ANTIBIOTICS RESISTANCE PATTERNS OF SOME COMMON GRAM-NEGATIVE UROPATHOGENS AMONG INPATIENTS AND OUTPATIENTS IN EKITI STATE UNIVERSITY TEACHING HOSPITAL ADO-EKITI, EKITI STATE.

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CERTIFICATION

This is to certify that this project work titled; Antibiotics resistance patterns of some **common** Gram-negative uropathogens among inpatients and outpatients in Ekiti **State** University Teaching Hospital Ado-Ekiti, Ekiti State was written and carried out **by OWOLABI, OLAJUMOKE VICTORIA** with matric number **MCB/14/2335**, a student of the Department of Microbiology, Faculty of science, Federal university Oye-Ekiti, Ekiti state, Nigeria.

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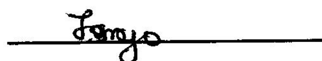

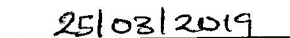

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DEDICATION

This research work is dedicated to God Almighty, who has been my strength and helped me through this work.

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I would like to express my special thanks and gratitude to the glorious God who gave me the golden opportunity for the completion of my program.

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ABSTRACT

Urinary tract infection (UTI) is one of the most common diseases in human societies which occur in women more than men. The aim of this study was to determine the antibiotics resistance pattern of some common Gram-negative uropathogens among inpatients and outpatients The UTI occurrence depends on several factors such as anatomy, sexual activities, catheterization, e.t.c. Consecutive clean-catch mid-stream urine samples were collected from 120 in and out-patients. Identification of the organisms was done and susceptibility testing using the Kirby Bauer's disc diffusion method.Out of the 120 urine samples collected, 46 were positive for significant bacteruria. Twenty-four uropathogen were isolated from females and twenty-two from males among which 34 were from outpatients and 12 from inpatients. Seventeen of the uropathogens were *Klebsiella pneumoniae,* 15(32.6%) were *E.coli,* 12(26.1) were *Proteus mirabilis* and 2(4.3%) were *Klebsiella oxytoca.* Antibiotics resistance was higher in ampicillin 44(95.7%) and pefloxacin 45(97.8%). Antibiotics prescription should be given after urine culture and sensitivity has been carried out and also people should be enlightened on the risks factors and means of prevention.

CHAPTER ONE

1.1 INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection (Rahimkhani *et al.*, 2008; Okonko *et al.*, 2009). In contrast to men, women are more susceptible to UTI, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with fecal flora (Haider *et al.*, 2010). A urinary tract infection (UTIs) is the one of the most common bacterial infection in women than in men, at a ratio of 8:1 and a major cause of morbidity.

UTI is mostly caused by gram negative aerobic bacilli found in gastro-intestinal tract. Included in this family are the *Escherichia coli (E.coli)*, *Klebsilla*, *Enterobacter*, *Citrobacter*, *Proteus* and *Serratia* species. Other common pathogens include *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Enterococcus Species*. *Escherichia coli* remains the predominant uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by *Staphylococcus saprophyticus*, *Klebsiella*, *Enterobacter*, and *Proteus* species (Jacoby, 2005)

1.2 Objective of study

The objective of this study is to isolate bacterial pathogens causing UTI and determine antimicrobial susceptibility patterns of the UTI pathogens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Urinary Tract Infection

Urinary tract infections have been described since ancient times with the first documented description in the Ebers Papyrus dated to 1550 BC.(Al-Achi and Antoine, 2008). It was described by the Egyptians as "sending forth heat from the bladder" (Wilson *et al.*, 2010). Effective treatment did not occur until the development and availability of antibiotics in the 1930s before which time herbs, bloodletting and rest were recommended (Al-Achi and Antoine, 2008).

A urinary tract infection (UTI) is an infection that affects part of the urinary tract (CDC, 2016). When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract, it is known as kidney infection (pyelonephritis) (Salvatore *et al.*, 2011). Symptoms from a bladder infection include pain with urination, frequent urination, and bacteriuria feeling the need to urinate despite having an empty bladder, (CDC, 2016). Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI (Lane and Takhar, 2011). Rarely the urine may appear bloody (Salvatore *et al.*, 2011). Among old people and young individuals, symptoms may be vague or non-specific(CDC, 2016; Woodford and Goerge, 2011).

2.2 Incidence and prevalence of UTIs

Urinary tract infections are common bacterial infection for females (Colgan and Williams 2011). They occur in most cases between the ages of 16 and 35 years, with 10 percent of females acquiring an infection each year and 60 percent having an infection at certain time of their lives (Nicolle, 2008; Salvatore *et al.,* 2011). Recurrences are common, with nearly half of people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than males (Salvatore *et al.,* 2011). Pyelonephritis occurs between 20-30 times less frequently (Nicolle, 2008). They are the common cause of hospital acquired infections and account for approximately 40 percent of all UTI. Rates of asymptomatic bacteria in the urine increase with age from two to seven percent in females of child bearing age to as high as 50 percent in older females in care-homes (Dielubanza and Schaeffer, 2011). Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10% (Woodford and George, 2011). Asymptomatic bacteria in the urine occur in 2% to 10% of pregnancies (Smaill and Vazquez, 2007). Urinary tract infections can affect up to 10 % of individuals during childhood (Salvatore *et al.,* 2011). Among children urinary tract infections are the most common in uncircumcised males less than three months of age, followed by females less than one year (Bhat *et al.,* 2011). Estimates of frequency of occurrence among children vary widely. In a group of children having fever, ranging in age between birth and two years, two to 20 percent were diagnosed with a UTI (Bhat *et al.,* 2011).

2.3 Risk factors for UTIs

The important predisposing factors for urinary infections in females are age, sex, pregnancy, sexual intercourse, menopause, use of birth control devices, catheterization, surgery, diabetes, use of calcium supplements, immunosupperession, renal transplantation and spinal cord injury

In addition, a number of predisposing factors render individuals susceptible to urinary tract infections (UTIs). Any obstruction in normal urine flow or complete emptying of bladder facilitates the access of organisms to the bladder and, in turn, predisposes an individual to infection (Jackson *et al.*, 2000). There are numerous possible structural abnormalities of urinary tract that are associated with a 'residual urine' which increases the chances of infection and may become associated with repeated attacks of UTI. Some of these are renal calculi, tumors, and urethral stricture. All these factors cause obstruction to complete emptying of bladder. Approximately 15% of the urinary stones diseases are infectious stones. These stones are composed of struvite and/or carbonate apatite. UTI caused by urease positive organisms is also a risk factor for the formation of infectious stones. If these infections are not treated and the stones are not removed, the kidney is damaged (Bichler *et al.*, 2002). Some important predisposing factors for urinary tract infections are:

(i) Sex

An important predisposing factor for UTI is the sex of patient. It is evident from substantial research that UTIs are more common in females as compared to males (Mohsin and Siddiqui, 2010; Dielubanza and Schaeffer, 2011). Most infections in women are uncomplicated, whereas in men complicated infections predominate. Women are especially prone to UTIs probably because of the shortness of urethra and closeness of urethra to opening of genital and intestinal tract (Nester *et al.*, 2004). The shorter length of the female urethra allows uropathogens easier access to the bladder. Men are less prone to get UTI, possibly because of their longer urethra and the presence of antimicrobial substances in the prostatic fluid. The relation of sex and incidence of UTI has been confirmed by the study of Laupland *et al.*, (2007).

(ii) Age

The incidence of UTIs in women tends to increase with increasing age. Symptomatic and asymptomatic UTIs are extremely common in the elderly population. The prevalence of bacteriuria in women is about 20% between ages 65 and 75, increasing to between 20-25% over the age of 80 years. Whereas, a significant number of infections occur in men only after the age of 50 years when prostatic hypertrophy or other urinary tract abnormalities occur. UTI in young men is unusual and requires further investigations (Shanson and Speller, 2004). The prevalence in men is 3% at age 65-70 years and about 20% at ages over 80 years. The geriatric (elderly) community is frequently affected by UTIs but these infections are usually asymptomatic. Approximately 25% of all infections in elderly are UTIs (Foxman, 2002). In a study

(Buonanno and Damweber, 2006), it was documented that 50% of elderly women are affected by asymptomatic UTI. In many cases bladder catheterization is a contributing factor and causes increasing incidence of UTIs in elderly population. A woman over 80 years with urinary incontinence and needing support to walk has 50% risk of asymptomatic UTI. The majority of symptomatic urinary tract infections occur in women after the age of 50 years (Shanson and Speller, 2004). In a study, an increased prevalence of UTI among women aged 18-30 years was found associated with sexual intercourse and pregnancy (Cunha *et al.,* 2007). It has been reported that complicated UTIs were found most frequent among females aged between 40-59 years, while, in other age groups, uncomplicated UTIs were most frequent. It has also been noted that the isolation frequency of *E. coli* gradually decreases with increasing age with both complicated and uncomplicated UTIs (Kumamoto *et al.*, 2001).

UTIs are generally asymptomatic among apparently healthy, sexually active young women. In contrast, UTIs are more complicated among elderly individuals, infants and young children. UTI in children younger than 2 years has been associated with significant morbidity and long term medical consequences (Shortliffe, 2003). Winiecka *et al* (2002), evaluated the bladder instability in children with recurrent UTIs. It was found that the most common disturbance of lower urinary tract functioning in the children with recurrent UTI was instability of the dtrusor muscles which occurred more often in children with vesicoureteral reflux.

(iii) Sexual activity

Another predisposing factor, sexual intercourse, is also a common cause of UTIs among women because during sexual intercourse bacteria in the vaginal area could be messaged into the urethra. This problem can be avoided by urinating after sexual intercourse (Cornforth, 2002). Women who change sexual partners or have sexual intercourse more frequently may experience more frequent bladder infections (Kontiokari *et al.,* 2003).

(iv) Use of birth control devices

Several studies have shown that women who use a diaphragm are more likely to develop UTIs than women who use other forms of birth control. More recently, investigators have demonstrated that women whose life partners use a condom with spermicidal foam also tend to have growth of *E.coli* in the vagina (Jancel and Dudas, 2002). UTI among IUCD/condom users may reflect existence of unhygienic conditions during application of procedure or spread of infection by the thread of IUCD. UTI in these women may have serious consequences of developing renal damage (Bhurt *et al.,* 2000).

(v) Pregnancy

Pregnancy also acts as a risk factor for UTI as it causes anatomic and hormonal changes which favour development of UTI (Schlembach, 2006; Marinade *et al.,* 2009). A history of current UTI, diabetes mellitus, analgestic nephropathy, hyperuricaemia and Fanconi's syndrome are predisposing factors for UTI during pregnancy (Krcmery *et al.,* 2001). Dietary habits seem to be an important risk factor for UTI recurrence in fertile

women, and dietary guidance could be a first step towards prevention (Kontiokari *et al.*, 2003). The physiological changes associated with pregnancy are the relaxation of ureter under the effect of hormones and increase urinary output. The chemical composition of urine is also affected and results in increased urinary substances e.g. glucose and amino acids, which may facilitate bacterial growth (Sheikh *et al.*, 2000). The pregnant women with kidney infection have a greater chance of delivering their babies prematurely with low weight (Mittal and Wing, 2005). Sometimes, it results in fetal and maternal morbidity (Lamyman *et al.*, 2005). Women with pregnancy also had somewhat shorter gestational age and a higher proportion of preterm birth. This preterm inducing effect of maternal UTI was preventable by antimicrobial therapy. In this connection, Hazir (2007) evaluated the frequency of asymptomatic UTI in pregnant women. Eleven hundred apparently healthy pregnant women were screened for significant bacteriuria. The prevalence of asymptomatic UTI was found to be 6.1%. However, asymptomatic UTI had no relationship with gestational age, parity, level of education, and body mass index. In a study (Al-Haddad, 2005) 500 pregnant women were screened for asymptomatic UTI in their first and second trimester. Out of them 8.4% were positive for culture. A control group of non-pregnant women was also screened for asymptomatic UTI. The control group yielded 3% positive cultures. The frequency of UTI in pregnant women was observed 30% of the women suffered from UTI. Of these infected women, 53.7% were in the age group of 15-24 years and 48.8% were in the third trimester. Primigravida had highest percent culture positivity i.e. 66.6%. The incidence was higher in less than 20 years of age group. The incidence of prematurity was 75% and that of low birth weight was 50% in untreated patients (Lavanya and Jogalakshmi, 2002).

During pregnancy symptomatic and asymptomatic UTI can trigger the development of serious complications affecting both the mother and the fetus. Thus, proper screening and treatment of bacteriuria is necessary to prevent complications during pregnancy. All women should be screened for bacteriuria in the first trimester. Women with a history of recurrent UTIs or urinary tract abnormalities should have repeated screening for bacteriuria during pregnancy (Macejko and Schaeffer, 2007).

(vi) Menopause

Postmenopausal women are also susceptible to UTI due to lack of estrogen which plays important role in pathogenesis (Hu *et al.*, 2004). The protective effect of estrogen replacement on ascending UTI is controversial. A study was designed using an experimental model of UTI. In that study surgically menopausal mice were supplemented with estrogen and the susceptibility of UTI was evaluated after experimental *E. coli* infection. Surprisingly, despite the hypothesis that estrogen would protect mice from infection, estrogen treatment significantly increased the susceptibility of the mice to ascending UTI (Curran *et al.*, 2007).

In postmenopausal women, sexual activity, history of UTI, treated diabetes and urinary incontinence are associated with a high risk of UTI. However, therapeutic role of oral estrogen remains uncertain. For instance, in a study (Hu *et al.*, 2004) which included the postmenopausal women aged between 55 and 75 years, development of UTI was noted in sexually active postmenopausal women, with a history of UTI, diabetes mellitus and urinary incontinence.

(vii) Catheterization

Another common source of infection is catheter or tube placed in the bladder (Ribby, 2006; Warde, 2010). The use of vesical catheter over 5 days is the cause of UTI. Bacteria on the outside of the catheter can climb up the device into the bladder and cause infection (Pawelczyk *et al.*, 2002). Infection associated with an indwelling catheter is a representative type of biofilm infection occuring in the urinary tract (Evans *et al.*, 2001). More than 90% of UTI in catheterized individuals are asymptomatic. These infections are rarely symptomatic and infrequently cause blood stream infection. (Tambyah and Maki, 2000).

Catheter associated UTI accounts for 40% of all nosocomial infections and are the most common source of Gram negative bacteremia in hospitalized patients. The risk of bacteriuria is approximately 5% per day in 10-20% of hospitalized patients who receive an indwelling foley catheter. With long term catheterization, bacteriuria is inevitable (Warren *et al.*, 2002).

(viii) Diabetes

It is evident from literature that diabetic subjects are also at high risk of UTIs. For instance, in a study (Goswami *et al.*, 2001; Litza and Brill, 2010), the prevalence of UTI in diabetic subjects were found to be higher when compared with non-diabetic subjects (9% vs. 0.78%). Symptomatic and asymptomatic UTIs occur more frequently in women with diabetes mellitus than women without diabetes mellitus (Daneshgari and Mooree, 2006). Women with diabetes who requires pharmacological treatment have approximately twice as high risk of cystitis as non-diabetic women (Boyko *et al.*, 2002)

because of the changes in the immune system secondary to the high sugar concentration (Geerlings *et al.*, 2002). However, gestational diabetes mellitus was not associated with increased risk of UTIs (Rizk *et al.*, 2001). Although asymptomatic bacteriuria is not associated with serious health outcomes in healthy persons, further research needs to be undertaken regarding the impact of asymptomatic bacteriuria in patients with diabetes (Geerlings *et al.*, 2002).

(ix) Surgery

Any surgery on the urinary tract increases the chances of UTI. Urological complications after renal transplantation are also frequently associated with UTIs (Senger *et al.*, 2007). UTI is also the most common bacterial infection occurring in the renal transplant recipients, particularly anatomic abnormalities of the native or transplanted kidneys and possible rejection and immunosupperession. The major risk factors for UTI in renal transplant recipients include indwelling bladder catheters, trauma to the kidney and ureter during surgery (Wilson *et al.*, 2005). Steroids or cytotoxic drugs, as given to renal transplant recipients, greatly increase the chances of recurrent UTIs and infections of kidney in the first few months of post-transplant (Shanson and Speller, 2004).

2.4 The Enterobacteriaceae

The Enterobacteriaceae are a large family of Gram-negative bacteria. This family is the only representative in the order Enterobacteriales of the class Gammaproteobacteria in the phylum Proteobacteria (Brenner *et al.*, 2005). Enterobacteriaceae includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella spp., Escherichia coli, Yersinia pestis, Klebsiella,* and *Shigella.* Other disease-causing bacteria in this family include Proteus, Enterobacter, *Serratia spp.,* and *Citrobacter spp.* (Williams *et al.*, 2010) Members of the Enterobacteriaceae can be trivially referred to as enterobacteria or "enteric bacteria", as several members live in the intestines of animals.

Most members of Enterobacteriaceae have peritrichous, type I fimbriae involved in the adhesion of the bacterial cells to their hosts. Some enterobacteria produce endotoxins. Endotoxins reside in the cell wall and are released when the cell dies and the cell wall disintegrates. Some members of the Enterobacteriaceae produce endotoxins that, when released into the bloodstream following cell lysis, cause a systemic inflammatory and vasodilatory response. The most severe form of this is known as endotoxic shock, which can be rapidly fatal.

A range of pathogens have been implicated in UTIs, include Gram-negative bacteria such as *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae.* However, the disease is predominantly caused by Uropathogenic *E. coli* (UPEC; Schulz, 2011; Flores-Mireles *et al.*, 2015; Su *et al.*, 2016b), which accounts for up to 75% of all cases (Foxman, 2002, 2003; Martinez and Hultgren, 2002) and 95% of community-acquired cases (Kucheria *et al.*, 2005).

2.5 Uropathogens and virulence properties

(i) Uropathogenic *E. coli*

UPEC are a pathotype of extraintestinal pathogenic E. coli (ExPEC) and originate from the intestinal microbiome. Within the intestine, UPEC rarely cause any complications and exist in a beneficial symbiotic relationship with intestinal microflora (Wiles *et al.*, 2008). However, UPEC have adapted the ability to disseminate and colonize other host environments such as the urinary tract and bloodstream. Virulence factors, such as toxins, modify, and damage the host to promote infection (Flores-Mireles *et al.*, 2015). In addition, physiological factors that do not directly damage the host but nevertheless are essential for UPEC growth and survival in the urinary tract are now being appreciated for their role in pathogenesis (Alteri and Mobley, 2012). The capacity of UPEC to utilize nutritionally-diverse environments such as the intestines, urine, bladder, kidneys, and bloodstream clearly plays a significant role in its pathogenesis (Brown *et al.*, 2008; Eisenreich *et al.*, 2010); UPEC metabolism is tightly regulated and highly responsive to nutrient availability, allowing survival with a wide range of nutrients in competitive, fluctuating environments.

UTIs are usually initiated when UPEC contaminate and colonize the urethra and migrate into the bladder lumen. Most of the characterized strains of UPEC invade the bladder epithelium and undergo an intracellular infection cycle (Martinez *et al.*, 2000; Justice *et al.*, 2004) and there is evidence that this occurs in most human UTIs (Rosen *et al.*, 2007). The infection cycle is a complex pathway involving epithelial attachment, invasion of host cells, and intracellular proliferation, leading to the eventual rupture of the bladder epithelial cell, dissemination and reinfection of surrounding epithelial cells

(Justice *et al.*, 2004, 2006a; Andersen *et al.*, 2012). Infections of the lower urinary tract have the potential to progress to the kidneys and enter the bloodstream causing potentially fatal urosepsis (Flores-Mireles *et al.*, 2015).

Many studies have focused on virulence factors that play a role in mediating UPEC colonization of the bladder, most notably the initial attachment of bacterial adhesive structures to the luminal surface of the bladder epithelium. This step of infection has been quite well characterized and has been a focus of attention in the development of therapies for UTIs (Langermann *et al.*, 2000; Asadi *et al.*, 2014). UPEC surface fibers, in particular Type 1 pili and their tip adhesin (FimH), recognize mannosylated glycoprotein receptors present on the host epithelium, mediating UPEC binding (Jones *et al.*, 1995; Martinez *et al.*, 2000). UPEC also express a variety of other proteins on their surface, which facilitates bacterial attachment (see Mulvey, 2002). Once bound, UPEC may become internalized via endocytosis (Mulvey *et al.*, 1998); activation of the host Rho-family proteins, such as RhoA, Cdc42, and Rac1 result in the rearrangement of the host cell membrane, engulfing the bacteria in endocytic vesicles (Martinez *et al.*, 2000; Martinez and Hultgren, 2002).

As UPEC proliferate and the cluster develops to overwhelm the host cell, subpopulations of differentiated bacteria develop, including highly motile bacteria and extensive bacterial filaments—cells that have continued growth with an arrest of cell division (Justice *et al.*, 2004, 2006b; Andersen *et al.*, 2012). The development of motile and filamentous bacteria accompanies host cell rupture, and promotes dissemination, adhesion and colonization of surrounding host surfaces, whilst helping avoid consumption by the innate immune system (Justice *et al.*, 2006b; Andersen *et al.*, 2012).

UPEC filaments have the potential to divide and reform to the regular rod-shaped cells that are able to invade new host cells (Rosen *et al.*, 2007; Andersen *et al.*, 2012). During these changes, UPEC would experience rapid substantial changes in nutritional status. Within the intestine, most UPEC strains are thought to exist like other strains of commensal E. coli, as they do not directly cause pathogenic infection there.

(ii) *Klebsiella pneumoniae*

Klebsiella pneumoniae, a member of the family Enterobacteriaceae, has become one of the most common causes of urinary tract infection (UTI), especially amongst patients with prolonged catheterization, spinal cord injuries–resulting in prolonged catheterization–and nosocomial infections (Jing-Jou *et al.*, 2001). Attachment to surfaces is typically mediated in Gram-negative enterobacteria by fimbrial adhesins. In *K. pneumoniae*, there are two well-characterized fimbrial adhesins that are often detected on the surfaces of clinical isolates, type 1 and type 3 fimbriae (Levrelli *et al.*, 2000).

Type 1 fimbriae are one of the best-characterized fimbrial adhesins and are found in many bacteria in the family Enterobacteriaceae (Klemm *et al.*, 2000). These fimbriae are encoded on a gene cluster (fim) containing all the genes required for the fimbrial structure and assembly, with assembly occurring via the chaperone-usher pathway (Klein *et al.*, 2010). The major component of the fimbrial appendage is made up of repeating FimA subunits with an adhesin molecule (FimH) at the tip of the fimbriae that confers adherence to mannose-containing glycoconjugates on host cells. Type 1 fimbriae in *K. pneumoniae* are regulated via phase variation in a manner similar to the regulation of type 1 fimbriae in Escherichia coli (Rosen *et al.*, 2008; Struve *et al.*,

2008). In uropathogenic E. coli, virulence in the urinary tract is correlated with the expression of type 1 fimbriae, which is attributed to the ability of the fimbriae to mediate adherence to epithelial cells of the urogenital tract (Struve *et al.*, 2008; Connell *et al.*, 2010). Additionally, in a murine model of uncomplicated UTI lacking any indwelling urinary device, a type 1 hyperfimbriate strain forms intracellular biofilm communities within bladder umbrella cells (Rosen *et al.*, 2008).

The type 3 fimbriae are characterized by their ability to agglutinate erythrocytes treated with tannic acid in vitro, and this phenotype has been referred to as the mannose-resistant Klebsiella-like hemagglutination (MR/K) reaction (Burmolle *et al.*, 2008). These fimbriae are encoded by the mrk operon and are predicted to also be assembled via the chaperone-usher pathway. While first identified and characterized in *Klebsiella*, type 3 fimbriae are commonly found in other Enterobacteriaceae, and the mrk gene cluster may be chromosome or plasmid borne (Ong *et al.*, 2008; Ong *et al.*, 2010). *In vitro* studies examining the role of type 3 fimbriae have shown that these fimbriae mediate attachment to endothelial and bladder epithelial cell lines and play a role in biofilm formation on abiotic surfaces, as well as surfaces coated with host-derived materials (Boddicker *et al.*, 2006; Schroll *et al.*, 2010). Variants of the adhesin MrkD can bind to type IV and/or type V collagen (Schurtz *et al.*, 2004). The crystal structure of one of these variants, MrkD1P, has recently been determined, and the collagen binding pocket has been described (Rego *et al.*, 2012). Thus far, *in vivo* models of UTI have not indicated a role for *K. pneumoniae* type 3 fimbriae in virulence. Given the *in vitro* evidence demonstrating that the type 3 fimbrial adhesin is necessary for biofilm formation on abiotic surfaces and surfaces coated with host-derived extracellular

matrix proteins, it has been suggested that type 3 fimbriae are important in biofilm-mediated infections on indwelling devices, including catether associated urinary tract infections (Mobley *et al.*, 2000).

(iii) *Proteus mirabilis*

Proteus mirabilis, a common uropathogen, is an infectious agent of pyelonephritis and catheter-associated urinary tract infections (UTIs) (Armbruster & Mobley, 2012; Jacobsen *et al.*, 2008). *P. mirabilis* has many virulence factors that contribute to UTIs (Nielubowicz & Mobley, 2010). These factors include fimbria-mediated adherence to host urothelial cells and the catheters (Jansen *et al.*, 2004), flagella mediated motility (swarming and swimming) (Jones *et al.*, 2004), hemolysin, and invasion of host tissues and immune evasion (Mathoera *et al.*, 2002). Flagella-dependent swarm cell formation contributes to establishing infections by migrating along the catheter. Hemolysin is also thought to facilitate bacterial spread within the kidney and the development of pyelonephritis by damaging host tissues. Bacteria must successfully evade immune responses to persist within the host. *P. mirabilis* uses several strategies to avoid immune attacks in the urinary tract. One is to vary the antigenic structures, such as flagellin by flagellar gene rearrangement, and fimbriae by fimbrial gene diversity or phase variation to prevent antibody recognition (Jansen *et al.*, 2004). Other immunoavoidance factors for *P. mirabilis* include capsules, IgA proteases (ZapA) (Belas *et al.*, 2004), and lipopolysaccharides (LPS). Capsules are effective at hiding many bacterial surfaces and preventing opsonization (Jacobsen *et al.*, 2008). *P. mirabilis* is an antigenically heterogeneous species due to structural differences of LPS. Modified LPS promotes bacterial survival by increasing resistance to cationic

antimicrobial peptides and by altering host recognition by Toll-like receptors (TLRs) (Ernst *et al.*, 2011). Moreover, capability of invading urothelial cells to survive intracellularly probably represents another mechanism for immune evasion and persistence (Mathoera *et al.*, 2002).

Ma ny studies have reported that the presence of mannose-resistant Proteus-like (MR/P) fimbriae of *P. mirabilis* is important in UTIs (Li *et al.*, 2002; Pearson *et al.*, 2011). MR/P fimbriae facilitate colonization of the urinary tract, and deficiency of the MR/P fimbriae decreased bacterial loads in the mouse model of UTIs (Zunino *et al.*, 2001). The mrp gene cluster contains two transcripts: mrpABCDEFGHJ (designated the mrp operon) and mrpI. The promoter for the mrp operon, which contains all of the genes required for MR/P fimbrial biogenesis, resides on a 251-bp invertible element (IE). The gene mrpI, transcribed divergently from the mrp operon and independent of the mrp promoter, encodes a recombinase capable of switching the IE from either "ON" to "OFF" or from "OFF" to "ON" to control MR/P fimbria expression (Li *et al.*, 2002).

Less is known about the host response to uropathogenic *P. mirabilis* but aspects of the host defense might be similar to uropathogenic E. coli (UPEC). Urothelial cells secreted soluble mediators such as soluble IgA, lactoferrin, and bactericidal antimicrobial peptides to inhibit attachment of UPEC (Weichhart *et al.*, 2008). Microbes that overwhelm these early defenses contact urothelia and activate an innate inflammatory response through TLRs (Mogensen, 2009). The inflammatory response consists of three principal steps: (i) urothelial cell activation and the production of distinct inflammatory cytokines, (ii) immune cell recruitment to the infectious site, and (iii) local

destruction and elimination of the invading bacteria (Weichhart *et al.*, 2008; Mulvey *et al.*, 2000). The bacterial envelope maintains cell homeostasis and is the site for crucial processes, such as metabolic energy transduction, the transport of nutrients and wastes, signal transduction, and cell-cell communication (Hurdle *et al.*, 2011). RpoE, an alternative sigma factor, is essential for the maintenance of cell envelope integrity in Gram-negative bacteria (Rowley *et al.*, 2006; Crouch *et al.*, 2005). In this regard, rpoE gene is important in pathogenesis and stress survival in many Gram-negative bacteria (Raivio, 2005), but the function of RpoE in uropathogenic P. mirabilis is still not known. RseA, belonging to the rpoE operon, is an anti-RpoE factor under nonstressed conditions. The release of RpoE from RseA binding allows it to combine core RNA polymerase to transcribe RpoE-dependent genes. In the present study, we characterized the roles of *P. mirabilis* RpoE in virulence by in vitro and in vivo assays. This is the first report to show that *P. mirabilis* RpoE affected multiple traits, including swarming motility, hemolysin activity, bacteria-mediated cytotoxicity, fimbria production, survival in macrophage, invasion ability, induction of cytokine expression, and colonization in mice. It is worth noting that *P. mirabilis* RpoE could not only regulate expression of MR/P fimbriae through mrpI but also modulate host immune responses. We noticed that fimbria expression, survival in macrophages, invasion ability, and colonization in mice were decreased in the rpoE mutant and that the induction of interleukin-8 (IL-8) by rpoE mutant was higher relative to the wild type. In addition, we found that RpoE was activated by urea, a component in urine. Altogether, RpoE of *P. mirabilis* could play important roles in establishing UTIs via modulating the appropriate production of virulence factors, including those associated with host immune responses. To our

knowledge, this is the first report describing the roles of RpoE in P. mirabilis. (Wang *et al.*, 2008)

2.6 Antibiotics susceptibility

Urinary tract infection (UTI) is one of the commonly reported microbial infections at healthcare and in the community. Effective management of UTI would usually require the use of antimicrobial agents. However, reports show increasing resistance among certain urinary pathogens (uropathogens) to some commonly used antimicrobial agents. A number of studies report resistance of uropathogens to some first line antibiotic therapy: 10 - 20% for trimethoprim and sulfamethoxazole (TMP-SMX), 40% for ampicillin, 15 - 20% for nitrofurantoin and less than 10% for the fluoroquinolones (Lienerrozos, 2004; McLaughlin, 2004). Among the various antimicrobials used in the treatment of UTIs, the fluoroquinolones remain the preferred class. This is due to the fact that fluoroquinolones have high bacteriologic and clinical cure rates, low resistance rates, and few adverse drug reactions (Schaeffer, 2002). Fluoroquinolones are bactericidal antibiotics that target specifically DNA gyrase of the microorganism. Amongst the most frequently prescribed fluoroquinolones for empirical treatment of UTIs are ciprofloxacin and levofloxacin (Hooton *et al.*, 2009; Fu *et al.*, 2013)). Ciprofloxacin, a second-generation fluoroquinolone, has greater activity against Gram-negative bacteria compared to nalidixic acid (a first-generation quinolone). Levofloxacin, a third-generation fluoroquinolone, is known to have broad spectrum of activity against Gram-positive and Gram-negative bacteria, and atypical pathogens (King *et al.*, 2000). Despite

the preference for fluoroquinolones in the management of UTIs, there are current reports that suggest emerg ence of uropathogen resistance to commonly prescribed fluoroquinolones (Lesho *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials used

Hand gloves, sterile universal bottles, ice packs, analytical weighing balance, aluminium foil paper, conical flask, cotton wool, masking tape, magnetic stirrer hot plate 78-1 (PEC Medical USA) and magnetic bar, measuring cylinder, test tubes, test tube rack, beakers, sterile swab sticks, spatula, inoculating loop, hypothermic syringe, bunsen burner, autoclave (Dixon), hot air oven (Genlab), incubator (Genlab), microscope, microscopic slides, Mccartney bottles, glass and disposable petri-dishes. Media used include: Nutrient agar, NA (Biomark, India), Eosin Methylene Blue Agar, EMB.

3.2 Study location and Collection of Sample

This study was carried out in Federal University Oye Ekiti, Ekiti State. Samples were collected from Ekiti State University Teaching Hospital (EKSUTH), Ado Ekiti, Ekiti State. Analysis was performed in the microbiology laboratory. A total number of 120 urine samples was collected from In-patients and Out-patients. Clean catch, mid-stream urine was collected into sterile universal bottle. Samples were transported in ice-packs and processed within two hours of collection.

3.3 Culture and Isolation of Organism

Samples were brought to the laboratory and processed within two hours of collection. Ten microlitre of each sample was innoculated using the spread plate method on a freshly prepared Cystine Lactose Electrolyte Defficient Agar (CLED), Eosin Methylene Blue Agar (EMB), and Mac Conkey Agar. The plates were inverted and incubated at 37 degree celcius for 18-24 hours

After 24hrs of incubation, plates with 10^5 CFU (colony forming units) were considered to be positive for significant bacteruria and distinct colonies were picked from either Mac Conkey or EMB agar into a sterile double strenght Nutrient Agar (NA) slant and incubated for 24hrs at 37 degree celcius. After incubation, the slants were kept in the refrigerator at 4 degree celcius for further use.

3.4 Identification of Uropathogens

(i) Gram's Staining

This technique is the most frequently used in the microbiology laboratory towards identifying microbial isolates based on cell wall constituent. A gram staining kit contains a primary stain (Crystal violet), a mordant (Gram's iodine), a de-colourizer (Ethyl alcohol) and a secondary stain (Safranin).

All organisms were subculture from slants onto freshly prepared Nutrient agar plates and incubated at 37 degree celcius for 18-24 hours. Grease-free glass microscopic slides were labelled such that staining reagents will not come in contact during the course of staining. A drop of distilled water was placed at the center of the slide. A

bacterial colony was picked and transferred onto the water and a smear was made. The smears were heat-fixed by briefly passing the slide through the flame. This was done in other to allow firm adherence to the slide and allow stains to be more readily taken up by the cell. The smear was gently flooded with crystal violet and let to stand for one minute and thereafter rinsed off under a running tap water. The smear was gently flooded with Gram's iodine and let to stand for one minute and thereafter rinsed off under a running tap water. Few drops of alcohol was added to the smear and was rinsed after five seconds to prevent decolourization. The smear was gently flooded with safranin to counter-stain and was left to stand for fourty-five seconds and thereafter rinsed off under a running tap water. The slides were allowed to dry and were thereafter mounted on a microscope and viewed under 1000x magnification with the aid of immersion oil.

3.5. Biochemical Identification

Twenty-four hours old cultures were used for all biochemical identification. The tests carried out during the course of characterization include: Citrate, Urease, Indole, Methyl red and Voges-Proskauer.

(i) Citrate Test

Citrate utilization test is commonly employed to distinguish between members of the Enterobacteriaceae family based on their ability to utilize citrate as carbon source. Citrate utilization can be used to distinguish between coliforms such as Enterobacter aerogenes (+ve) which occur naturally in the soil and in aquatic environments and fecal coliforms such as Escherichia coli (-ve) whose presence would be indicative of fecal

contamination.

Citrate utlilization test is used to determine the ability of bacteria to utilize sodium citrate as it's only carbon source and inorganic ($NH_4H_2PO_4$) is the sole fixed nitrogen source.

The simmons citrate agar lightly in the slant was inoculated by touching the tip of a needle to a colony that is 18 to 24 hours old. The sample was incubated at 35 degree celcius to 37 degree celcius for 18 to 24 hours. The development of colour change was observed, a change from green to blue color denotes alkalinization.

(ii) Urease Test

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Many organisms especially those that infect the urinary tract, have an urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink.

The medium was inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant was streaked with the test organism which is not more than twenty-four hours old. The test tube was innoculated at 35 degree celcius in ambient air for 18 to 24 hours.

(iii) Indole Test

Indole test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole. Tryptophan is hydrolysed by tryptophanase to

produce three possible end products – one of which is indole. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylamino benzaldehyde, this reacts with indole to produce a red coloured compound. Indole test is a commonly used biochemical test which helps to differentiate Enterobacteriaceae and other genera.

A conventional tube method was done which required an overnight incubation, which identifies weak indole producing organisms. The tryptophan broth was inoculated with a loop full of emulsify isolated colony of the test organism. It was then incubated at 37 degree celcius for 24-28 hours. 0.5 ml of Kovac's reagent was added to the broth culture.

(iv) Methyl-Red

Methyl Red (MR) test determines whether the microbe performs mixed acids fermentation when supplied glucose. Types and proportion of fermentation products produced by anaerobic fermentation of glucose is one of the key taxonomic characteristics which help to differentiate various genera of enteric bacteria. It is visualized using pH indicator, methyl red (p-dimethylaminoaeobenzene-O-carboxylic acid), which is yellow above pH 5.1 and red at pH 4.4.

The tubes containing Methyl red broth was inoculated with a pure culture of the microorganisms under investigation and incubated at 35 °C for 24 hours. 5 drops of the methyl red indicator solution was added to the tubes. The tubes were observed for colour change.

(v) Voges Proskauer Test

Voges–Proskauer is a test used to detect acetoin in a bacterial broth culture. The test depends on the digestion of glucose to acetylmethylcarbinol. In the presence of oxygen and strong base, the acetylmethylcarbinol is oxidized to diacetyl, which then reacts with guanidine compounds commonly found in the peptone medium of the broth. Alpha-naphthol acts as a color enhancer, but the color change to red can occur without it. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result.

The tubes of Voges Proskauer broth was inoculated with a pure culture of the test organism and incubated for 24 hours at 35 degree celcius. 6 drops of 5% alpha naphthol was added and mixed to aerate followed by the addition of 4 drops of 40% KOH. The tubes were shaked vigorously for 30 minutes and were observed for colour change.

3.6. VIRULENCE ASSAY TESTS

(i) 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).

(ii) 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).

3.7. Antibiotics Susceptibility Assay

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The method used during this study is the disc diffusion method.

The disk diffusion susceptibility method is simple and practical and has been well-standardized. The test was performed by applying a bacterial inoculum of approximately 1−2×108CFU/mL to the surface of a Mueller-Hinton agar plate. Nine commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface. Plates were incubated for 24 hours at 35 degree celcius prior

to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI).

3.8. Extended spectrum beta-Lactamase production

Extended spectrum beta-lactamase (ESBL) producers are Gram-negative bacteria that produce enzymes that bestow resistance to most beta-lactam antibiotics like penicillins and cephalosporins. ESBL producers have been noticed mainly in the Enterobacteriaceae family which habours several antibiotic resistance determinant.

A 24 hour old culture of test organism was inoculated using 1 µl (a standard loopful) of a 0.5 McFarland standard suspension on a freshly prepared Muller Hinton agar. Three commercially prepared, fixed concentration, paper antibiotics disc were used which included Clavulanic acid, Ceftriaxone (30 microgram) and Ceftazidime (30 microgram) were placed in a specific pattern on the plates and was then inoculated at 37 degree celcius for 24 hours. The plates were observed for clear zones and patterns and the zone diameter for each drug were interpreted using the criteria published by the Clinical and Laboratory Standard Institute (CLSI).

CHAPTER FOUR

RESULTS

4.1 Demographics

In this study, 120 urine samples, comprising of 54 samples from males and 66 samples from females, were collected from the Ekiti State University Teaching Hospital. Forty-six of the urine samples showed significant bacterial growth. Twenty-two samples from males grew significant bacteria while 24 from females grew gignificant bacteria. Sixty-six urine samples were collected from outpatients and 54 samples were collected from inpatients. (Table 1)

4.2 Distribution of Outpatients and Inpatients based on age groups and frequency of patients with significant bacteuria

Among the 46 isolates obtained, 34 isolates were obtained from outpatients and 12 isolates were obtained from inpatients. Nineteen and seven isolates were obtained from females in outpatient and inpatient, 13 and 5 isolates were obtained from males in outpatients and inpatients. (Table 2)

4.3 Total number of samples from each unit

A total of 120 urine samples was collected across various units in EKSUTH, more samples (10%) were obtained from the surgical outpatient department, 9.2% were obtained from accident and emergency, 7.5% were obtained from National health insurance scheme, 5.% was obtained from General outpatient unit. (Table 3)

4.4 Percentage of bacteria isolated

Among the 46 isolates, 37% were *Klebsiella pneumoniae*, 4.3% were *Klebsiella oxytoca*, 32.6% were *Escherichia coli*, and 26.1% were *Proteus mirabilis*. (Table 4)

4.5 Distribution of the isolates on the basis of patient types and departments

In this study, *Klebsiella pneumoniae* was the most isolated uropathogen among outpatients and inpatients with 13 (36.1%) and 4 (40%) respectively, followed by *Escherichia coli* 12 (33.3%) among outpatients and 3 (30%) among inpatients. 9 (25%) and 3 (30%) of isolates were *Proteus mirabilis* from outpatients and inpatients and the least isolated uropathogen was *Klebsiella oxytoca* 2 (5.6) from outpatients and non was isolated from inpatients. (Table 5)

4.6 Distribution of bacteria recovered based on demographics and age groups

Among the 46 isolated Gram's negative uropathogens, 10(45.5%) and 7(29.2) *Klebsiella pneumoniae,* 5(22.7) and 10(41.7) *Escherichia coli,* 7(31.8) and 5(20.8) *Proteus mirabilis* and 0 and 2(8.3) was obtained from males and females respectively, *Escherichia coli* was the most isolated uropathogen from the age groups; 11-40 and 41-70 with a value of 4(8.7%) each. (Table 6)

4.7 Antibiotics Susceptibility Test of isolated uropathogens

In this study, more isolated uropathogens were resistant to selected groups of antibiotics. Forty-six of the isolates showed resistance to pefloxacin, followed by 44 (95.7%) which showed resistance to Ampicilin; 41(89.1%) showed resistance to tetracyclin, 38(82.6%) were resistant to Gentamicin. Thirty (65.2%) of the 46 isolates were sensitive to Meropenem, 29(63%) were sensitive to Ertapenem and 20(43.5%) were sensitive to Ceftazidime. (Table 7)

4.8 Percentage of Multi-Drug Resistance among Outpatients and Inpatients

The 46 isolated uropathogens were tested against six different classes of antibiotics which included; Cephalosporins, Cabapenems, Quinolones, Amynoglycoside,tetracyclin and Ampicilin. Multidrug resistant isolates were more

among the outpatients 24 than among inpatients 13. Out of the 15 uropathogens that showed resistance to the six classes of antibiotics used, 11(73.3) was obtained from outpatients while 4(26.7) was obtained from inpatients. (Table 8)

4.9 Distribution of Multi-Drug Resistance across isolates

Forty-one uropathogens were multidrug resistance among which only 6(40%) *E. coli* of the isolates were resistant to 3 classes of antibiotics, 49(26.7%) *E. coli* and 2(16.7%) *P. mirabilis* were resistant to 4 classes of antibiotics, more *K. pneumoniae* were resistant to 5 classes of antibiotics 8(47.1%) than 6 classes of antibiotics 7(41.2%). (Table 9)

4.1.0 Antibiotics resistance pattern across isolates

In this study, the pattern of resistance of the 46 isolates were observed. Nine uropathogens were resistance to seven of the antibiotics used which were pefloxacin, gentamicin, tetracyclin, ampicilin, ceftazidime, ceftriaxone and norfloxacin with *Klebsiella pneumoniae* with the highest prevalence (5). Nine isolate were resistant to the nine antibiotics used with *Proteus mirabilis* with the highest prevalence (5) (Table 10).

4.1.1 Haemolysis test

Among the 46 isolates, seven (15.2) lysed blood agar partially, 1(2.2) lysed blood agar completely and 38(82.6) did not lyse the erythrolytes in blood cell. One (8.3) of *Proteus mirabilis* was the isolate that showed complete lysis on blood agar. (Table 11)

4.1.2 Biofilm Production

In this study, all the 46 isolates were tested for biofilm production. Twenty-three s(50%) of the isolates were strong biofilm producers with *E. coli* 11(47.8%) as the

highest frequency, 17(37%) were weak biofilm producers with frequency of 7(41.2%) among *Klebsiella pneumoniae,* 6(13%) were non biofilm producers. (Table 12)

4.1.3 ESBL Production by some selected isolates across department

Among the 46 isolates, 13(28.2) of the isolates were ESBL producers with prevalence among *klebsiella pneumoniae* (7) were from outpatients, 7(15.2) were ESBL producers from inpatients from with prevalence in *Klebsiella Pneumoniae.* (Table 13)

4.1.4 Antibiotics Resistance of isolates based on Outpatients and Ipatients

In this study, 54.3% of the isolates were resistant to Ertapenem with more resistance in the outpatient department, 20% were resistance to Meropenem, 25% were resistance to Ceftazidime, 24% resistance to Ceftriaxone, 46% were resistant to Gentamycin, 44% were resistant to ampicillin, 42% were resistance to Tetracyclin, 36% were resistance to norfloxacin and 43% were resistant to Pefloxacin. All *Klebsiella oxytoca* were sensitive to Ceftazidime, Ceftriaxone and Norfloxacin. Resistance in outpatients was higher than resistance in inpatients.

Table 1: Demographics

Patients demographic characteristics		Number
SEX	**Male**	22
Positive samples	**Female**	24
Negative samples	Male	32
	Female	42
AGE	≥10	1
Positive samples	11—40	7
	41-70	10
	≤71	7
	Adult	8
	Others	10
Negative sample	≥10	2
	11—40	22
	41-70	13
	≤71	12
	Adult	9
	Others	19
LOCATION	Outpatient	34
Positive samples	Inpatient	12
Negative samples	Outpatient	30
	Inpatient	44

Table 2: Distribution of Outpatients and Inpatients based on age groups and frequency of patients with significant bacteuria

Age range (years)	Inpatient			Outpatients		
	Male	Female	**Total**	Male	**Female**	**Total**
≤ 10	0	1	**1**	0	**0**	**0**
11 – 40	0	3	**3**	2	**3**	**5**
41 – 70	2	2	**4**	5	1	**6**
≥ 71	2	0	**2**	3	2	**5**
ADULT	1	1	**2**	2	6	**8**
OTHERS	0	0	**0**	3	7	**10**

Keys

- Some patients' demographics were not properly documented and they were classified as adults or others.

Table 3: Total number of samples from each unit

Patient type	Department	Number of isolates n (%)
Outpatient	SOP	12 (10.0)
	NHIS	9 (7.5)
	GYNAE	5 (4.2)
	MED	1 (0.8)
	HDU	1 (0.8)
	ANC	2 (1.7)
	A&E	11 (9.2)
	GOPD	7 (5.8)
	OTHERS	49 (40.8)
Inpatient	MMW	3 (2.5)
	MSW	3 (2.5)
	FMW	1 (0.8)
	FSW	2 (1.7)
	CW	5 (4.2)
	ER	1 (0.8)
	UROLOGY	1 (0.8)
	PALPI	3 (2.5)
	OHW	1 (0.8)
	ANW	3 (2.5)

Keys

- SOP- surgical outpatient, NHIS- national health insurance scheme, GYNAE- gynaecology, MED- medical, HDU-high dependency unit, ANC- anti-natal care, A&E- accident and emergency, GOPD- general outpatient department, MMW- male medical ward, MSW- male surgical ward, FMW- female medical ward, FSW- female surgical ward, CW- children ward, ER-emergency room, Urology, PALPI- palpition, OHW-organisational health and wellness , ANW- anti-natal ward

Table 4: Percentage of bacteria isolated

Isolated uropathogens	Total number (n=46) n (%)
Klebsiella pneumoniae	17 (37)
Klebsiella oxytoca	2 (4.3)
Escherichia coli	15 (32.6)
Proteus mirabilis	12 (26.1)

Table 5: Distribution of the isolates on the basis of patient types and departments

Patient type	Depart-ment	*E. coli* n (%)	*K. pnenmoniae* n (%)	*K. oxytoca* n (%)	*P. mirabilis* n (%)	Total n (%)
Outpatient	SOP	4 (26.7)	2 (11.8)	0 (0)	1 (8.3)	7(15.2)
	NHIS	0 (0)	0 (0)	1 (50)	1 (8.3)	2 (4.3)
	GYNAE	2 (13.3)	0 (0)	0 (0)	1(8.3)	3 (6.5)
	MED	0 (0)	1 (5.9)	0 (0)	0 (0)	1 (2.2)
	HDU	0 (0)	1 (5.9)	0 (0)	0 (0)	1 (2.2)
	ANC	0 (0)	0 (0)	0 (0)	1 (8.3)	1 (2.2)
	A&E	0 (0)	2 (11.8)	0 (0)	2 (16.7)	4 (8.7)
	OTHERS	6 (40)	7 (41.2)	1 (50)	3 (25)	17 (37)
	Sub total	12 (33.3)	13 (36.1)	2 (5.6)	9 (25.0)	36(100)
Inpatients	MMW	0 (0)	2 (11.8)	0 (0)	1 (8.3)	3 (6.5)
	MSW	2 (13.3)	0 (0)	0 (0)	0 (0)	2 (4.3)
	CW	0 (0)	2 (11.8)	0 (0)	0 (0)	2 (4.3)
	FMW	0 (0)	0 (0)	0 (0)	1 (8.3)	1 (2.2)
	FSW	0 (0)	0 (0)	0 (0)	1 (8.3)	1 (2.2)
	PALPI	1 (6.7)	0 (0)	0 (0)	0 (0)	1 (2.2)
	Sub total	3 (30)	4 (40)	0 (0)	3 (30)	10 (100)

Keys

- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis.*
- Some patients demographics were not properly documented and were classified as others.
- SOP- surgical outpatient, NHIS- national health insurance scheme, GYNAE- gynaecology, MED- medical, HDU-high dependency unit, ANC- anti-natal care, A&E- accident and emergency, GOPD- general outpatient department, MMW- male medical ward, MSW- male surgical ward, FMW- female medical ward, FSW- female surgical ward, CW- children ward, ER- emergency room, Urology, PALPI- palpition, OHW-organisational health and wellness , ANW- anti-natal ward

Table 6: Distribution of bacteria recovered based on demographics and age groups

	Isolated uropathogens				
	E. coli (n=15) n (%)	*K. pnenmoniae*(n=17) n (%)	*K. oxytoca*(n=2) n (%)	*P. mirabilis*(n=12) n (%)	Total n (%)
SEX					
Male	5 (22.7)	10 (45.5)	0 (0)	7 (31.8)	22 (47.8)
Female	10 (41.7)	7 (29.2)	2 (8.3)	5 (20.8)	24 (52.2)
AGE GROUPS					
≤ 10	0 (0)	1 (2.2)	0 (0)	0 (0)	1 (2.2)
11 – 40	4 (8.7)	2 (4.3)	0 (0)	3 (6.5)	9 (19.6)
41 – 70	4 (8.7)	3 (6.5)	0 (0)	2 (4.3)	9 (19.6)
≥ 71	2 (4.3)	3 (6.5)	0 (0)	2 (4.3)	7 (15.2)
ADULT	3 (6.5)	2 (4.3)	1 (2.2)	4 (8.7)	10 (21.3)
OTHERS	2 (4.3)	6 (13.0)	1 (2.2)	1 (2.2)	10 (21.3)

Key

- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis.*

Table 7: Antibiotics Susceptibility Test of isolated uropathogens

Antibiotics	Susceptible	Resistance
Norfloxacin	13 (28.3)	33 (71.7)
Gentamicin	8 (17.4)	38 (82.6)
Tetracyclin	5 (10.9)	41 (89.1)
Pefloxacin	1 (2.2)	45 (97.8)
Meropenem	30 (65.2)	16 (34.8)
Ampicillin	2 (4.3)	44 (95.7)
Ceftazidime	20 (43.5)	26 (56.5)
Ceftriaxone	16 (34.8)	30 (65.2)
Ertapenem	29 (63)	17 (37)

Table 8: Percentage of Multi-Drug Resistance among Outpatients and Inpatients

MDR	Total number (n=41) n (%)	Outpatient (n=36) n (%)	Inpatient (n=10) n (%)
3	6 (14.7)	4 (66.7)	2 (33.3)
4	6 (14.7)	4 (66.7)	2 (33.3)
5	14 (34.1)	9 (64.3)	5 (35.7)
Resistance to 6 groups	15 (36.6)	11 (73.3)	4 (26.7)

Key

- MDR- Multi-drug Resistance

Table 9: Distribution of Multi-Drug Resistance across isolates

Number of drug groups	*K. pnenmoniae* (n=17) n (%)	*K. oxytoca* (n=2) n (%)	*E. coli* (n=15) n (%)	*P. mirabilis* (n=12) n (%)	**Total (n=41) n (%)**
3	0 (0)	0 (0)	6 (40)	0 (0)	**6 (14.7)**
4	0 (0)	0 (0)	4 (26.7)	2 (16.7)	**6 (14.7)**
5	8 (47.1)	1 (50)	2 (13.3)	3 (25)	**14 (34.1)**
6	7 (41.2)	0 (0)	1(6.7)	7 (58.3)	**15 (36.6)**

Key

- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis.*

Table 10: Antibiotics resistance pattern across isolates

No. of antibio-tics	Resistant pattern	*K. pnenmoniae*	*K. oxytoca*	*E. coli*	*P. mirabilis*	**Total**
2	TE/AMP	0	0	2	0	2
	AMP/PEF	0	1	0	0	1
3	TE/PEF/AMP	0	0	2	0	2
4	PEF/NOR/AMP/ETP	0	0	1	0	1
	PEF/CN/TE/AMP	0	0	0	2	2
	PEF/CN/TE/NOR	0	0	3	0	3
5	PEF/CN/TE/AMP/MEM	0	1	0	0	1
	PEF/CN/TE/AMP/NOR	0	0	3	0	3
6	PEF/CN/TE/AMP/CAZ/CRO	1	0	0	0	1
	PEF/CN/TE/AMP/CRO/MEM	1	0	0	0	1
	PEF/CN/TE/AMP/CRO/NOR	1	0	1	0	2
7	PEF/CN/TE/AMP/CAZ/CRO/NOR	5	0	1	3	9
	PEF/CN/TE/AMP/CRO/MEM/ETP	0	0	0	1	1
8	PEF/CN/TE/AMP/CAZ/CRO/NOR/ETP	1	0	0	0	1
	PEF/CN/TE/AMP/CAZ/CRO/MEM/ETP	1	0	0	0	1
	PEF/CN/AMP/CAZ/CRO/NOR/MEM/ETP	1	0	0	0	1
	PEF/CN/TE/AMP/CAZ/CRO/NOR/ETP	1	0	1	1	3
9	PEF/CN/TE/AMP/CAZ/CRO/NOR/ETP/MEM	4	0	0	5	9

Keys

- CN=Gentamicin PEF=Pefloxacin NO=Norfloxacin TE=Tetracycline ETP=Ertapenem CAZ=Ceftazidime CRO=Ceftriaxone AMP=Ampicilin MEM=Meropenem
- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis.*

Table 11: Haemolysis test

	K. pneumoniae *n (%)*	*K. oxytoca* *n (%)*	*E. coli* *n (%)*	*P. mirabilis* *n (%)*	Total n (%)
Beta-Hemolysis	2 (11.8)	1 (50)	3 (20)	1 (8.3)	7 (15.2)
Alpha-Hemolysis	0 (0)	0 (0)	0 (0)	1 (8.3)	1 (2.2)
Gamma-Hemolysis	15 (88.2)	1 (50)	12 (80)	10 (83.4)	38 (82.6)

Key

- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis.*

Table 12: Biofilm Production

	K. pneumoniae n (%)	*K. oxytoca n (%)*	*E. coli n (%)*	*P. mirabilis n (%)*	Total n (%)
Strong Biofilm	10 (43.5)	0 (0)	11 (47.8)	2 (8.7)	23 (50)
Weak Biofilm	7 (41.2)	2 (11.8)	3 (17.6)	5 (29.4)	17 (37)
Non Biofilm	0 (0)	0 (0)	1 (16.7)	5 (83.3)	6 (13)

Key

- *E. coli- Escherichia coli, K. pneumoniae- Klebsiella pneumoniae, K. oxytoca- Klebsiella oxytoca, P. mirabilis- Proteus mirabilis*

Table 13: ESBL Production by some selected isolates across department

Patient type	Department	*E. coli* n (%)	*K. pnenmoniae* n (%)	*K. oxytoca* n (%)	*P. mirabilis* n (%)	Total
Outpatient	MED	0 (0)	1 (5.9)	0 (0)	0 (0)	1 (2.2)
	NHIS	0 (0)	1 (5.9)	0 (0)	1 (8.3)	2 (4.3)
	SOP	0 (0)	1 (5.9)	0 (0)	1 (8.3)	2 (4.3)
	OTHERS	1 (6.7)	4 (23.5)	0 (0)	3 (25)	8(17.4)
Inpatient	CW	0 (0)	2 (11.8)	0 (0)	0 (0)	2 (4.3)
	FSW	0 (0)	0 (0)	1 (50)	0 (0)	1 (2.2)
	FMW	0 (0)	0 (0)	1 (50)	0 (0)	1 (2.2)
	MMW	0 (0)	1 (5.9)	0 (0)	1 (8.3)	2 (4.3)
	PALPI	1 (6.7)	0 (0)	0 (0)	0 (0)	1 (2.2)

Keys

- SOP- surgical outpatient, NHIS- national health insurance scheme, GYNAE- gynaecology, MED- medical, HDU-high dependency unit, ANC- anti-natal care, A&E- accident and emergency, GOPD- general outpatient department, MMW- male medical ward, MSW- male surgical ward, FMW- female medical ward, FSW- female surgical ward, CW- children ward, ER- emergency room, Urology, PALPI- palpition, OHW-organisational health and wellness , ANW- anti-natal ward

Table 14: Antibiotics Resistance of isolates based on Outpatients and Ipatients

Antibiotics	*E. coli* (n=15) n (%)	*K. pnenmoniae* (n=17) n (%)	*K. oxytoca* (n=2) n (%)	*P. mirabilis* (n=12) n (%)	Total n (%)
Ertapenem	3 (20)	12 (41.2)	1 (50)	9 (75)	25 (54.3)
Outpatient	2 (5.6)	9 (25)	1 (2.8)	6 (16.7)	18 (50)
Inpatient	1 (10)	3 (30)	0 (0)	3 (30)	7 (70)
Meropenem	2 (13.3)	9 (52.9)	1 (50)	8 (66.7)	20 (43.5)
outpatient	1 (2.8)	7 (19.4)	1 (2.8)	5 (13.9)	14 (38.9)
Inpatient	1 (10)	2 (20)	0 (0)	4 (40)	7 (70)
Ceftazidime	5 (33.3)	17 (100)	0 (0)	8 (66.7)	25 (54.3)
Outpatient	4 (11.1)	14 (38.9)	0 (0)	4 (11.1)	22 (61.1)
Inpatient	1 (10)	3 (30)	0 (0)	4 (40)	8 (80)
Ceftriaxone	4 (26.7)	17 (100)	0 (0)	8 (66.7)	24 (52.2)
Outpatient	3 (8.3)	14 (38.9)	0 (0)	4 (11.1)	21 (58.3)
Inpatient	1 (10)	3 (30)	0 (0)	4 (40)	8 (80)
Gentamicin	15 (100)	17 (100)	2 (100)	12 (100)	46 (100)
Outpatient	12 (33.3)	13 (36.1)	2 (5.6)	9 (25)	36 (100)
Inpatient	3 (30)	4 (40)	0 (0)	3 (30)	10 (100)
Ampicilin	14 (93.3)	17 (100)	2 (100)	11 (91.7)	44 (95.7)
Outpatient	11 (30.6)	13 (36.1)	2 (5.6)	8 (22.2)	34 (94.4)
Inpatient	3 (30)	4 (40)	0 (0)	3 (30)	10 (100)
Tetracyclin	13 (86.7)	16 (94.1)	2 (100)	11 (91.7)	42 (91.3)
Outpatient	10 (27.8)	12 (33.3)	2 (5.6)	8 (22.2)	32 (88.9)
Inpatient	3 (30)	4 (40)	0 (0)	3 (30)	10 (100)
Norfloxacin	11 (73.3)	15 (88.2)	0 (0)	10 (83.3)	36 (78.3)
Outpatient	8 (22.2)	12 (33.3)	0 (0)	6 (16.7)	26 (72.2)
Inpatient	3 (30)	3 (30)	0 (0)	4 (40)	10 (100)
Pefloxacin	13 (86.7)	16 (94.1)	2 (100)	12 (100)	43 (93.5)
Outpatient	10 (27.8)	13 (36.1)	2 (5.6)	8 (22.2)	33 (91.7)
Inpatient	3 (30)	3 (30)	0 (0)	4 (40)	10 (100)

CHAPTER FIVE

5.1 DISCUSSION

In this study, isolation of some common Gram-negative bacterial uropathogens among outpatients and inpatients in Ekiti state University Teaching Hospital (EKSUTH) causing UTI was carried out and the determination of their antimicrobial susceptibility patterns was also investigated. Majority of urine samples tested were obtained from outpatients during the period of collection.

The occurrence of UTI and isolated pathogens with respect to the age groups and sex shows that uropathogen has the highest occurring UTI pathogen in females than in male [24(52.2%) and 22(47.8%) respectively]. This concur with the study of Agbagwa and Ifeanacho, 2015 which also reported that bacteruria was higher in females (55.1%) than in males (32.6%). The high incidence of UTI in females could be attributed to the Physiological and anatomical differences in males and females (Laupland *et al.*, 2007). With respect to anatomical differences, the female urethra and vagina are liable to trauma during sexual intercourse as well as bacteria been travels up the urethra into the bladder during pregnancy and child birth (Swetha *et al.*, 2014). More samples were obtained from the surgical oupatients department unit than in other units among inpatients and outpatients. This may be due to the use of invasive devices such as urinary catheter. The pathogen prevalence of the UTI's shows that *Klebsiella pneumoniae* had the highest frequency of 37%, followed by *E. coli* and *Proteus mirabilis* (32.6% and 26.1% respectively), the least occurring pathogen was *Klebsiella oxytoca* (4.3%) which is similar to the study done by Agbagwa and Ifeanacho, (2015) with *Klebiella pneumoniae* as the highest followed *Escherichia coli.*

Antibiotics resistance has become a major clinical problem worldwide and has increased over the years (Kaur *et al.*, 2014). Antimicrobial susceptibility patterns of the pathogens vary widely by region, patient population and type of healthcare facility (Sood and Gupta, 2012). Resistance to pefloxacin and ampicillin was seen most among the Gram-negative uropathogens. This is similar to the study of Bhuvanesh and Savitha, 2016. *Klebsiella pneumoniae* showed the highest level of multiple resistance. This organism has the ability to acquire resistance gene by mutations and commonly by transmissible plasmids (Lombardi *et al.,* 2015). Carbapenems showed the highest efficacy on uropathogens among all 9 tested antibiotics, with a susceptibility rate of 64.1% as compared to 39.1% in cephalosporin.

Multidrug resistance which is resistance to greater than two groups of antibiotics was observed among the 46 uropathogens. Forty-one (89.1%) of the uropathogens were resistant to more than two group of antibiotics. This observation is similar to the study carried out by Yeshwondm (2016) which also reported a high multidrug resistance prevalence rate (77.6%).

Biofilms are microbial communities of organisms adherent to each other and/or a target surface. Biofilm formation protects bacteria from hydrodynamic flow conditions, for example in the urinary tract, and against phagocytosis and host defence mechanisms, as well as antibiotics (Hanna *et al.*, 2003). Forty (87%) of the total isolates were biofilm producers out of which 50% were strong biofilm producers (43.5% *Klebsiella pneumoniae* and 47.8% *Escherichia coli*). Hemolysin production is associated with pathogenicity of the organism. In the present study, few isolates produced

hemolysin. One (8.3) isolate which was *Proteus mirabilis* showed complete haemolysis (Alpha haemolysis) of the 46 isolated uropathogens. Seven (15.2) showed incomplete lysis (Beta haemolysis) and 38 (82.6%) did not show any haemolysis (Gamma haemolysis). A total of 8 (17.4%) produced haemolysis which concurs with the study of Delcaru *et al.* (2017) (27%). Extended spectrum beta-lactamase which was carried out on 20 isolated uropathogen had a high prevalence of 13(65%) which is similar to the study of Nahla *et al.*, 2018 (77.6%).

5.2 CONCLUSION AND RECOMMENDATION

The relatively high prevalence of UTI among female subjects and between the reproductive age group may be due to unsafe sexual practices, unclean hygiene and or underlying immune related problems. The uropathogenss showed high levels of resistance to multiple urinary antimicrobial agents. Therefore, it is recommended that therapy should only be advocated after culture and sensitivity has been performed. It is also recommended that awareness on UTI, factors leading to UTI and prevention should be increased to reduce the high rate occurrence.

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APPENDIX

Preparing Reagents and Culture Media

Blood agar..No. 1

Contents: 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 25ºC

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 (121ºC) for 15 minutes. After allowing the sterilized medium to cool (45ºC), 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 25ºC

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 (121ºC) for 15 minutes. After allowing the sterilized medium to cool (45ºC), 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 25ºC

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 (121ºC) for 15 minutes. After allowing the sterilized medium to cool (45ºC), 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 25ºC

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.

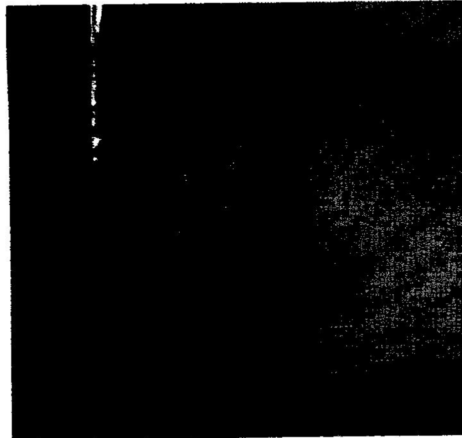

Fig., 1: Test tube showing positive and negative result to citrate test

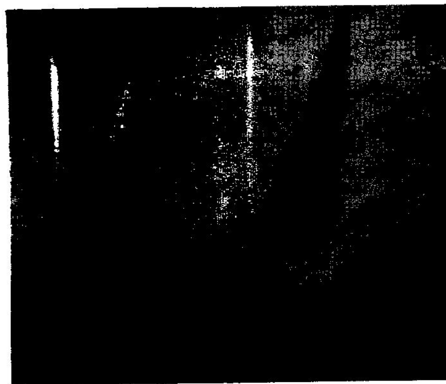

Fig., 2: Test tube showing positive and negative result for urease test

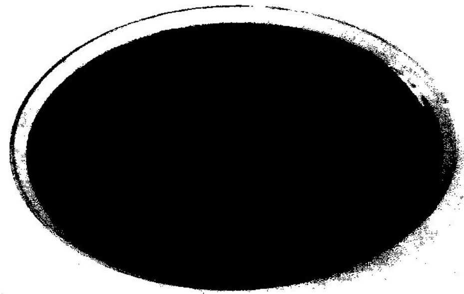

Fig., 3: Agar plate showing biofilm formation

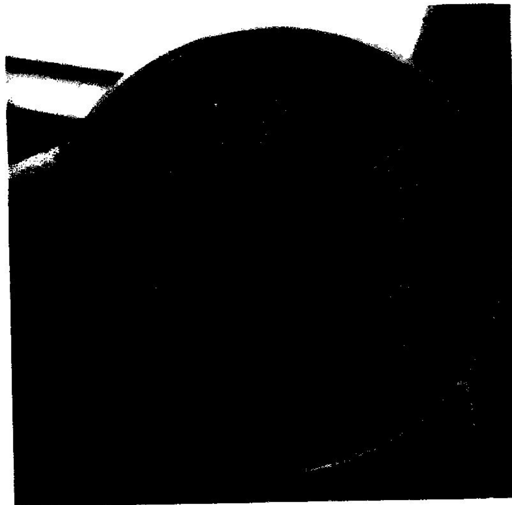

Fig., 4: Agar plate showing haemolysis of blood