Okonkwo *et al*., 2009). However, majority of the treatments are done completely empirically, especially in developing countries. Multi-drug resistant bacteria have been reported over the past three decades and this has become a major challenge in the treatment of UTIs worldwide (Mitta *et al*., 2009). Also, the indiscriminate use of antibiotics remains the leading force to emergence of drug resistant bacteria. Indiscriminate use of antibiotics has led to the emergence of resistance pathogens which has contributed immensely to morbidity and mortality (Idris *et al*., 2014).

1.3 Justification for the Study

Previous studies by Adabara *et al*. (2012), Idakwo *et al*. (2015) and Ogbukagu *et al*. (2016) were focused on the prevalence of bacteria in urine samples without much emphasis on socio demographic information as well as their Multi-drug resistant. Such information would assist on its prevention and management of this fatal health challenge in General Hospital Minna, Niger State. The antimicrobial susceptibility study of any pathogen is vital in effective treatment of the disease caused by such pathogen. This study is necessary in order to establish the need of treating patients with urinary tract infection based on antimicrobial susceptibility test result and not based on prescriptive diagnosis.

1.4 Aim and Objectives of the Study

The study aimed at investigating antibiotic susceptibility pattern of bacterial isolates from urine samples of patients attending General Hospital, Minna, Niger State, Nigeria.

The objectives of this study were to:

i. isolate and identify bacterial pathogens from urine samples of the study population.
ii. determine the socio demographic factors associated with urinary tract infection in the study population.
iii. determine the antibiotic susceptibility profile of the bacterial isolates from the study population.

CHAPTERTWO

2.0 LITERATURE REVIEW

2.1 Overview of Urinary Tract Infections

Urinary tract infection (UTI) is a microbial colonization of the urinary epithelial cells as well as tissue invasion and increased number of uropathogens (Otajevwo *et al*., 2013). The urinary cells are major sites of bacterial invasion and a high proportion of women have persistent urinary tract infections at different points in their life. Microorganisms such as bacteria, fungi, protozoa and viruses, with bacteria having higher prevalence and invasiveness than others (Momoh *et al*., 2011). Typical symptoms associated with UTIs include painful urination (dysuria), the enhanced desire to void the bladder (urgency) and increased rate of urination (frequency). Other symptoms may include, flank pain, fever or chills, nausea, polyuria; urine is milky, bloody and may have odious smell (Momoh *et al*., 2011). Urinary tract infection is a public health challenge occurring among patients of all ages in most parts of the developing countries and particularly with Sub-Saharan Africa carrying the larger burden (Tula and Lyoha, 2014).

2.2 Modes of Bacterial Entry

Bacterium enters the genitourinary tract through either of the following; ascending or haematogenous spread (Kalantar *et al*., 2008) in a study reported that microbial normal flora in the rectum; enter the urinary tract via the urethra into the bladder in healthy humans. Uropathogens therefore colonize epithelium of the urethra in the ascending

route. A report by Foxman, (2010) showed that most urinary tract infection cases are caused by bacteria ascending from the perineum. About half of the infections ascend into the upper urinary tracts in patient with cystitis and infections of pyelonephritis which are caused by ascension of the bacteria from the bladder through the urethra and into the renal pelvic region (Patel *et al*., 2012).

Pregnancy and urethral obstruction are vital factors in attachment of uropathogens inhibiting urethral peristalsis. Manikandan *et al*. (2011) went further to report that after microbial attachment, they enter the renal parenchymal cells through the collecting ducts and colonize the pelvic region causing inflammation of the urinary tract. Haematogenous route entry of bacteria into the urinary tract is seen in neonates and immuno-compromised patients as reported by Dulczack and Kirk (2005). Schlager (2001) in a related study reported that UTI may be secondary to haematogenous source within the first eight to 12 weeks of life and at such; early diagnosis of UTI in children is of great importance because it serves as a marker urinary tract abnormalities in neonates. The frequently occurring pathogens involved in the haematogenous route are *Staphylococcus aureus, Candida* species and *Mycobacterium tuberculosis* Tonagho and Mcaninch (2004).

2.3 Bacterial Associated with Urinary Tract Infections

Over the past three decades, there have been drastic changes in features of pathogens implicated for urinary tract infections as a result of drug resistance, some underlying host factors such as personal hygiene, age, pregnancy, health history, spinal cord injury, and catheterization (Antwi *et al*., 2008). As a result, complicated urinary tract infection has a more varied etiology than uncomplicated urinary tract infection. Bennett *et al*. (2014) reported that UTI causing bacteria are more likely to infect individuals with

underlying health complications that affect the optimal anatomic, metabolic or immunologic functions than in healthy individuals. The major bacteria implicated for urinary tract infections are most particularly the Gram negative bacteria such as *Pseudomonas, Escherichia coli, Serratia, Proteus* spp., and *Klebsiella* spp. (Shaikh *et al*., 2008; Manikandan *et al*., 2011; Onuoha and Fatokun, 2014).

Studies have also reported some few important Gram positive bacteria such as Staphylococci, Enterococci and Streptococci in some cases (Foxman, 2003; Adabara *et al*., 2012; Ogbukagu *et al*., 2016). Foxman (2002) reported that the most common organisms isolated in children with uncomplicated urinary tract infections are the Enterobacteriaceae. In diabetic patients, pathogens implicated for urinary tract infections include *Klebsiella* spp., *Streptococcus* spp., *Enterococcus* spp., and *E. coli* (Whitinga *et al*., 2011).

2.4 Prevalence of Urinary Tract Infections

According to Fofana *et al*. (2016) reported that the most common bacteria isolated from urine samples of patients was *E. coli*, with 45.7 % followed by coagulase negative *Staphylococcus* which had 17.1 %.

In another related study conducted in India, Thomas *et al*. (2018), reported that of 96 urine samples collected, 24 were positive for urinary tract infection. It was also reported from the study that the most common causative organism was *E. coli* which accounted for 12 (50 %), followed by *P. aeruginosa* 6 (25 %), *Enterococcus faecalis* 4 (17 %), and *Klebsiella pneumoniae* 2 (8 %) respectively. The study also reported that patients ranging from 18 to 26 years showed a high prevalence of urinary tract infection. 41.6 % of the patients with bacteriuria were in the second trimester followed by third (37.5 %) and first trimester (20.8 %) of pregnancy. Kenechukwu *et al*. (2005) in a study

conducted at the Imo State University Teaching Hospital, Orlu, Nigeria, stated that *E. coli* accounted for 52.5 %, *S. aureus* 33.9 %, *P. mirabilis* 8.8 %, *Enterococcus* spp. 5.0 % and *N. gonorrhea* 1.7 %, of urinary tract infection cases. In Kano State, Nigeria, it was reported that UTI was predominantly caused by *Staphylococcus aureus* (67.9 %), *Klebsiella* species (7.9 %,) and *Pseudomonas* (14.2 %) Adeleke and Asani (2009).

Adabara *et al*. (2012) reported that of the total number of samples investigated in the study, 75 (75.0 %) were found to be positive for bacterial urinary tract infection. On the basis of age, the distribution of infection revealed prevalence rates of 100.0 %, 94.4 % and 64.0 % for age groups 30-39, 20-29 and 40-49 respectively. One hundred and ten bacterial agents were isolated, characterized and identified. *Klebsiella* sp showed the highest frequency of occurrence of 43 (39.1 %) and followed in descending order by *Escherichia coli*, *Staphylococcus aureus, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella* sp with 31 (28.2 %), 23 (20.9 %), 11(10.0 %) 1 (0.9 %) and 1 (0.9 %) respectively.

In a study conducted by Idris *et al*. (2014), 200 clinically diagnosed cases of UTI in pregnancy were recorded between January, 2012 and June, 2012, out of three thousand four hundred and forty-two obstetric patients seen at the department of Obstetrics and Gynecology of university of Ilorin teaching hospital, Ilorin, giving a prevalence of 5.8 %. Of the 200 subjects, 112 (56 %) had clinical cystitis and 88 (44 %) had pyelonephritis. There were 90 (45 %) laboratory confirmed cases of UTI in pregnancy with significant bacterial growth. Over half of the pregnant women with significant bacterial growth from urine was in the age group 21-30 years, while those less than or equals to 20 years had the least frequency (4.4 %). *Escherichia coli* were the most common isolated uropathogen and it accounted for 46.7 % of the laboratory confirmed cases. Other organisms isolated in order of frequency were *Staphylococcus aureus* (17.9

%), *Proteus* species (13.3 %), *Klebsiella* (11.1 %), *Pseudomonas* (4.4 %) and *Candida* species (2.2 %). Co-amoxyclav had the widest coverage, was effective against 81 % of all the organisms isolated. Other antimicrobial sensitivity pattern in order of frequency were Gentamicin (68.8 %), Cefuroxime (54.4 %), Ciprofloxacin (49.8 %), ceftriaxone (28.8 %), Nitrofurantoin (25.5 %), Erythromycin (15.5 %), Amoxicillin (5.6 %) and Cotrimoxazole (4.4 %). Ampicillin didn`t show any degree of effectiveness to all uropathogens isolated in this study, probably because of its indiscriminate use among the general populace. Age bracket 21-30 years had highest frequency of significant bacteriuria. Low social status and third trimester of pregnancy were identified risk factors for UTI in pregnancy. The Gram-negative Rods *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas aeruginosa* and other Enterobacteriaceae are frequently found in hospital. They are common cause of UTI in hospital because of their resistance to antibiotics (Manikandan *et al*., 2011; Onuoha and Fatokun, 2014).

In hospitalized and long term catheterized patients, the risk of *S. aureus* carriage to the urinary tract is increased, leading to urinary tract infection which may also result in staphylococcal bacteremia (Muder *et al*., 2006; Ikeagwu *et al*., 2008). Staphylococcal urinary tract infections may lead to septicaemia which affects about 10 % of the people (Sarathbabu *et al*., 2013).

Mitchell *et al*. (2016) reported an incidence of 1.73 % from eight hospitals during a four-year period also for healthcare-associated urinary tract infections. In another study from 82 hospitals and 17 aged care facilities in Australia, it a point prevalence of 1.4 % and 1.5 % respectively was reported for healthcare-associated urinary tract infection (Mitchell *et al*., 2016).

2.5 Epidemiology of Urinary Tract Infection

In a Study by Khoshbakht *et al*. (2013), it was reported that the problem of drug resistance in Africa comes from factors such as, inappropriate advertisement of medicines, indiscriminate use of antibiotics, lack of correct awareness and prescription by quacks. The incidence of bacteriuria is higher among the very young and very old in both men and women, but the prevalence of Urinary Tract Infection is extensively higher for women than men until men attain the age of 60 (Foxman, 2010).

The high prevalence in females has been traced to the nature of the urino-genetal tract; the urethra of the female is much shorter and at the extreme of the anus than in males and it also lacks the bacteriostatic properties of prostratic secretions (Ogbukagu *et al*., 2016). The sexually active age group has the highest occurrence of urinary tract infections; this is perceived to be because of tissue injury during sex and also due to the increased sexual activities with different people thereby predisposing them to urinary tract infection. Urinary tract infections are broadly divided into two categories, namely; hospital acquired and community-acquired. The hospital-acquired is associated with catheterization. There has been increase in multidrug resistance both in developing and developed countries and this has become a global health challenge. The distribution of antimicrobial resistance among different uropathogens differs between and within countries leading to selection of superior regimen of treatment in developed countries and raising the cost of treatment unaffordable to developing countries (Oladeinde *et al*., 2011: Annpurna and Lakshmi, 2013).

Inadequate access to antibiotics, poor health care services, poverty, malnutrition and incomplete doses of medicines that are routinely used are some of the factors that contribute to the rapid emergence and spread of antimicrobial resistance (Oladeinde *et al*., 2011). Urinary tract infections are more prevalent among young women who have

not attained the age of menopause than postmenopausal women (Fihn, 2003; Henn, 2010).

Recurrent urinary tract infections are also common among healthy women with structurally normal urinary tracts, with as many as 5 % of women experiencing it at some stage during their life (Scholes *et al*., 2000). Recurrent urinary tract infections could be as a result of the following; persistence of the original organism, re-infection with the original organism and re-infection with a different strain of bacteria (Dwyer and O'Reilly, 2002). In women, the majority of urinary tract infections are as a result of re-infection of the initial bacteria due to bacterial persistence in the faecal flora and subsequent re-colonisation of the urethra (Dwyer and O'Reilly, 2002).

2.6 Bacterial Virulence Factors

For any pathogenic organism to effectively establish an infection and subsequently cause a disease, there are four main attributes the pathogen must possess; attachment, invasion, ability to damage the tissues of the host by toxins and evasion of host defense mechanism. The virulence of bacteria determines the level of infection and this determines its ability to invade the urinary tract (Alemu *et al*., 2012).

Bacteria attachment to the urinary tract is by adhesions which are found on the bacterial cell membrane (Foxman, 2010). Members of the Enterobacteriaceae adhere to host cells with the help of two major adherence factors namely, type 1 fimbriae and P fimbriae (Oladeinde *et al*., 2011). Type 1 fimbriae protrude from the surface of *E. coli* and other genera of the Enterobacteriaceae (Alemu *et al*., 2012). Invasion of the urinary tract requires the binding of fimbriae to Mannose-containing oligosaccharide by means of the Fim H adhesive tip protein. In addition to their primary function as adhesion molecules,

type 1 fimbriae and P fimbriae also send signals to the epithelial cells leading to inflammation of host's epithelial cells as reported by Ejaz *et al*. (2006).

Haemolysin and aerobactin which are resistant to the bactericidal action of urinary tract tissues are produced by some *E. coli* strains, these molecules are responsible for acute cystitis and acute pyelonephritis. Patients with functional or structural deformities of the urinary tract become susceptible to infections caused by bacterial strains that possess haemolysin and aerobactin (Oladeinde *et al*., 2011). Rare voiding, partial voiding, sexual activity, personal hygiene, hormonal status, use of spermicidal contraception, genetics, diabetes and immunosuppressant substances are notable factors that may increase the risk of urinary tract infection (Komala and Sampath, 2013).

2.7 Pathogenesis

Sterile urine is produced in the kidney of healthy individuals which passes through the renal pelvic region and ureters where an infection is acquired by the ascending route from the urethra to the urinary bladder proceeding to the kidney leading to pyelonephritis (Oladeinde *et al*., 2011). Women have a shorter urethra which predisposes them to infection than the males. In adolescent males, the uncircumcised stand at a high risk of UTI due to colonization of the inside of the prepuce and urethra (Kathleen, 2008; Alemu *et al*., 2013).

Invasion and multiplication of urinary pathogens in the underlying epithelial cells leads to the establishment of dormant bacterial reservoir within the bladder tissue (Lewis, 2013). Uropathogens stays in the bladder epithelial cells for weeks therefore causing infection. The symptoms of UTI include dysuria, pain on urination, incontinence and polyuria. A full sensation in the rectum is experienced by men. Children with UTIs

present with symptoms, such as irritability, incontinence, diarrhea, poor appetite, and fever (Kathleen, 2008).

2.8 Clinical Manifestations of Urinary Tract Infections

Various clinical manifestations of urinary tract infection exist; these include asymptomatic bacteriuria, cystitis and acute pyelonephritis.

2.8.1 Asymptomatic bacteriuria

The presence of bacteria in urine (bacteriuria) is a clinical feature that is associated with all types of the types of urinary tract infection. Bacteriuria is often accompanied with no symptoms (asymptomatic bacteriuria). A diagnosis of asymptomatic bacteriuria requires ≥105 colony forming unit per milliliter (cfu/mL) from a mid-stream, clean catch urine, or a minimum of 100 cfu/mL for a catheterized urine (Initiative and WHO, 2003). Although other studies have suggested that lower levels of bacteriuria (10^2 to 10^4 cfu/mL) should be indicative of UTI (Fihn, 2003; Wilson and Gaido, 2004). Pregnant women are at risk of asymptomatic bacteriuria, due to decrease in immuno-competence. Untreated bacteriuria in pregnancy has been associated with prematurity and low birth weight.

2.8.2 Cystitis

Cystitis primarily involves colonization of the bladder, otherwise defined as symptomatic infection of the urinary bladder in healthy person with a normal genitourinary tract (Oladeinde *et al*., 2011). Cystitis typically present with supra-pubic pain, urgency, haematuria, dysuria and frequency (Medina-Bombardo *et al*., 2003). Also, patients may experience (blood in urine), suprapubic pain or tenderness, and a change in the odor of the urine (Medina-Bombardo *et al*., 2003; Vasudevan, 2014).

2.8.3 Acute pyelonephritis

Acute pyelonephritis is an abnormal urinary condition commonly referred to as upper urinary tract infection. The condition is more severe than the asymptomatic bacteriuria and cystitis. It involves colonization of the kidneys and it is capable of progressing to bacteremia (Rajabnia-Chenari *et al*., 2012). Symptoms generally develop rapidly over few hours of colonization and may include chills, vomiting, fever, nausea and diarrhea. Symptoms of cystitis may or may not be present. leukocytosis is another marked symptom in most patients. Leukocyte casts are seen in the urine of some patients, and the detection of these casts is pathogenic. Hematuria manifests at the acute stage of the disease but if it persists beyond the acute phase, it develops into a stone or a tumor (Emiru *et al*., 2013). However, clinical manifestation in children may be non-specific, such as poor feeding, irritability and jaundice in newborns.

2.9 Laboratory Diagnosis of Urinary Tract Infections

There are three major laboratories diagnostic methods employed to detect the presence of uropathogens; dipstick urinalysis and microbiological urine culture and most recently, the Polymerized Chain Reaction (PCR). The preliminary diagnosis of urinary tract infection is physical screening of urine to observe its appearance, this is followed by further examination of urine for the presence of uropathogens; UTI is established when a single bacterium is detected in urine culture with 10^5 colony forming unit (WHO, 2000; Annpurna and Lakshmi, 2013; Mehta *et al*., 2013).

In order to limit morbidity, mortality and to avoid prolonged use of antibiotics, arising from UTIs, accurate diagnosis and treatment of UTI is essential (Piranfar *et al*., 2014). With the use of a sterile container, midstream urine sample is collected and used in the diagnosis of UTI (Annpurna and Lakshmi, 2013; Mehta et *al*., 2013). Following

preliminary urine analysis, urine culture is carried out for the confirmatory diagnosis of urinary tract infections in patients (Piranfar *et al*., 2014).

2.10 Treatment of Urinary Tract Infections

To avoid antibiotic resistance, antimicrobial sensitivity test ought to be conducted before commencement of treatment (Beyene and Tsegaye, 2011). The common antibiotics used in the management of urinary tract infection include, Cefuroxime, Amoxicillin/clavulanic acid, Trimethoprim/Sulpmethoxazole and Fluoroquinolones (Franco, 2005).

In a study, it was indicated that most uropathogens isolated were resistant to Tetracycline, Ampicillin and Cotrimoxazole but showed susceptibility to Nitrofurantoin, Gentamycin and Nalidixic Acid. Ogbukagu *et al*. (2016) in a study reported that all *E. coli, Klebsiella* species, *Citrobacter intermedius*, *Pseudomonas aeruginosa* isolates were resistant to Cephalexin at all concentrations, *Staphylococcus aureus* was sensitive to Cephalexin at high concentrations but resistant at low concentrations. The study also provided that all *E. coli, Klebsiella* species, *Pseudomonas aeruginosa* isolates were resistant to Penicillin V, *Staphylococcus* and *Citrobacter* showed sensitivity at high concentrations but were resistant at higher concentrations.

The study also reported that all *E. coli, Klebsiella* species and *Pseudomonas aeruginosa* isolates were resistant erythromycin at all concentrations while *Staphylococcus aureus* showed sensitivity at very high concentrations but resistant at medium and low concentrations. In the report, it was also reported that *Pseudomonas aeruginosa* was completely resistant to Gentamicin, *E. coli, Klebsiella* species and *Citrobacter* species were all resistant to Gentamicin at low concentrations but sensitive to same at very high concentration. In another study, Thomas *et al*. (2018) also reported that Nitrofurantoin,

Ciprofloxacin, Norfloxacin, Amoxicillin-Clavulanate (amoxiclav), Cefotaxime, Gentamicin, and Ceftriaxone antibiotics were tested for resistance. The resistance pattern of the isolates in the study revealed that *E. coli* had 8.3 % resistance to Nitrofurantoin, 8.3 % resistance to Ciprofloxacin, 25 % resistance to Norfloxacin, 83.3 % resistance to Amoxiclav, 75 % resistance to Gentamicin, and 8.3 % resistance to Ceftriaxone.

The highest resistance was seen with the use of Gentamicin (66.6 %). *Klebsiella pneumoniae* was found to be completely resistant to Gentamicin was observed with. *E. faecalis* showed 25 % resistance to Nitrofurantin, Ciprofloxacin, Norfloxacin, and Amoxiclav 83.3 and 66.6 % of *P. aeruginosa* were resistant to Gentamicin and Ciprofloxacin respectively. Debabrata *et al.* (2018) reported that Amikacin and Meropenem had high sensitivity to Enterobacter, *E. coli*, *Citrobacter* sp and *Klebsiella* sp, while the four (4) organisms were resistant to Ampicillin and Cefotaxime. The following factors should be considered in the selection of antibiotics for treatment: pharmacokinetics, spectrum of activity, resistance to community, potency of the drug, side effects, adverse effects and duration of therapy (Manikandan *et al*., 2011).

Non-antimicrobial treatment of uncomplicated UTI has been investigated as a promising means to reducing superfluous antimicrobial prescriptions and consequent resistance. Published evidence from randomised controlled trials comparing antimicrobial treatment with placebo or alternative treatment options such as delayed (48 hours) antimicrobials or ibuprofen showed that patients in the placebo and delayed antimicrobial groups had significant delays in improvement of symptoms and bacteriological cure (Little *et al*., 2010; Gágyor *et al*., 2012). Although patients in the ibuprofen group had significantly fewer antimicrobial courses, they had a significantly higher total symptom burden with more patients having pyelonephritis (Gágyor *et al*.,

2015). To further investigate the effectiveness of non-antimicrobial approaches in treatment of urinary tract infection, further research with larger sample sizes are recommended.

2.11 Antimicrobial Resistance

The world health organization (WHO) has termed antimicrobial resistance as an international threat to public health, with great threats arising from the phenomenon in the successful prevention and treatment of bacterial, viral, parasitic and fungal infections (World Health Organization, 2012, 2014). Therefore, misuse and indiscriminate use of antimicrobial agents resulted in the post antibiotic era which has become a worldwide challenge (Vila and Pau, 2010).

Basically, the Factors influencing microbial resistance to antibiotics are mutations, acquiring new genetic material, exposure to cells with new genetic material and use of antimicrobial agents as growth promoters in animal feeds destined for human consumption give rise to multidrug resistance. Many studies have indicated the presence of multidrug resistance in etiologic agents of urinary tract infection (Tula and Iyoha, 2014). Omulo *et al*. (2015) describes the emergence of antimicrobial resistance as interplay of human being, ecological and pathogen-related factor. Inadequacies in the healthcare environment ranging from inadequate diagnostic capacity and resources, over the counter access to antibiotics, constrained access to health facilities and limited education with respect to antibiotic use have greatly contributed to the demand for antibiotics (Shears, 2001).

Khan *et al*. (2018) reported that out of 500, 105 isolates were identified as multi-drug resistant uropathogenic *E. coli*, the multidrug resistant isolates showed the highest resistance to Aztreonam, Amoxil/Clavulanic acid, Ampicillin, Cotrimoxazole,

Ceftriaxone, Cefipime, and Cefuroxime. It was also reported from the genetic analysis that the majority of the multidrug resistant *E. coli* carried extended spectrum β-lactamase (ESBL) in the following proportions, CTX M1 (57.1 %) followed by TEM (33.3 %) and SHV (9.5 %). The study however revealed that 79 % of multidrug resistant *E. coli* possessed class 1 integrons, whereas all three conserved genes for class 1 integrons were present in 58 % of multidrug resistant *E. coli.*

2.12 Antimicrobial Susceptibility

Antimicrobial susceptibility is termed as any drug that works effectively in the treatment of pathogenic infections. In some health facilities clinician prescribe drugs based on the symptoms instead of performing diagnostic tests. Hence, antibiotics susceptibility testing plays an important role in determining the effectiveness of the drug against the bacteria (Huang *et al*., 2015).

Out of 215 urine samples, 100 isolates were identified. The overall susceptible isolates against antibiotics tested, *Streptococcus* sp recorded the highest sensitive isolates to Nitrofurantoin, Ciprofloxacin and Ofloxacin. Antibiotic usage has proven to be beneficial in counteracting infection, plant source like cranberry juice is equally effective in eliminating infection and can also be used as an alternative to counteract the pathogen causing urinary tract infections (Perpetua *et al*., 2016).

2.13 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing (AST) is a widely-used technique of evaluating antibiotic resistance and determine the treatment in clinical settings. Antimicrobial susceptibility testing (AST) is done in determining drug of choice in the treatment of pathogenic infections and also to determine the conceivable drug resistance in pathogenic microbes. Antimicrobial susceptibility testing is used for epidemiology, drug

discovery and prediction of therapeutic outcome. It was discovered that the recent use of antimicrobial testing methods is the in vitro investigation of samples and pure drugs as potential antimicrobial agents. The revolution in the 'golden era', reported that all groups of antibiotics are important (Tetracycline, Aminoglycosides, Macrolides and Cephalosporins) (Huang *et al*., 2015).

2.14 Methods of Antimicrobial Susceptibility Testing

Various methods of antimicrobial susceptibility testing exist; these include disc diffusion assays, agar dilution, antimicrobial gradient and broth dilution methods.

2.14.1 Disc diffusion assay

The disc diffusion (Kirby-Bauer) technique involves spreading of bacteria inoculum (approximately $1\text{-}2\times10^8$ cfu/mL) using swab stick on the Mueller-Hinton agar. The antibiotic disc was placed on Mueller-Hinton agar medium with the use of sterilize forceps. Each disc must be press down in order to ensure complete contact with the agar surface. The plate was incubated at 37 ^{o}C for 18 hours. The antibiotic diffuses from the disc into the agar in decreasing amounts from the disc. The killing or inhibiting of organisms by the concentration of the antibiotic there will be no growth around the disc represented as zone of inhibition. The zone of inhibition was measured in millimeters by either radius or diameter which compares the efficacy of antibiotics and monitoring antimicrobial resistance. The disc diffusion test result is reported as susceptible, intermediate and resistant (CLSI, 2018).

2.14.2 Agar dilution test

Agar diffusion test is one of the oldest methods for tedious antimicrobial susceptibility testing and remains one of the most common manual techniques for AST in clinical microbiological laboratories (Matuschek *et al.,* 2014). The test organisms are inoculated with a standardized inoculum on 90 mm diameter Mueller-Hinton agar plate (corresponding to 0.5 McFarland turbidity standard). Hence, 12 commercially prepared paper disc with the concentrations of the tests agent are placed on the inoculated agar surface. Agar plates are incubated at 37 ^{o}C for 16 hours. The diameter of the growth on zone of inhibition around each antibiotic disc is then measured in millimeter. The zone of inhibition is measured in millimeters and diameter (CLSI, 2018).

2.14.3 Broth dilution test

Broth dilution tests or micro-broth is one of basic antimicrobial susceptibility testing techniques. The procedure involves preparing two-fold dilutions of antibiotics (1, 2, 4, 8 and 16 µg/mL) in a liquid growth medium dispensed in tubes containing a minimum volume of 2 mL. Then each tube is inoculated with a prepared microbial inoculum in the medium after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale. The inoculated tubes are incubated at 37 ^{o}C for 20 hours. The tubes are examined for visible bacterial growth as evidenced by turbidity that prevented growth represented as minimal inhibitory (CLSI, 2016).

2.14.4 Antimicrobial gradient test

The antimicrobial gradient tests used to establish an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility using thin plastic

test strips that are impregnated on the underside with dried antibiotic concentration scale. As many as 5 strips may be placed in a radial fashion on the surface of an appropriate 150 mm agar plate that has been inoculated with standardized organism suspension and then, incubated at 37 ^{o}C for 20 hours. The result is interpreted by viewing the strip from tip of the plate. The MIC is determined by the intersection of lower part of the ellipse shape growth inhibition area with test strip (CLSI, 2018).

2.15 Mechanisms of Action of Antimicrobial Agents

Antimicrobial agents act in different ways, understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways resistance against bacteria. Generally, antimicrobial agents may either be bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system (Samuel, 2012). Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead. Conversely, the mechanism of action of antimicrobial agents can be further categorized on the basis of the structure of the bacteria or the function that is affected by the agents. These include generally the following: inhibition of the cell wall synthesis, inhibition of ribosome function, inhibition of nucleic acid synthesis, inhibition of foliate metabolism and inhibition of cell membrane function (Samuel, 2012).

2.16 Prevention and Control of Urinary Tract Infections

Creating awareness to women on the effects of frequently using low dose antibiotics to treat symptomatic UTIs and prevent recurrent infections will be of great importance.

Other antibiotics used for prophylaxis for recurrent UTIs are Norfloxacin and Fluoroquinolone. They can only be used after bacteriuria has been eradicated with a full dose treatment regimen (Saint and Chenoweth, 2003).

UTI can be prevented by regular intake of fluids which can help flush microorganisms from the urinary system. The individual should urinate when the urge arises to avoid multiplication of microorganisms when urine stays for long period in the bladder. Females should wipe from front to back after visiting toilet to prevent feacal flora microorganisms entering urethra. Tight-fitting jeans and nylon under wears trap a lot of moisture and hence encourage multiplication of microorganisms leading to UTI instead cotton underwear (Sood and Gupta, 2012).

Patients with urinary tract infections are subjected to several factors that may be associated with multidrug resistant microorganism carriage such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission (Kandemir *et al*., 2007) Continuous surveillance of antimicrobial susceptibility pattern culminating in rational use of antibiotics is known to improve treatment outcome, shortens duration of hospital stay and reduces the cost of treatment.

2.17 Health and Economic Implications of UTIs

Generally, there are numerous and significant health implications of antimicrobial resistance on the society. Increased patient morbidity and mortality are both as a result of antimicrobial resistance. Antimicrobial resistance may lead to treatment failure, resulting in death, especially in already critically unwell patients who are more at risk because of their relative immune deficiency and high exposure to antimicrobial agents (Tenover, 2006).

Approximately 25,000 patients died in 2007 from antimicrobial resistant infections in the European Union, Iceland and Norway. About two-thirds of these deaths were due to Gram- negative bacterial infections from third-generation Cephalosporin-resistant *E. coli, Klebsiella pneumoniae* and Carbapenem-resistant *Pseudomonas aeruginosa* (European Centre for Disease Prevention and Control, 2009). However, antimicrobial resistance is also a problem for Gram-positive bacteria such as Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus faecium* (European Centre for Disease Prevention and Control, 2009).

Infection control problems may arise from spread of resistant bacteria in both healthcare facilities and in the community. Spread within the community creates significant concerns for infection control in long-term care facilities and day care centers, due to increased population mobility (Tenover, 2006). Antimicrobial resistance prolongs the duration of illness, increases the risks of complications and leads to longer hospital stay, thereby leading to greater healthcare costs for patients.

O'Neill (2016) estimated that by 2050 the global financial cost of antimicrobial resistance will be approximately US$100 trillion, with ten million lives at risk of developing a resistant infection each year if the issue of antimicrobial resistance is not addressed. The economic burden of antimicrobial resistance also includes loss of productivity and increased cost of diagnostics and treatment (World Health Organization, 2012).

Direct healthcare costs in the US have been estimated to be as high as $20 billion with loss of productivity costing $35 billion per year (Centers for Disease Control and Prevention, 2013). In the European Union the estimated total cost to society of antimicrobial resistance is €1.5 billion each year (European Centre for Disease

Prevention and Control, 2009). In Australia, the estimated cost of antimicrobial resistance to the health budget is over $250 million annually (Shaban *et al*., 2013). Most determinations of costs attributable to antimicrobial resistance are approximate values because they have been derived from small, often non-representative databases. Urinary tract infections pose significant health and economic implications to society. They are a cause of morbidity in the community and also in the hospital (Rogers and Peterson, 2011).

Urinary tract infections impact considerably on the quality of life of those affected. An investigation of the impact of UTI on the health-related quality of life of female nurses in Taiwan showed that symptoms of UTI, such as urinary frequency and urgency, negatively impacted on the quality of life of the participants, especially in relation to their physical health (Liao *et al*., 2009). Recurrent infection may be either re-infection, caused by a new infecting organism, or relapsing infection, caused by the same organism present before therapy. Relapse may occur either because the infecting organism was not completely eradicated from the genitourinary tract by antimicrobial therapy or because of re-infection by a persistent colonizing strain in the gut reservoir (Nicolle, 2013). In a cohort study of 113 women enrolled at the University of Michigan in the US, 27 % experienced a recurrent infection within six months of the first infection. Determination of recurrence was based on review of medical records which may not have sufficiently documented information on urinary tract infections, thereby underestimating the recurrence rates. Significant adverse health outcomes may occur, especially in people who have a higher risk of developing urinary tract infections, such as pregnant women and immuno-compromised patients (Matuszkiewicz-Rowińska *et al*., 2015).

Immuno-compromised patients, undergoing organ transplant and those with HIV, have a higher rate of bacteremia which occurs when urinary tract infections spread to the bloodstream. A retrospective study of UTI patients presenting to an emergency department in Israel in 2004 reported the presence of bacteremia in 15 % of patients with a UTI (Bahagon *et al*., 2007). In patients presenting with bacteremic UTI, the 30 day-all-cause mortality rate can be as high as 25 % (Hounsom *et al*., 2011). This may be an underestimation as patients with septicaemia who did not have a blood culture taken were excluded from the study (Hounsom *et al*., 2011).

Factors such as age of patient and presence of underlying medical conditions influence the progression to mortality in patients with bacteremic urinary tract infections. If antimicrobial treatment is delayed, this may negatively affect the patient's outcome (Foxman, 2002; Van Nieuwkoop *et al*., 2010; Hounsom *et al*., 2011). When UTI is not associated with mortality, patients may require additional stay in hospital of up to four days, which places a significant economic burden on the health system (Mitchell *et al*., 2016). The economic burden of urinary tract infection is substantial, primarily due to the frequency of occurrence of urinary tract infection (Foxman, 2002). Estimation of costs is based on physician visits, antimicrobial therapy, laboratory diagnosis, hospitalisation as well as non-medical costs attributed to work days lost and morbidity (Foxman, 2002). In the United State, it is estimated that over $1 billion is expended for community-acquired UTI and $451 million for healthcare-associated UTI respectively (Jacobsen, *et al*., 2008; Hsueh *et al*., 2011).

In Italy, the mean yearly cost per patient for the diagnosis and treatment of UTI was estimated to be €229, with antimicrobial therapy identified as contributing the most to the total cost (Ciani *et al*., 2013). This cost estimate was deemed to be conservative given the model assumption that consumption of healthcare resources was constant over

the study period as well as the exclusion of indirect costs including loss of productivity (Ciani *et al*., 2013). Annual estimates from Ireland are approximately €19.2 million at the national level and this is said to cover general practice consultations, antimicrobial therapy and laboratory costs (Callan *et al*., 2014). The cost estimates for UTI reported in this section should be interpreted in relation to the varying population sizes for the countries mentioned. In Australia, it is estimated that approximately 380,600 extra bed-days are used in public hospitals each year by patients acquiring a UTI in hospital (Mitchell *et al*., 2016).

The health and economic implications of a potentially preventable disease such as UTI are considerable, which demands further investigation. Especially of importance is the use of antimicrobials for the treatment of this common infection, with an increase in the risk of patients developing antimicrobial resistance. This research program, which evaluates antimicrobial resistance in urinary tract infection, has the potential to inform policy making that may improve health and economic outcomes for patients and the health system as a whole.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area /Population

The study was carried out in Minna, Niger State. The state is located in the North Central geopolitical zone of Nigeria and covers a land mass of 76,363 square kilometres. It lies between Latitude 8°.00-11°.30´N and Longitude 4º.00-8.00´E (Edogun *et al*., 2017).

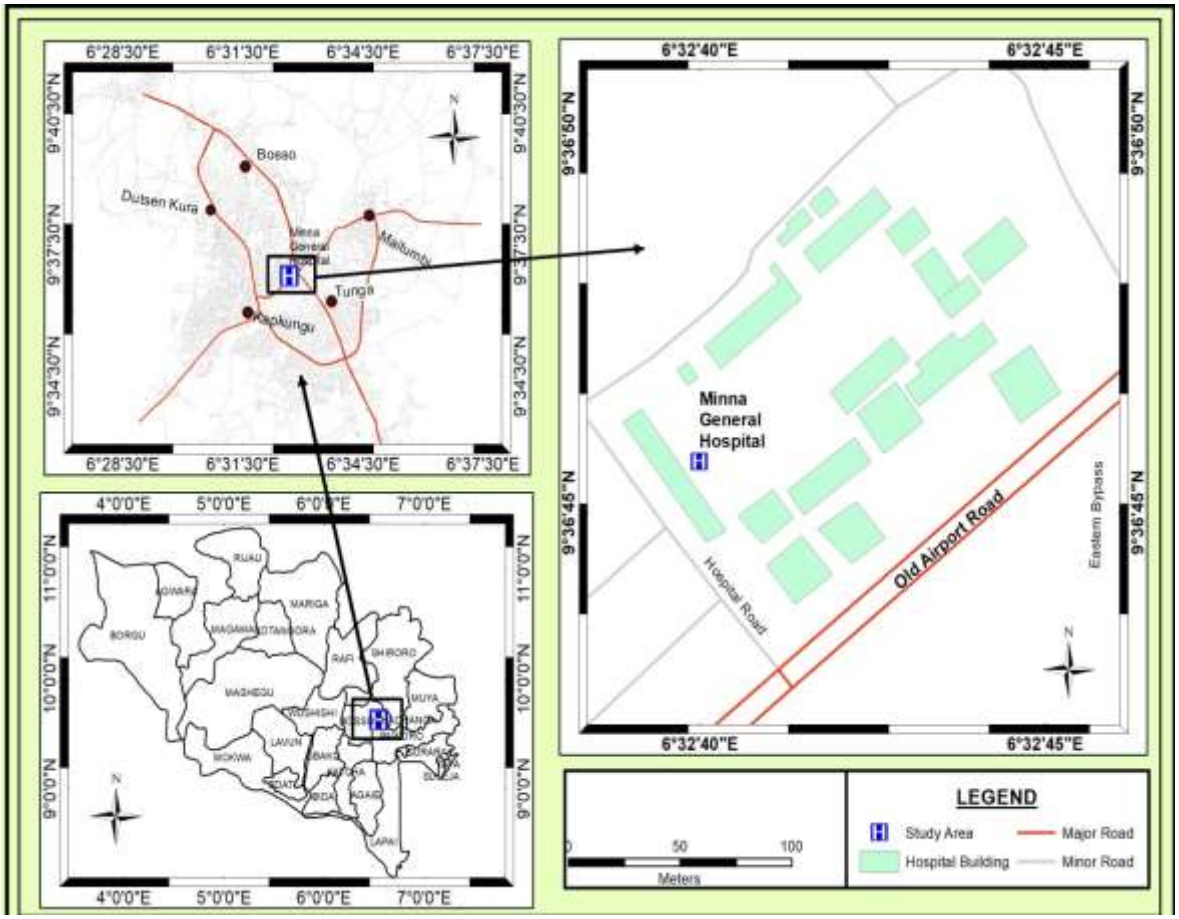

Figure 3.1: Map of the Study Area
Source: (GIS, 2021).

3.2 Study Population

Urine samples were collected from 400 (male and female) patients attending General Hospital Minna, Niger State. Verbal informed consent was obtained from all the

patients prior to specimen collection. Ethical approval was obtained from Niger State Hospitals Management Board.

3.3 Sample Size Determination

The sample size (n) was determined using the formula below:

Where;

$$N=\frac{Z^2\,Q(1-P)}{I^2}$$

N = Sample size

Q= (1-P)

Z= Level of significance (1.96) for confidence level of 95 %

P= Previous prevalence of UTI reported in Minna 38.5 % (Sani, *et al*., 2019)

I= Margin of error of setting a significance level of 0.05 (i.e. 5 %) at confidence level of 95 %

$$N=\frac{(1.9)^2(1-0385)\ (0.385)}{0.05^2}$$

$$N=\quad\frac{3.8416(0.615)(0.385)}{0.0025}$$

$$N=\frac{0.90959}{0.0025}=363\quad\cong 400$$

3.4 Sample Collection

Five milliliters (5 mL) of midstream urine samples were collected from in-and-out patients into a sterile screw capped container. The urine samples were labeled and then

transported to the Microbiology Laboratory, Federal University of Technology, Minna, Niger State, Nigeria in ice pack for standard microbiological analysis. The urine samples were processed within 2 hours of collection, to ensure maximum recovery of the organisms. Baseline data of patients such as age, gender, marital status, educational background and clinical history were recorded at the time of sampling (Thomas *et al*., 2018).

3.5 Bacterial Isolation from Urine Samples

The urine samples were cultured for bacteria according to the methods described by Thomas *et al*. (2018). A loopful of thoroughly mixed urine sample was inoculated by streaking method on MacConkey agar, Cysteine Lactose Electrolyte Deficient (CLED) and Nutrient agar freshly prepared plates. The plates were incubated at 37 ^{o}C for 24 hours. Discrete colonies that emerged at 24 hours incubation were sub-cultured onto nutrient agar at 37 ^{o}C for 24 hours in order to obtain pure culture. Pure cultures were stocked using Nutrient agar slants and stored at 4 ˚C for further analysis as described by Cheesbrough (2009).

3.6 Total Bacterial Counts

To determine the total viable bacterial count, samples was prepared using serial dilution. Four (4) 9.9 mL saline tube was label as 10^{-2}, 10^{-4}, 10^{-6},10^{-8} and 10^{-9}. 0.1 mL of urine sample was remove with sterile pipette and transfer it to the 10^{-2} dilution tube. Vortex the 10^{-2} tube and transfer 0.1ml to the 10^{-4} tube. Vortex, the10^{-4} tube and transfer 0.1 mL to the 10^{-6} tube. 10^{-6} tube and transfer 0.1 mL to the 10^{-8} and the least vortex the 10^{-8} and transfer to the 10^{-9}. Using a sterile pipette, transfer 1.0 mL from 10^{-4} dilution tube to the plate label 10^{-4} and 0.1 mL to the plate labelled 10^{-5} spread the inoculum on the surface of the agar in each plate using an alcohol dipped, flamed, metal spreader. Dip

the spreader into the alcohol jar and quickly take through the flame after each spreading for total coliform bacteria count and total viable bacteria count. The plates were incubated at 37 ^{o}C for 24 hours. All samples were placed on duplicate plates. The colonies that appeared at the end of incubation period were counted using digital illuminated colony counter and result was expressed in colony forming units per mL (cfu/mL) of the samples (Fardows *et al.,* 2016).

3.7 Identification of Bacterial Isolates from Urine Samples

3.7.1 Gram staining

A thin smear of a colony of the test isolate was placed on a clean grease free glass slide. The slide was air dried and heat-fixed by passing over the flame three times. The fixed smear was covered with 3 drops of crystal violet for 60 seconds and rinsed with distilled water. 2 drops of Logol's iodine was added for 60 seconds and rinsed with distilled water. The stained smear was decolorized with 95 % acetone and rinsed immediately with distilled water. The smear was covered with safranin for 30 second before it was rinsed with distilled water and air dried. The slide was viewed with oil immersion under light microscope (Cheesborough, 2017).

3.7.2 Catalase test

A drop of 3 % Hydrogen peroxide was placed on cleaned glass slide. Using a sterile inoculating loop, a small amount of bacteria from 24 hours pure culture was placed onto the reagent on the slide. An immediate bubble formation indicates a positive result while no bubble formation represents negative for catalase (Cheesbrough, 2009).

3.7.3 Coagulase test

Coagulase test was detected according to the procedure outlined by Cheesbrough, (2007). A drop of normal saline was placed on a slide (for both test and negative control), colonies of the test organism was emulsified in each of the drops to make two thick suspensions. A drop of human plasma was added to one of the suspensions, and mixed gently. Agglutination appears within 10 seconds indicate coagulase positive and absence of agglutination indicate coagulase negative.

3.7.4 Oxidase test

Two (2) drops of oxidase reagent (p-Amino dimethyl aniline oxalate) was placed on the left side of the filter paper, bacteria isolate was then mixed with the oxidase reagent on the filter paper and observed for color change (Thomas *et al*., 2018).

3.7.5 Triple sugar iron (TSI) agar test

In carrying out this test, TSI agar was prepared and dispensed into test tubes and allowed to solidify. Using sterile technique, small amount of the bacteria isolate from fresh culture was inoculated into the tubes by means of stab inoculation method with an inoculating needle. After which the tubes were incubated for 24 hours. Examine for color change in slant and butt, blackening and cracks in the medium (Tula and Iyoha 2014).

3.7.6 Indole test

The test organism was cultured in a medium containing tryptophan. Indole production was detected by Kovac's reagent which contains 4 (p)-dimethylamino- benzaldehyde. The test organisms were inoculated in a bijou bottle containing 3 mL of sterile tryptone water, and then it was incubated at 37 ºC for up to 48 hours. Test for indole was performed by adding 0.5 mL of Kovac's reagent then the bottle was gently shaken and

examine for colour change in the surface layer within 10 minutes. The result observes for the presence or absence of red ring colour (Cheesbrough 2009; Kolawole *et al*., 2010).

3.7.7 Urease test

The bacteria isolates were inoculated into urea broth medium containing phenol red indicator and it was incubated for 24 hours. The presence of colour change to pink at the end of the tab-inoculation negative organisms produce no colour change (Kolawole *et al*., 2010; Jaiswal *et al*., 2013).

3.7.8 Citrate test

The citrate test was done using Simmons' citrate agar, the medium was prepared and dispensed into bijou bottles and kept as slants. Using a sterile nickel wire loop, the slope was first streaked with a saline suspension of the test organism and then stabbed to the butt. After which it was incubated at 35 ºC for 48 hours. The medium observes a color change from green to blue along the slant (Cheesbrough, 2009).

3.7.9 Motility test

The medium was prepared according to the manufacturer's guide, and then 5 mL of the medium was dispensed into tubes and autoclaved at a temperature of 121 degrees Celsius for about 15 minutes. After cooling of the medium, the test organisms were inoculated by stab using sterile wire loop then the culture was incubated for 18 hours at 35 degree Celcius. Observation of the pattern of growth by the organism around the stab was recorded as either motile or non-motile (Jaiswal *et al*., 2013; Tula and Iyoha 2014).

3.7.10 Sugar fermentation

Seven milliliter (7 mL) of peptone water was dispensed into test tubes and Durham's tubes were inverted in each of the test tubes. 0.5 g of different test sugars was added to the peptone water in each of the test tube. 0.01 g phenol red (an indicator) was also added. These were sterilizing by autoclaving at 121 ˚C for 15 minutes. Each was allowed to cool and inoculated with loopful of the bacteria isolates. Two test tubes containing peptone water and the test sugars served as controls without the addition of the bacteria isolate. The test tubes were incubated for 72 hours. Test tubes were observed for acid and gas production by comparing them with the control and results were recorded (Shahzad and Rajoka 2011).

3.8 Antibiotic Susceptibility Test

Antimicrobial susceptibility test of the 83 bacteria isolated from urine samples was carried out using the disc diffusion techniques in accordance with the Clinical and Laboratory Standards Institute guideline. Antimicrobial agents tested include Ciprofloxacin (10 µg), Norfloxacin (10 µg), Gentamicin (10 µg), Amoxil (20 µg), Streptomycin (30 µg), Rifampicin (20 µg), Erythromycin (30 µg), Chloramphenicol (30 µg), Ampiclox (20 µg), Levofloxacin (20 µg), Streptomycin (30 µg), Sulfamethoxazole-trimepthoprim (30 µg), Nalidixic (30 µg), Ceporex (10 µg), Tarivid (10 µg), Reflacine (10 µg), Gentamicin (10 µg), Amoxicillin and clavulanic acid (30 µg), Ciprofloxcin (10 µg) and Ampilicin (30 µg).Using a sterile wire loop, the test organisms were emulsified in 4 mL of sterile nutrient broth using a sterile swab, a plate of Mueller Hinton agar was inoculated with the test organism by streaking the swab evenly over the surface of the medium in three directions rotating the plates approximately 60 ^{o}C to ensure even distribution. Using sterile forceps, the antibiotics discs were placed on the plate at about

15 mm from the edge of the plate and not less than 25 mm from disc to disc. Each disc was slightly pressed down to ensure its contact with the agar. The plates were inverted after 30 minutes of applying the disc and were incubated at an inverted position for 18 hours at 35 ^{o}C. The results were interpreted using antimicrobial susceptibility testing standards charts (CLSI, 2018).

3.9 Analysis for Multi-Drug Resistant (MDR) Bacteria

Analysis of multi-drug resistant bacteria isolated was carried out using cross-sectional study on the patient samples with chronic sinusitis. Based on the susceptibility pattern of the isolated bacteria, bacteria resistant to ≥ 3 classes of antibiotic were considered as Multi-Drug Resistant. Bacterial susceptibility to antimicrobial agent was determined according to the Clinical Laboratory Standard Institute (CLSI, 2016).

3.10 Statistical Analysis

Data analysis was done using statistical package for social sciences (SPSS) version 20.0 for windows and Chi-square (χ^2) tests. Also, to ascertain the correlation that exists among infection, age, gender and resistance or susceptibility to the different antimicrobial agent tested. The significant level was $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Biochemical identification of bacterial isolates

The isolated bacteria were identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Salmonella enterica*, *Streptococcus pneumoniae*, *Enterobacter faecalis* and *Serratia marcescens* (Table 4.1).

4.1.2 Frequency of occurrence of bacterial isolates from urine samples

Out of the nine different bacterial isolates identified, *Escherichia coli* had the highest frequency of occurrence (30.1 %) followed by *Klebsiella pneumoniae* (24.0%) and *Staphylococcus aureus* (19.3 %) while *Enterobacter faecalis* and *Serratia marcescens* had the least frequency of occurrence (2.4 %) (Table 4.2). *Pseudomonas aeruginosa* had frequency of occurrence of 9.6 %. Similarly, *Micrococcus luteus* and *Salmonella enterica* had 5.0 % and 3.6 % frequency of occurrence respectively (Table 4.2).

4.1.3 Distribution of bacterial isolates according to gender of the patients

Female patients had the highest prevalence of 43 (51.8 %) while the male counterparts had the least bacterial isolates 40 (48.1 %) *Staphylococcus aureus* had highest frequency of occurrence (16.8 %). *Enterobacter faecalis* and *Serratia marcescens* had the least frequency of occurrence of 1.2 % recorded in female patients. *Escherichia coli* had the highest frequency of occurrence of 20.4 % followed by *Klebsiella pneumoniae* with 15.6 %. *Enterobacter feacalis*, *Micrococcus luteus* and *Salmonella enterica* had least prevalence (1.2%) recorded in male patients (Table 4.3).

Table 4.1: Morphological and biochemical characteristics of Gram negative and Gram positive bacteria isolated from urine samples

Isolate No	Gram reaction	Shape	Catalase	Citrate	Methyl-red	Indole Test	Urease	Oxidase	Slant/Butt	H_2S	Gas Production	Motility	G	L	S	V.P	Coagulase	Identified bacterial
U6	–	Rod	+	–	+	+	–	–	R/Y	–	+	+	+	+	+	–	–	*Escherichia coli*
U21	–	Rod	+	+	+	+	+	–	Y/R	+	–		+	–	+	–	–	*Enterobacter faecalis*
U30	–	Rod	+	+	–	–	–	–	R/Y	–	+	+	+	+	–	–	–	*Serratia marcescens*
U36	–	Rod	+	+	+	–	–	–	R/R	+	+	+	+	–	–	–	–	*Salmonella enterica*
U41	–	Rod	+	+	–	–	+	–	Y/R	–	–	–	+	–	–	–	–	*Klebsiella pneumoniae*
U127	–	Rod	+	+	–	–	–	+	R/Y	–	+	+	+	–	–	–	–	*Pseudomonas aeruginosa*
U4	+	Cocci	+	+	+	-	-	-	R/R	-	-	+	-	-	-	-	+	*Staphylococcus aureus*
U12	+	Cocci	+	-	+	-	+	-	R/R	–	–	+	-	-	-	+	–	*Micrococcus luteus*
U215	+	Cocci	–	+	-	-	+	-	R/R	–	–	+	+	-	+	+	–	*Streptococcus pneumoniae*

Key: + (Positive), - (Negative), R/Y Red/Yellow (Alkaline slant/Alkaline butt), Y/R, Yellow/Red (Acidic slant/Alkaline butt), R/R, Red/Red (Alkaline slant/Alkaline butt), G (Glucose), L (Lactose), S (Sucrose), V.P (Voges Proskeur)

Table 4.2: Frequency of occurrence of bacterial isolates in urine samples

Bacterial Isolates	**No of Isolates**	**Percentage (%)**
Escherichia coli	25	30.1
Klebsiella pneumoniae	20	24.0
Pseudomonas aeruginosa	8	9.6
Salmonella enterica	3	3.6
Enterobacter faecalis	2	2.4
Serratia marcescens	2	2.4
Staphylococcus aureus	16	19.3
Micrococcus luteus	4	5.0
Streptococcus pneumoniae	3	3.6
Total	**83**	**100**

Table 4.3: Distribution of bacterial isolates according to gender of the patients

Bacterial isolates	Female	Male	Total	P-value
Enterobacter faecalis	1(1.2)	1(1.2)	2(2.4)	
Escherichia coli	8(9.6)	17(20.4)	25(30.1)	
Klebsiella pneumoniae	7(8.4)	13(15.6)	20(24.0)	
Micrococcus luteus	3(3.6)	1(1.2)	4(4.8)	
Pseudomonas aeruginosa	5(6.0)	3(3.6)	8(9.6)	0.250
Salmonella enterica	2(2.4)	1(1.2)	3(3.6)	
Serratia marcescens	1(1.2)	1(1.2)	2(2.4)	
Staphylococcus aureus	14(16.8)	2(2.4)	16(19.6)	
Streptococcus pneumoniae	2(2.4)	1(1.2)	3(3.6)	
Total	**43(51.8 %)**	**40(48.1 %)**	**83 (100.0 %)**	

The subject, gender is not a significant risk factor influencing bacterial infection at *p> 0.05*

4.1.4 Distribution of bacterial isolates according to age of the patients

It was observed that patients within the age group 15-24 years were infected with diverse bacterial pathogens (36.1 %) followed by age group 25-34 years (21.6 %) and 35-44 years (13.2 %). While the age groups ≥ 55 years had the least bacterial infections (Table 4.4). *Escherichia coli* and *Klebsiella pneumoniae* had the highest prevalence (12.0-10.8 %). While *Enterobacter faecalis*, *Micrococcus luteus* and *Streptococcus pneumoniae* had the least prevalence (1.2 %) recorded in the age group 15-24 years. *Escherichia coli* and *Klebsiella pneumoniae* had the least bacterial isolates (1.2 %) (Table 4.4).

4.1.5 Frequency of bacterial occurrence with respect to toilet facilities

Patients using pit latrine (39.7 %) had the highest bacterial infections followed by patients using water closet (31.3 %) and other toilet facilities (28.8 %). *Escherichia coli* recorded the highest prevalence (18.0 %). While *Enterobacter faecalis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Streptococcus pneumoniae* had the least bacterial prevalence (1.2 %) in patients using pit latrine. *Klebsiella pneumoniae* had the highest prevalence (12.0 %). While *Salmonella enterica and Streptococcus pneumoniae* had the least bacterial infections (1.2 %) in patients using water closet. Similarly, *Staphylococcus aureus* had the highest prevalence (7.2 %). While *Enterobacter faecalis* and *Streptococcus pneumoniae* recorded the least bacterial infections (1.2 %) in other toilet facilities (Table 4.5).

Table 4.4: Distribution of bacterial isolates with respect to age of the patients

	Age(Years)							P value
Bacterial Isolates	**1-14**	**15-24**	**25-34**	**35-44**	**45-54**	**55**	**Total**	
Enterobacter faecalis	0(0.0)	1(1.2)	1(1.2)	0(0.0)	0(0.0)	0(0.0)	2(2.4)	
Escherichia coli	2(2.4)	10(12.0)	5(6.0)	5(6.0)	2(2.4)	1(1.2)	25(30.1)	
Klebsiella pneumonia	1(1.2)	9(10.8)	3(3.6)	3(3.6)	3(3.6)	1(1.2)	20(24.5)	
Micrococcus luteus	0(0.0)	1(1.2)	1(1.2)	2(2.4)	0(0.0)	0(0.0)	4(4.8)	
Pseudomonas aeruginosa	0(0.0)	3(3.6)	3(3.6)	0(0.0)	2(2.4)	0(0.0)	8(9.6)	0.050
Salmonella enterica	0(0.0)	2(2.4)	0(0.0)	0(0.0)	1(1.2)	0(0.0)	3(3.6)	
Serratia marcescens	0(0.0)	0(0.0)	1(1.2)	1(1.2)	0(0.0)	0(0.0)	2(2.4)	
Staphylococcus aureus	5(6.0)	3(3.6)	2(2.4)	3(3.6)	3(3.6)	0(0.0)	16(19.2)	
Streptococcus pneumoniae	0(0.0)	1(1.2)	2(2.4)	0(0.0)	0(0.0)	0(0.0)	3(3.6)	
Total	**8(9.6 %)**	**30(36.1 %)**	**18(21.6 %)**	**14(16.8 %)**	**11(13.2 %)**	**2(2.4 %)**	**83(100.0 %)**	

The subject, age is a significant risk factor influencing bacterial infection at $p \leq 0.05$.

Table 4.5: Frequency of occurrence of bacterial isolates with respect to toilet facilities

	Toilet facilities				
Bacterial isolates	**Others**	**Pit latrine**	**Water closet**	**Total**	**P-value**
Enterobacter faecalis	1(1.2)	1(1.2)	0(0.0)	2(2.4)	
Escherichia coli	3(3.6)	15(18.0)	7(8.4)	25(30.1)	
Klebsiella pneumoniae	5(6.0)	5(6.0)	10(12.0)	20(24.0)	
Micrococcus luteus	3(3.6)	1(1.2)	0(0.0)	4(4.8)	0.196
Pseudomonas aeruginosa	4(4.8)	1(1.2)	3(3.6)	8(9.6)	
Salmonella enterica	1(1.2)	1(1.2)	1(1.2)	3(3.6)	
Serratia marcescens	0(0.0)	2(2.4)	0(0.0)	2(2.4)	
Staphylococcus aureus	6(7.2)	6(7.2)	0(0.0)	16(19.2)	
Streptococcus pneumoniae	1(1.2)	1(1.2)	4(4.8)	3(3.6)	
Total	**24(28.8 %)**	**33(39.7 %)**	**26(31.3 %)**	**83(100 %)**	

Toilet facility is not a significant risk factor influencing bacterial infection at $p>0.05$.

4.1.6 Frequency of bacterial occurrence with respect to marital status of the patients

It was observed that married patients recorded higher prevalence of bacterial infections (55.4 %) when compared to patients that were single (44.5 %). Among the married patients, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* had higher prevalence (14.4-9.6 %) than the rest of the bacterial isolates. Among the single patients, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* had higher prevalence (15.6 – 9.6 %) than the rest of the bacterial isolates (Table 4.6)

4.1.7 Frequency of bacterial occurrence with respect to education status of the patients

The result (Table 4.7) recorded that patients with non-formal education were infected with diverse bacteria (42.1 %), when compared to patient with formal education (13.2 %). For patients with non-formal education, *Staphylococcus aureus* and *Escherichia coli* had higher prevalence (9.6-10.8 %) than, the rest bacterial isolates. For patients with primary and secondary education, *Escherichia coli* and *Klebsiella pneumoniae* had higher prevalence (7.2-10.8%). While *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* constituted the least bacterial infections (2.4-4.8 %) in patients with tertiary education (Table 4.7).

Table 4.6: Frequency of occurrence of bacterial isolates with respect to marital status of the patients

	Marital status			
Bacterial isolates	**Single**	**Married**	**Total**	**P-value**
Enterobacter faecalis	1(1.2)	1(1.2)	2(2.4)	
Escherichia coli	13(15.6)	12(14.4)	25(30.1)	
Klebsiella pneumoniae	8(9.6)	12(14.4)	20(24.0)	
Micrococcus luteus	0(0.0)	4(4.8)	4(4.8)	
Pseudomonas aeruginosa	3(3.6)	5(6.0)	8(9.6)	0.524
Salmonella enterica	2(2.4)	1(1.2)	3(3.6)	
Serratia marcescens	0(0.0)	2(2.4)	2(2.4)	
Staphylococcus aureus	8(9.7)	8(9.6)	16(19.2)	
Streptococcus pneumoniae	2(2.4)	1(1.2)	3(3.6)	
Total	**37(44.5 %)**	**46(55.4 %)**	**83(100.0 %)**	

Marital status is not a significant risk factor influencing bacterial infection at p> *0.05.*

Table 4.7: Frequency of occurrence of bacterial isolates with respect to education status of the patients

			Educational status			
Bacterial isolates	**N.F.E**	**P.E**	**S.E**	**T.E**	**Total**	**P-value**
Enterobacter faecalis	2(2.4)	0(0.0)	0(0.0)	0(0.0)	2(2.4)	
Escherichia coli	8(9.6)	6(7.2)	9(10.8)	2(2.4)	25(30.1)	
Klebsiella pneumoniae	4(4.8)	6(7.2)	6(7.2)	4(4.8)	20(24.0)	
Micrococcus luteus	2(2.4)	0(0.0)	1(1.2)	1(1.2)	4(4.8)	
Pseudomonas aeruginosa	5(6.0)	1(1.2)	0(0.0)	2(2.4)	8(9.6)	0.050
Salmonella enterica	2(2.4)	0(0.0)	0(0.0)	1(1.2)	3(3.6)	
Serratia marcescens	2(2.4)	0(0.0)	0(0.0)	0(0.0)	2(2.4)	
Staphylococcus aureus	9(10.8)	4(4.8)	2(2.4)	1(1.2)	16(19.2)	
Streptococcus pneumoniae	1(1.2)	1(1.2)	1(1.2)	0(0.0)	3(3.6)	
Total	**35(42.1 %)**	**18(21.6 %)**	**19(22.8 %)**	**11(13.2 %)**	**83(100.0 %)**	

The subject, educational status is a significant risk factor influencing bacterial infection at $p \leq 0.05$.
Key: N.F.E (Non formal education), P.E (Primary education), S.E (Secondary education) and T.E (Tertiary education).

4.1.8 Antibiotics susceptibility pattern of Gram negative bacterial isolates from urine samples

All the isolated Gram negative bacterial strains were intermediately resistant to Cefalexin, Gentamicin, Ciprofloxacin, Reflacine, Ofloxacin, Amoxycillin and clavulanic. Similarly, the Gram negative bacterial strains (25-50 %) exhibited resistance to all antibiotics tested except *Escherichia coli* with 8 % to Ofloxacin, Ampicilin, Gentamicin, Ciprofloxacin, Amoxycillin and clavulanic acid. *Salmonella enterica* (66.7 %) recorded the highest susceptibility to Nalidixic acid, Gentamicin and Sulfamethoxazole-trimepthoprim. Therefore, Nalidixic acid, Gentamicin and Sulfamethoxazole-trimepthoprim are the most effective antibiotics tested against Gram negative bacteria isolates (Table 4.8).

4.1.9 Antibiotics susceptibility pattern of Gram positive bacterial isolates from urine samples

The Gram positive bacterial strains exhibited intermediate resistance to Rifampicin and Ampiclox. All the Gram positive bacterial strains were resistant to Norfloxacin, Streptomycin, Erythromycin and Ciprofloxacin. Of all the three Gram positive bacterial strains recovered from patients. *Micrococcus luteus* recorded the highest resistance (100.0 %) to all antibiotics tested. *Staphylococcus aureus* (62.5 %) and *Streptococcus pneumoniae* (66.7 %) from this study recorded the highest susceptibility to Levofloxacin, Chloramphenicol and Amoxil. Therefore, Levofloxacin, Chloramphenicol and Amoxil are the most effective antibiotics tested against Gram positive bacterial isolates (Table 4.9).

Table 4.8: Antibiotics susceptibility pattern of Gram negative bacterial isolates from urine samples

Bacterial isolates	Number of isolates	Pattern	CEP	S	NA	OFX	AU	PEF	PN	CN	CPX	SXT
		I	2(100.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)	2(100.0)	1(50.0)
Enterobacter faecalis	2	R	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)
		S	0(0.0)	1(50.0)	1(50.0)	0(0.0)	1(50.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)
		I	25(100.0)	21(84.0)	22(88.0)	14(56.0)	19(76.0	24(96.0)	19(76.0)	14(56.)	16(64.0)	15(60.0)
Escherichia coli	25	R	0(0.0)	1(4.0)	1(4.0)	2(8.0)	2(8.0)	0(0.0)	2(8.0)	2(8.0)	2(8.00	1(4.0)
		S	0(0.0)	3(12.0)	2(8.0)	9(36.0)	4(16.0)	1(4.0)	4(16.0)	9(36.0)	7(28.0)	9(36.0)
		I	19(95.0)	13(65.0)	13(65.0)	11(55.0)	15(75.0	19(95.0)	15(75.0)	11(55.0)	9(45.0)	13(65.0)
Klebsiella pneumoniae	20	R	0(0.0)	2(10.0)	3(15.0)	2(10.0)	2(10.0)	0(0.0)	1(5.0)	2(10.0)	5(25.0)	3(15.0)
		S	1(5.0)	5(25.0)	4(20.0)	7(35.0)	3(15.0)	1(5.0)	4(20.0)	7(35.0)	6(30.0)	4(20.0)
		I	7(87.5)	6(75.0)	7(87.5)	4(50.0)	7(87.5	8(100.0)	6(75.0)	3(37.5)	5(62.5)	4(50.0)
Pseudomonas aeruginosa	8	R	1(12.5)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)	2(25.0)	1(12.5)	2(25.0)
		S	0(0.0)	2(25.0)	1(12.5)	3(37.5)	1(12.50	0(0.0)	2(25.0)	3(37.5)	2(25.0)	2(25.0)
		I	3(100.0)	2(66.70)	1(33.3)	2(66.7)	3(100.0	3(100.0)	3(100.0)	1(33.3)	1(33.3)	1(33.3)
Salmonella enterica	3	R	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)
		S	0(0.0)	1(33.3)	2(66.7)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	2(66.7)	1(33.3)	2(66.7)
		I	2(100.0)	1(50.0)	1(50.0)	2(100.0)	2(100.0	2(100.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Serratia marcescens	2	R	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
		S	0(0.0)	1(50.0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
p-value			0.569	0.849	0.088	0.807	0.924	0.099	0.481	0.844	0.801	0.703

KEY: S (Sensitive), R (Resistance) and I (Intermediates) S: Streptomycin, NA: Nalidixic acid, SXT: Sulfamethoxazole-trimethoprim, CEP: Cefalexine, PEF: Reflacine, OFX: Ofloxacin, AU: Amoxicillin and clavulanic acid, PN: Ampicilin CN: Gentamicin and CPX: Ciprofloxacin.

Table 4.9: Antibiotics susceptibility pattern of Gram positive bacterial isolates from urine samples.

Bacterial isolates	Number of isolates	Pattern	LEV	APX	S	CH	E	RD	NB	CN	CPX	AML

Micrococcus luteus	4	I	2(50.0)	4(100.0)	3(75.0)	3(75.0)	3(75.0)	4(100.0)	0(0.0)	3(75.0)	3(75.0)	3(75.0)
		R	2(50.0)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	4(100.0)	1(25.0)	1(25.0)	1(25.0)
		S	0(0.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Staphylococcus aureus	16	I	3(18.8)	12(75.0)	6(37.5)	8(50.0)	5(31.3)	13(81.3)	1(6.3)	9(56.3)	4(25.0)	10(62.5)
		R	3(18.8)	1(6.3)	6(37.5)	6(37.5)	8(50.0)	3(18.7)	14(87.4)	3(18.8)	8(50.0)	4(25.0)
		S	10(62.5)	3(18.8)	4(25.0)	2(12.5)	3(18.7)	0(0.0)	1(6.3)	4(25.0)	4(25.0)	2(12.5)
Streptococcus pneumoniae	3	I	2(66.7)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	3(100.0)	0(00.0)	1(33.3)	0(0.0)	1(33.3)
		R	0(0.0)	1(33.3)	2(66.7)	1(0.0)	2(66.7)	0(0.0)	2(66.7)	1(33.3)	2(66.7)	0(0.0)
		S	1(33.3)	0(0.0)	0(00.0)	2(66.7)	0(0.0)	0(0.0)	1(33.3)	1(33.3)	1(33.3)	2(66.7)
p-value			0.113	0.382	0.533	0.154	0.466	0.47	0.523	0.746	0.241	0.197

KEY: S (Sensitive), R (Resistance) and I (Intermediates) S: Streptomycin, NA: Nalidixic acid, SXT: Sulfamethoxazole-trimethoprim, CEP: Cefalexine, PEF: Reflacine, OFX: Ofloxacin, AU: Amoxicillin and clavulanic acid, PN: Ampicilin CN: Gentamicin and CPX: Ciprofloxacin

4.1.10 Multi-drug resistance of bacterial isolates from urine samples

Table 4.10 revealed that the bacterial isolates were resistant to at least three (3) antibiotics tested (MDRI=0.3). For instance, *E. coli* strains was resistant to more antibiotics (3-10 antibiotics) than, the rest isolates tested. Similarly, *Klebsiella pneumoniae* and *Micrococcus luteus* strains were resistant to 3-6 antibiotics tested. (Table 4.10).

Table 4.10: Multidrug resistance pattern of bacterial isolates from urine samples.

ID	RP	MDRI	ID	RP	MDRI
U33	CEP/S/PEF	0.3	U17	APX/CH/RD/NB/CN/AMX	0.6
U105	CEP/NA/PEF	0.3	U60	APX/S/CH/RD/NB/AMX	0.6
U2	CEP/CN/CPX	0.3	U70	APX/S/RD/NB/CN/AMX	0.6
U151	CEP/S/PEF	0.3	U215	APX/S/E/RD/NB/AMX	0.6
U192	CEP/NA/PEF	0.3	U81	CEP/S/PEF/AU/PN/CPX	0.6
U4	S/RD/NB	0.3	U141	CEP/NA/OFX/PEF/AU/PN	0.6
U9	APX/NB/AMX	0.3	U30	CEP/S/NA/PEF/AU/PN	0.6
U19	APX/RD/NB	0.3	U114	CEP/S/NA/PEF/AU/CN	0.6
U40	RD/NB/CN	0.3	U110	CEP/S/PEF/AU/PN/CN/SXT	0.7
U15	APX/RD/NB/CN	0.4	U3	CEP/S/NA/PEF/AU/PN/CN	0.7
U6	CEP/S/NA/PEF	0.4	U127	CEP/S/PEF/AU/PN/CN/CPX	0.7
U41	CEP/S/PEF/PN	0.4	U35	APX/CH/E/RD/NB/CPX/AMX	0.7
U8	CEP/PEF/PN/SXT	0.4	U62	APX/S/CH/RD/NB/CN/AMX	0.7
U179	CEP/CN/CPX/SXT	0.4	U225	LEV/APX/CH/RD/NB/CN/AMX	0.7
U46	CEP/NA/PEF/AU	0.4	U250	LEV/APX/E/RD/CN/CPX/AMX	0.7
U130	CEP/OFX/PEF/AU	0.4	U36	CEP/S/NA/OFX/PEF/AU/PN/CPX	0.8
U143	CEP/NA/PEF/PN	0.4	U144	CEP/NA/PEF/AU/PN/CN/CPX/SXT	0.8
U158	NA/PEF/AU/CN	0.4	U5	CEP/S/NA/OFX/PEF/AU/CN/SXT	0.8
U204	NA/PEF/AU/PN	0.4	U67	CEP/S/OFX/PEF/AU/PN/CPX/SXT	0.8
U42	CEP/OFX/PEF/AU/PN	0.5	U126	CEP/S/NA/OFX/PEF/AU/PN/CPX/SXT	0.9
U57	CEP/S/NA/OFX/PEF/AU/PN/CPX/SXT	0.9	U166	CEP/S/NA/OFX/PEF/AU/PN/CN/CPX/SXT	1.0
U170	LEV/APX/S/CH/E/RD/NB/CN/CPX/AMX	1.0	U39	CEP/S/NA/OFX/PEF/AU/PN/CN/CPX/SXT	1.0

KEY: S: Streptomycin, CN: Gentamicin, NB: Norfloxacin, E:Erythromycin, CPX:Ciprofloxacin, CH: Chloramphenicol, RD: Riframpin, LEV: Levofloxacin, AML:AmoxilandAPX:Ampiclox, MDRI: Multidrug resistant index and RP: Resistant pattern.

4.2 Discussion

Multi-drug resistant urinary tract infection has assumed a public health significance in the past three decades (WHO, 2012, 2014), particularly in the developing countries. However, efficient management of bacterial UTIs rest on the identification of the type of bacteria that colonize the urinary tract as well as the choice of a valuable antibiotic agents.

Among the isolated bacteria from this study, Gram-negative organisms constituted 72.3 % while Gram-positive organisms accounted for 27.7 %. This is consistent with the report of Ebie *et al.* (2001), Onifade *et al*. (2005), Idakwo *et al*. (2015), Ogbukagu *et al.* (2016) and Sani *et al*. (2019) which recorded high prevalence of Gram negative bacilli and reiterated the fact that most organisms causing urinary tract infections were from lower gastrointestinal tract.

Although, the result in which at 37 ^{o}C Table 4.3 which reported *Escherichia coli* as the predominant pathogen in urine is in contrast to the findings of Adabara *et al*. (2012) and Otajevwo (2013) who reported *Klebsiella pneumoniae* to be more predominant than *Escherichia coli* in urine culture. Hence, higher prevalence of *Escherichia coli* and *Klebsiella pneumonia*e reported in this study brings to bear that both have become predominant bacteria in urine culture.

Bacteria occurred more in females (32.0 %), (Table 4.4). Different studies have reported female predominance (Oladeinde *et al*., 2011; Idakwo *et al*., 2015; Ogbukagu *et al*., 2016). However, gender was not an influencing factor to bacterial colonization ($P=0.25$, $P>0.05$). The higher prevalence rate recorded in females could be due to the proximity of the urethral to the anus, shorter urethra, contraception, pregnancy, and sexual intercourse which may introduces bacteria into the female urinary tract as described by

Oluwafemi *et al.* (2018). Furthermore, the spread of normal flora in feces from the anus to the vagina could result from poor anal hygiene.

Age was a significant risk factor influencing urinary bacterial infection (Table 4.5). Higher bacteria occurred in urine of patient within 15-24 and 25-34 years. Therefore, the findings of the present study are in agreement with the previous studies by Idakwo *et al*. (2015) and Ogbukagu *et al*. (2016) who reported the highest prevalence among 26-38 and 30-39 years respectively. This could be because patients within the age group 15-24 years are sexually active. Nalidixic acid, Gentamicin and Sulfamethoxazole trimethoprim was the most effective drug (66.7 %) against Gram negative uropathogens isolated. Streptomycin, Reflacine, Ampicilin, Gentamicin, Ciprofloxacin, Amoxicilin and clavulanic acid were active against (50 %) of the isolated bacteria while Ofloxacin, Ampicilin and Nalidixic acid (50 %) recorded the highest resistance rate in the antibiogram test. This result is in contrast to the findings of Idakwo *et al.* (2015) and Thomas *et al.* (2018). The high rate of resistance of Reflacine, Gentamicin, Amoxicilin and clavulanic acid observed in this study may support the fact that these are the commonly prescribed antibiotics in the hospitals and also the easiest available over the counter in the community.

Furthermore, *Salmonella enterica*, *Streptococcus pneumoniae* and *Staphylococcus aureus* were susceptible to Nalidixic acid, Gentamicin, Sulfamethoxazole trimethoprim, Chloramphenicol and Amoxil (Table 4.8 and Table 4.9) this finding conform to previous studies by Idris *et al*. (2014), Idakwo *et al*. (2015) and Ogbukagu *et al*. (2016). The result however is not in agreement with the result of Thomas *et al*. (2018) and Ogbukagu *et al*. (2016) who reported highest resistance with the use of Gentamicin. Although diverse studies in different parts of the country and the world reported different resistances rates to different drugs, it is of great importance that attention be

paid to local resistance patterns since they have great impact on health care. Raza *et al*. (2011) and Iregbu and Nwajiobi-Princewill (2013) reported that variations in susceptibility may be as a result of presumptive treatment practices common in developing countries like Nigeria as well as inappropriate exposure to antibiotics. Furthermore, accessibility of the drugs over the counter without the need of a prescription encourages the abuse of drugs. In addition, the use of fake and substandard drugs in Nigeria may also be a contributory factor to the emergence of resistant strains.

It is evident from the results analysis that MDR bacterial isolate was 97.6 % (82/83) (Table 4.11). The prevalence rate of MDR bacteria from this study is higher than those described by Adebayo *et al.* (2012) and Tek *et al.* (2015). Therefore, this is a situation requiring serious attention as potential community transmission of these MDR bacteria is conceivable. Anthropogenic pollutant such as antibiotic in sub-therapeutic doses, toxic or recalcitrant chemicals in the soil are known to increase antibiotic resistant genes selection in the environment (Adebayo *et al*., 2012) Thus, high MARI obtained for most bacterial isolates pointed out the consequences of anthropogenic pollution water sources in settings lacking adequate sewage management system like the study area.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study has revealed that the total number of 83 bacterial isolates from urine samples of patients attending General Hospital Minna, Niger State. *Escherichia coli* (30.1 %) had the highest frequency of occurrence while the least bacteria encounter was *Enterobacter faecalis* and *Serratia marcescens* 2 (2.4 %). Other bacterial encountered was *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

It has been established that marital status and toilet facility were not the predisposing risk factors with occurrence of bacteria associated with urinary tract infections. Hence, the Age groups 15-24 years were the most vulnerable groups affected by bacterial infections which could be attributed to the high sexual activity that characterize this age group. Those with no formal education had the highest occurrence of bacterial pathogens when compared to formal education.

Out of the nine (9) bacteria identified from urine samples *Salmonella enterica* 2 (66.7 %), *Staphylococcus aureus* 10 (62.5 %) and *Streptococcus pneumoniae* 2 (66.7 %) were susceptible to Nalidixic acid, Gentamicin, Sulfamethoxazole trimethoprim, Levofloxacin, Chloramphenicol and Amoxil while other bacteria were resistant to antibiotics tested. All isolated bacteria were resistant to at least three (3) antibiotics tested (MDRI=0.3). Therefore, multidrug resistant uropathogens exist in this study area.

5.2 Recommendations

The following recommendations are drawn from this research study:

i. There is a need for awareness to people on the prevalence of bacterial isolates from urine samples of patients attending General Hospital Minna, Niger State. Likely, people need to be enlightened on the mode of transmission of the bacterial infections.

ii. Socio demographic factors plays a major role in urinary tract infections. Thus, parameters such as age, groups within 15-24 years should be given priority, screening of both genders should be considered.

iii. Public health education on personal hygiene to reduce the high rate of contacting bacterial infections should be improved.

iv. Gentimicin, Nalidixic acid, Sulfamethoxazole trimethoprim, Chloramphenicol and Amoxil are the most effective antibiotics in the treatment of Urinary tract infections.

v. Policies should be enforced to prevent indiscriminate use of antibiotics by sick individuals, as this could be one of the reasons for multidrug resistance recorded in this study.

vi. There is need for continuous surveillance of antibiotics resistance in order to identify the dynamic of antibiotic resistance on the population.

vii. Prior to the commencement of antibiotics therapy, antimicrobial susceptibility test is vital in effective treatment and management of bacterial infections.

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APPENDIX A : QUESTIONNAIRE

Sample ID No……

Date…….

Introduction: I, Yusuf Zainab, an MTech student of the Department of Microbiology, Federal University of Technology Minna. I am conducting a research on Antimicrobial Susceptibility Pattern of Bacteria Isolated from Urine Samples of Patients attending General Hospital, Minna, Niger State.

Purpose of the research: The research will help in determining the antibiotic susceptibility and resistance profile of the isolates.

Procedure: If you agree with the purpose of the research, please answer the following questions which might take approximately 5-10 minutes.

Benefits: There are no direct benefits for you being part of this research. However, your contribution will help immensely in the research. You are free not to participate in this research or not to answer any question you feel uncomfortable with. Confidentiality is guaranteed, your name will not appear in any oral or written report of this study. There are no wrong or right answers. Your openness and honest opinions are extremely important. In case you do not understand any question please ask me to clarify.

Please tick the following questions as appropriate

Would you like to participate in this study? Yes ( ) No ( )

1. Age in years: 1-14 ( ) 15-24 ( ) 25-34 ( ) 35-44 ( ) 45-54 ( ) ≥ 55 ( )

2. Sex: Male ( ) Female ( )

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

4. Family type: Monogamy ( ) Polygamy ( )

5. Educational status: None ( ) Primary ( ) Secondary ( ) Tertiary ( )

6. Occupation: Trader ( ) Farmer ( ) Civil servant ( ) Unskilled Pensioner ( )

7. Out-patient ( ) In-patient ( )

8. Residence: Urban ( ) Rural ( )

9. Toilet type: Pit latrine ( ) Water closet ( ) others ( )

10. Do you know about urinary tract infection (UTI)? Yes ( ) No ( )

11. Have you been treated of UTI in the recent past? Yes ( ) No ( )

12. Which are the common signs and symptoms of UTI that you know? Dysuria ( ) urgency ( ) increased rate of urination ( ) don't know ( )

13. How important will you rate going for laboratory test before treatment? Very important ( ) Barely important ( ) Not important ( )

14. Are you presently on any antibiotics? Yes ( ) No ( )

APPENDIX B

Colonial characteristics of gram negative bacteria isolated from urine samples

S/No	Isolate No	Morphology		
		CLED Agar	**MacConkey Agar**	**Suspected Organisms**
1.	U6	Opaque yellow colonies with slightly deeper yellow center	Flat, dry, pink colonies with surrounding darker pink area of precipitated bile salt	*Escherichiacoli*
2.	U23	Golden yellow colonies	Flat, dry pink colonies	*Escherichia coli*
3.	U29	Opaque yellow colonies with deeper yellow center	Flat, pink colonies	*Escherichia coli*
4.	U33	Yellow colonies with slightly deeper yellow center	Flat, dry, pink colonies	*Escherichia coli*
5.	U34	Golden yellow colonies	Flat dry, pink colonies	*Escherichia coli*
6.	U36	Flat blue colonies	Colourless transparent colonies	*Salmonellaenterica*
7.	U41	Yellow-whitish colonies, extremely mucoid	Pink colonies, extremely mucoid	*Klebsiella pneumoniae*
8.	U42	Flat blue colonies	Colorless transport colonies	*Salmonella enterica*
9.	U47	Opaque yellow colonies with slightly deeper yellow center	Flat, dry, pink colonies	*Escherichia coli*
10.	U82	Opaque yellow colonies with deeper yellow center	Flat, pink colonies non-mucoid	*Escherichia coli*
11.	U88	Widths colonies extremely mucoid	Pink colonies extremely mucoid	*Klebsiella pneumoniae*
12.	U91	Opaque yellow colonies with deeper yellow center	Flat,dry, pink colonies non-mucoid	*Escherichia coli*
13.	U101	Opaque yellowish colonies with deeper yellow center	Flat, pink colonies non-mucoid	*Escherichia coli*

Appendix B: Continues

S/No	Isolate No	Morphology		
		CLED Agar	**MacConkey Agar**	**Suspected Organisms**
14.	U102		Minute red colonies	*Enterobacter faecalis*
15.	U105	Opaque yellowish colonies with deeper yellow center	Flat, pink colonies non-mucoid	*Escherichia coli*
16.	U106	Yellow-whitish, extremely mucoid colonies	Pink extremely mucoid colonies	*Klebsiella pneumoniae*
17.	U110	Flat blue colonies	Colourless transparent colonies	*Salmonella enterica*
18.	U71	Opaque yellow with slightly deeper yellow center	Flat, pink colonies, non-mucoid	*Escherichia coli*
19.	U78	Opaque yellow slightly deeper yellow center colonies	Flat pink colonies, non-mucoid	*Escherichia coli*
20.	U141	Whitish extremely mucoidcolonies	Pink, extremely mucoid colonies	*Klebsiella pneumoniae*
21.	U120	Non-transparent yellow colonies	Flat, pink, non-mucoid colonies	*Escherichia coli*
22.	U166	Opaque yellow, slightly deeper yellow center colonies	Flat, pink, non-mucoid colonies with deeper centers	*Escherichia coli*
23.	U30		Red pigmented colonies	*Serratia marcescens*
24.	U172	Opaque yellow colonies with deeper yellow center	Flat, pink, non-mucoid colonies	*Escherichia coli*
25.	U39	Red Pigmented colonies	Red pigmented colonies	*Serratia marcescens*
26.	U2		Minute red colonies	*Enterobacter faecalis*
27.	U1	Opaque yellow colonies with deeper yellow center	Flat, pink, non-mucoid colonies	*Escherichia coli*

Appendix B: Continues

S/No	Isolate No	Morphology		
		CLED Agar	**MacConkey Agar**	**Suspected Organisms**
28.	U17	Opaque yellow colonies with deeper yellow center	Flat, pink, non-mucoid colonies	*Escherichia coli*
29.	U175	Opaque yellow colonies with deeper yellow centers	Flat, pink non-mucoid colonies	*Escherichia coli*
30.	U144	Opaque yellow colonies with deeper yellow center	Flat, pink, non-mucoid colonies	*Escherichia coli*
31.	U179	Yellow-whitish, extremely mucoid colonies	Pink, extremely mucoid colonies	*Klebsiella pneumoniae*
32.	U154	Non-transparent yellow colonies	Non-mucoid, pink, flat colonies	*Escherichia coli*
33.	U156	Opaque yellow colonies	Pink, flat, non-mucoid colonies	*Escherichia coli*
34.	U151	Opaque yellow colonies	Pink, flat, non-mucoid colonies	*Escherichia coli*
35.	U59	Yellow extremely mucoid	Large,shinyand dark pink mucoid	*Klebsiella pneumoniae*
36.	U46	Opaque yellow colonies	Flat, pink, dry colonies non-mucoid	*Escherichia pneumoniae*
37.	U5	Yellow-whitish mucoid colonies	Large, shiny and dark pink mucoid colonies	*Klebsiella pneumoniae*
38.	U3	Yellow-whitish, extremely mucoid colonies	Shiny, large and dark pink mucoid colonies	*Klebsiella pneumoniae*
39.	U22	Yellow-whitish, extremely mucoid colonies	Large, shiny and dark pink mucoid colonies	*Klebsiella pneumoniae*

Appendix B: Continues

S/No	Isolate No	Morphology		
		CLED Agar	**MacConkey Agar**	**Suspected Organisms**
40.	U67	Yellow-whitish mucoid colonies	Pink extremely mucoid colonies	*Klebsiella pneumoniae*
41.	U100	Yellow-whitish, extremely mucoid colonies	Large, shiny and dark pink mucoid colonies	*Klebsiella pneumoniae*
42.	U110	Yellow-whitish mucoid colonies	Pink and extremely mucoid colonies	*Klebsiella pneumoniae*
43.	U130	Yellow-whitish mucoid colonies	Pink and shiny mucoid colonies	*Klebsiella pneumoniae*
44.	U132	Yellow-whitish mucoid colonies	Dark pink, extremely mucoid colonies	*Klebsiella pneumoniae.*
45.	U126	Whitish-bluish extremely mucoid colonies	Pink mucoid colonies	*Klebsiela pneumoniae.*
46.	U158	Whitish extremely mucoid colonies	Shiny, large, dark pink extremely mucoid	*Klebsiella pneumoniae*
47.	U127	Green colonies with matted surface	Fluorescent green brown colonies	*Pseudomonas aeruginosa*
48	U129	Green colonies with matted surface and rough periphery	Transparent green brown colonies	*Pseudomonas aeruginosa*
49.	U57	Green and matted surface	Transparent clear colonies	*Pseudomonas aeruginosa*
50.	U198	Green colonies with matted surface	Green-brown colonies	*Pseudomonas aeruginosa*
51.	U192	Green colonies with matted surface	Green-brown colonies	*Pseudomonas aeruginosa*
52.	U204	Green pigmented colonies with rough periphery	Fluorescent green brown colonies	*Pseudomonas aeruginosa*
53.	U114	Green pigmented, matted surface colonies	Fluorescent green-brown colonies	*Pseudomonas aeruginosa*

54.	U48	Green pigmented colonies with matted surface	Transparent green-brown colonies	*Pseudomonas aeruginosa*

Appendix C: Colonial characteristics of Gram positive bacteria isolated from urine samples

	Morphology	
Isolate No	**Nutrient Agar**	**Suspected organisms**
U4	Deep golden yellow /white colonies.	*Staphylococcus aureus*
U9	Deep golden yellow /white colonies.	*Staphylococcus aureus*
U11	Deep golden yellow /white colonies.	*Staphylococcus aureus*
U12	Circular tetrads and bright yellow colonies	*Micrococcus luteus*
U14	Deep golden yellow/white colonies.	*Staphylococcus aureu*s
U19	Circular tetrads and bright yellow colonies.	*Micrococcus luteus*
U75	Circular tetrads and bright yellow colonies.	*Micrococcus luteus*
U170		*Micrococcus luteus*
U200	Circular tetrads and bright yellow colonies.	*Staphylococcus* aureus
U215	Glistening and mucoid form colonies.	*Streptococcus pneumoniae*
U225	Glistening and mucoid form colonies.	*Streptococcus pneumoniae*

Appendix D: Bacterial load from urine samples

The total viable bacteria count (TVBC) ranges from 1.61×10^3 - 5.2×10^5cfu/ml.

Total viable bacterial counts

S/NO	SAMPLE CODE	TVBC(cfu/mL)
1	U33	1.72×10^5
2	U6	1.61×10^5
3	U23	2.01×10^5
4	U105	3.0×10^6
5	U106	2.4×10^7
6	U71	2.1×10^6
7	U154	5.2×10^5
8	U151	2.4×10^5
9	U46	2.5×10^5
10	U127	3.2×10^5
11	U67	5.1×10^6
12	U132	4.1×10^6
13	U198	2.4×10^5
14	U17	3.1×10^5
15	U51	4.5×10^5
16	U21	5.0×10^5
17	U120	4.1×10^5
18	U172	2.4×10^5

Key: TVBC = Total viable Bacterial Counts

U=Urine

Appendix E: Distribution of isolate according to Gram reaction

Out of eighty-three (83) bacteria isolates obtained from urine samples 60 (72.3 %) were Gram negative bacteria while Gram positive bacteria had 23 (27.7 %). *Escherichia coli*25 (30.1%) had the highest frequency. This is followed in descending order by *Klebsiella pneumoniae* 20 (24.0 %), *Staphylococcus aureus* 16 (19.3%), *Pseudomonas aeruginosa* 8 (9.6 %), *Micrococcus luteus* 4 (5.0 %), *Salmonella enterica* 3 (3.6 %), *Streptococcus pneumoniae* 3 (3.6 %) the least are *Enterobacter faecalis* 2 (2.4 %) and *Serratia marcescens* 2 (2.4 %).

Distribution of isolate according to Gram reaction

Gram reaction	Number of isolate	Percentage
Gram negative	60	72.3 %
Gram positive	23	27.7 %
Total	83	100

Appendix F: Distribution of bacterial isolates according to family type of the patients

The result recorded that monogamous family had the highest prevalence of 53(58.3 %) while the polygamous family had the least bacterial isolates 30(36.1 %) *Escherichia coli, Klebsiella pnuemoniae* and *Staphylococcus aureus* had the highest frequency (20.4-13.2 %) and *Salmonella enterica* and *Streptococcus pneumoniae* had the least frequency of occurrence of 2.4 % recorded in monogamous family. *Escherichia coli* and *Klebsiella pnuemoniae* had the highest frequency of occurrence (9.6-8.4 %). *Micrococcus luteus* and *Salmonella enterica* had the least prevalence 1.2 % recorded in polygamous family.

Distribution of bacterial isolates according to family type of the patients

	Family type			
Bacterial isolates	**M.F**	**P.F**	**Total**	**P-value**
Enterobacter faecalis	0(0.0)	2(2.4)	2(2.4)	
Escherichia coli	17(20.4)	8(9.6)	25(30.1)	
Klebsiella pneumoniae	13(15.6)	7(8.4)	20(24.0)	
Micrococcus luteus	3(3.6)	1(1.2)	4(4.8)	
Pseudomonas aeruginosa	5(6.0)	3(3.6)	8(9.6)	0.466
Salmonella enterica	2(2.4)	1(1.2)	3(3.6)	
Serratia marcescens	0(0.0)	2(2.4)	2(2.4)	
Staphylococcus aureus	11(13.2)	5(6.0)	16(19.2)	
Streptococcus pneumonia	2(2.4)	1(1.2)	3(3.6)	
Total	**53(63.8 %)**	**30(36.1 %)**	**83(100.0 %)**	

Family type is not a significant risk factor influencing bacterial infection at $p > 0.05$.

Key: M.F: Monogamous family and P.F: Polygamous family.

Appendix G: Distribution of bacterial isolates according to in and out-patients

The out-patients had the highest bacterial isolates (61.4 %) while the in-patients had the least bacterial isolates (38.5 %). *Escherichia coli*, *Klebsiella pnuemoniae* had the highest frequency of occurrence (22.8-18.0 %) while *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Salmonella enterica* and *Streptococcus pneumoniae* had the least frequency of occurrence of 2.4 % recorded in out-patients. *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pnuemoniae* had the highest frequency of occurrence (8.4-6.0 %). *Salmonella enterica* and *Streptococcus pneumonia* had the least frequency of occurrence of 1.2 % recorded in in-patients.

Distribution bacterial isolates according to in and out-patients

	Patients status			
Bacterial isolates	**I**	**O**	**Total**	**P-value**
Enterobacter faecalis	2(2.4)	0(0.0)	2(2.4)	
Escherichia coli	6(7.2)	19(22.8)	25(30.1)	
Klebsiella pneumoniae	5(6.0)	15(18.0)	20(24.0)	
Micrococcus luteus	2(2.4)	2(2.4)	4(4.8)	
Pseudomonas aeruginosa	6(7.2)	2(2.4)	8(9.6)	0.05
Salmonella enterica	1(1.2)	2(2.4)	3(3.6)	
Serratia marcescens	2(2.4)	0(0.0)	2(2.4)	
Staphylococcus aureus	7(8.4)	9(10.8)	16(19.2)	
Streptococcus pneumoniae	1(1.2)	2(2.4)	3(3.6)	
Total	**32(38.5 %)**	**51(61.4 %)**	**83(100.0 %)**	

Patient type is a significant risk factor influencing bacterial infection at $p \leq 0.05$.
Key: I: In-patients and O: out-patient

Appendix H: Frequency of bacterial occurrence with respect to residence of the patients

Rural residence had the highest bacterial isolates (59.0 %) while the urban residence had the least bacterial isolates (40.9 %). *Escherichia coli* and *Klebsiella pnuemoniae* had the highest frequency of occurrence (19.2-16.8 %) while *Enterobacter faecalis* had the least frequency of occurrence of 1.2 % recorded in rural residence. *Escherichia coli* and *Staphylococcus aureus* had the highest frequency of occurrence (10.8-9.6 %) while *Enterobacter faecalis, Micrococcus luteus, Salmonella enterica and Streptococcus pnuemoniae* had the least frequency of occurrence 1.2 % recorded in urban residence patients.

Frequency of bacterial occurrence with respect to residence of the patients

	Residence			
Bacterial isolates	**U.R**	**R.R**	**Total**	**P-value**
Enterobacter faecalis	1(1.2)	1(1.2)	2(2.4)	
Escherichia coli	9(10.8)	16(19.2)	25(30.1)	
Klebsiella pneumoniae	6(7.2)	14(16.8)	20(24.0)	
Micrococcus luteus	1(1.2)	3(3.6)	4(4.8)	
Pseudomonas aeruginosa	5(6.0)	3(3.6)	8(9.6)	0.05
Salmonella enterica	1(1.2)	2(2.4)	3(3.6)	
Serratia marcescens	2(2.4)	0(0.0)	2(2.4)	
Staphylococcus aureus	8(9.6)	8(9.6)	16(19.2)	
Streptococcus pneumoniae	1(1.2)	2(2.4)	3(3.6)	
Total	**34(40.9 %)**	**49(59.0 %)**	**83(100.0 %)**	

Residence location is not a significant risk factor influencing bacterial infection at $p \leq 0.05$.
Key: R: Rural Residence and U: Urban Residence.

Appendix I: Zone of inhibition

The diameters of the zone of inhibition were compared with the clinical laboratory standard institute (CLSI, 2018) for antimicrobial susceptibility test and results were recorded accordingly as sensitive, intermediate and resistant.

Standard antimicrobial inhibition zones according to clinical laboratory standards institute for gram positive bacteria

Antibiotics	Resistant	Intermediate	Sensitive
Ciprofloxacin (10 μg)	≤ 15 mm	16–20 mm	≥21 mm
Norfloxacin (10 μg)	≤12 mm	13–16 mm	≥17 mm
Gentamicin (10 μg)	≤12 mm	13–14 mm	≥15 mm
Amoxil (20 μg)	≤19 mm	14–19 mm	≥18 mm
Streptomycin (30 μg)	≤10 mm	11–12 mm	≥15 mm
Riframpicin (20 μg)	≤16 mm	17–19 mm	≥20 mm
Erythromycin (30 μg)	≤13 mm	14–22 mm	≥23 mm
Chloramphenicol (30 μg)	≤12 mm	13–17 mm	≥18 mm
Ampiclox (20 μg)	≤11 mm	12–13 mm	≥14 mm
Levofloxacin (20 μg)	≤13 mm	14–16 mm	≥17 mm

Standard antimicrobial inhibition zones according to clinical laboratory standards institute for gram negative bacteria

Antibiotics	Resistant	Intermediate	Sensitive
Streptomycin (30 μg)	≤ 10 mm	11–12 mm	≥15 mm
Sulfamethoxazol-trimehoprim (30 μg)	≤10 mm	11–15 mm	≥16 mm
Nalidixic acid(30 μg)	≤13 mm	14–18 mm	≥19 mm
Cefalexin (10 μg)	≤10 mm	11–12 mm	≥19 mm
Ofloxacin (10 μg)	≤12 mm	13–15 mm	≥16 mm
Reflacine (10 μg)	≤23 mm	24 mm	≥25 mm
Gentamicin (10 μg)	≤12 mm	13–14 mm	≥15 mm
Amoxicillinand clavulanic(30 μg)	≤13 mm	14–17 mm	≥18 mm
Ciprofloxacin (10 μg)	≤15 mm	16–17 mm	≥21 mm
Ampilicin (30 μg)	≤11 mm	12–13 mm	≥14 mm

Appendix J: Antibiotic susceptibility test result of Gram negative bacterial isolates

Isolates ID	CEP	S	NA	OFX	PEF	AU	PN	CN	CPX	SXT
U6	R	R	R	S	R	S	S	S	S	S
U23	R	R	R	R	R	R	R	R	R	R
U29	R	R	R	I	R	R	R	S	I	S
U33	R	R	S	S	R	S	S	I	S	S
U34	R	R	R	R	R	R	R	R	R	R
U36	R	R	R	R	R	R	R	S	R	S
U41	R	R	S	S	R	S	R	S	S	S
U42	R	S	I	R	R	R	S	S	S	S
U81	R	R	S	S	R	R	R	S	R	S
U82	R	R	R	R	R	R	R	R	R	R
U88	R	S	I	S	R	S	R	I	I	R
U91	R	S	I	S	R	S	S	S	S	S
U101	R	R	R	R	R	R	R	R	R	R
U102	R	R	R	R	R	R	R	R	R	R
U105	R	S	R	S	R	I	I	S	S	S
U106	R	R	R	R	R	R	R	R	R	R
U110	R	R	R	R	R	R	R	R	R	R
U71	R	R	R	R	R	R	R	R	R	R
U78	R	R	R	S	R	R	R	R	R	R
U141	R	R	R	S	S	I	S	S	S	S
U12O	R	S	R	S	S	I	S	S	S	S
U166	R	R	R	R	R	R	R	R	R	R

Appendix J: Continues

U30	R	R	R	I	R	R	R	S	I	S
U172	R	R	R	R	R	R	R	R	R	R
U39	R	R	R	R	R	R	R	R	R	R
U2	R	S	S	I	S	S	I	R	R	S
U1	R	R	R	R	R	R	R	R	R	R
U17	R	R	R	R	R	R	R	R	R	R
U175	R	R	R	R	R	R	R	S	R	R
U144	R	I	R	I	R	R	R	R	R	R
U179	R	S	S	I	S	I	S	R	R	R
U154	R	R	R	R	R	R	R	R	R	R
U156	R	R	R	R	R	R	R	R	R	R
U151	R	R	I	S	R	S	S	S	S	S
U59	R	R	R	R	R	R	R	R	R	R
U46	R	S	R	S	R	R	I	S	I	S
U5	R	R	R	R	R	R	R	R	S	S
U3	R	R	R	I	R	R	R	R	I	S
U22	R	R	R	R	R	R	R	R	R	R
U67	R	R	I	R	R	R	R	S	R	R
U100	R	R	R	R	R	R	R	R	R	R
U110	R	R	S	S	R	R	R	R	I	R
U130	R	S	I	R	R	R	S	S	S	S
U132	R	I	R	R	R	R	R	R	S	R
U126	R	R	R	R	R	R	R	S	R	R
U150	R	R	R	R	R	R	R	R	R	R

Appendix J: Continues

U143	R	S	R	S	R	S	R	S	S	I
U158	S	S	R	S	R	R	S	R	S	I
U127	R	R	S	S	R	R	R	R	R	I
U129	R	R	R	R	R	R	R	S	R	R
U57	R	R	R	R	R	R	R	I	R	R
U198	R	R	R	R	R	R	R	S	R	R
U192	R	S	R	I	R	S	S	I	S	S
U204	I	S	R	S	R	R	R	S	I	I
U114	R	R	R	S	R	R	S	R	S	S
U48	R	R	R	R	R	R	R	R	R	R
U97	R	R	R	R	R	R	R	R	R	R
U119	R	R	R	R	R	S	R	R	R	R
U106	R	R	R	R	R	R	R	R	R	R
U66	R	S	R	S	R	R	S	S	S	S

KEY: S (Sensitive), R (Resistance) and I (Intermediates) S: Streptomycin, NA: Nalidixic acid, SXT: Sulfamethoxazole-trimethoprim, CEP: Cefalexine, PEF: Reflacine, OFX: Ofloxacin, AU: Amoxicillin and clavulanic acid, PN: Ampicilin CN: Gentamicin and CPX: Ciprofloxacin9999

Appendix K: Antibiotic susceptibility test result of Gram positive bacterial isolates

Isolates ID	LEV	APX	S	CH	E	RD	NB	CN	CPX	AMX
U4	S	S	R	I	S	R	R	S	I	I
U9	S	R	S	I	I	S	R	S	S	R
U11	R	R	I	R	R	R	R	R	I	R
U12	I	R	R	R	R	R	R	R	R	R
U14	S	I	I	S	I	I	I	S	R	R
U19	I	R	S	S	I	R	R	I	I	I
U75	R	R	R	R	R	R	R	R	R	R
U170	R	R	R	R	R	R	R	R	R	R
U90	I	R	I	R	R	R	R	R	R	R
U7	S	R	S	S	S	S	S	I	I	S
U60	S	R	R	R	I	R	R	I	S	R
U38	S	R	R	R	I	R	R	I	S	R
UI5	S	R	I	I	I	R	R	R	S	S
U23	R	R	I	I	R	R	I	R	R	R
U35	I	R	S	R	R	R	R	I	R	R
U70	S	R	R	I	I	R	R	R	I	R
U40	I	I	S	I	I	R	R	R	I	I
U17	S	R	I	R	I	R	R	R	I	R
U62	S	R	R	R	I	R	R	R	I	R
U200	R	R	R	R	R	R	R	R	R	R
U215	S	R	R	S	R	R	R	I	I	S
U225	R	R	I	R	I	R	R	R	I	R
U268	S	S	I	S	I	S	R	S	S	I

KEY: S (Sensitive), R (Resistance) and I (Intermediates) S: Streptomycin, NA: Nalidixic acid, SXT: Sulfamethoxazole-trimethoprim, CEP: Cefalexine, PEF: Reflacine, OFX: Ofloxacin, AU: Amoxicillin and clavulanic acid, PN: Ampicilin CN: Gentamicin and CPX: Ciprofloxacin

Appendix L: Biochemical Identification and Antimicrobial Susceptibility Test

Triple iron sugar test

TSI Test: Gas production

Hydrogen sulfide (H_2S) production

Antimicrobial susceptibility test

Appendix M: Ethical Approval to conduct research

NIGER STATE HOSPITALS MANAGEMENT BOARD
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107th June, 2019
HMB/GHM/STA/136/VOL.IV/547

The Head of Department,
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Federal University of Technology,
Minna.

Through:
Yusuf Zainab
Microbiology Department,
Federal University of Technology,
Minna.
Sir,

YUSUF ZAINAB (M TECH/SLS/2017/6891) RESEARCH, ETHICS AND PUBLICATION COMMITTEE'S APPROVAL TO CONDUCT RESEARCH

The bearer a student of your department sought for permission to conduct research on "Antimicrobial susceptibility pattern of Bacteria Isolated from Urine Samples of patients attending General Hospital, Minna Niger State".

The committee after going through her proposal has given her the approval to conduct the research.

A copy of her final findings must be submitted to the committee as a pre-requisite to this approval.

Thank you for your cooperation

MEDICAL OFFICER
GENERAL HOSPITAL, MINNA
SIGN

Amuda O. Amuda BDS, Mph, Msc, Phd
Chairman Research ethics and publication committee