

**ANTIBIOTIC RESISTANCE PATTERNS OF SOME COMMON GRAM-NEGATIVE
UROPATHOGENS AMONG PATIENTS ATTENDING FEDERAL TEACHING
HOSPITAL IDO-EKITI, EKITI STATE**

BY

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**BEING A PROJECT WRITE-UP SUBMITTED TO THE DEPARTMENT OF
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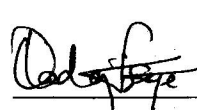
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CERTIFICATION

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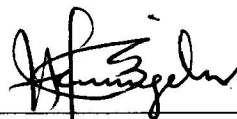
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DEDICATION

This research work is dedicated to God Almighty, the giver and sustainer of life, for His unconditional love and mercy over my life. May His name be glorified in Jesus name. Amen.

ACKNOWLEDGEMENTS

I give thanks to God Almighty for giving me the grace, mercy, favour, and wisdom to complete this research study. May His name be glorified in Jesus name, Amen.

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ABSTRACT

Urinary tract infections (UTIs) are among the most common human infections with the distribution of causative agents and resistance patterns to antibiotics varying from region to region. The aim of this study was to determine the antibiotic resistance profiles of some common Gram-negative uropathogens among patients with UTI symptoms at Federal Teaching Hospital, Ido-Ekiti, Ekiti State. Clean catch midstream urine samples were obtained and analyzed within 2 hours of collection for the detection of Gram-negative uropathogens. The isolated organisms were identified by standard biochemical tests and antibiotic susceptibility test was carried out using Kirby-Bauer disc diffusion technique. Extended Spectrum Beta-lactamase producing enterobacteria were detected using double-disc synergy test. Of the total 122 urine samples collected, 64 (52.5 %) samples were positive for Gram-negative uropathogen with significant bacteriuria of which 37 (57.8%) were from females and 27 (42.2%) from males. *Klebsiella* spp. was the most commonly isolated Gram-negative uropathogen 32 (50%), followed *Proteus* spp. 20 (31.3%), *Escherichia coli* 9 (14.1%) and *Pseudomonas aeruginosa* 3 (4.7%). Resistance rate to meropenem and ertapenem was generally very low compared to other tested antibiotics. Multidrug resistance was observed in 95.3% of the isolated bacterial uropathogens in this study. Moreover, ESBL-producing enterobacteria were detected among both in-and out-patient with a prevalence rate of 95%. In conclusion, choosing a particular antibiotic for empirical treatment will be challenging as the two antibiotics that showed highest susceptibility are not often deployed for empirical treatment of uncomplicated UTI, hence antibiotic prescription should be done only after urine culture and sensitivity is conducted.

CHAPTER ONE

1.1 INTRODUCTION

Gram-negative bacteria are bacteria that have outer membrane that is made up of phospholipids and lipopolysaccharides linked to peptidoglycan layer by lipoproteins (Nikaido, 2003). These bacteria stain pink to red when subjected to gram-staining due to their ability to retain the colour of the counter stain. They are different from Gram-positive bacteria in that Gram-positive bacteria generally lack outer membrane. The cell envelope of Gram-negative bacteria is a complex structure. It consists of four different components: the inner membrane, periplasm, cell wall, and outer membrane (Nikaido, 2003; Ruiz *et al.*, 2006). The inner membrane consists of a phospholipid bilayer and regulates the transport of materials in and out of the bacterial cell via specific transport proteins. The periplasm is the hydrophilic layer between the inner and outer membranes and contains the thin mesh of the peptidoglycan cell wall that maintains cell shape and rigidity. Finally, there is the outer membrane. The outer membrane is a highly asymmetric bilayer with phospholipids on the inner leaflet and lipopolysaccharides (LPS) on the outer (Nikaido, 2003) and serves as a selectively permeable barrier. The outer membrane also contains a large variety of proteins that include porins, which facilitate the general diffusion of small molecules across the membrane, specialized channels and pumps for the transport of specific molecules, lipoproteins that anchor the outer membrane to the peptidoglycan layer, enzymes, and secretion complexes that assemble the outer membrane (Caroff and Karibian, 2003).

Urinary tract infections are one of the most common diseases and can be defined as the presence of infectious microbes within urinary tract. UTI is the second most widespread infectious disease after respiratory tract infection in most communities (Sohail *et al.*, 2015) and it is regarded as a major public health problem due to increased costs with an estimated 150 million cases per annum worldwide (Arjuman *et al.*, 2010). Theodoros (2010) reported that most UTIs are caused by Gram-negative bacteria like *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas*

aeruginosa, *Acinetobacter* spp., and *Serratia* spp. and Gram-positive bacteria such as *Enterococcus* spp. and *Staphylococcus* spp. Risk factors associated with UTIs include indwelling catheter, immunosuppression, diabetes, family history, sexual intercourse, social class, age of patients among others. Recent studies have shown that microbes causing UTIs are classified by their target sites, such as urethra infection (urethritis), bladder infection (cystitis), and kidney infection (pyelonephritis), which can be either asymptomatic or symptomatic (Piranfar, *et al.*, 2014).

The most important treatment for UTIs is the antimicrobial therapy, with the main objective of eradicating bacterial growth in the urinary tract through efficacious, safe and cost-effective antimicrobial agent. However, widespread use of antibiotics has intensified the problem of antibiotic resistance in bacteria (Paterson and Bonomo, 2005). The production of extended-spectrum beta-lactamases (ESBLs) is an important mechanism of antimicrobial resistance in *Enterobacteriaceae* especially *Escherichia coli* and *Klebsiella pneumonia*, and the enzyme can hydrolyze penicillin, cephalosporin, and monocyclic amide antibiotics, but its activity is usually inhibited by beta-lactamase inhibitors, such as sulbactam, clavulanic acid, and tazobactam (Bradford, 2001). Various studies have reported the production of ESBL and concomitant multidrug resistance (MDR) among Gram-negative uropathogens such as uropathogenic *E. coli* (Hyle *et al.*, 2005). This is of great concern as it affects the treatment modalities. The present study seeks to determine the resistance patterns of some common Gram-negative uropathogens in order to generate data that will guide appropriate use of antimicrobial therapy.

CHAPTER TWO

LITERATURE REVIEW

2.1 What are Urinary Tract Infections?

Urinary tract infection is one of the most common diseases and can be defined as the presence of infectious microbes within urinary tract. It is regarded as a major public health problem due to increased costs with an estimated 150 million cases per annum worldwide (Arjuman *et al.*, 2010). It has been estimated that globally, symptomatic UTIs result in as many as seven million visits of outpatients to clinics, one million visits to emergency departments, and 100, 000 hospitalizations, annually (Ronald and Pattulo, 1991; Foxman, 2002; Wilson and Gaido, 2004). Urinary tract infections are classified on the basis of the affected part in the urinary tract, such as urethra infection (urethritis), bladder infection (cystitis), and kidney infection (pyelonephritis). UTIs can be either asymptomatic or symptomatic (Piranfar *et al.*, 2014). Asymptomatic patients are those that do not exhibit clinical symptoms while symptomatic patients exhibit clinical manifestation of the disease. Approximately 50%-60% of women have at least one urinary tract infection in their lifetime, and one-third will have at least one symptomatic urinary tract infection necessitating antibiotic treatment by age 24 (Rahn, 2008; Foxman *et al.*, 2000; Foxman, 2002).

UTIs are more predominant in women than in men at a ratio of 8:1 (Ahmed and Ghadeer, 2013). Urinary tract is sterile naturally but bacteria may ascend from the perianal region, possibly resulting into UTI. Microbial pathogen in the bladder may be hidden or can cause irritative symptoms like increased urinary frequency and urgency (Mignini *et al.*, 2009; Mohsin and Siddiqui, 2010). Common symptoms associated with bladder infection include burning with urination and having to urinate frequently in the absence of vaginal discharge and significant pain (Mignini *et al.*, 2009) while symptoms associated with pyelonephritis include flank pain, fever, or nausea, and vomiting in addition to the symptoms of bladder infection (Ramakrishnan and Scheid, 2005).

2.1.2 Aetiology

The community-acquired UTIs are also known as iatrogenic UTIs, meaning that they are contracted in the community while hospital-acquired (nosocomial infection) UTIs are contracted in the hospital environment. Published data regarding changes in the frequency of causative agents among outpatients is limited (Wilson and Gaido, 2004). Enteric bacteria (particularly *E.coli*) have been the most frequent cause of UTIs in automatically normal unobstructed urinary tracts (Elahe *et al.*, 2015). The more common UTI-pathogens apart from *E. coli* include *Staphylococcus saprophyticus*, *Enterobacter* spp, *Pseudomonas aeruginosa*, *Candida* spp, *Klebsiella pneumonia*, *Proteus* spp, and *Enterococcus* spp (Beyene and Tsegaye, 2011; EI Naggat *et al.*, 2001; Behzadi and Behzadi, 2008; Patel, 2001). *Escherichia coli*, the most common uropathogen is typically isolated from over 80% of outpatients with acute uncomplicated cystitis across the various regions of the world in uncomplicated UTI (Gupta *et al.*, 2001). *S. Saprophyticus* accounts for 5% to 15% of these infections and is especially prevalent in younger women with cystitis (Wagenlehner and Naber, 2004). Causative pathogens in the remaining 5% to 10% of cases include aerobic gram-negative rods such as *Klebsiella* spp. and *Proteus* spp. and other enteric bacteria (Wagenlehner and Naber, 2004). Amidst the fungal agents, *Candida albicans* is the most common cause of funguria, followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and other yeasts (Wagenlehner and Naber, 2004).

2.1.3 Epidemiology of Urinary Tract Infections

Urinary tract infections (UTIs) are one of the most common bacterial infections, affecting 150 million people each year worldwide (Flores-Mireles *et al.*, 2015). In 2007, in the United States alone, there were an estimated 10.5 million office visits for UTI symptoms (constituting 0.9% of all ambulatory visits) and 2–3 million emergency department visits (Foxman, 2014; Foxman, 2010). Currently, the societal costs of these infections, including health care costs and time missed from work, are approximately US\$3.5 billion per year in the United States alone. UTIs are a significant cause of morbidity in infant boys, older men and females of all ages. Serious sequelae include

frequent recurrences, pyelonephritis with sepsis, renal damage in young children, pre-term birth and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance and *Clostridium difficile* colitis (Flores-Mireles *et al.*, 2015).

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities; these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hooton, 2012; Nielubowicz and Mobley, 2010). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (Hooton, 2012; Hannan, 2012). Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices (Hooton, 2012; Nielubowicz and Mobley, 2010).

In the United States, 70–80% of complicated UTIs are attributable to in-dwelling catheters, accounting for 1 million cases per year (Foxman, 2010). Catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes (Chenoweth *et al.*, 2014).

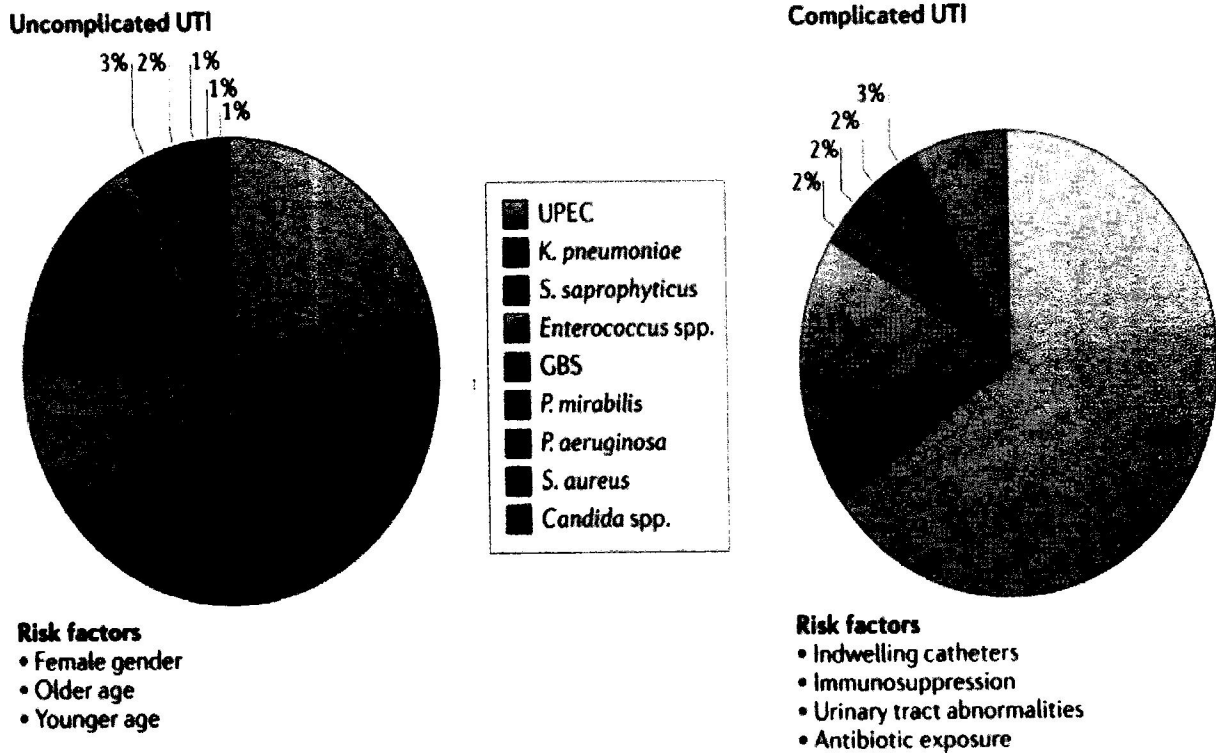


Fig. 1: Epidemiology of urinary tract infection.

Source: (Flores-Mireles *et al.*, 2015).

2.1.4 Antimicrobial regimen of Gram-negative Uropathogens

The most important treatment for UTIs is the antimicrobial therapy, with the main objective of eradicating bacterial growth in the urinary tract through efficacious, safe and cost-effective antimicrobial agent. The prescription of antimicrobial agents should be based on the susceptibility of the infecting bacteria, the concentration of uropathogens in the urine and the urinary complaint (Ahmed and Ghadeer, 2008). As a result of widespread emergence of resistance in 15-20% of *E. coli* in several areas of the USA and other countries, ampicillin, amoxicillin and sulphonamides are no longer the drugs of choice for empirical treatment (Gupta *et al.*, 2011; Drekonja and Johnson, 2008). Penicillins and cephalosporins are considered safe during pregnancy, but trimethoprim, sulphonamides and fluoroquinolones should be avoided (Ahmed and Ghadeer, 2013). Oral antibiotic

therapy resolves 94% of uncomplicated UTIs, although recurrence is not common. In the recently published *International Clinical Practice Guidelines for the Treatment of Acute Cystitis*, a 3-day regimen of trimethoprim-sulfamethoxazole (TMP-SMX) and a 5-day course of nitrofurantoin are recommended as a first-line therapy for the management of uncomplicated UTIs. A 5-day course of nitrofurantoin has an efficacy equivalent to a 3-day TMP-SMX course (Gupta *et al.*, 2007). A 3- to 7-day regimen of beta-lactams, such as cefaclor or amoxicillin/clavulanic acid, is appropriate when first-line therapies cannot be used (Gupta *et al.*, 2007). Although a 3-day course of fluoroquinolones can be quite effective, it is not usually recommended as first-line therapy because of the emerging resistance to them and their potential side effects, as well as the high cost; nevertheless, fluoroquinolones are the drug of choice in women who are experiencing low tolerance or an allergic reaction after empirical therapy (Gupta *et al.*, 2007). In a meta-analysis, a single-dose regimen of fosfomycin trometamol has been shown to be a safe and effective alternative for the treatment of UTIs in both pregnant and non-pregnant women, as well as in elderly and paediatric patients, but it seems to be slightly less effective than the above mentioned therapies (Falagas *et al.*; 2010). Pivmecillinam in a 3- to 7-day course is also effective, but not available in most regions. Because of the resistance of majority of uropathogens, amoxicillin and ampicillin should not be employed for the empirical treatment of UTIs.

2.1.5 Classification of Urinary Tract Infections based on the target site of infection

There are three different types of urinary tract infections on the basis of target site of infection and these include pyelonephritis, cystitis, and urethritis.

2.1.5.1 Pyelonephritis

This is one of the types of urinary tract infection and is characterized by the inflammation of the kidney, calyces, and renal pelvis. It is commonly associated with bacterial infection and results when bacteria have spread up the urinary tract or travelled through the bloodstream to the kidney, the pelvis, and calyces. Severe cases of pyelonephritis can lead to pyonephritis (pus accumulation

around the kidney), sepsis (a systemic inflammatory response of the body to infection), kidney failure, and even death. Signs and symptoms associated with acute pyelonephritis generally develop rapidly over a few hours or a day. It can cause high fever, pain on passing urine, and abdominal pain that radiates along the flank towards the back. There is often associated vomiting (Ramakrishnan and Scheid, 2005).

Signs and symptoms associated with chronic pyelonephritis include persistent flank or abdominal pain, signs of infection (fever, unintentional weight loss, malaise, decreased appetite), lower urinary tract symptoms and blood in the urine (Korkes *et al.*, 2008). In addition, chronic pyelonephritis can cause fever of unknown origin, accumulation of inflammation-related proteins in organs and the condition known as amyloidosis (Herrera and Picken, 2007). Community-acquired pyelonephritis is mostly associated with bowel organisms that enter the urinary tract. *E. coli* and *Enterococcus faecalis* are the common organism with *E. coli*, causing 70%-80% of community-acquired pyelonephritis. Hospital-acquired infections may be due to coliform bacteria and enterococci, as well as other organisms uncommon in the community (e.g. *Pseudomonas aeruginosa* and various species of *Klebsiella*). Pyelonephritis in most cases starts off as lower urinary tract infections, mainly cystitis and prostatitis (Ramakrishnan and Scheid, 2005).

2.1.5.2 Cystitis

This is another type of UTI characterized by the inflammation of the bladder, particularly in women and is usually more of a nuisance than a cause for serious concern (U. S National Health Service, 2018). Mild cases are often self-limiting within a few days. However, some people experience episodes of cystitis frequently and may need regular or long-term treatment. In addition, there is also a chance that cystitis could lead to a more serious kidney infection in some cases. The main symptoms of cystitis include: (i) pain, burning or stinging when pee (ii) needing to pee more often and urgently than normal (iii) blood in the urine (iv) urine that is dark, cloudy, or strong smelling (v) pain down the tummy (vi) feeling generally unwell, achy, sick and tired (U. K National Health

Service, 2018). Furthermore, possible symptoms in young children include a high temperature (fever) of 38° c or above, weakness, irritability, reduced appetite, and vomiting.

Most cases are thought to occur when bacteria that live harmlessly in the bowel or on the skin get into bladder through the urethra (tube that carries urine out of the body). In addition, other predisposing factors include: (i) having unprotected sex (ii) wiping bottom after going to the toilet- particularly wiping from back to front (iii) inserting a tampon or urinary catheter (a tube inserted into the urethra to drain the bladder) (iv) using a diaphragm for contraception (*U. K National Health Service*, 2018).

2.1.5.3 Urethritis

This is the third type of UTI, characterized by the inflammation of urethra. The most common symptom associated with urethritis is painful or difficult urination (*U.S. National Library of Medicine*, 2010; CDC, 2012). Urethritis is usually caused by infection with bacteria. Furthermore, the infection is often a sexually transmitted infection (STI), however some are just non-STI urinary tract infections. Urethritis can be caused by bacteria (UPEC, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* etc.) and viruses (Adenoviridae, Herpes simplex, Cytomegalovirus etc). The disease is classified as either gonococcal urethritis, caused by *Neisseria gonorrhoeae*, or non- gonococcal urethritis (NGU), most commonly caused by *Chlamydia trachomatis* (CDC, 2012)

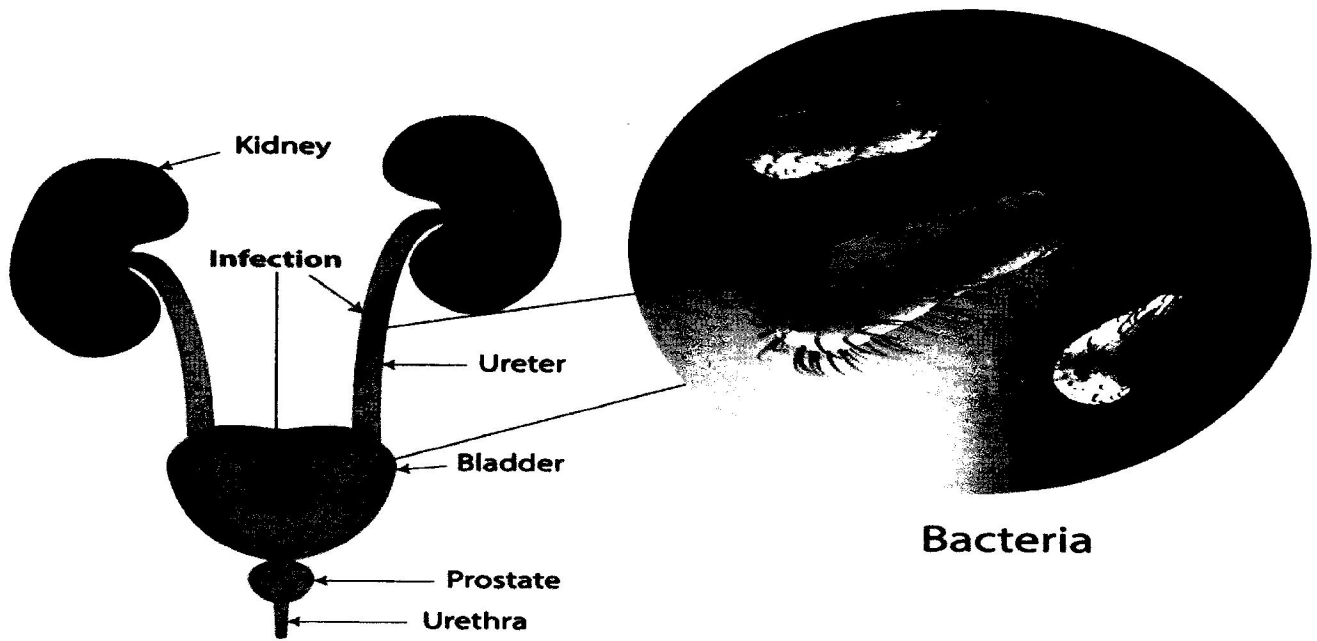


Figure 2: Urinary tract and site of infection.

Source: (Terlizzi *et al.*, 2017).

2.1.6 Virulence Factors of Gram-negative Uropathogens

There are varieties of virulence factors encoded by Gram-negative uropathogens which enable them to colonize the urinary tract and also facilitate their persistence in face of highly effective host defence. These Gram-negative uropathogens strains are equipped with specialized virulence genes located on mobile genetic elements called pathogenicity islands, hence exhibit a high degree of genetic diversity (Oelschlaeger *et al.*, 2002). Virulence factors of Gram-negative uropathogens that have been found to be associated with UTIs can be divided into two groups: (i) virulence factors associated with the surface of bacterial cell and (ii) virulence factors, which are secreted and exported to the site of action (Mulvey, 2002).

2.1.6.1 Surface Virulence Factors

These include a number of different types of adhesive organelles (fimbriae), which facilitate the attachment of bacteria to host tissues within the urinary tract. The most important determinant of gram-negative uropathogens pathogenicity is the presentation of adhesive molecules (adhesins). The

Gram-negative uropathogens adhesins can contribute to virulence in different ways: (i) directly triggering host and bacterial cell signalling pathways, (ii) facilitating the delivery of other bacterial products to host tissues, and (iii) promoting bacterial invasion (Emody *et al.*, 2003).

i. Fimbriae

Type 1 fimbriae have been found to be associated with UTI in animal models, but their function in human pathology remains unclear (Bergsten *et al.*, 2007). Type 1 fimbriae consist of heteropolymeric fibres composed of different protein subunit encoded by *fim* gene operon. The role of the type 1 fimbriae in human disease is difficult to reconcile because they are expressed in both pathogenic and commensal strains. However, in the murine UTIs model, the type 1 fimbriae have been shown to augment bacterial survival, to stimulate mucosal inflammation and to promote invasion and growth as a biofilm (Anderson *et al.*, 2003; Oelschlaeger *et al.*, 2002; Schembri and Klemm, 2001). A protein called Tamm-Horsfall (THP) is produced by kidney cells into human urine and can act as a soluble FimH receptor, thereby obstructing bacterial-host cell interaction and limiting the ability of some gram-negative uropathogen to colonize the UTI (Batès *et al.*, 2004; Pak *et al.*, 2001). Similarly to UPEC, *K. pneumoniae* uses type 1 pili for biofilm formation and bladder colonization. *K. pneumoniae* FimH-mediated biofilm formation is inhibited by heptyl mannose, as opposed to the methyl mannose-mediated inhibition of UPEC FimH. Moreover, *K. pneumoniae* FimH has a weaker adherence to the bladder than UPEC FimH, resulting in significantly lower *K. pneumoniae* titers in the mouse bladder and fewer IBCs than are seen for UPEC (Flores-Mireles *et al.*, 2015).

P fimbriae are common virulence factor of some gram-negative uropathogens that have been found to play a crucial role in the pathogenesis of ascending UTIs and pyelonephritis in humans. They are responsible for adhesion to mucosal and tissue matrix and for the production of cytokines (Godaly *et al.*, 2000). These fimbriae recognize and bind to kidney glycosphingolipids carrying the Gal α (1-4) Gal determinant on renal epithelia through its papG adhesion (Kaper *et al.*, 2004). These interactions

result in the release of ceramide, which acts as an agonist of Toll-like receptor 4 (TLR4), a receptor involved in the activation of the immune response (Fischer *et al.*, 2007). This, in turn, leads to the development of the local inflammation and pain associated with UTIs. P fimbriae have been shown to enhance early colonization of the tubular epithelium, while the type 1 fimbriae mediate colonization of the center of the tubule via a mechanism that involves inter-bacterial binding and biofilm formation.

ii. Lipopolysaccharide (LPS)

The LPS is an integral component of the cell wall of Gram-negative bacteria. LPS has been implicated in the activation of host response and induction of nitric oxide and cytokine production (Bäckhed *et al.*, 2001). Lipopolysaccharide (also known as endotoxin) unlike exotoxin is not secreted in soluble form but may be released into the environment during growth or when Gram-negative bacterial cell becomes lysed, thereby stimulating host immune response. In addition, LPS are molecules with amphipathic properties consisting of fatty acids lined to an oligosaccharide core, which in turn is bound to a long polysaccharide chain commonly called O antigen (Simpson *et al.*, 2015).

Although LPS of gram-negative uropathogens is important in the activation of proinflammatory response in uncomplicated UTIs but it is not clear whether LPS plays a role in mediating a renal failure and acute allograft injury in patients with ascending UTIs. It has been demonstrated in an animal model that the acute renal failure due to LPS depends on the systemic response to LPS and does not depend on expression of functional LPS receptor, TLR4, in the kidney (Cunningham *et al.*, 2004). However, TLR4 is expressed in renal epithelia and in the renal pelvis, and these findings suggest that the ascending infection due to *E. coli* may stimulate the innate immune response associated with the acute allograft injury in patients with UTIs (Wolfs *et al.*, 2002; Samuelsson *et al.*, 2004).

iii. Capsule

This is a membranous structure (mainly a polysaccharide) that surrounds and protects the bacterium from the host immune system. The capsule provides protection against phagocytic engulfment and complement-mediated bactericidal effect in the host. Certain capsular types, for example, K1 and K5, show a molecular mimicry to tissue components, prevents a proper humoral immune response of the infected host (Chapman/ *et al.*, 2002).

iv. Flagella

This is the organelle of motility among gram-negative uropathogens. It has been discovered that this organelle is involved in the interaction of various pathogenic *E. coli* strains with epithelial cells. Flagellated UPEC causes 70–90% of all urinary tract infections, and their pathogenesis involves contact between the bacteria and epithelial cell surface of the urinary tract. The pyelonephritis-associated *E. coli* strains may invade renal collecting duct (CD) cells through flagellin, and the flagellin acts as an invasin in this process (Pichon *et al.*, 2009). Other studies have suggested that *E. coli* flagella may be of importance in allowing the bacteria to ascend from the bladder and to initiate kidney infection in humans. The use of an antibody against the flagella could prevent the spread of UPEC into the kidneys.

v. Siderophore receptors

These receptors are associated with iron acquisition system among gram-negative uropathogens and help to scavenge ferric iron (Fe^{3+}). In addition, iron acquisition is essential for the survival of in an environment that is iron-limited as the urinary tract (Skaar, 2010). Thus, IBC (intracellular bacterial communities) of gram-negative uropathogens show up-regulation of redundant systems for the acquisition of iron (Reigstad *et al.*, 2017). For this iron acquisition to be ensured, some gram-negative uropathogens produce siderophores, which are small molecule iron chelators that scavenge ferric iron (Fe^{3+}), thus expressing yersiniabactin, salmochelin, aerobactin, enterobactin, pyochelin and pyoverdin. Siderophore receptors require the TonB cytoplasmic membrane-localized complex, a

high-affinity iron acquisition system that allows binding and chelation of iron at the cell surface to promote its uptake (O'Brien *et al.*, 2016).

vi. Curli

Curli are bacterial surface appendages that secrete subunits from the cell as soluble monomeric proteins and possess the typical structure and physical characteristics of amyloid fibrils. The bacterial amyloids may facilitate biofilm formation (Goyal *et al.*, 2014). In UPEC, curli formation is coordinated by proteins encoded in the operons *csg* DEFG. The operon accessory proteins CsgE, CsgF, and CsgG are required to facilitate the secretion of CsgA whereas CsgB nucleates CsgA subunits into curli fibers (Chapman *et al.*, 2002; Barnhart and Chapman, 2006).

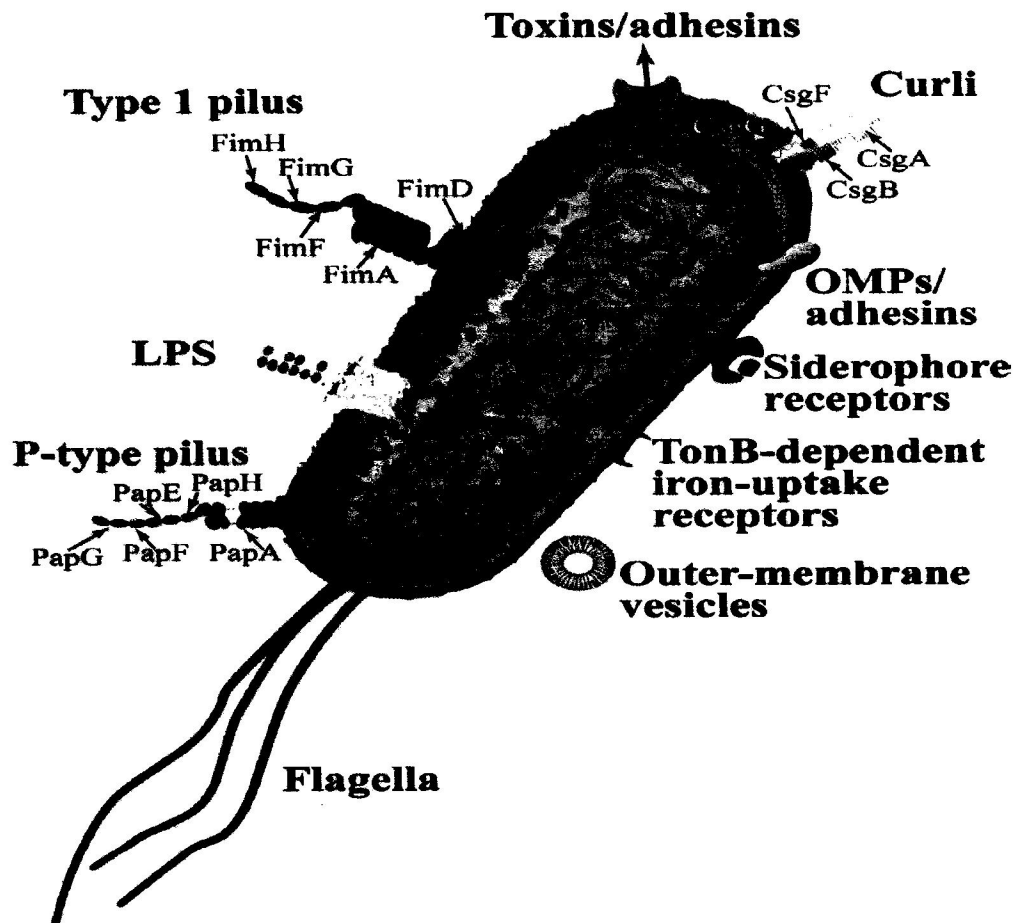


Fig 3: *Escherichia coli* adhesins and harboring/motile structures.

Source: (Chapman *et al.*, 2002; Barnhart and Chapman, 2006).

2.1.6.2 Secreted Virulence Factors

These are the virulence factors secreted in soluble form and exported to the site of action. Examples of secreted virulence factors include α -hemolysin, cytotoxic necrotising factor 1 and secreted auto-transporter toxin (Chapman *et al.*, 2002; Barnhart and Chapman, 2006).

i. Hemolysin

Toxins are important virulence factors in a variety of gram-negative uropathogen-mediated diseases. Production of toxins by colonizing gram-negative uropathogens may cause an inflammatory response, a possible pathway for UTIs symptoms. The α -hemolysin (a lipoprotein) is the most important secreted virulence factor (toxin) of UPEC and is associated with upper UTIs such as pyelonephritis. *P. mirabilis* produces two toxins, haemolysin (HpmA) and *Proteus* toxic agglutinin (Pta), which are implicated in tissue damage and dissemination to the kidneys, initiating acute pyelonephritis (Jacobsen *et al.*, 2008). *Pseudomonas aeruginosa* produces elastases, exoenzyme S (ExoS) and haemolytic phospholipase C, all of which have been implicated in UTI initiation and dissemination, and subsequent pyelonephritis.

It has been shown that this toxin exerts dual concentration-dependent activities on primary epithelial cells originating from renal proximal tubules (Laestadius *et al.*, 2002). At high concentrations, HlyA is able to lyse erythrocytes and nucleated host cells, a process that may enable extraintestinal pathogens like UPEC to better cross mucosal barriers, damage effector immune cells, and gain enhanced access to host nutrients and iron stores. At low concentrations, HlyA can induce the apoptosis of target host cells, including neutrophils, T lymphocytes, and renal cells, and promote the exfoliation of bladder epithelial cells (Chen *et al.*, 2006; Smith *et al.*, 2006; Russo *et al.*, 2005). Approximately 50% of all cases of pyelonephritis, which leads to renal complications, are caused by HlyA. The HlyA and other *E. coli* toxins have been shown to cause inducible nitric-oxide-synthase-(iNOS-) mediated cell membrane injury and apoptosis, a process that is regulated by extracellular signal regulated kinase (ERK) independently of the p53 pathway (Chen *et al.*, 2006).

ii. Cytotoxic Necrotising Factor 1 (CNF1)

The CNF1 is produced by one-third of all pyelonephritis strains and may also be involved in kidney invasion. *In vitro*, this protein is secreted by *E. coli* and stimulates actin stress fibers formation and membrane ruffle formation in a Rho GTPase-dependent manner, resulting in the entry of *E. coli* into the cells. However, the detailed role of CNF1 in invasion processes during pyelonephritis remains unclear and is a matter of debate. *In vitro* studies have also showed that CNF1 interferes with polymorphonuclear phagocytosis and evokes apoptotic death of bladder epithelial cells (Mills *et al.*, 2000). *In vivo*, CNF1 may lead to bladder cell exfoliation and to enhanced bacterial access to underlying tissue (Mills *et al.*, 2000).

iii. Secreted Autotransporter Toxin (SAT)

Secreted autotransporter toxin (SAT) is a virulence factor of pyelonephritis *E. coli* strains. SAT has a toxic activity against cell lines of bladder or kidney origin and, thus, may be important for pathogenesis of UTIs (Guyer *et al.*, 2002; Guyer *et al.*, 2000).

2.1.7 Mechanisms of Antibiotic Resistance among Gram-negative Uropathogens

Antibiotic resistance can be defined as the ability of bacteria to grow under achievable therapeutic concentrations of antibiotic substances at the site of infection (Wagenlehner and Naber, 2004). Resistance is divided into primary or intrinsic resistance of bacteria, if bacteria are constitutively resistant against an antibacterial substance and secondary or acquired resistance, if resistance emerges in intrinsically susceptible bacteria (Wagenlehner and Naber, 2004). Epidemiologically important genomes are; transferable resistance located on plasmids (extrachromosomal autonomous mobile genetic element transferable to other cells) or transposons (mobile genetic element transposable to plasmids or other chromosomal sites) (Wagenlehner and Naber, 2004).

i. Alterations of permeability and efflux mechanisms

Probably all antibiotics have to get into the bacterial cells in order to act. Intrinsic resistance for example of Gram-negative bacteria against macrolides is due to impermeability of the outer

membrane to these hydrophilic compounds. Efflux mechanisms can potentially pump antibiotic substances, such as quinolones or tetracyclines out of the cell. There are five superfamilies of efflux transport systems known so far: ABC (ATP-binding cassette), MFS (major facilitator superfamily), RND (resistance nodulation- division), SMR (small multidrug resistance) and MATE (multidrug and toxic compound extrusion) family. Efflux systems are responsible for low level resistance, and thus may promote selection of mutations responsible for higher level resistance (Lehn, 2004).

A powerful efflux mechanism in *Pseudomonas* spp. is one constitutively produced system (MexAB-OprM- RND superfamily) that generates intrinsic resistance against most beta-lactams, quinolones, tetracycline, chloramphenicol, trimethoprim and sulfamethoxazole. On the other side non-constitutive systems (i.e. MexCD-OprJ; MexEF-OprN) can be expressed by mutation.

ii. Alterations of target structures

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

iii. Mutations

Fluoroquinolone resistance is mediated by target modifications (DNA gyrase and/or topoisomerase IV) and/or decreased intracellular accumulation. Whereas in Gram-negative bacteria like *E. coli*, the DNA gyrase is the primary target. With clinically relevant concentrations newer quinolones like moxifloxacin and gemifloxacin inhibit both targets, the DNA gyrase as well as topoisomerase IV (Wagenlehner and Naber, 2004).

iv. Acquisition of genetic material

Resistance to TMP/SMX arises from a variety of mechanisms, involving enzyme alteration, cellular impermeability, enzyme overproduction, inhibitor modification or loss of binding capacity. The mechanism of greatest clinical importance is the production of plasmid encoded, trimethoprim resistant forms of dihydrofolate reductase (Park *et al.*, 1997).

v. Inactivation of antibiotics

Beta-lactamases are enzymes produced by bacteria that inactivate beta-lactam antibiotics by cleavage of the beta-lactam ring. More than 80 different enzymes so far are identified and the substrates comprise penicillines, cephalosporins or other beta-lactam antibiotics (Lehn, 2004).

Resistance to penicillin is mediated by a penicillinase that hydrolyses the beta-lactam ring of penicillin. A frequent resistance mechanism in *E. coli* and *Proteus* spp. is the production of TEM-1, a plasmid-mediated beta-lactamase that is inhibitor resistant. It therefore confers resistance in strains having acquired the resistance plasmid, for example to ampicillin as well as ampicillin/sulbactam. The SHV-1 beta-lactamase of *Klebsiella pneumoniae*, as well as the K1-beta lactamase of *Klebsiella oxytoca* strains are chromosomally encoded but are inhibitor- sensitive (Wagenlehner and Naber, 2004).. It therefore encodes intrinsic resistance in all *Klebsiella* strains, for example to-ampicillin but not to ampicillin/sulbactam.

2.1.8 Emergence of Antibiotic Resistance among Gram-negative Uropathogens

Antibiotic resistance in general is related to the amount of application of an antibiotic substance or a related substance or an unrelated substance with an identical resistance mechanism (Wagenlehner and Naber, 2004).

i. Frequent application of antimicrobials in clinical practice

It is generally assumed that the antibiotic prescription policy of a hospital has a significant impact on emergence of bacterial resistance. It was reported in a three-year surveillance study conducted by Lepper *et al.* (2002) that shown the consumption of imipenem correlated significantly with beta-lactam resistance in nosocomial *Pseudomonas aeruginosa* isolates, while consumption of ceftazidime or piperacillin/tazobactam had no apparent association with resistance (Lepper *et al.*, 2002). Another example is the resistance rate of *E. coli* against fluoroquinolones in isolates of hospitalised children, which is ten-fold less than in adults, because fluoroquinolones are generally not used for children (Wagenlehner and Naber, 2004).

ii. Antibacterial substances other than for treatment of human infections

Antibiotics are not only used for treatment of human infections. A great proportion of antibiotic substances or related compounds are used in animal husbandry as food adjunctive or in household products, such as aseptic soaps or lotions (Wagenlehner and Naber, 2004). Avoparcin for example is a glycopeptide antibiotic and therefore related to vancomycin, used exclusively in animal husbandry. Avoparcin was prohibited to be used in husbandry in Denmark since 1995.

2.2 JUSTIFICATION

Aetiology of urinary tract infections (UTI) and their antibiotic resistance patterns vary from time to time and across different areas. In Nigeria, studies on the prevalence and antimicrobial resistance patterns of clinical isolates from urinary tract infections are inadequate (Abdu *et al.*, 2018). Hence, this study is conducted to ascertain the prevalence and resistance patterns of some common Gram-negative uropathogens in our area, thereby forestalling the problem of antibiotic resistance among patients in hospitals and in the community, since antibiotic misuse in developing countries is the major reason for the development of high resistance rates in bacterial isolates.

2.3 AIMS OF STUDY

The aims of this study are isolate and identify some common Gram-negative uropathogens from patients referred to microbiology laboratory for urine microscopy, culture and sensitivity at Federal Teaching Hospital, Ido-Ekiti, Ekiti State.

2.4 OBJECTIVES

To determine the prevalence rate of urinary tract infection and antibiotic resistance patterns of some common Gram-negative uropathogens at Federal Teaching Hospital, Ido-Ekiti, Ekiti State.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials and equipments used: The following materials were used: hand gloves, masking tape, cotton wool, wire loops, disinfectant (hand wash), ethanol, standard antibiotic discs, petri dishes, aluminum foil, dry ice pack, MacConkey agar, Cystine Lactose Electrolyte Deficient agar (CLED), Eosin Methylene blue agar (EMB), Nutrient agar, Muller Hinton agar, MR-VP broth, Simmon's citrate agar, Gram's iodine, crystal violet stain, acetone-alcohol decolorizer, safranin, blood agar, Kovac's reagent, micropipettes, microscope, bunsen burner, autoclave, centrifuge, McCartney bottle, urease agar, sterile universal bottle.

3.2 Methods

3.2.1 Study location

This study was carried out at Federal Teaching Hospital Ido-Ekiti, Ekiti State. Urine samples of only the patients that were specifically referred to microbiology laboratory for urine microscopy, culture and sensitivity were obtained from Federal Teaching Hospital, Ido-Ekiti. The study subjects were from out-patient and in-patient departments. Appropriate ethical approval (ERC/2017/10/18/84B) was obtained before conducting the research.

3.2.2 Sample collection

Urine specimens were collected in accordance to the standardised protocols as described by Cheesbrough (2006) and modified by Prakash and Saxena (2013) and Ochada *et al.* (2014). Clean catch midstream urine (MSU) was collected from each patient into a 20 mL calibrated sterile screw-capped universal container which was distributed to the patients. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Samples collected were transported to the laboratory in ice pack and processed within 2hrs of collection, for Gram-negative uropathogen isolation (Miao *et al.*, 2017).

3.2.3 Culture and Isolation of Organism

The Urine samples (10 µl) were cultured on Cysteine Lactose Defficient agar, MacConkey, and Eosin-Methylene blue simultaneously (EMB) agar using micropipette and incubated in aerobic conditions for 24 hours at 37°C. Cultures without any colony at the end of 24hrs of incubation were further incubated for 48hrs. Plates with colony count equal to or more than 10⁵ Cfu/ml were considered significant culture positive (Pooja *et al.*, 2017). The organisms were further subcultured on fresh MacConkey agar plate in order to get a pure culture. The isolates were identified and confirmed using standard methods including: Gram staining, colonial morphology on media, growth on selective media, indole, citrate utilization, MR-VP tests and urease test.

3.2.4 Identification of Gram-negative Uropathogens

❖ Gram staining:

This was done by transferring a little amount of 18- 24 hrs old culture onto a grease-free microscopic slide. The culture homogenized in 1ml of normal saline at the centre of the microscopic slide and was air-dried. The smear was then heat fixed by the passing the slide through flame several times. Crystal violet solution was applied on the smear for one minute and then washed with running water. Lugol's iodine was added to act as mordant for one minute and then washed with running water. Subsequently, acetone alcohol was added, which acted as a decolorizer, for 5 seconds. After washing with water, safranin was added as counter stain and allowed to stay for one minute. The microscopic slide was washed with water, dried in air and then examined under microscope with high power objectives (100X) using immersion oil (Merchant and Packer, 1967).

3.2.5 Biochemical Identification of Gram-negative Uropathogens

❖ Indole test

This test is based on the principle that bacteria that have the enzyme tryptophanase, can convert the amino acid, tryptophane to indole. Indole reacts with added Kovac's reagent to form rosindole dye which is red in colour (indole +). Distinct colonies of 24 hours old culture were subcultured into

individual tube containing 5 ml tryptone water, incubated at 37°C for 24 hours and tested for indole production with Kovac's reagent. The appearance of cherry red color layer confirms positive test (Cheesbrough, 1984).

❖ **Methyl-Red test**

This test uses the ability of bacteria to utilize glucose through Embden-Meyerhof fermentation pathway and the organisms either produce a single acid or mixture of acids as the end products. The bacteria produce large amounts of acid resulting in significant decrease in the pH of the medium below 4.4. This acidic nature is indicated by methyl red (p-dimethylaminoacetic acid) indicator which is yellow above pH 5.1 and red at pH 4.4. The test was performed by transferring a distinct colony of 24 hours old culture in MR-VP broth. After overnight incubation at 37°C, 3 drops of methyl red solution was added. A positive methyl red test was shown by the appearance of bright red colouration (Cheesbrough, 1984).

❖ **Voges Proskauer test**

Voges-Proskauer tests for the ability of bacteria to produce butylene products. Acetoin (3 hydroxybutanone) is an intermediate in the reaction which is tested for using 40% KOH and alpha-naphthol. If acetoin is present, it is oxidised in the presence of air and KOH to diacetyl which reacts with guanidine components of peptone, in the presence of alpha-naphthol to produce a red colour. MR-VP broth was inoculated with 24 hours old culture incubated for 24 hours at 37°C. After incubation, the broth was removed from the incubator and 10 drops of Barritt's A reagent (alpha-naphthol and potassium hydroxide) was added to the broth. The tube containing the broth was shaken gently for several minutes to expose the medium to atmospheric oxygen and allowed to remain undisturbed for 10 to 15 minutes. Red colour formation within 15 to 20 minutes is a positive result. No red colour formation after 15 to 20 minutes is a negative result (Cheesbrough, 1984).

❖ Citrate utilization test

The test looks for the ability of a bacterium to utilise citrate as a sole source of carbon. For the bacteria to be able to do so, it requires 2 components- Citrate permease and citrate lyase. Citrate permease is a group of uptake proteins that allows the cell to take up citrate and then lyase which converts citrate to oxaloacetate and acetate. The oxaloacetate is then metabolised to pyruvate and CO₂. Twenty-four hours old culture was inoculated onto Simmon citrate agar lightly on the slant and incubated at 37°C for 24 hours. The utilization of the citrate by the organism produces ammonia and ammonium hydroxide from conversion of ammonium dihydrogen phosphate (component of Simmon's citrate agar) which create an alkaline environment in the medium. At pH 7.5 or above, bromthymol blue turns royal blue which is otherwise green.

❖ Urease test

Urease is an enzyme belonging to belong to the superfamily of amidohydrolases and phosphotriesterases. It catalyzes the hydrolysis of urea into ammonia and carbon dioxide. The formation of ammonia causes alkalinization of the medium, and the pH change is indicated by a change to pink at pH 8.1. Urea medium was inoculated with 24 hours old culture and incubated at 37°C for 24 hours. A change in colour of the medium from orange yellow to bright pink is a positive test while no change in the colour of the medium represents a negative test.

3.2.6 Virulence Assay Tests

3.2.6.1 Biofilm Formation Test

Detection of biofilm formation was investigated basically using the qualitative method of Congo red agar technique (CRA). The CRA method described by Freeman *et al.* (1989) was used in this study. Congo red powder (0.8g/L) was prepared as concentrated aqueous solution and sterilized in the autoclave at 121°C for 15 minutes, separately from other medium constituents [MHA (38 g/L)] and Sucrose [(50 g/L)]. The solution of Congo red powder was then added to a sterilized Muller Hinton agar solution (containing sucrose) when the agar had cooled to 55°C. Plates were inoculated with pure

isolates and incubated aerobically for 24hr at 37°C. Colour changes were observed after incubation (Freeman *et al.*, 1989).

3.2.6.2 Haemolysis test

Eighteen hours old cultures were subcultured on 5% blood agar plates to detect the production of hemolysin by the isolates. The plates were incubated aerobically for 24 hours at 37°C. The presence of complete clearing zone around the colony on blood agar plate indicates complete lysis of erythrocytes (β -hemolysis). The appearance of green colouration around the colony indicates partial lysis of erythrocytes (α -hemolysis) while no lysis of erythrocytes indicates that the isolate is non-hemolysin producing organism (Tabasi *et al.*, 2015).

3.2.7 Antibiotic Susceptibility Assay

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar using disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs and their concentrations consisted of ceftriaxone (CRO, 30 μ g), ceftazidime (CAZ, 30 μ g), gentamicin (CN, 10 μ g), ampicillin (AM, 10 μ g), norfloxacin (NOR, 10 μ g), pefloxacin (PEF, 5 μ g), tetracycline (TE, 30 μ g), ertapenem (ETP, 10 μ g) and meropenem (MEM, 10 μ g).

3.2.8 Testing for Extended Spectrum Beta-Lactamase (ESBL) Production

Some of the isolates which showed a diameter of less than 20mm for ceftriaxone and 18mm for ceftazidime, were selected for checking the ESBL production. The ESBL production was tested by Double Disc Synergy Test (DDST) by using a disc of amoxicillin-clavulanate (30 μ g) along with two cephalosporins; ceftriaxone (30 μ g) and ceftazidime (30 μ g). A lawn culture of each isolate was made on a Mueller-Hinton agar plate, as recommended by CLSI. A disc which contained amoxicillin-clavulanate (30 μ g) was placed in the centre of the plate. The discs of ceftriaxone (30 μ g) and ceftazidime (30 μ g) were placed 20mm apart, centre to centre to that of the amoxicillin-clavulanate

disc (Paterson and Bonomo, 2005). Any enhanced zone of inhibition towards the disc of amoxicillin-clavulanate was considered positive for the ESBL production.

CHAPTER FOUR

4.1 RESULTS

A total of 122 urine specimens recommended for Urine Microscopy, Culture and Sensitivity at Federal Teaching Hospital Ido-Ekiti were collected and processed. A total of 67 (54.9%) urine specimens were collected from females while 55 (45.1%) urine specimens were collected from males. Urine specimens of only 27 (42.2%) males and 37 (57.8%) females showed demonstrable bacteriuria and were all positive for Gram-negative uropathogens. Out of the 64 positive samples, 48 (75%) urine specimens were from outpatients while 16 (25%) urine specimens were from inpatients (Table 1). The Gram-negative uropathogens were more isolated from patients between 11-40 years and > 70 years among in-patients and out-patients. Among in-patients and out-patients, significant bacteriuria was detected in 50% and 43.8% of patients between 11 and 40 years and 31.3% and 29.2% of patients > 70 years respectively. Males were mostly affected in their late ages above 50 but females were mostly affected in their reproductive age group (11-40 years) (Table 2).

Of the total 122 urine specimens collected, 94 (77%) urine samples were obtained from outpatients while 28 (23%) samples were from inpatients. Most of the urine samples were from Obstetrics and Gynecology Department 34 (27.9%), General Outpatient Department 23 (18.6%), Accident and Emergency 16 (13.1%), Surgical Outpatient Department 12 (9.8%), Urology 8 (6.6%), Female Medical Ward 7 (5.7%), Male Surgical Ward 6 (4.9%), and Antenatal Care Unit 5 (4.1%) (Table 3).

The most isolated Gram-negative uropathogens were *Klebsiella pneumoniae* 17 (26.6%), *K. oxytoca* 15 (23.4%), *Proteus vulgaris* 14 (21.9%), and *Escherichia coli* 9 (14.1%). Others were *Proteus mirabilis* 6 (9.4%) and *Pseudomonas aeruginosa* 3 (4.7%) (Table 4).

The predominant bacterial isolates among both out-patients and in-patients were *Klebsiella pneumoniae* 14 (29.2%) and 3 (18.8%), *K. oxytoca* 11 (22.9%) and 4 (25%) and *Proteus vulgaris* 10 (20.8%) and 4 (25%) respectively. Other isolates were *Escherichia coli* 7 (14.6%) and 2 (12.5%), *P.*

mirabilis 5 (10.4%) and 1 (6.3%) and *Pseudomonas aeruginosa* 1 (2.1%) and 2 (12.5%) among out-patients and in-patients respectively. Moreover, most of the uropathogens were isolated from Obstetrics and Gynaecology Department 15 (23.5%), Surgical Outpatient Department 10 (15.6%), Accident and Emergency Department 8 (12.5%), General Outpatient Department 7 (10.9%), Female Medical Ward 6 (9.4%), and Antenatal Care Department 5 (7.8%) (Table 5).

Klebsiella pneumoniae 9 (24.3%), *K. oxytoca* 10 (27%), *Proteus vulgaris* 8 (21.6%), *Proteus mirabilis* 4 (10.8%) and *Pseudomonas aeruginosa* 2 (5.4%) were more isolated from female patients while only *E. coli* isolates 5 (18.5%) were more predominant among male patients. The bacteria were more isolated from females 37 (57.8%) than males 27 (42.2%). Also, the organisms were most prevalent among 11-40 years 29 (45.3%), > 70 years 19 (29.7%) followed by 41-70 years 15 (23.4%) (Table 6). During the study period, sixty one (95.3%) MDR Gram-negative uropathogens were isolated. There was high resistance to beta-lactams with ampicillin 60 (93.8%), as compared to aminoglycoside with gentamicin 56 (87.5%). Resistance to the quinolone was 51 (79.7%) to pefloxacin and tetracyclines was 50 (78.1%) while resistance to cephalosporins was 46 (71.9%) to ceftriaxone. The Gram-negative uropathogens in this study were highly susceptible to meropenem (71.9%) followed by ertapenem (59.4%) but moderately sensitive to ceftazidime (39.1%), followed by norfloxacin (31.2%) (Table 7).

Resistance of *Klebsiella pneumoniae* among in-patients as against out-patients was 100% versus 92.9%, 100% versus 78.6% and 66.7% versus 64.3% respectively for ampicillin, gentamicin and norfloxacin. For meropenem, ertapenem, ceftazidime and ceftriaxone, the values were 33.3% versus 35.7%, 66.7% versus 42.9%, 100% versus 64.3% and 66.7% versus 78.6% respectively. In the case of *Escherichia coli*, resistance was 0% versus 14.3% to meropenem, 100% versus 100% to ampicillin, 0% versus 71.4% to ceftazidime, 50% versus 0% to ertapenem, 100% versus 85.7% to norfloxacin, 100% versus 85.7% to gentamicin and 50% versus 57.1% to gentamicin. Resistance of *Proteus vulgaris* was 0% versus 40% to meropenem, 100% versus 80% to ampicillin, 75% versus 50% to ceftazidime, 25% versus 50% to ertapenem, 75% versus 50% to norfloxacin, 100% versus 90% to

gentamicin and 75% versus 60% to ceftriaxone (Table 8). The degree of resistance to each antibiotic among in-patient was found to be higher throughout than that of out-patient (Table 9).

Out of the 64 (52.5%) positive samples, 61 (95.3%) isolates showed multidrug resistance. Majority of the isolates were resistant to 5 drug groups followed by 6 drug groups. All the 16 (25%) positive samples from in-patients showed multidrug resistance while 45 (93.8%) showed multidrug resistance out of 48 (75%) positive samples from out-patients. Multidrug resistance in this study is defined as resistance of uropathogen to more than two different classes of antibiotics (Table 10). All the *K. oxytoca* isolates 15 (100%), *E. coli* isolates 9 (100%), *Proteus mirabilis* isolates 6 (100%) and *Pseudomonas aeruginosa* 3 (100%) showed multiple drug resistance. Majority of the isolates were resistant to five drug groups 25 (41%), six drug groups 19 (31.1%) followed by three drug groups 9 (14.8%) (Table 11).

A total of 26 multidrug resistant phenotypes were exhibited by the isolated Gram-negative uropathogens with highest resistance to six antibiotics with a value of 19 (30.2%) followed by 9 antibiotics with a value of 10 (15.9%). Majority of the isolates showed multiple drug resistance phenotypes of gentamicin, pefloxacin, norfloxacin, tetracycline, ceftazidime, ceftriaxone and ampicillin with a prevalence rate of 16 (25.4%), followed by MDR phenotypes of gentamicin, pefloxacin, norfloxacin, tetracycline, ertapenem, ceftazidime, ceftriaxone, ampicillin and meropenem with a prevalence rate of 10 (15.9%) (Table 12). A total of 13 (20.3%) isolates showed complete lysis of erythrocytes on blood agar plate, 6 (6.9%) isolates showed green colouration on blood agar plate due to partial lysis of erythrocytes while 45 (70.3%) isolates showed neither complete lysis nor partial lysis after incubation. Beta-hemolytic uropathogens were more isolated among patients from Accident and Emergency (2), General Outpatient Department (2), Clinic (2) and Surgical outpatient Department (2). Alpha-hemolytic uropathogens were more isolated from Surgical Outpatient Department (2) (Table 13).

Of the 64 positive samples, 51 (79.7%) were biofilm producers while 13 (20.3%) were non-biofilm producers. Out of the 51 (79.7%) biofilm producers, 25 (49%) were strong biofilm producers while 26 (51%) were weak biofilm producers. The most predominant strong biofilm producers were *E. coli* 6 (66.7%), *Proteus mirabilis* 3 (50%) and *P. vulgaris* 6 (42.9%). Others were *Klebsiella pneumoniae* 6 (35.3%), *Pseudomonas aeruginosa* 1 (33.3%) and *K. oxytoca* 3 (20%). Majority of the strong-biofilm producers were isolated from Surgical Outpatient Department (5), Obstetrics and Gynecology Department (4), Female Medical Ward (4) and Accident and Emergency (4). Weak-biofilm producers were more prevalent among patients from Obstetrics and Gynaecology Department (6), and Surgical Outpatient Department (5) (Table 14).

Out of the 61 (95.3%) isolates that showed multidrug resistance most especially to the cephalosporins used in this study with a diameter less than 20mm for ceftriaxone and less than 18mm for ceftazidime, 20 isolates were selected for checking the ESBL production. Out of the 13 (65%) isolates selected from out-patients, 12 (92.3%) isolates showed enhanced growth towards the amoxicillin-clavulanate disc while all the 7 (35%) isolates selected from in-patients showed enhancement or increase in the zone towards the amoxicillin-clavulanate disc. Majority of the ESBL producers were discovered to be isolated from General Outpatient Department 3 (15%), Obstetrics and Gynaecology 3 (15%), Accident and Emergency 3 (15%), Female Medical Ward 3 (15%) and Male Surgical Ward 3 (15%) (Table 15).

Table 1: Percentage of patients' demographic information.

Patient demographic characteristics		Frequency (%)
Sex		
Positive samples	Male	27 (42.2%)
	Female	37 (57.8%)
	Total	64 (52.5%)
Negative samples	Male	28 (48.3%)
	Female	30 (51.7%)
	Total	58 (47.5%)
Location		
Positive samples	Outpatient	48 (75%)
	Inpatient	16 (25%)
	Total	64 (52.5%)
Negative samples	Outpatient	46 (79.3%)
	Inpatients	12 (20.7%)
	Total	58 (47.5%)
Total sample collected		122

Table 2: Age-group and frequency of patient with significant bacteruria.

Age-group Years	In-patients n (%)			Out-patients n (%)		
	Male	Female	Total	Male	Female	Total
≤10	1 (100%)	0 (0%)	1(6.3%)	0 (0%)	0 (0%)	0 (0%)
11-40	2 (25%)	6 (75%)	8 (50%)	1 (4.8%)	20 (95.2%)	21(43.8%)
41-70	1 (50%)	1 (50%)	2 (12.5%)	7 (53.8%)	6 (46.2%)	13 (27.1%)
> 70	3 (60%)	2 (40%)	5 (31.3%)	12 (85.7%)	2 (14.3%)	14 (29.2%)
Total	7 (43.75%)	9 (56.25%)	16 (25%)	20 (41.7%)	28 (58.3%)	48 (75%)

Table 3: Total number of samples from each department.

Patient type	Department	Total number of samples n (%)
Outpatient	SOPD	12 (9.8%)
	GOPD	23 (18.6%)
	A/E	16 (13.1%)
	O/G	34 (27.9%)
	Clinic	4 (3.3%)
	ANC	5 (4.1%)
Sub-total		94 (77%)
Inpatient	EPU	1 (0.8%)
	Urology	8 (6.6%)
	FMW	7 (5.7%)
	MSW	6 (4.9%)
	ARD	2 (1.6%)
	ICU	2 (1.6%)
	ORTHO	2 (1.6%)
Sub-total		28 (23%)
Total		122

Keys: SOPD= Surgical Out-patient Department GOPD= General Out-patient Department

A/E= Accident and Emergency Department O/G= Obstetrics and Gynaecology Department

ANC=Antenatal Care EPU= Emergency Paediatric Unit MSW= Male Surgical Ward FMW=

Female Surgical Ward ARD=Ascites Reinfusion Dialysis ICU= Intensive Care Unit

ORTHO= Orthopaedic Unit

Table 4: Percentage of isolated uropathogens.

Isolated Uropathogens	Frequency (%)
<i>Klebsiella pneumoniae</i>	17 (26.6%)
<i>Klebsiella oxytoca</i>	15 (23.4%)
<i>Proteus vulgaris</i>	14 (21.9%)
<i>Escherichia coli</i>	9 (14.1%)
<i>Proteus mirabilis</i>	6 (9.4%)
<i>Pseudomonas aeruginosa</i>	3 (4.7%)
Total	64 (52.5%)

Table 5: Distribution of the isolates on the basis of type and department of the patient.

Patient types	Ward	Bacterial isolates (N=64)						Total (%)
		<i>K. pne</i> 17 (26.6%)	<i>K. oxy</i> 15 (23.4%)	<i>P. vul</i> 14 (21.9%)	<i>E. col</i> 9 (14.1%)	<i>P. mir</i> 6 (9.4%)	<i>P. aer</i> 3 (4.7%)	
Outpatient	SOPD	0 (0%)	4 (26.7%)	2 (14.3%)	3 (33.3%)	1 (16.7%)	0 (0%)	10 (15.6%)
	GOPD	5 (29.4%)	0 (0%)	2 (14.3%)	0 (0%)	0 (0%)	0 (0%)	7 (10.9%)
	A/E	4 (23.5%)	0 (0%)	4 (28.6%)	0 (0%)	0 (0%)	0 (0%)	8 (12.5%)
	O/G	2 (11.8%)	5 (33.3%)	2 (14.3%)	3 (33.3%)	3 (50%)	0 (0%)	15 (23.5%)
	CLINIC	2 (11.8%)	0 (0%)	0(0%)	1(11.1%)	0 (0%)	0 (0%)	3 (4.7%)
	ANC	1 (5.9%)	2 (13.3%)	0 (0%)	0 (0%)	1 (16.7%)	1 (33.3%)	5 (7.8%)
Sub-Total		14 (29.2%)	11 (22.9%)	10 (20.8%)	7 (14.6%)	5 (10.4%)	1 (2.1%)	48 (75%)
Inpatient	EPU	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)
	UROLOGY	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	1 (33.3%)	2 (3.1%)
	MSW	2 (11.8%)	2 (13.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (6.3%)
	FMW	1 (5.9%)	2 (13.3%)	1 (7.1%)	1 (11.1%)	0 (0%)	1 (33.3%)	6 (9.4%)
	ARD	0 (0%)	0 (0%)	1(7.1%)	1 (11.1%)	0 (0%)	0 (0%)	2 (3.1%)
	ICU	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)
Sub-Total		3 (18.8%)	4 (25%)	4 (25%)	2 (12.5%)	1 (6.3%)	2 (12.5%)	16 (25%)
Total		17 (26.6%)	15 (23.4%)	14 (21.9%)	9 (14.1%)	6 (9.4%)	3 (4.7%)	64 (52.5%)

K. pne= *Klebsiella pneumoniae* *K. oxy*= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*= *Escherichia coli* *P. mir*=*Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa* SOPD= Surgical Out-patient Department GOPD= General Out-patient Department A/E= Accident and Emergency Department O/G= Obstetrics and Gynaecology Department ANC=Antenatal Care EPU= Emergency Paediatric MSW= Male Surgical Ward FMW= Female Surgical Ward ARD=Ascites Reinfusion Dialysis ICU= Intensive Care Unit

Table 6: Distribution of bacteria recovered based on demographics and age-group.

	Isolated Uropathogens N (%)						Total n (%)
	<i>K. pne</i> n (%)	<i>K. oxy</i> n (%)	<i>P. vul</i> n (%)	<i>E. col</i> n (%)	<i>P. mir</i> n (%)	<i>P. aer</i> n (%)	
Sex							
Male	8 (29.6)	5 (18.5)	6 (22.2)	5 (18.5)	2 (7.4)	1 (3.7)	27 (42.2)
Female	9 (24.3)	10 (27.0)	8 (21.6)	4 (10.8)	4 (10.8)	2 (5.4)	37 (57.8)
Age-group							
≤10	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	0 (0)	1 (1.6)
11-40	7 (10.9)	8 (12.5)	5 (7.8)	3 (4.7)	4 (6.3)	2 (3.1)	29 (45.3)
41-70	4 (6.3)	2 (3.1)	6 (9.4)	3 (4.7)	0 (0)	0 (0)	15 (23.4)
> 70	6 (9.4)	5 (7.8)	2 (3.1)	3 (4.7)	2 (3.1)	1 (1.6)	19 (29.7)

K. pne= *Klebsiella pneumoniae* *K. oxy*= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*=
Escherichia coli *P. mir*=*Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa*

Table 7: Overall antibiotic resistance patterns among the isolated uropathogens.

Antibiotics	Susceptible n (%)	Resistant n (%)
Meropenem	46 (71.9%)	18 (28.1%)
Ertapenem	38 (59.4%)	26 (40.6%)
Ceftazidime	25 (39.1%)	39 (60.9%)
Ceftriaxone	18 (28.1%)	46 (71.9%)
Ampicilin	4 (6.2%)	60 (93.8%)
Norfloxacin	20 (31.2%)	44 (68.8%)
Pefloxacin	13 (20.3%)	51 (79.7%)
Gentamicin	8 (12.5%)	56 (87.5%)
Tetracycline	14 (21.9%)	50 (78.1%)

Total number of Bacterial isolate = 64

Table 8: Antibiotic resistance patterns of the isolated uropathogens among in-patients and out-patients.

		ISOLATED UROPATHOGENS n (%)					
		<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>
		17 (26.6%)	15 (23.4%)	14 (21.9%)	9 (14.1%)	6 (9.4%)	3 (4.7%)
Antibiotic Resistance n (%)							
Meropenem	Inpatient	1 (33.3%)	2 (50%)	0 (0%)	0 (0%)	1 (100%)	1 (50%)
	Outpatient	5 (35.7%)	1 (9.1%)	4 (40%)	1 (14.3%)	2 (40%)	0 (0%)
Ertapenem	Inpatient	2 (66.7%)	2 (50%)	1 (25%)	1 (50%)	1 (100%)	2 (100%)
	Outpatient	6 (42.9%)	2 (18.2%)	5 (50%)	0 (0%)	4 (80%)	0 (0%)
Ampicillin	Inpatient	3 (100%)	4 (100%)	4 (100%)	2 (100%)	1 (100%)	2 (100%)
	Outpatient	13 (92.9%)	11 (100%)	8 (80%)	7 (100%)	4 (80%)	1 (100%)
Ceftazidime	Inpatient	3 (100%)	3 (75%)	3 (75%)	0 (0%)	1 (100%)	1 (50%)
	Outpatient	9 (64.3%)	6 (54.5%)	5 (50%)	5 (71.4%)	2 (40%)	1 (100%)
Ceftriaxone	Inpatient	2 (66.7%)	3 (75%)	3 (75%)	1 (50%)	1 (100%)	2 (100%)
	Outpatient	11 (78.6%)	8 (72.7%)	6 (60%)	4 (57.1%)	4 (80%)	1 (100%)
Norfloxacin	Inpatient	2 (66.7%)	4 (100%)	3 (75%)	2 (100%)	1 (100%)	2 (100%)
	Outpatient	9 (64.3%)	7 (63.6%)	5 (50%)	6 (85.7%)	2 (40%)	1 (100%)
Pefloxacin	Inpatient	3 (100%)	4 (100%)	4 (100%)	2 (100%)	1 (100%)	2 (100%)
	Outpatient	12 (85.7%)	11 (100%)	8 (80%)	7 (100%)	5 (100%)	1 (100%)
Gentamicin	Inpatient	3 (100%)	4 (100%)	4 (100%)	2 (100%)	1 (100%)	2 (100%)
	Outpatient	11 (78.6%)	9 (81.8%)	9 (90%)	6 (85.7%)	4 (80%)	2 (100%)
Tetracycline	Inpatient	3 (100%)	3 (75%)	3 (75%)	2 (100%)	1 (100%)	1 (50%)
	Outpatient	10 (71.4%)	10 (90.1%)	6 (60%)	7 (100%)	3 (60%)	1 (50%)

Table 9: Resistance Rate among In-patients and Out-patient to each antibiotic

Antibiotics	In-patient 16 (25%)	Out-patient 48 (75%)
Meropenem	5 (31.3%)	13 (27.1%)
Ertapenem	9 (56.3%)	17 (35.4%)
Ampicillin	16 (100%)	44 (91.7%)
Ceftazidime	11 (68.8%)	28 (58.3%)
Ceftriaxone	12 (75%)	34 (70.8%)
Norfloracin	14 (87.5%)	30 (62.5%)
Pefloxacin	16 (100%)	44 (91.7%)
Gentamicin	16 (100%)	41 (85.4%)
Tetracycline	13 (81.3%)	37 (77.1%)

Total number of In-patient=16, Total number of Out-patient=48

Table 10: Percentage of Multidrug (MDR) resistant isolates among out-patients and in-patients.

No. of Antibiotic classes	Total number n (%)	Outpatient n (%)	Inpatient n (%)
3	9 (14.8%)	9 (100%)	0 (%)
4	8 (13.1%)	5 (62.5%)	3 (37.5%)
5	25 (41%)	19 (76%)	6 (24%)
6	19 (31.1%)	12 (63.2%)	7 (36.8%)
Total	61 (95.3%)	45 (93.8%)	16 (100%)

No. =Number Total number of out-patient that showed MDR =45 Total number of in-patient that showed MDR=16

Table 11: Multidrug resistance across the isolates.

ISOLATED UROPATHOGENS N (%)							
No. of Antibiotic classes	<i>K. pne</i> 17 (26.6%)	<i>K. oxy</i> 15 (23.4%)	<i>P. vul</i> 14 (21.9%)	<i>E. col</i> 9 (14.1%)	<i>P. mir</i> 6 (9.4%)	<i>P. aer</i> 3 (4.7%)	Total (%)
3	1 (5.9%)	2 (13.3%)	3 (21.4%)	1 (11.1)	2(33.3%)	0 (0%)	9 (14.8%)
4	0 (0%)	3 (20.0%)	3 (21.4%)	2 (22.2)	0 (0%)	0 (0%)	8 (13.1%)
5	7 (41.2%)	7 (46.7%)	5 (35.7%)	4 (44.4)	0 (0%)	2(66.7%)	25 (41%)
6	7 (41.2%)	3 (20.0%)	2 (14.3%)	2(22.2%)	4(66.7%)	1(33.3%)	19 (31.1%)
Total	15 (88.2%)	15 (100%)	13 (92.9%)	9 (100%)	6 (100%)	3 (100%)	61 (95.3%)

K. pne= *Klebsiella pneumoniae* *K. oxy*= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*=
Escherichia coli *P. mir*=*Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa* No.=Number

Table 12: Multiple resistance pattern across the isolates.

No. Of Antibiotics	Multidrug resistance pattern	ISOLATED UROPATHOGENS N (%)						Total N (%)
		<i>K. pne</i> N (%)	<i>K. oxy</i> N (%)	<i>P. vul</i> N (%)	<i>E. col</i> N (%)	<i>P. mir</i> N (%)	<i>P. aer</i> N (%)	
2	Gen-Pef			1 (100)				1 (1.6)
	Pef-Amp	1 (100)						1 (1.6)
3	Gen-Pef-Amp		1 (33.3)	1 (33.3)		1 (33.3)		3 (4.8)
	Pef-Ert-Ceftx					1 (100)		1 (1.6)
	Pef-Te-Amp		1 (100)					1 (1.6)
4	Pef-Nor-Te-Amp			1 (50)	1 (50)			2 (3.2)
	Gen-Ert-Amp-Mer			1 (100)				1 (1.6)
	Nor-Ceftz-Ceftx-Mer	1 (100)						1 (1.6)
	Gen-Pef-Te-Amp		1 (100)					1 (1.6)
	Pef-Te-Ceftx-Amp		1 (100)					1 (1.6)
5	Gen-Pef-Te-Ceftx-Amp			1 (100)				1 (1.6)
	Gen-Pef-Nor-Te-Amp		1 (25)	1 (25)	2 (50)			4 (6.3)
	Gen-Pef-Ert-Ceftx-Amp	1 (100)						1 (1.6)
	Gen-Pef-Te-Ert-Amp	1 (100)						1 (1.6)
	Gen-Pef-Te-Ceftz-Amp				1 (100)			1 (1.6)
6	Gen-Te-Ert-Ceftz-Ceftx-Mer			1 (100)				1 (1.6)
	Gen-Pef-Ert-Ceftz-Ceftx-Amp			1 (100)				1 (1.6)
	Gen-Pef-Nor-Te-Ceftx-Amp	1 (100)						1 (1.6)
	Gen-Pef-Te-Ceftz-Ceftx-Amp	1 (100)						1 (1.6)
	Gen-Pef-Te-Ceftz-Amp-Mer	1 (100)						1 (1.6)
	Gen-Pef-Nor-Ert-Ceftx-Amp						1 (100)	1 (1.6)
7	Gen-Pef-Nor-Te-Ceftz-Ceftx-Amp	3 (18.8)	6 (37.5)	3 (18.8)	3 (18.8)		1 (6.3)	16 (25.4)
	Gen-Pef-Nor-Te-Ert-Ceftx-Amp		1 (33.3)		1 (33.3)	1 (33.3)		3 (4.8)
8	Gen-Pef-Nor-Ert-Ceftz-Ceftx-Amp-Mer		1 (50)	1 (50)				2 (3.2)
	Gen-Pef-Te-Ert-Ceftz-Ceftx-Amp-Mer					1 (100)		1 (1.6)
	Gen-Pef-Nor-Te-Ert-Ceftz-Ceftx-Amp				1 (100)			1 (1.6)
	Gen-Pef-Nor-Te-Ert-Ceftz-Ceftx-Amp	2 (66.7)		1 (33.3)				3 (4.8)
9	Gen-Pef-Nor-Te-Ert-Ceftz-Ceftx-Amp-Mer	4 (40)	2 (20)	1 (10)		2 (20)	1 (10)	10 (15.9)
Total								63

Keys: Gen=Gentamicin Pef=Pefloxacin Nor=Norfloxacin Te=Tetracycline Ert=Ertapenem
 Ceftz=Ceftazidime Ceftx=Ceftriaxone Amp=Ampicilin Mer=Meropenem *K. pne*= *Klebsiella pneumoniae*
K. oxy= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*= *Escherichia coli* *P. mir*= *Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa*

Table 13: Percentage of Hemolysin production among the isolated uropathogens.

ISOLATED UROPATHOGENS N (%)

Types of Hemolysis	<i>K. pne</i> 17 (26.6%)	<i>K. oxy</i> 15 (23.4%)	<i>P. vul</i> 14 (21.9%)	<i>E. col</i> 9 (14.1%)	<i>P. mir</i> 6 (9.4%)	<i>P. aer</i> 3 (4.7%)	Total (%)
Beta-Hemolysis	3 (17.7%)	3 (20%)	4 (28.6%)	2 (22.2%)	1 (16.7%)	0 (0%)	13 (20.3%)
Gamma-Hemolysis	11 (64.7%)	12 (80%)	9 (64.3%)	7 (77.8%)	3 (50%)	3 (100%)	45 (70.3%)
Total	17 (26.6%)	15 (23.4%)	14 (21.9%)	9 (14.1%)	6 (9.4%)	3 (4.7%)	64 (52.5%)

K. pne= *Klebsiella pneumoniae* *K. oxy*= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*=
Escherichia coli *P. mir*=*Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa*

Table 14: Percentage of biofilm production among the isolated uropathogens.

Biofilm Production	ISOLATED UROPATHOGENS N (%)						Total (%)
	<i>K. pne</i>	<i>K. oxy</i>	<i>P. vul</i>	<i>E. col</i>	<i>P. mir</i>	<i>P. aer</i>	
Biofilm Production	17 (26.6%)	15 (23.4%)	14 (21.9%)	9 (14.1%)	6 (9.4%)	3 (4.7%)	
Strong Biofilm P.	6 (35.3%)	3 (20%)	6 (42.9%)	6 (66.7%)	3 (50%)	1 (33.3%)	25 (39.1%)
Weak Biofilm P.	8 (47.1%)	8 (53.3%)	4 (28.6%)	2 (22.2%)	2 (33.3%)	2 (66.7%)	26 (40.6%)
Non-Biofilm P.	3 (17.7%)	4 (26.7%)	4 (28.6%)	1 (11.1%)	1 (16.7%)	0 (0%)	13 (20.3%)
Total	17	15	14	9	6	3	64

K. pne= *Klebsiella pneumoniae* *K. oxy*= *Klebsiella oxytoca* *P. vul*= *Proteus vulgaris* *E. col*=
Escherichia coli *P. mir*=*Proteus mirabilis* *P. aer*= *Pseudomonas aeruginosa* P= Producer

Table 15: ESBL production by some selected isolates across wards.

Patient type	Ward	Tested Uropathogens (N=20)				Total n (%)
		<i>K. p</i> n (%)	<i>K. o</i> n (%)	<i>P. v</i> n (%)	<i>E. c</i> n (%)	
Outpatient						
	SOPD	0 (0%)	1 (5%)	0 (0%)	1 (5%)	2 (10%)
	GOPD	1 (5%)	0 (0%)	2 (10%)	0 (0%)	3 (15%)
	O/G	0 (0%)	2 (10%)	0 (0%)	1 (5%)	3 (15%)
	A/E	2 (10%)	0 (0%)	1 (5%)	0 (0%)	3 (15%)
	Clinic	1 (5%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)
Inpatient						
	FMW	0 (0%)	1 (5%)	1 (5%)	1 (5%)	3 (15%)
	MSW	1 (5%)	2 (10%)	0 (0%)	0 (0%)	3 (15%)
	ICU	0 (0%)	0 (0%)	1 (5%)	0 (0%)	1 (5%)

K. p= *Klebsiella pneumoniae* *K. o*= *Klebsiella oxytoca* *P. v*= *Proteus vulgaris* *E. c*= *Escherichia coli* *P. m*=*Proteus mirabilis* *P. a*= *Pseudomonas aeruginosa* SOPD= Surgical Out-patient Department GOPD= General Out-patient Department A/E= Accident and Emergency Department O/G= Obstetrics and Gynaecology Department MSW= Male Surgical Ward FMW= Female Surgical Ward ICU= Intensive Care Unit

CHAPTER FIVE

5.1 DISCUSSION

Urinary tract infection is the most widespread infectious disease after respiratory tract infection in most communities (Sohail *et al.*, 2015). It is regarded as a major public health problem due to increased costs with an estimated 150 million cases per annum worldwide (Arjuman *et al.*, 2010). In this study, urine samples were collected aseptically from 122 patients referred to microbiology laboratory for urine culture and sensitivity. These samples were cultured on cysteine lactose electrolyte deficient agar (CLED), MacConkey agar and Eosin methylene agar simultaneously and the Gram-negative uropathogens were enumerated on CLED agar. This study is sought to ascertain the extent to which Gram-negative uropathogen isolates are susceptible to various classes of antibiotics, thereby forestalling the problem of antibiotic resistance among patients in hospitals and in the community, since antibiotic misuse in developing countries is the major reason for the development of high resistance rates in bacterial isolates.

A total of 122 urine samples from patients suspected of UTI were analyzed, 55 (45.1%) urine samples were from males while 67 (54.9%) were from females. Sixty-four (52.5%) urine samples were positive for Gram-negative uropathogens with significant bacteriuria. This prevalence rate 64 (52.5%) is comparable to UTI prevalence rates reported by various authors in India 53.5% (Prakash and Saxena, 2013) and Cameroon 59.8% (Nzaline *et al.*, 2016). However the prevalence rate from this study is higher than the prevalence rates reported in previous studies which account for 4.2% (Bigwan and Eliyah, 2013), 11% (Ibeneme *et al.*, 2011), 17.19% (Akram *et al.*, 2003) and 29.3% (Ochada *et al.*, 2014). The differences in the prevalence rate in different studies may be explained by differences in methodology used, the environment and education (Yeshwondm, 2016). The frequency rate of UTI in female samples 37 (57.8%) was higher than that of males 27 (42.2%). This finding corroborates with the studies carried out by Agbagwa and Ifeanacho (2015) in Rivers State, Nigeria, Ibadin (2006) and Okonko (2009). This also correlates with other studies by Swetha (2014), Kibret and Abera (2004), where UTI was higher in females compared to males. The high prevalence of UTI in females could be attributed to the physiological and anatomical differences in males and females (Agbagwa

and Ifeanacho, 2015). In line with the anatomical differences, females' urethra and vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up the urethra into the bladder during pregnancy and child birth (Swetha *et al.*, 2014; Nicolle, 2008; Hooton and Stamm, 1997). Females having a higher rate of UTI could also be attributed to menopause and the use of contraceptives. Males have a drier environment in the urethra which reduces microbial growth and longer distance between the anus and urethra meatus (Kibret and Abera, 2014; Prakasam *et al.*, 2012; Anthony, 2011; Okonko *et al.*, 2010) contributes to the reduction of UTI in males. Reduced rate of UTI in males could also be attributed to longer length of urethra in males where the uropathogen would have to travel longer distance before they reach the bladder and during which the uropathogen may be flushed out of the urethra during urination.

The highest rate of infection was recorded in subjects between the age group (11-40) with a frequency rate of 29 (45.3%) and the least rate of infection was recorded in subjects between the age group (≤ 10) with a frequency rate of 1 (1.6%). There was higher frequency rate of UTI among females between age bracket 11-40 years in both in-patients and out-patients as compared to males while higher frequency rate of UTI was recorded among males above 70 years as compared to females. These findings are in accordance with the findings from the studies carried out by Agbagwa and Ifeanacho (2013) in Rivers State, Nigeria, Iregbu and Nwajiobi-Princewill (2013) in Abuja, Nigeria. Similar findings were reported by Chedi *et al.*, (2009) in Kano, Nigeria where female patients between 21-30 years and males > 60 years had highest frequency rate. In addition, Devi and Rajkumar (2012) reported highest rate of infection among female patients between the age group 15-40 years in their study. The high frequency of UTI between the ages of 11-40 maybe due to the relatively higher sexual activity that is observed in the age group and multiple sex partners. These findings are in contrast with the study carried out by Krieger *et al.* (1993), Ani and Mgbечи (2008), and Nicolle (2011) where the prevalence of UTI increases with increasing age for both sexes. The high frequency rate observed in the present study in males above 70 years could be attributed to the presence of a number of risk factors that arise due to age advancement. Such factors include prostatic enlargement found in males, diabetes mellitus, interventional instrumentations like catheterization and weak bladder sphincter (Moore *et al.*, 2002).

Urine specimens were mostly obtained from outpatients 94 (77%) as compared to inpatients 28 (23%), meaning that most cases were coming in directly from the community. This is in agreement with the study carried out by Iregbu and Nwajiobi-Princewill (2013) in Abuja, Nigeria and the studies from Botswana and the United States (Renuart, 2013; Doyle *et al.*, 2001). The occurrence of UTI and isolated pathogens with respect to the age group and sex shows that *Klebsiella* spp. was the highest occurring UTI pathogen in both male and female (13 and 19 respectively) followed by *Proteus* spp. with a frequency rate of 8 (40%) and 12 (60%) and *E. coli* with a frequency rate of 5 (55.6%) and 4 (44.4%) among males and females respectively. The pathogen and sex specific prevalence of the UTI's shows that *Klebsiella* spp. had the highest frequency of 50%, followed by *Proteus* sp (31.3%) and *E. coli* (14.1%) while the least occurring uropathogen was *Pseudomonas aeruginosa* (4.7%). This is comparable with a finding from the study carried out by Agbagwa and Ifeancha (2015) in Rivers State, Nigeria, where *Klebsiella* spp. was the most isolated Gram-negative uropathogen with a frequency rate of 30.9% followed by *E. coli* with a frequency rate of 22.6%. However, this contradicts the reports in other studies (Okonko *et al.*, 2009; Dielubanza *et al.*, 2011; Salwa *et al.*, 2014) where it was reported that *E. coli* is the predominant organism. This finding is also in contradiction to the finding from the study carried out by Ehinmidu (2003) in Zaria, Nigeria who recorded *Pseudomonas aeruginosa* isolates as the most predominant bacteria with a frequency rate of 53.4%. The higher frequency rate of *Proteus* spp. as compared to *E. coli* in this study may be attributed to the presence of *Proteus* spp. as normal flora of gastrointestinal tract of humans, although the predominant resident is *E. coli*. *Proteus* spp. were mostly isolated from females and it may be attributed to the ability of the organisms to leave the gastrointestinal tract using their virulence factors such as flagella and contaminate faeces which can further contaminate the urethra of females when the anus is wiped from back to front. While in males, higher frequency of *Proteus* spp. as compared to *E. coli* may be attributed to factors such as indwelling catheter and urinary tract obstruction.

The disparity observed in the frequency of pathogen isolated from urine specimen by various researchers could be due to antibiotics taken by patients before visiting the hospital due to persistence of UTI, which can

affect the frequency of pathogens isolated (Kolawole *et al.*, 2009; Moore *et al.*, 2002; Ekrem *et al.*, 2015). The similarities and differences in the type and distribution of uropathogens in the current study and other studies conducted thus far in different countries may be due to source of the specimens, patients involved and the various environmental conditions and host factors. Standard hygiene practices, education, population and the socioeconomic standards of the different sources of specimen collected may be responsible for the differences observed in uropathogens from the different studies (Ani and Mgbechi, 2008).

Of the total 122 urine specimens collected from both outpatients and inpatients, 94 (77%) urine specimens were obtained from outpatient departments while 28 (23%) urine specimens were obtained from inpatient departments. Of these 122 urine specimens, 64 (52.2%) yielded Gram-negative uropathogens while 58 (47.5%) yielded no Gram-negative uropathogen and were considered negative cultures. Similarly, urine specimens from outpatient departments yielded the highest uropathogens 48 (75%) as compared to urine specimens from inpatient departments 16 (25%). Most of the urine specimens were obtained from Obstetrics and Gynaecology department 34 (27.9%), General Outpatient department 23 (18.6%), Accident and Emergency 16 (13.1%), Surgical Outpatient department 12 (9.8%), Urology 8 (6.6%), Female Medical ward 7 (5.7%), and Male Surgical ward 6 (4.9%). However, urine specimens from Obstetrics and Gynaecology department 15 (23.5%), Surgical Outpatient department 10 (15.6%), Accident and Emergency 8 (12.5%), General Outpatient department 7 (10.9%) and Female Medical ward 6 (9.4%) yielded the highest uropathogen. This corroborates with the findings from the study carried out by Devi and Rajkumar (2012) where it was reported that the risk of UTI is most prevalent among patients with gynaecological problems.

The observed higher degree of resistance among inpatients as compared to outpatients in the present study corroborates with the findings from previous studies which reported higher resistance rate among hospitalized patients as compared to out-patients (Khamenah and Afshar, 2009; Biswas *et al.*, 2006). However, it contradicts the findings of Inwadioha *et al.* (2010) who reported that the degree of resistance among inpatients was almost similar to that of out-patients. This made Inwadioha *et al.* (2010) to state that the drug resistant strains have spread in the community. In the present study, majority of the isolates showed resistance

to commonly employed drugs in the treatment of UTIs. However, meropenem was broadly the most sensitive drug, followed by ertapenem and ceftazidime, and these are not drugs often deployed as first line of treatment of uncomplicated UTI. This is in agreement with the study carried out by Iregbu and Nwajiobi-Princewill (2013) which reported similar antibiotic resistance patterns. However, this contradicts the findings in various studies from different part of the world and in different parts of the same country where resistant rates reported were different (Ehinmidu, 2003; Raza *et al.*, 2011; Ghorbani *et al.*, 2012). It is therefore paramount that emphasis should be laid on local resistance patterns as these have the greatest impact on care (Iregbu and Nwajiobi-Princewill, 2013). The differences in susceptibility rates may be attributed to the use of antibiotics prior to urine culture and indiscriminate use of antibiotics enhances the development of resistance.

Multidrug resistance (MDR= resistance in > 2 drug groups) was observed in 95.3% of the isolated bacterial uropathogens in this study. This is comparable with the findings from the study carried out by Yeshwondm (2016) which also reported a high MDR prevalence rate (77.6%). However, the MDR prevalence rate reported in this study was higher than the prevalence rate reported in the study carried out in Gondar (59.8%) (Yismaw *et al.*, 2012). Findings from this study showed a high prevalence of MDR UTIs in both inpatients 16 (100%) and outpatients 45 (93.8%). Similar result was reported in the study carried out by Pankaj *et al.* (2012) in which MDR UTIs in inpatients (85%) was higher than that of outpatients (36.7%). In this study, majority of the isolated uropathogens showed multiple antibiotic resistance to five 25 (41%) and six 19 (31.1%) drug groups. This contradicts the findings from the study carried out by Yeshwondm (2016) where it was reported that majority of the isolated uropathogens showed multiple drug resistance to two drug 26 (30.6%) and three 17 (20%) drug groups. In this study, all the *Pseudomonas aeruginosa* isolates 3 (100%) showed multidrug resistance (MDR) to five and six drug groups, followed by *Klebsiella* spp. with MDR rate of 24 (80%); *E. coli* with MDR rate of 6 (66.7%) and *Proteus* spp. with MDR rate of 11 (57.9%) to five and six drug groups. Yeshwondm (2016) also reported a high MDR rate (66.7%) for *Pseudomonas aeruginosa*, 42.9% MDR rate for *Klebsiella* spp. to 5 and ≥ 6 drug groups, however, *E. coli* isolates showed 9.5% MDR rate and *Proteus* spp. showed 12.5% to 5 and 5 and ≥ 6 drug groups. These variations in MDR rates could be attributed to the

intensity of indiscriminate use of antibiotics by patients in our area compared to other geographical locations. Findings from this study showed that the most prevalent MDR pattern shown by most of isolated bacterial uropathogens, including *E. coli* includes resistance to: ampicillin, pefloxacin, norfloxacin, ceftazidime, ceftriaxone, tetracycline and gentamicin. This is comparable to the findings from the study carried out by Pankaj *et al.* (2012) who reported that the most prevalent MDR pattern shown by *E. coli* isolates in their study includes resistance to: amoxicillin, ciprofloxacin, cephotaxime, cefixime, co-trimoxazole, ofloxacin, norfloxacin, ceftazidime, gentamicin and ceftriaxone.

Findings from this study showed that 13 (20.3%) isolated bacterial uropathogens were beta-hemolysin producing uropathogens, 6 (9.4%) uropathogens were alpha-hemolytic organisms and 45 (70.3%) were non-hemolytic organisms. It was also discovered that 8 (40%) *Proteus* spp., 9 (28.1%) *Klebsiella* spp., and 2 (22.2%) *E. coli* isolates were hemolysin-producing organisms while none of the *Pseudomonas aeruginosa* isolates produced hemolysin. The prevalence rate of hemolysin production 19 (29.7%) in this study is almost similar to the prevalence rate reported in the study carried out by Delcaru *et al.* (2017) (27%) and Johnson *et al.* (1991) (38%). However, the prevalence rate is higher than the prevalence rate 14 (13.33%) reported by Vaishali *et al.* (2015).

In this study, it was discovered that 51 (79.7%) isolated bacterial uropathogens were biofilm producers. Twenty-five (39.1%) uropathogens were strong biofilm producers, twenty-six (40.6%) uropathogens were weak biofilm producers and thirteen (20.3%) uropathogens were non-biofilm producers. The most predominant biofilm producers in this study were *Pseudomonas aeruginosa* isolates 3 (100%), followed by *E. coli* 8 (88.9%), *Klebsiella* sp. 28 (87.5%) and *Proteus* sp. 15 (75%). The prevalence rate of biofilm production among the isolates in this study corroborates with the findings of Niveditha *et al.* (2012), Reid *et al.* (1992), and Ponnusamy *et al.* (2012), which reported a prevalence rate greater than 60% (>60%). However, it is higher than the prevalence rate 58 (37.2%) reported by Alves *et al.* (2014).

The prevalence rate of ESBL production among the selected bacterial isolates was 19 (95%). This prevalence rate is comparable to the prevalence rate (90%) reported in the study carried out by Fattahi *et al.* (2017). All the isolates selected from inpatients for checking ESBL production produced extended spectrum beta-lactamases (ESBL) 7 (100%) while 12 (92.3%) out of 13 isolates selected from outpatients produced ESBL. These prevalence rates among inpatients and outpatients could be ascribed to the indiscriminate use of cephalosporins by the patients. Majority of the ESBL producers were discovered to be isolated from General Outpatient Department 3 (15%), Obstetrics and Gynaecology 3 (15%), Accident and Emergency 3 (15%), Female Medical Ward 3 (15%) and Male Surgical Ward 3 (15%).

5.2 CONCLUSION AND RECOMMENDATION

Klebsiella spp. was the most common cause of UTI among the study population in the present study. Moreover, from the results in the present study, it is obvious that choosing a particular drug for empirical treatment will be challenging as the two antibiotics that showed highest susceptibility are not often deployed for empirical treatment of uncomplicated UTI. These antibiotics are usually deployed when the first line and second line of treatment of UTI failed. Consequently, the treatment of UTIs among the study population in this study should involve antibiotic prescription only after urine culture and sensitivity is conducted by medical personnel. The public should also be educated on the consequences of indiscriminate use of antibiotics. All these will go a long way in curbing the emergence of antibiotic resistance.

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APPENDIX

Preparing Reagents and Culture Media

Blood agar.....No. 1

Contents: Peptic digest of animal tissue (5g/l), beef extract (1.5g/l), yeast extract (1.5g/l), sodium chloride (5g/l), agar (15g/l), Sheep Blood (5%)

pH of medium: 7.4 ± 0.2 at 25°C

Nutrient agar powder was weighed in measure of 28g and dissolved into 1000ml of distilled water. The preparation was properly homogenized on the hot plate magnetic stirrer and thereafter autoclaved at 15 lbs pressure (121°C) for 15 minutes. The sterilized agar was allowed to cool to 45°C-50°C and 5% of sheep blood was added.

Congo red agar..... No. 2

Contents: Congo red powder (0.8g/l), Mueller Hinton agar (38g/l), sucrose (50g/l)

Congo red powder was prepared as concentrated aqueous solution and sterilized in the autoclave at 121°C for 15min under 15 psi pressure. MHA powder and sucrose powder were weighed and dissolved appropriately in distilled water according to manufacturers' specifications; medium was thereafter autoclaved at 121°C for 15min under 15psi pressure. Prepared aqueous congo red solution was poured into the sterile medium at 55°C.

Cystine Lactose Electrolyte Deficient Agar (CLED Agar)..... No. 3

Contents: Peptone (4g/l), Trypsic peptone (4g/l), Meat extract (3g/l), Lactose (10g/l), L-Cystine (0.128g/l), Bromothymol blue (0.02g/l), Agar (15g/l).

pH of medium: 7.4 ± 0.2 at 25°C

CLED agar powder was weighed in measure of 36g and dissolved into 1000ml of distilled water. The preparation was homogenized and thereafter autoclaved at 15 lbs (121°C) for 15 minutes. After allowing the sterilized medium to cool (45°C), it was then dispensed aseptically into sterile petri dishes in the required amounts (15ml). Plates were thereafter left to allow proper gelling of the medium.

Eosin Methylene Blue Agar (EMB agar)..... No. 4

Contents: Peptone (10g/l), Lactose (5g/l), Sucrose (5g/l), Dipotassium hydrogen phosphate (2g/l), Eosin Y (0.4g/l), Methylene blue (0.065g/l), Agar (13.5g/l).

pH of medium: 7.2 ± 0.2 at 25°C

EMB agar powder was weighed in measure of 35.96g and dissolved into 1000ml of distilled water. The preparation was homogenized and thereafter autoclaved at 15 lbs pressure (121°C) for 15 minutes. After allowing the sterilized medium to cool (45°C), it was then dispensed aseptically into sterile petri dishes in the required amounts (15ml). Plates were thereafter left to allow proper gelling of the medium.

Kovac's Reagent.....No. 5

Components: p-dimethylamino benzaldehyde (5g/l), Amyl alcohol (75g/l), Hydrochloric acid, concentrated (25g/l)

MacConkey agar..... No. 6

Contents: Peptones (meat and casein) (3g/l), Pancreatic digest of gelatin (17g/l), Lactose monohydrate (10g/l), Bile salts (1.5g/l), Sodium chloride (5g/l), Crystal violet (0.001g/l), Neutral red (0.03g/l), Agar (13.5g/l).

pH of medium: 7.1 ± 0.2 at 25°C

MacConkey agar powder was weighed in measure of 49.53g and dissolved into 1000ml of distilled water. The preparation was homogenized and thereafter autoclaved at 15 lbs pressure (121°C) for 15 minutes. After allowing the sterilized medium to cool (45°C), it was then dispensed aseptically into sterile petri dishes in the required amounts (15ml). Plates were thereafter left to allow proper gelling of the medium.

Methyl red Reagent.....No. 7

Components: Methyl red (0.2g/l), Ethyl alcohol (60ml), Distilled water (40ml)

Mueller Hinton agar..... No. 8

Contents: Beef (300g/l), infusion from casein acid hydrolysate (17g/l), starch (1.5g/l), agar (17g/l).

pH of medium: 7.3 ± 0.1 at 25°C

Mueller Hinton agar powder was weighed in measure of 38g and dissolved into 1000ml of distilled water. The preparation was homogenized and thereafter autoclaved at 15 lbs pressure (121°C) for 15 minutes. After allowing the sterilized medium to cool (45°C), it was then dispensed aseptically into sterile petri dishes in the required amounts (15ml).. Plates were thereafter left to allow proper gelling of the medium.

MR-VP broth..... No. 9

Contents: Buffered peptone (7g/l), Dextrose (5g/l), Dipotassium phosphate (5g/l).

pH of medium: 6.9 ± 0.2 at 25°C

MR-VP broth powder was weighed in measure of 17g and dissolved into 1000ml of distilled water. The preparation was measured appropriately (10ml) and dispensed into test tubes and thereafter autoclaved at 15 lbs pressure (121°C) for 15minutes.

Nutrient agar..... No. 10

Contents: Peptic digest of animal tissue (5g/l), beef extract (1.5g/l), yeast extract (1.5g/l), sodium chloride (5g/l), agar (15g/l).

pH of medium: 7.4 ± 0.2 at 25°C

Nutrient agar powder was weighed in measure of 28g and dissolved into 1000ml of distilled water. The preparation was properly homogenized on the hot plate magnetic stirrer and thereafter autoclaved at 15 lbs pressure (121°C) for 15 minutes. Sterilized agar was made to cool (45°C) and dispensed aseptically into sterile petri dishes in the required amounts (15ml). Plates were thereafter left to allow proper gelling of the medium. To prepare nutrient agar slopes, appropriate measurement (10ml) and dispensation into Bijou bottles was done immediately after homogenization right before sterilization; bottles containing N.A were slanted immediately after sterilization and allowed to gel while forming slopes.

Simmon's Citrate agar..... No. 11

Contents: Sodium Chloride (5g/l), Sodium Citrate (dehydrate) (2g/l), Ammonium Dihydrogen Phosphate (1g/l), Dipotassium Phosphate (1g/l), Magnesium Sulfate (heptahydrate) (0.2g/l), Bromothymol blue (0.08g/l), Agar (15g/l).

pH of medium: 6.9 ± 0.2 at 25°C

To prepare Simmon's citrate agar slope, Simmon's citrate agar powder was weighed in measure of 24.28g into 1000ml of distilled water. Appropriate measurement (5ml) and dispensation into sterile test tubes was done immediately after homogenization before sterilization; test tubes containing Simmon's citrate agar were slanted immediately after sterilization and allowed to solidify while forming slopes.

Tryptone soy broth..... No. 12

Contents: Pancreatic digest of casein (17g/l), enzymatic digest of soya bean (3g/l), sodium chloride (5g/l), di-potassium hydrogen phosphate (2.5g/l), glucose (2.5g/l).

pH of medium: 7.3 ± 0.2 at 25°C

Tryptone soy broth powder was weighed in measure of 30g and dissolved into 1000ml of distilled water. The preparation was measured appropriately (10ml) and dispensed into test tubes and thereafter autoclaved at 15 lbs pressure (121°C) for 15minutes.

Urea agar..... No. 13

Contents: Peptone (1g/l), Dextrose (Glucose) (1g/l), Sodium chloride (5g/l), Disodium phosphate (1.2g/l), Monopotassium phosphate (0.8g/l), Phenol red (0.012g/l), Agar (15g/l).

pH of medium: 6.8 ± 0.2 at 25°C

To prepare urea agar slope, urea agar powder was weighed in measure of 24.01g into 950ml of distilled water. Appropriate measurement (5ml) and dispensation into sterile test tubes was done immediately after homogenization before sterilization; test tubes containing urea agar were slanted immediately after sterilization and allowed to solidify while forming slopes.

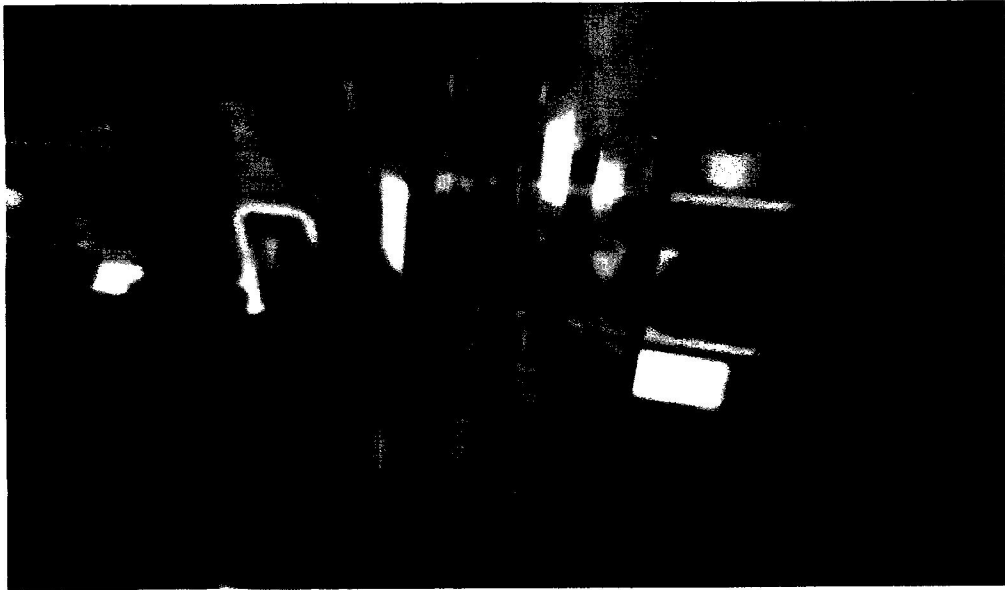


Fig., 4: Test tubes showing positive result

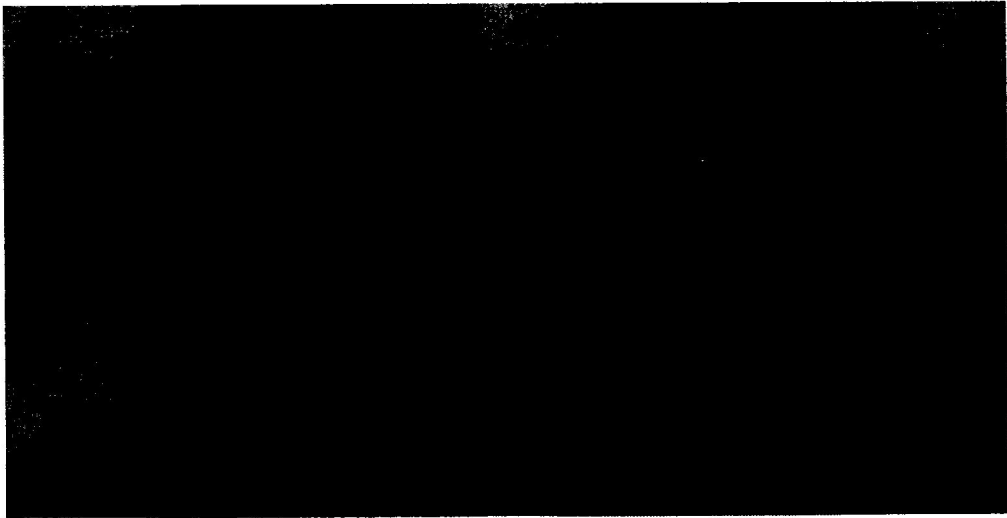


Fig., 5: Test tubes showing negative and positive result

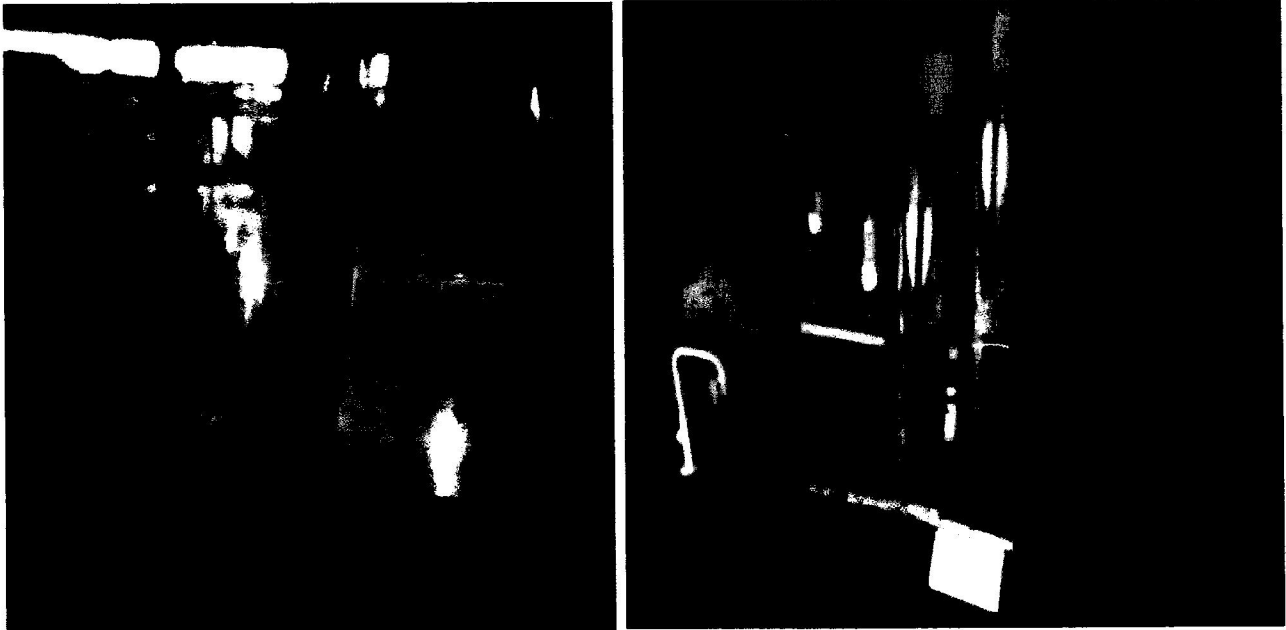


Fig., 6: Test tubes showing positive and negative results

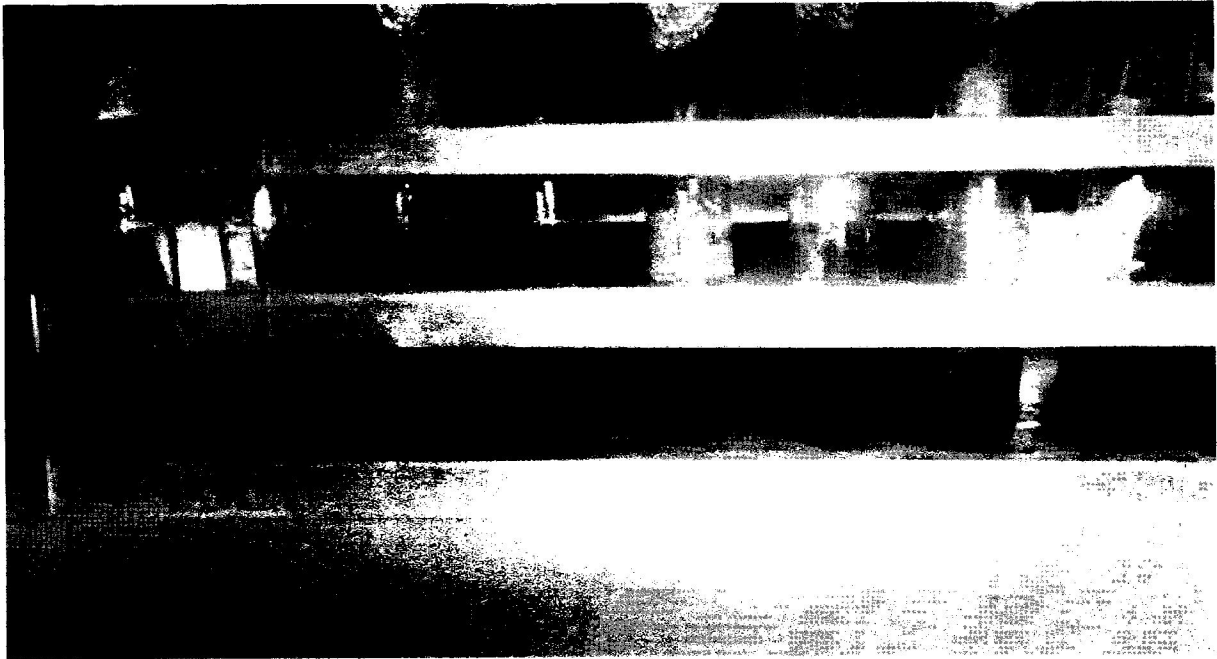


Fig., 7: Test tubes showing both positive and negative results for citrate utilization test

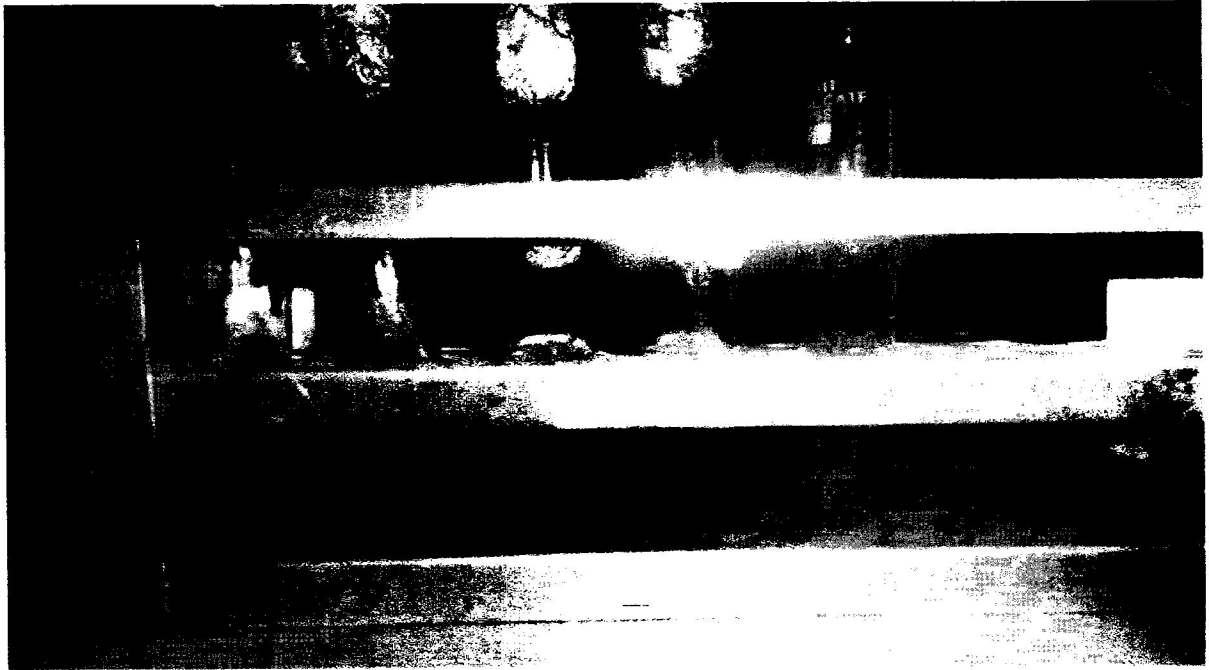


Fig., 8: Test tubes showing both positive and negative results

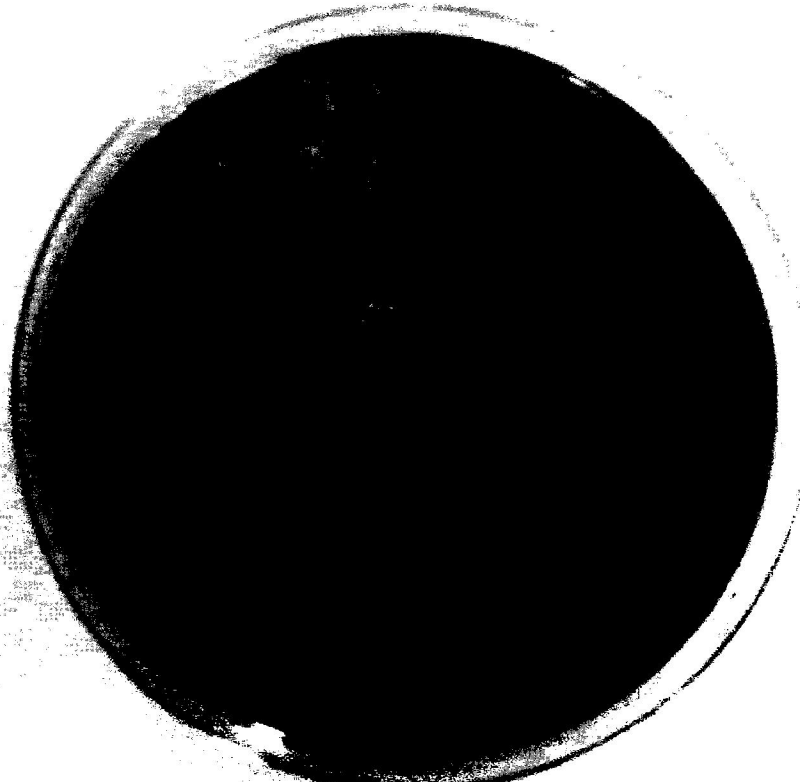


Fig., 9: Agar plate showing biofilm formation by the isolated Gram-negative uropathogen

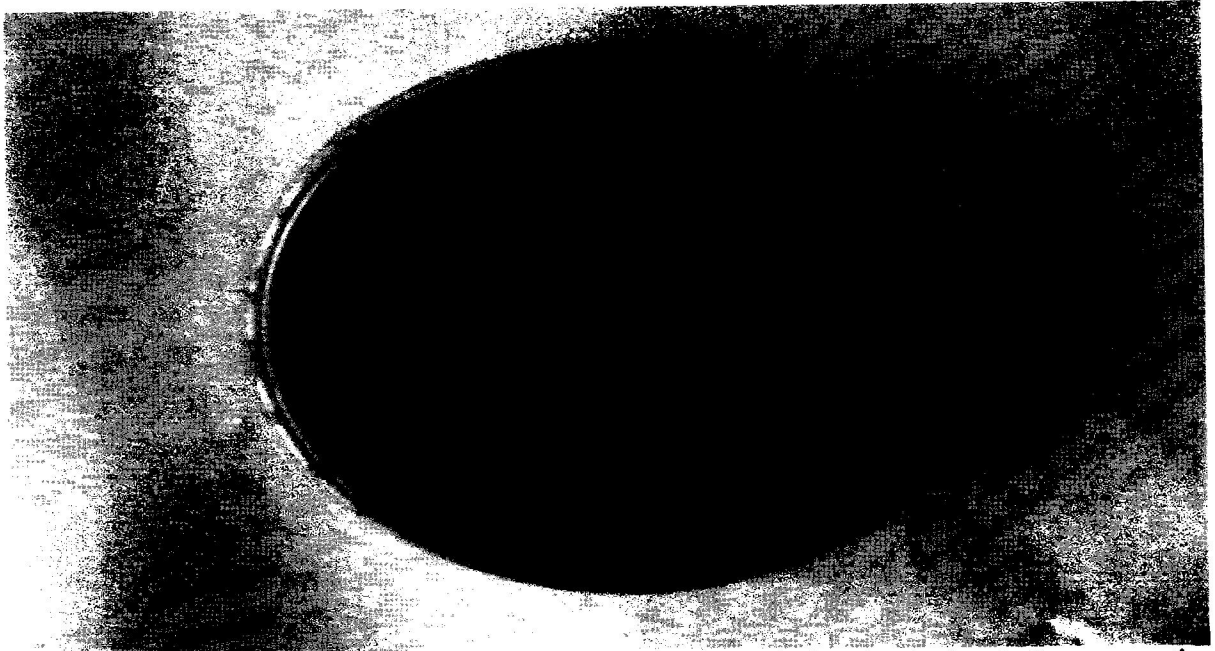


Fig., 10: Agar plate showing hamolysis of red blood cells by the isolated Gram-negative uropathogen

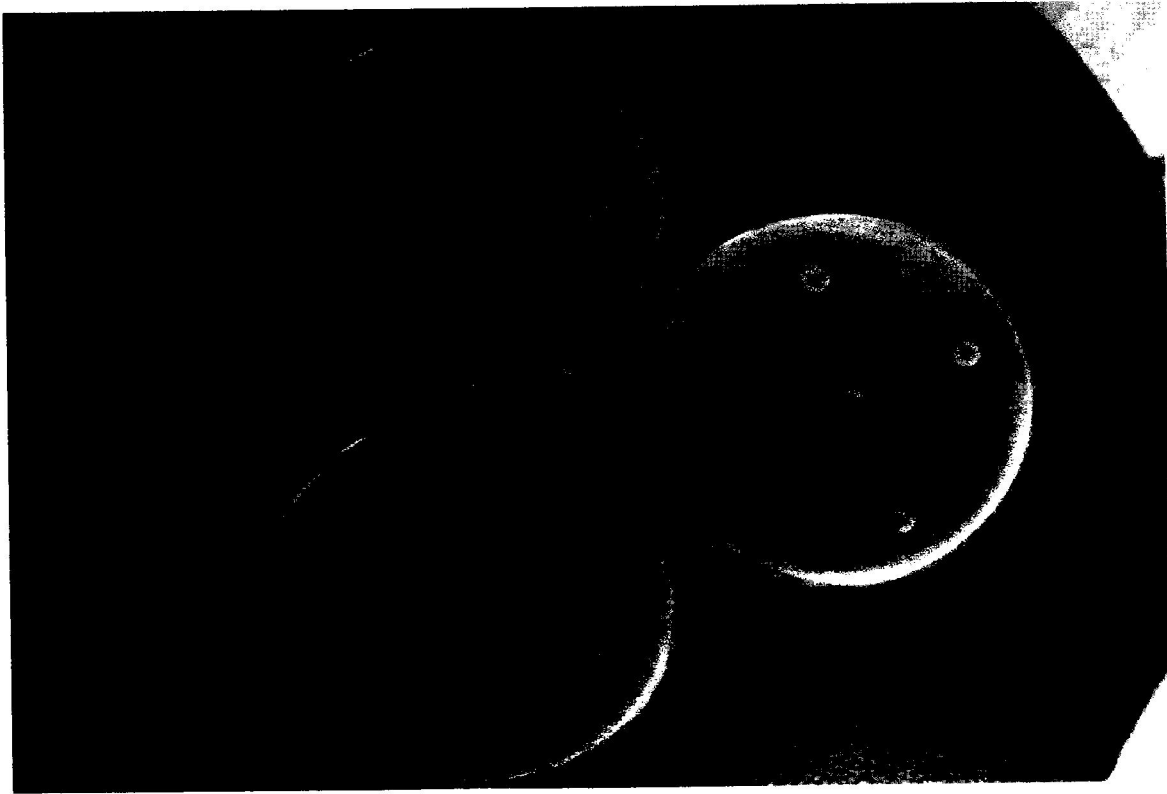


Fig., 11: Agar plate showing the susceptibility of Gram-negative uropathogen to antibiotics

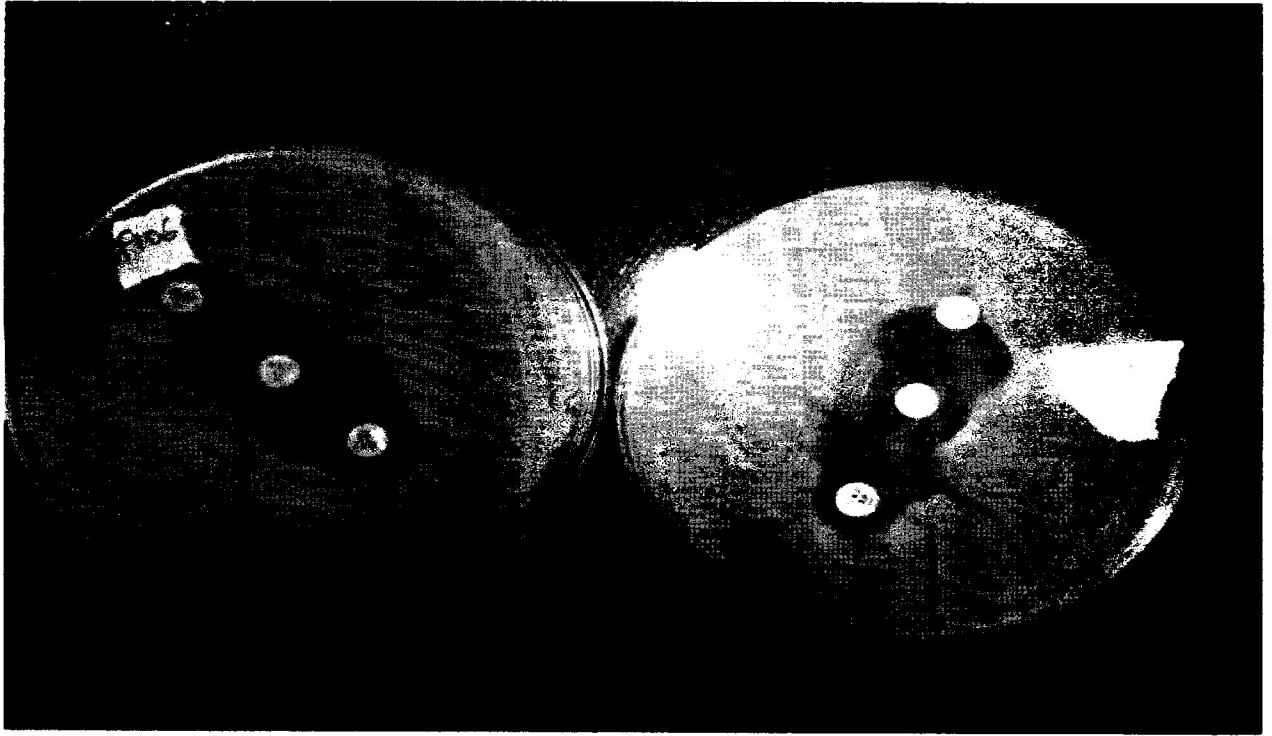


Fig., 12: Agar plate showing ESBL formation by the isolated Gram-negative uropathogen