TITLE PAGE

EPIDEMIOLOGY AND PATHOPHYSIOLOGICAL DETERMINANTS OF STAPHYLOCOCCAL URINARY TRACT INFECTIONS IN ENUGU STATE

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CERTIFICATION

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The result embodied in the work have not been submitted in part or full to any diploma or degree of this or any other University.

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SIGNATURE.....

DEDICATION

This work is dedicated to my children Arc. Chukwuemeka Onyebueke, Chinaza Onyebueke, Chidiogo Onyebueke and also to my late mother Mrs. Mabel Uzoamaka Izuakor, who was a source of inspiration, encouragement and financial support to me during the course of this doctorate degree research work but died on the 11th day of February 2015 when I was completing this study. She could not live to see the end of the work. May her kind and generous soul rest in perfect peace, Amen.

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ONYEBUEKE, EBERE ADAEZE

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ABSTRACT

In a study to investigate the epidemiology and pathophysiological determinants of staphylococcal urinary tract infection (UTI) in Enugu state, a total of 818 participants (290 males and 528 females) randomly selected, submitted freshly voided urine samples for laboratory investigation between 2013-2015. Candidates aged between 3-87 years comprised of apparently healthy individual (558) were selected from schools and various occupational groups and classes, while 119 pregnant women and 141 candidates with various medical conditions seen in the two tertiary hospitals serving the area of study were included. Samples were processed using standard microbiological techniques and confirmed with API identification systems (Biomerieux France). The major staphylococcal isolates were investigated for any pathological effects using albino Wistar rats. Results showed that 307 urine samples (37.5%) yielded significant bacterial growth of which 89 (10.9%) yielded staphylococcal species with *Staph aureus* 40 (44.9%) ranking highest, followed by *S. xylosus* 25 (28.1%), *S. lentus* 9 (10.1%), with *S. capre*, S. *sciuri*, *S. heamolyticus* and *S. epidermidis* yielding 3 (3.4%) each while *S. hominis*, *S. capitis* and *S. saprophyticus* ranked least with 1(1.1%) isolate each which difference was statistically significant ($P < 0.05$). Sex-wise distribution showed a female preponderance 69 (77.5%) as against their male counter parts 20 (22.5%) which difference was also statistically significant ($P < 0.05$) using fishers exact test for contingency. Age-wise distribution showed no statistically significant difference in the distribution of positive cases ($P > 0.05$) though the age group 22-32 years recorded the highest with 37 (41.6%) positives. These also apply to each of the different staphylococcal species except *S. heamolyticus* for which all 3 isolates were from males. According to occupational grouping of the study population, traders ranked highest in prevalence for staphylococcal spp UTI 18.7% followed by house wives 15.6%, tertiary students 11.5%, secondary students 9.2%, pupils 8.0%, civil servants 6.7%, with artisans 5.4% being the least which difference was statistically significant ($P < 0.05$). Of the 558 apparently healthy candidates,

60 (10.8%) were positive for staph spp UTI, pregnant women 24 (20.2%) of the 119 candidates while for candidate with different medical conditions (141), only 5 (3.5%) yielded staphylococcal organisms. Age and sex distribution for the positive staphylococcal UTI cases among the apparently healthy individuals showed a higher female positive group (40) as against their male counter parts (20) which was statistically significant (P-< 0.05), with the age group 22-32 recording the highest. For the 24 positive pregnant women, the age group 22-32 recorded the highest positive cases for staphylococcal UTI with 19 (79.2%) followed by the age group 33-43 with 4 (16.7%) while the age group 11-21 recorded only 1 (4.7%), which difference was also statistically significant (P < 0.05). Of the 141 cases sampled with different medical conditions, only 5 (3.5%) were positive for staphylococcal UTI (3.*S. aureus* and 2. *S. sciuri*) while for the apparently healthy and pregnant groups, *S.aureus S. xylosus* and S. *lentus* ranked highest. Antibiogram results showed a range of sensitivity (47.5 -72.5%) for *S. aureus,* (44 -72%) for *S. xylosus*, (44.4 -77.8%) for *S. lentus* while *S. saprophyticus* was generally resistant apart from levoflaxacin. Pathological studies using the 3 commonest *Staph* species revealed varying degree of toxicity effects for the kidney, liver and bladder with *S. aureus* showing a higher degree of toxicity especially with the kidney and effects were more significant than when compared with *S. xylosus* and *S. lentus* with effects generally increasing with increased dosing.

CHAPTER ONE

INTRODUCTION

Staphylococcus is a Greek word, Staphyle means grape while kokkos means granule. *Staphylococicus* is a genus of Gram positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters (Ryan and Ray, 2004). They are about 1mm in diameter. Single cocci, pairs, tetrads and chains are seen in liquid cultures. Young cocci stain Gram positive; on aging, many cells become Gram negative.

Staphylococci are motile and do not form spores. Under the influence of drugs, such as penicillin, staphylococci are lysed. They grow readily on many types of bacteriological media under aerobic or micro-aerobic conditions and are active metabolically, fermenting carbohydrates slowly, producing lactic acid but no gas, and producing pigments that vary from white to deep yellow. Staphylococci grow most rapidly at 37^{o}C but form pigment best at room temperature (20-25^{o}C). Colonies on solid media are round, smooth, raised and glistening. Staphylococci produce catalase which differentiates them from the streptococci (Brooks *et al*., 2013).

The genus *Staphylococcus* includes at least 40 species (Harris *et al*., 2002; Brooks *et al*., 2013). Members of the genus *Staphylococcus* frequently colonise the skin and upper

respiratory tracts of mammals and birds (Kloos, 1980). They are small components of soil microbial flora (Madigan and Matinko, 2005). *Staphylococcus aureus* occurs harmlessly as a commensal parasite in the anterior nares and moist areas of the skin in 20 ï 30 percent of healthy persons (Duguid *et al*., 2012). *Staphylococcus saprophyticus* is found in the normal flora of the female genital tract (Levinson, 2010) and perineum (Widerstrom *et al*., 2012). *Staphylococcus saprophyticus* have also been isolated from meat, cheese, vegatables, the environment and animal gastrointestinal tract (Widerstrom *et al.*, 2012). *Staphylococcus epidermidis* is part of the normal flora on the skin and mucous membrances (Levinson, 2010).

Staphylococcus aureus (a coagulase positive *Staphylococcus*) causes pyogenic (pus producing) infections and toxin-mediated diseases (Levinson, 2013) and has been reported in some literatures to cause urinary tract infections (Pragesh *et al*., 2014; Omojasola and Omojasola, 2001). Other staphylocci apart from *Staphylococcus aureus* termed coagulase negative staphylococci (CoNS) were said to lack primary pathogenicity and were reported to clinicians as albus staphylococci or *S. albus*. Generally, their presence in clinical samples is said to be ñnot clinically significant. Cogulase negative staphylococci sometimes act as opportunistic pathogens and cause infection in the urinary tract or in debilitied or immune deficient subjects and bacteremic infections (Collee *et al*., 1989).

The coagulase negative staphylococci (CoNS) are normal human microbiota and sometimes cause infections often associated with implanted devices, such as joint prostheses, shunts and intravascular catheters (medical devices) (Brooks *et al.*, 2013) e.g *S. haemolyticus* (Falcone *et al.*, 2006; Poyert *et al.*, 2001; Viale and Stefani, 2006), *S. capitis* (Van Der Zivet *et al.*, 2002; Dômello *et al.*, 2008; Iwase *et al.*, 2007). *S. epidemidis* (Levinson, 2012) especially in very young, old (Brooks *et al.*, 2013) and immunocompromised patients e.g. *S. hominis* (Brooks *et al.*, 2013).

Staphylococcus saprophyticus is a relatively common cause of UTI in young women, although it rarely causes infections in hospitalized patients (Brooks *et al.*, 2013). *Staphylococcus saprophyticus* is found primarily on the mucosa of the genital tract in young women (normal flora) and from that, it can ascend into the urinary bladder to cause

UTI (Levinson, 2012). This occurs mostly within 24 hours of sex (Levinson, 2010) earning it a nick name ñhoney moon cystitisò (WEB MD, 2013).

Other staphylococcal species that have been implicated in UTI in various studies include *S. xylosus* (Schleifer and Kloos *et al.*, 1995; Tselenis-Kotsowilis *et al.,* 1982; Al-matkhury *et al.*, 2008), *S. sciuri* (Dromigny *et al.*, 2002; Marsou *et al.,* 1999), *S. lentus* (Guirguitzova *et al.*, 2002), *S. capre* (Carretto *et al.*, 2005). Other species of staphylococci are important in veterinary medicine (Brooks *et al.*, 2013).

Urinary tract infection (UTI) can be defined as colonization of a pathogen anywhere along the urinary tract: Kidney, ureter, bladder and urethra (Chang and Shortliffe, 2006).Traditionally, UTIôs have been classified by the site of infection [ie. pyelonephritis (kidney), cystitis (bladder), urethritis (urethra)] and by the severity (i.e. complicated versus uncomplicated). Although UTI may be caused by any pathogen that colonizes the urinary tract (e.g. fungi, parasites and viruses), most causative agents are bacteria of enteric origin and the causative agents vary based on age and associated comorbidities with *E. coli* being the most frequently documented uropathogen (Chang and Shortliffe, 2006). Bacterial clonal studies strongly support entry into the urinary tract by the fecal-perineal-urethral route with subsequent retrograde ascent into the bladder (Yamamoto *et al.*, 1997).Once the uropathogen reaches the bladder, it may ascend to the urethers and then to the kidney by some yet undefined mechanism. Additional pathways of infection include nosocomial infections through instrumentation, hematogeneous seeding in the setting of systemic infection or a compromised immune system and direct extension caused by the presence of fistulae from the bowel or vagina (Chang and Shortliffe, 2006).

Predisposing factors or risk factors to UTI includes shorter urethra of women, sexual intercourse, use of diaphragm, menopause, diabetes, advanced age and conditions that affect personal care habits (such as Alzheimerôs disease and delirium), problems emptying the bladder completely, having a urinary catheter, bowel incontinence, enlarged prostrate, narrow urethra or anything that blocks the flow of urine, kidney stone, staying still (immobile) for a long period of time, pregnancy, surgery (Vorvick, 2013). Risk factors for paediatric UTI includes being a neonate/infant, gender, foreskin, fecal and perenial colonization, urinary tract anomalies, functional anomalies, immuno-

compromised states, sexual activity (Chon *et al.*, 2001), wiping from back (near the anus) to the front after going to the restroom (Vorvick, 2013), etc.

Symptoms of bladder infection (Cystitis) include: cloudy or bloody urine which may have a strong odour, low fever in some people, pain or burning with urination (dysuria), pressure or cramping in the lower abdomen or back, strong need to urinate often (frequency and urgency). Symptoms of kidney infections (pyelonephritis) may include the following in addition to the aforementioned: chills and shaking or night sweats, fatigue and general ill feeling, fever above 101 degrees fahrenheit, pain in the side, back or groin, flushed, warm or reddened skin, mental changes or confusion (in the elderly), nausea and vomiting, and very bad abdominal pain (Vorvick, 2013).

Bacteria that cause UTI in otherwise healthy hosts often exhibit distinctive properties known as virulent factors to overcome the normal defenses of the urinary system (Johnson 2003; Sussman and Gally, 1999; Bower *et al.*, 2005). This includes adhesins, often fimbrae (pili) which enhances adherence, toxins (Uhlen *et al.*, 2000; Guyer *et al.*, 2002, Toth *et al.*, 2003). Also to promote, survival, various uropathogens posses siderophore system capable of acquiring iron, an essential bacterial micronutrient from heme (Rosso *et al.*, 2001). They also have a defensive mechanism that consists of a glycosylated polysaccharide capsule that interferes with phagocytosis and complement ï mediated destruction (Russo *et al.*, 1996).

Complications of UTI includes life ï threatening blood infection (sepsis), kidney damage or scarring, kidney infection (pyelonephritis) (Vorvick,2013), renal failure (Cheesbrough, 2006), preterm babies, low birth weight and higher fetal mortality rates (Connoly and Throp, 1999), intrauterine growth retardation, maternal anemia and the chance of recurrent infection (Kladensky, 2012). Serious kidney disease due to UTI can lead to shock and death (Ochei and Kolhatkar,2000).

Literatures abound in different parts of the world which have implicated bacterial organisms as the major cause of UTI with *Escherichea coli* often as the leading cause of UTI. Sowmya and Lekshmidevi (2013) investigated the prevalence and incidence of

urinary tract infection among 1085 diabetic patients (565 males and 429 females) with signs and symptoms of UTI attending both outpatient and inpatient department in the Government hospital Mysore, Karnataka. About 900 samples were culture-positive and 936 isolates were obtained. *Escherichia coli* was the major cause for UTI in both type 1 (34%) and type 2 (32%) diabetic patients followed by methycillin-resistant *Staphylococcus aureus* (11.4% and 12.6%), *Enterobacter* spp (10.3% and 9.7%), *Klebsiella* spp (8.6% and 6.8%, S*taphylococcus aureus* (7.4% and 8.7%), *Pseudomonas aeruginosa* (6.6% and 5.8%), *Proteus mirabilis* (6.0% and 4.9%) *Citrobacter* spp (5.4% and 4.8%), *Candida* spp (3.7% and 3.9%), *Streptococcus* spp. (2.9% and 3.9%), *Enterococcus faecalis* (2.3% and 1.2%), *Staphylococcus saprophyticus* (2.0% and 1.0%) and *Serratia macescens* (1.4% and 0.4%).

In another study, Alemu *et al.* (2012) carried out a study on bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women attending University of Gonder Teaching Hospital, North West Ethiopia from March 22 to April 30, 2011 using their midstream urine samples. The overall prevalence of UTI in these pregnant women was 10.4%. The predominant bacterial pathogens were *Escherchia coli* 47.5%, followed by coagulase ï negative staphylococci 22.5%, *Staphylococcus aureus* 10% and *Klebsiella pneumonia* 10%. Significant bacteriuria was observed in asymptomatic pregnant women.The work showed that all their urinary isolates were sensitive to chloramphenicol 27(100%). Most of Gram- negative isolates were sensitive to ceftriaxon 26(96.3%), ciprofloxacin 26 (96.3%), norfloxacin 25 (92.6%), gentamycin 25 (92.6%), amoxicillin-clavulamic acid 16 (59.3%), co-trimoxazole 14 (51.9%), and tetracycline 11(40.7%). However, all isolates of Gram negative bacteria were resistant to ampicillin and amoxicillin 27 (100%). Majority of the Gram positives were resistant to most of the antibiotics tested compared to Gram negatives. Among the Gram positives, 11(84.6%), 12 (92.3%) and 12 (92.3%) of the isolates were sensitive to ceftriaxon, gentamycin and amoxicillin-clavulanic acid respectively. Coagulase nagetive staphylococci which were predominant isolates from Gram positives 9 (69.2%) were resistant to most of the antibiotics tested. The resistant patterns of the isolates were found to be 8 (88.9%) for ampicillin, 7(77.9%) for cotrimoxazole and tetracycline and 6 (66.7%) for amoxacillin

and chloramplenced. Gentamycin and amoxicillinï clavulinic acid were found to be effective against 8 (88.9%) coagulase negative staphylococci.

Theodore (2006) conducted a study on the prevalence and antibiogram of urinary tract infection among prison inmates in Nigeria using 181 urine samples of prison inmates attending University of Nigeria Teaching Hospital Bacteriological Laboratory Centre from July 30 to August, 2005. Of the number, 141 (77.9%) gave significant bacteruria. *Escherichia coli* was mostly isolated with a frequency of 47 (33.3%) followed by *Klebsiella pneumoniae* 28(19.9%), *Staphylococcus aureus* 21(14.9%), *Proteus mirabilis* 21 (14.9%), *Citrobacter freundi* 10 (7.1%), *Staphylococcus epidermidis* 7(5.0%), *Streptococcus faecalis* 5(3.5%) and *Pseudomonas aeruginosa* 2 (1.4%). Gram positive isolates *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus faecalis* were mostly sensitive to erythromycin, chloramphenicol and gentamycin. These isolates were resistant to the following antibiotics as follows: ciprofloxacin (cf), ampicillin (amp), nalidixic acid (nal) and tetracycline (tet). The Gram negative isolates were mainly sensitive to ciprofloxacin (cf), gentamycin (gen) and nalidixic acid (nal). The in vitro sensitive testing shows that all Gram negative isolates were senstivie to ciprofloxacin (cf). Erythromycin and chloramphenicol were effective against the Gram positive organisms. Very high proportions of the organisms (Gram positive and Gram negative) were resistant to the antibiotics: tetracyclin, ampicillin, chloramphenicol and nalidixic acid.

Staphylococci possess some virulence factors that enable it to establish infection such as polysaccharides and proteins, ability to multiply and spread widely in tissues and through their production of many extracellular substances, their variability too many antimcrobial drugs and development of resistance to them. *Staphylococcus aureus* produces the enzyme coagulase which is usually associated with virulence as well as other enzymes and toxins that confer on it further virulence and ability to cause a lot of human diseases.

The clinical feature (signs and symptoms) of staphylococcal UTI is almost same as those for general UTI for urethritis, cystitis, and pyelonephritis. In addition, urinary tract infection caused by *S. saprophyticus* usually present with symptomatic cystitis and symptoms include burning sensation when passing urine, the urge to urinate quickly (urgency) more often than normal (frequency), a dripping effect after urination, weak

bladder, a bloated feeling with sharp razor pains in the lower abdomen around the bladder and ovary areas and razor-like pains during sexual intercourse (Jordan *et al.*, 1980).

Literatures exist that showed staphylococcal species as causes of UTI and *Staphylococcus aureus* as a leading cause of UTI contrary to the popular opinion that *Escherichia coli* is always the leading cause of UTI. Otajevwo and Eriagbo (2014) conducted a study on asymptomatic UTI infection occurence among students of Western Delta University, Oghara, Nigeria. Urine samples were collected from 291 students of the University and examined for UTI (both symptomatic and asymptomatic). Of this number, 225 (77.3%) yielded bacterial growth. *Staphylococcus aureus* had the highest number of isolates (34.8%), followed by *Escherichia coli* (24.4%), *Klebsiella aerogenes* (13.9%), *Candida albicans* (7.8%), coliform organisms (6.1%), *Proteus* spp (4.4%), *Enterobacter* spp (4.4%), *Serratia* spp (1.7%), *Pseudomonas aeruginosa* (1.7%) and *Providencia* spp 0.9%.

In another study on urinary tract infection among adult subjects in Ilorin metropolis, Nigeria conducted from June, 1999 to June 2000 by Omojasola and Omojasola (2001), 167 early morning midstream urine sample were collected from the subjects (84 males and 83 females) and examined for UTI. Of this number, 139(83.2%) were positive for UTI. *Staphylococcus aureus* had the highest number of isolates 73(52.5%) followed by *Escherichia coli* 39(28.1%), *Klebsiella* spp 7(5.0%), *Pseudomonas aeruginosa* 7(5.0%), *Proteus* spp 6(4.3%), faecal streptococci 4(2.9%) and a mixed culture of *S. aureus* and faecal coliform 3(2.2%).

Staphylococcus aureus has also been isolated from UTI in several other studies in addition to the ones previously shown for UTI in this study. From general UTI (Oladeinde *et al*., 2011 at Okada town, Ovia East Local Government Area, Edo State Nigeria), from pregnant women (Hamdan *et al.,* 2011 at Khartoun North Hospital, Khartoun, Sudan; Obiogbolu *et al.,* 2009 at Awka metropolis South Eastern Nigeria), from females (Humyan and Igbal, 2012 at Sharif Medical City Hospital, Lahore), from children (Anigilaje and Bitto, 2013 at Federal Medical Centre, Markudi, Nigeria), from diabetic patients (Pragash *et al.,* 2014) at rural South India, (Samuel *et al.*, 2014) at Ago-Iwoye Ogun State, Nigeria), from catherized patients (Abaeze and Abasiama, 2011) at Federal Medical Centre, Abeokuta, Nigeria).

Other staphylococcal species have also been isolated in UTI and these include; *Staphylococcus saprophyticus* (Lo *et al*., 2013 at Teaching Hospital of University of Sao Paulo; Orhue, 2014 at Univeirtsy Teaching Hospital, Benin City, Nigeria), *S. epidermides* (Obiogbolu *et al.,* 2009 at Awka metropolis, South Eastern Nigeria), *S. xylosus* (Alrmethkhury *et al*., 2008 at Baghad), *S. heamolyticus* (Simago, 2005 at Harare Zimbabwe), *S. sciuri* (Dromigny *et al*., 2002 at Dakar Senegal), *S. lentus* (Guirgiutzova *et al*., 2002) cited in Stepanovic *et al.,* 2003 at Pribram, Czech republic), *S. capre* (Carreto *et al.*, 2015).

JUSTIFICATION

Reports from laboratories may largely be based on coagulase test to acknowledge only the aetiological role of *S. aureus* while other staphylococcal species are usually ignored as not relvant to the UTI. Debility and technological development and advancement have opened routes to invasion by the hitherto ignored CoNS thought to be incapable of establishing an infectious process because they lack the coagulase enzyme long assumed to be major virulence factor for the staphylococcal group. Today, implanted devices such as various types of prosthesis, shunts, and catheters have opened a way to the involment of CoNS as serious agents of UTI being compounded by debilitating conditions and long hospital stay. Many have evolved armories that assets their invasion which include different attachment and adhesion factors by which they can firmly attach onto plastic prosthetic devices. Complications of UTI include life threatening blood infection (sepsis), kidney damage or scarring, kidney infection (pyelonephritis), renal failure. Pregnancy enhances the progression from asymptomatic bacteriuria to symptomatic bacteriuria if untreated which could lead to pyelonephritis, and adverse obstetric outcomes such as premature babies, low birth weight and higher mortality rates, intrauterine growth retardation, maternal anaemia and the chance of recurrent infection. Serious kidney disease due to UTI can lead to shock and death. The exact epidemiology,

risk factors and pathological effects of staphylococcal spectrum of UTI is largely unknown especially in this part of the world. The few literature existing are scanty and inconsistent, hence creating a gap for the study. This therefore deserves closer research emphasis with a view of curtailing its adverse health effects on the populace.

AIM

The aim of the study was to determine the aetiological spectrum of staphylococcal species in cases of asymptomatic urinary tract infection in parts of Enugu state and also study the pathophysiological effects on animal models.

SPECIFIC OBJECTIVES:

1. To isolate staphylococcal species involved in significant bacteriuria in Enugu in the apparently healthy population.
2. To determine other bacterial agents involved in significant bacteriuria.
3. To monitor same in those that showed signs and symptoms suggestive of UTI.
4. To delineate their prevalences in specific groupings (pregnant women premenopausal women, menopausal women, candidates with medical conditions e.g diabetes mellitus hypertension and different occupational groups).
5. To carryout detailed antibiogram on isolates using commonly used antibiotics in the areas of study.
6. To determine any pathological features using experimental animal models for the commonest species of staphylococcal isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 DEFINITION OF *Staphylococcus* AND MORPHOLOGY

The word *Staphylococcus* is from Greek work. Staphle ñgrapeò and kokkoôs ñgranuleò. It is a genus of Gram positive bacteria. Under the microscope they appear round (cocci) and form in grape ï like clusters (Ryan and Ray, 2004). Staphylococci are therefore Gram-positive spherical cells usually arranged in grape-like clusters (Brooks *et al.*, 2013). Staphylococci are about 1mm in diameter. Single cocci, pairs, tetrads and chains are seen in liquid cultures. Young cocci stain Gram positive; on aging, many cells become Gram negative. Staphylococci are mobile and do not form spores. Under the influence of drugs such as penicillin, staphylococci are lysed. They grow readily on many types of bacteriological media under aerobic or microaerobic conditions and are active metabolically, fermenting carbohydrates slowly, producing lactic acid but no gas and producing pigments that vary from white to deep yellow. Staphylococci grow most rapidly at 37^{o}Cbut form pigments best at room temperature (20-25^{o}C). Colonies on solid media are round, smooth, raised and glistening. *Staphylococcus aureus* usually forms gray to deep golden yellow colonies. *Staphylococcus epidermidis* colonies usually are gray to white on primary isolation. Many colonies develop pigment only upon prolonged

incubation. No pigment is produced anaerobically or in both. The staphylococci produce catalase which differentiates them from the streptococci. Protelytic activity varies greatly from one strain to another and pathogenic staphylococci produce many extracellular substances. Staphylococci are relatively resistant to drying, heat (they withstand 50^{o}c for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemical (e.g. 3% hexachlorophene) (Brooks *et al.*, 2013).

2.2 CLASSIFICATION OF *Staphylococcus*

The genus *Staphylococcus* includes at least 40 species (Harris *et al.*, 2002; Brooks *et al.*, 2013). Of these, nine have two subspecies and one has three sub species (Harries *et al.*, 2002). The four most frequently encountered species of clinical importance are *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdenensis* and *Staphylococcus saprophyticus* (Brooks *et. al.*, 2013)

Scientific classification of *staphylococcus*

Kingdom	-	Bacteria
Phylum	-	Firmicutes
Class	-	Bacilli
Order	-	Bacillales
Family	-	Staphylococcaceae
Genus	-	*Staphylococcus*
Species	-	*S. arlettae, S. agnetis, S. aureus, S. auricularis, S. capitis, S. caprae, S. carnosus, S. caseolyticeus, S. chromogenes, S. cohnii, S. condiment, S. delphini, S. devriesei, S. epidermidis, S. equorum, S. felis, S. fleurettii, S. gallinarum, S. haemolyticus, S. hominis, S.*

hyicus, S. intermedius, S. kloosii, S. leei, S. lentus,S. lugdenensis, S. lutrae, S.massiliensis, S. microti, S. muscae, S. nepalensis, S. pasteuri, S. pettenkoferi, S.piscifermentans, S.pseudintermedius, S.pseudolugdunensis, S. pulvereri, S. rostra, S.saccharolyticus, S. saprophyticus, S. schleiferi, S. sciuri, S. simiae, S. simulans, S. stepanovicci, S. succinus, S. vitulinus, S. warneri, S. xylosus

The taxonomy is based on 16s rRNA sequences (Madigan and Martinko, 2005) and most of the staphylococcal species fall into 11 clusters as follows:

(1) *S. aureus* group ï *S. aureus, S. simiae*

(2) *S. auricularis* group ï *S. auricularis*

(3) *S. carnosus* group ï *S. carnosus,*
S. condimenti, S. massilensis, S. piscifermentaus, S. simulans

(4) *S. epidermidis* group - *S. capitis, S. capre, S. epidermidis, S. saccharolyticus.*

(5) *S. haemolyticus* group ï *S. devriesei, S. haemolyticus, S. hominis*

(6) *S. hyicus* – intermedius group - *S. chromogenes, S. felis, S.delphni, S. hyicus, S.intermedius, S. lutrae, S. microti, S. musae, S. pseudintermedius, S. rostri, S. schleiferi.*

(7) *S. lugdunensis* group ï *S. lugdunensis*

(8) *S. saprophyticus* group ï *S. artettae, S. cohnii, S. equorum, S. gallinarum, S. kloosii, S. leei, S. nepalensis, S. saprophyticus, S. scuccinus, S. xylosus.*

(9) *S. sciuri* group ï *S. flauretti, S. lentus, S. sciuri, S. stepanovicci, S. vitulinus*

(10) *S. simulans* group ï *S. simulans*

(11) *S. warneri* group ï *S. pasteuri, S. warneri.*

A twelfth group ï that of *S. caseolyticus* ï has now been moved to a new genus Micrococcus, the species of which are currently closest known relatives of the staphylococci (Kloos *et al.*, 1998).

Subspecies of the *Staphylococcus* species

S. aureus sub sp. *aureus*

S. aureus sub sp. *anaerobius*

S. capitis sub sp. *capitis*

S. capitis sub sp. *urealyticus*

S. carnosus sub sp. *carnosus*

S. carnosus sub sp. *utilis*

S. cohnii sub sp. *cohnii*

S. cohnii sub sp. *urealyticus*

S. equorum sub sp. *equorum*

S. equorum sub sp. *linens*

S. hominis sub sp. *hominis*

S. homnis sub sp. *novobiosepticus*

S. saprophyticus sub sp. *bovis*

S. saprophyticus sub sp. *saprophyticus*

S. schleiferi sub sp. *coagulans*

S. schleiferi sub sp. *schleiferi*

S. sciuri sub sp. *caranticu*

S. sciuri sub sp. *rodentum*

S. sciuri sub sp. *sciuri*

S. succinus sub sp. *casei*

S. succinus sub sp. *succinus*

2.3 SOURCE OF *Staphylococcus* (HABITAT)

Members of the genus *Staphylococcus* frequently colonize the skin and upper respiratory tracts of mammals and birds (Kloss, 1980). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are small component of soil microbial flora (Madigan and Martinko, 2005).

Staphylococcus aureus occurs harmlessly as a commensal parasite in the anterior nares and on moist areas of the skin in 20 to 30 percent of healthy persons (Carriers) (Duguid *et al.*, 1978). *Staphylococcus saprophyticus* is found in the normal flora of the female genital tract (Levinson, 2010) and perineum (Widerstrom *et al.*, 2012). It has been isolated from other sources too including meat and cheese products, vegetables, the environment and human and animal gastrointestinal tracts (Widerstrom *et al.*, 2012). *Staphylococcus epidermidis* is part of the normal flora on the skin and mucous membranes (Levinson, 2010).

Host Range: Some species specificity has been observed in host range, such that the *Staphylococcus* species observed on some animals appear more rarely on more distantly related host species (Kloss, 1980). Some of the observed host specificity includes: *S. artalla* – *Chickens, goats, S. auricularis* – deer, dogs, humans, *S. capitis* ï humans, *S. caprae* ï goats, humans, *S. cohnii* ï Chickens, humans, *S. delphini* ï dolphins, *S. devriesei* ï Cattle, *S. epidermidis* ï humans, *S. equorum* ï horses, *S. felix* ï cats, *S. flaureth* - goats, *S. gallinarum* - Chicken, goat, pheasants, *S. haemolyticus* ï humans, cerococebus, eythrocebus, lemur, macca, microcebus, pan, *S. hyicus* ï pigs, *S. leei* ï Humans, *S. lentus* – goats, rabbits, sheep, *S. lugdunensis* ï humans, goats, *S. lutrae* ï others, *S. microti* ï volves (Microtus arvalis), *S. nepalensis* ï goats, *S. pasteuri* ï humans, goats, *S. koferi* – humans, *S. pseudintermiduis* ï dogs, *S. rotri* - pigs, *S. schleiferi* ï humans, dogs, goats, *S. simiae* ï South American squirrel, monkeys (*saimiri sciureus*)*S. simulans* ï humans, *S. warneri* ï humans, cercopithecoidea pongidae, *S. xylosus* ï humans, (Kloss, 1980).

2.4 DISEASES CAUSED BY *STAPHYLOCOCCUS*

Staphylococcus genus is important because it includes the common and versatile pathogenic species *Staphylococcus aureus* which causes a wide range of different kinds of major and minor pyogenic infections as well as toxin-mediated diseases. Other staphylococci apart from *S. aureus,* were said to lack primary pathogenicity and were reported to the physician as óalbus staphylocociô or *'S. albus'*. Of these, *S. epidermidis* and *S. saprophyticus* are commonly present and other species less commonly present as commensals on the body surfaces. They are thus often found as contaminants in clinical

specimens e.g. in Swabs from the skin, nose, throat, wounds, burns and bed sores. Generally, their presence is said to be ñnot clinically significantò but they sometimes act as opportunistic pathogens and cause infection in the urinary tract or in debilitated or immune-deficient subjects, more serious, bacteriaemic infections (Collee *et al*., 1989).

2.4.1 Staphylococcus aureus

Staphylococcus aureus is coagulase-positive (i.e produces the enzymes coagulase) which differentiates it from other species of *Staphylococcus* which are coagulase negative (CoNS). The important clinical manifestations caused by *S. aureus* can be divided into two groups: pyogenic (pus producing) and toxin-mediated. *S. aureus* is a major cause of skin, soft tissue, bone, joint, lung, heart and kidney infections.

Pyogenic diseases caused by *S. aureus* includes: Skin infections which are very common and includes impetigo, furuncles, carbuncles, paronychia, cellulitis, folliculitis, hydradenitis suppurativa, conjuctivitis, eyelid infections (Blepharitis and hordeolum), and post partum breast infections (mastitis), lymphangitis. Other pyogenic diseases of *S. aureus* are: Septicaemia, endocarditis, osteomylitis, post surgical wound infections, pneumonia, conjunctivitis, and abcesses. *Staphylococcus aureus* toxin-mediated diseases includes: food poisoning (gastroenteritis), toxic shock syndrome, scalded skin syndrome, (Levinson, 2012).

Staphylococcus aureus causes Kawasaki syndrome (Ks), a disease of unknown etiology that is discussed here because several of its features resemble toxic shock syndrome caused by the super antigen of *S. aureus* (and Strept pyogenes). Ks is a vasculitis involving small and medium size arteries, especially coronary arteries (Levinson, 2012). *Staphylococcus aureus* have also been implicated as a cause of UTI in several literatures (Omojasola and Omojasola, 2001; Pragesh *et al.*, 2014).

2.4.2 *Staphylococcus epidermidis*

Is a coagulase-negative staphylococci. *Staphylococcus epidermidis* infections are almost always hospital ï acquired. *Staphylococcus epidermidis* is part of the normal human flora on the skin and mucous membranes but can enter the blood stream (bacteremia) and cause

metastatic infections especially at the site of implants. It commonly infects intravenous catheters and prostatic implants e.g. prosthetic heart valves (Endocarditis), vascular grafts and prosthetic joints (arthritis or osteomyelitis).*Staphylococcus epidermidis* is also a major cause of sepsis in neonates and of peritonitis. In patients with renal failure who are under going peritional dialysis through an undwelling catheter, it is the most common bacterium to cause cerebrospinal fliud shunt infections (Levinson, 2012). *Staphylococcus epidermidis* have also been isolated from urine samples in signficant numbers and documented in some literatures as a cause of UTI (Obiogbolu *et al*., 2009).

2.4.3 *Staphylococcus saprophyticus*

Staphylococcus saprophyticus is a coagulase-negative *Staphylococcus* which causes UTI (Levinson, 2012) especially in young women (Brooks *et al.*, 2013). It causes 10 - 20% of UTI in females 17 ï 27 years old and it is the second most common cause of community acquired UTI, after *Escherichia coli* (Rupp *et al.,* 1992). *Staphylococcus saprophyticus* is associated with cystits, pyelonephritis and acute urethritis in young women (Ochei and Kolhatkar, 2000). Cheesbrough (2000) also stated that *Staphylococcus saprophyticus* causes UTI in sexually-active women.

2.4.4 *Staphylococcus xylosus*

Staphylococcus xylosus is a coagulase-negative *Staphylococcus*. *Staphylococcus xylosus* has very occasionally been identified as a cause of human infection. It has been associated with the following conditions: nasal dermatitis in gerbils, pyelonephritis in humans, avian staphylococcosis, bovine intermammary infection, (Schleifer and Kloos, 1975).Tselenis-Kotsowilis *et al.* (1982) here also reported *S. xylosus* as a cause of pyelonephritis in humans. Kuboskova (2004) has also isolated *S. xylosus* from urinary tract infection. *Staphylococcus xylosus* may cause dermatits, UTI, bacteremia.

2.4.5 *Staphylococcus Scuiri*

Staphylococcus sciuri is a coagulase-negative *Staphylococcus*. *Staphylococcus sciuri* has been associated with serious infections in human such as endocarditis (Hedin, 1998), peritonitis (Wallet, 2000), septic shock (Horri *et al.*, 2001) and wound infections (Stepanovic *et al.*, 2002). Several investigators have reported isolation of *S. sciuri* from Urine (Dromigny *et al.*, 2002; Guirguitzova *et al*., 2002; Marsour *et al.,* 1999).

2.4.6 Staphylococcus lentus

Staphylococcus lentus is coagulase-negative. *Staphylococcus* lentus has also been isolated from urine (Guirgutzova *et al.,* 2002).

2.4.7 *Staphylococcus haemolyticus*

Staphylococcus haemolyticus is a coagulase-negative staphylococci (Devos *et al.,* 2009). It is part of the skin flora of humans (de Silva *et al.,* 2002). *Staphylococcus haemolyticus* is a well known opportunistic pathogen and is the second most frequently isolated CoNS (*S. epidermidis* is the first) (de Allor *et al.*, 2006). Infections can be localized or systematic and are often associated with medical devices (Falcone *et al.;* 2006; Poyert *et al.,* 2001; Viale and Stefani, 2006). *Staphylococcus haemolyticus* is considered an important nosocomial pathogen (Vignaroli *et al.*, 2006). Human infections include: native value endocarditis, septicemia, peritonitis and urinary tract, wound, bone and joint infections (de Silva *et al*., 2002; Fischetti *et al*., 2000; de Allori *et al.*, 2006; Flahaut *et al*., 2008). Infrequent soft ï tissue infections usually occur in immunocompromised patients (Rolston and Bodey, 2003). Like other CoNS, *S. haemolyticus* is often associated with the insertion of foreign bodies, such as prosthetic valves, cerebrospinal fluid shunts, orthopedic prostheses and intravascular, urinary and dialysis catheters, (Falcon *et al.*, 2006; Poyert *et al.*, 2001; Viale and Stefani, 2006). *Staphylococcus* haemolyticus is multi- drug resistant (Froggatt *et al.,* 1989) and able to form biofilms which makes infections especially difficult to treat (Klingenberg *et al.*, 2007). *Staphylococcus haemolyticus* has the highest level of antibiotic resistance among the CoNS (Fredheim *et al.,* 2009).

2.4.8 ***Staphylococcus hominis:*** Is coagulase-negative staphylococci. It occurs as harmless commensal on human and animal skin but may occasionally cause infection in patients whose immune system are compromised, for example by chemotherapy or predisposing illness.

2.4.9 ***Staphylococcus capre:*** Is a coagulase-negative staphylococci. It was originally isolated from goats but members of this species have also been isolated from human samples. *S. capre* occurs as a commensal on human skin but has also been implicated in infection of the blood steam, urinary tract, bones and joints (Carretto *et al.,* 2005).

2.4.10 ***Staphylococcus capitis:*** Is a coagulase-negative species (CoNS) of *Staphylococcus*. It is part of the normal flora of the skin of the human scalp, face, neck and ears and has been associated with prosthetic valve endocarditis but rarely associated with native valve infection.

2.5 URINARY TRACT INFECTION

2.5.1 The Urinary Tract

The urinary tract (or system) is a system in the body for removing waste and extra water. It consist of the bladder, the kidneys, the ureters and the urethra. The kidneys filter the blood and remove waste and extra water which becomes a component of urine.

Urine passes from the kidneys through the ureters and is stored in bladder until it is ready to be passed through the urethra. The opening of the urethra is the end of the penis in males and at the front of the vagina in females. The urinary tract can be divided into the upper urinary tract and the lower urinary tract. The upper urinary tract consists of the kidneys and the ureters and the lower urinary tract consists of bladder and the urethra.

2.5.2 Definition of Urinary Tract Infection

Urinary tract infection (UTI) is the microbial invasion of any of the tissues of the urinary tract extending from the renal context to the urethral meatus (Otajevwo and Eriabor, 2014). It can also be defined as the colonization of a pathogen any where along the urinary tract: kidney, ureter, bladder and urethra. Traditionally, UTIôs have been

classified by the site of infection (ie pyelonephritis (kidney), Cystitis (bladder), Urethritis (Urethra), and by the severity (ie complicated versus uncomplicated (Chang and shortliffe, 2006). A complicated UTI describes infections in urinary tract with structural or functional abnormalities or the presence of foreign objects, such as an indwelling catheter.

2.5.3 Causes of UTI

Although UTI may be caused by any pathogen that colonized the urinary tract (e.g fungi, parasite and viruses), in most cases agents are bacteria of enteric origin and the causative agents varies based on age and associated comorbidites with *E. coli* being the most frequently documented uropathogen (Chang and shortliffe, 2006). *Escherichia coli* is said to be the commonest urinary pathogen causing 60-90% of UTI (Cheesbrough, 2006). The urinary pathogens include the followings:

- Gram-negative rods (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Citrobacter* spp, *Enterobacter cloacae*, *Morgenella morganii*, *Proteus mirabilis*, *Providentia stuartii*, *Serratia* spp)
- Gram negative cocci (*Neisseria gonorrhoea*)
- Gram positive cocci (*Enterococuss* spp), *Streptococcus* group B, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus* group D, *Streptococcus faecalis*).
- Other pathogens (*Candida* spp, *Chlamydia trachomatis*, *Adenovirus*) (data from Chon *et al.*, 2001 cited in Chang and Shortly, 2006).

2.5.4 Mode of Entry

Bacterial clonal studies strongly support entry into urinary tract by the fecal- perineal-Urethral route with subsequent retrograde ascent into bladder (Yamamoto *et al.*, 1997). Because of differences in anatomy, girls are at a higher risk of UTI than boys beyond the first year of life. In girls, the moist periurethral and vaginal areas promote the growth of uropathogens. The shorter urethral length increases the chance for ascending infection into the urinary tract. Once the uropathogen reaches the bladder, it may ascend to the ureters and then to the kidneys by some as yet undefined mechanism. Additional

pathways of infection include nosocomial infection through instrumentation, hematogenous seeding into the setting of systematic infection or a compromised immune system and direct extension caused by the presence of fistulae from the bowel or vagina. (Chang and Shortliffe, 2006). The urinary tract is a closed, normally sterile space lined with mucosa composed of epithelium known as transitional cells. The main defence mechanism against UTI is constant antegrade flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra. This wash out effect of the urinary flow usually clears the urinary tract of pathogens (Cox and Hinman, 1961). The urine itself also has specific antimicrobial characteristics including low urine pH, polymorphonuclear cells, and tammhorse fall glycoprotein which inhibits bacterial adherence to the bladder mucosal wall (Sobel, 1997). UTI occurs when the introduction of pathogens into the space is associated with adherence to the mucosa of the urinary tract. If uropathogens are cleared inadequately by the washout effect of voiding, then microbial colonization potentially develops. Colonization may be followed by microbial multiplication and associated inflammatory response (Chang and Shortliffe, 2006)

2.5.5 Predisposing Factors to UTI

Many factors can put someone at risk of having UTI. They include:

- Obstructions: Blockage that makes it difficult to empty the bladder can cause a UTI. Obstruction can be caused by an enlarged prostate, kidney stones and certain forms of cancer.
- Gender: Woman are more likely to get UTIs. This is because their Urethras are shorter. Urinary Tract Infection in men are less common and more serious.
- Sexual activity: Pressure on the urinary tract during sex can move bacteria from the colon into the bladder.
- Bathroom hygiene: Wiping from back to front after going to the bathroom can lead to UTI. This motion drags bacteria from the rectal area towards the Urethra.
- Spermicides: Spermicides can increase UTI risk. They may cause skin irritation in some women. This increases the risk of bacteria entering into the bladder.
- Condons, diaphragms, diabetes, loss of estrogen after menopause, prolonged use of bladder catheters (Lights and Boskey, 2012).

- Poor personal care, pregnancy, procedures involving the urinary tract, suppressed immune system, and immobility for long period (Mclntosh, 2014). Risk factors for pediatric urinary tract infection includes: A neonate/infant, gender, foreskin, faecal and perenial colonization, urinary tract anomalies, functional anomalies, Immunocompromised states, sexual activity (Chon *et al.*, 2001).

2.5.6 Symptoms of UTI

The symptoms of a UTI can depend on the age, gender, the presence of a catheter and what part of the urinary tract has been infected (Mclntosh, 2014).

Symptoms of a bladder infection (cystitis) include: Cloudy or bloody urine which may have a foul or strong odour, low fever in some people, pain or burning with urination (dysuria), pressure or cramping in the lower abdomen or back, strong need to urinate often (frequency) and urgency. Symptoms of kidney infection (pyelonephritis) may include the following in addition to the aforementioned; chills and shaking or nights sweats, fatigue and a general ill feeling, fever above I01 degrees Fahrenheit, pain in side, back or groin, flushed, warm or reddened skin, mental changes or confusion (in the elderly, these symptoms often are the only sign of a UTI), nausea and vomiting, very bad abdominal pain sometimes (Vorvick, 2013).

According to Cheesbrough (2006), symptoms of cystitis include frequency, dysuria (pain on passing urine), suprapubic pain, sometimes haematuria and usually pyuria (increased number of puscells in urine). There could be acute urethral syndromes (dysuria-pyuria) during acute cystitis, where there is pyuria but no bacteria are detected by routine culture and if infection reaches the kidney (pyelonephritis), it causes loin pain, pyuria, rigors, fever and often bacteremia).

2.5.7 Virulence Factors of UTI Organisms

Bacteria that cause UTI in otherwise healthy hosts, often exhibit distinctive properties-known as virulence factors to overcome the normal defenses of the urinary system (Johnson 2003; Sussman and Gally, 1999; Bower *et al.*, 2005). In serotypes of *E.coli* frequently isolated in UTI, bacterial adherence to the uroepithelium is enhanced by adhesins often fimbrae (pili), which bind to specific receptors of the uroepithelium

(Sussman and Gally, 1999; Bower *et al.*, 2005; Wullt *et al.*, 2000). The interaction of fimbrae with the mucosal receptor triggers internalization of the bacterium into the epithelial cells, which leads to apoptosis, hyperinfection and invasion into surrounding epithelial cells or establishment of a bacterial focus for recurrent UTI (Bower *et al.*, 2005; *Wullt et al.*, 2000; Mulvey *et al.*, 2000). Uropathogenic strains of *E.coli* have been recognized to release toxins including cytolethal distending toxin, alpha hemolysin, cytotoxic necrotizing factor-1 , secreted auto transporter toxin that causes cellular lysis, cause cell cycle arrest and promote changes in cellular morphology and function (Uhlen *et al*., 2003; Guyer *et al.*, 2002; Toth *et al.*, 2003). To promote survival, various uropathogens possess siderophore systems capable of acquiring iron an essential bacterial micronutrient from heme (Russo *et al*., 2001). Uropathogenic strains of *E.coli* have a defensive mechanism that consists of a glycosylated polysaccharide capsule that interfers with phagocytosis and complement mediated destruction (Russo *et al.,* 1996).
Cheesbrough (2006), also stated that some strains of *E.coli* are more invasive e.g capsulated stains of *E.coli* are able to resist phagocytosis, other strains are more adhesive.

2.5.8 Complications of UTI

This includes life- threatening blood infection (Sepsis), kidney damage or scaring, kidney infection (pyelonephritis) (Vorvic, 2013), renal failure (Cheesbrough, 2006). Pregnancy enhances the progression from asymptomatic bacteriuria to symptomatic bacteriuria if untreated which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low birth weight and higher fetal mortality rates (Connoly and Throp, 1999), intrauterine or recurrent infection (Kladensky, 2012). Urinary tract infection due to *S.xylosus* can cause pyelonephritis (Tselenis-Kotsowilis *et al.*, 1982).
Ochci and Kolhatkar (2000), stated that UTI range from asymptomatic infections to serious kidney diseases which can lead to shock and death.

2.5.9 Prevalence of UTI World Wide

Literatures abound in different parts of the world which have implicated bacterial organisms as the major causes of UTI with *Escherichia coli* often as the leading cause of

UTI. This has been observed in persons of diverse ages in both sexes with signs and symptoms of UTI and in asymptomatic cases of UTI, catheterized patients, immunosuppressed patients e.g diabetic patients, pregnant woman etc.

A study on the culture and sensitivity pattern of urinary tract infections in females of reproductive age group was conducted at the microbiology dept Sharif Medical City, Lahore, India between June 2009 to June 2010. A total of 181 mid steam urine specimens were collected from suspected urinary tract infected cases of women of reproductive age group from out door and in door female patients. The patients included were between the ages of 15-50 years who came through out patient department or emergency and whose routine urine examination revealed numerous pus cells on microscopy. Of the181 urine samples examined, 70 (38.6%) were found to have significant bacteriuria. Out of the 70 Isolated pathogens, the most common Isolate was *Escherichia coli* (70%), followed by *Klebsiella pneumoniae* (14%), *Streptococcus faecalis* (5.7%), *Acinetobacter* (4.2%), *Staphylococcus aureus* (2.8%), *Candida* (1.4%), *Pseudomonas* (1.4%) and *Proteus* (1.4%) (Humayun and Igbal, 2012).

A study carried out by Mahesh *et al.* (2011) aimed at finding out the common presenting symptomatology and risk factors associated with UTI and distribution of isolated uropathogen and their resistant pattern among the elderly patients aged 65 years and above who were admitted or visited the out patient department in a tertiary care centre in Bangalore, India from January to December 2008. One urine sample was collected from each patient and examined. Overall, 194 subjects were included in the study (116 males and 78 females). Result showed that *Extended* Spectrum Beta-Lactamase (ESBL) positive E. coli was the most frequently isolated pathogen for UTI in their study (47.94%) followed by extended spectrum bêtalactamase (ESBL) negative *E. coli* (23.2%), ESBL Positive *Klebsiella* and *Pseudomonas* spp (8.25%), ESBL negative *Klebsiella* (5.15%), *Citrobacter freundi* (3.09%), *Enterococcus faecalis* (2.58%), *Enterobacter* spp (1.03%), *Morganelle* (0.52%).

Sowmya and Lakshmidevi, (2013) also investigated the prevalence and incidence of urinary tract infection among diabetic patients in Mysore, Karnataka. The study was conducted for a period of two years from March 2011 to February 2013. The age range

of the study participants was from 20 to 65 years and above. Overall 1085 urine samples from 429 female and 565 males were collected from diabetic patients with signs and symptoms of urinary tract infection attending both out patient and inpatient department in the government hospital, Mysore, Karnataka. About 900 samples were culture positive (330 males and 570 females) and 936 isolates were obtained. *Escherichia coli* was the major cause for UTI in both types 1 (34%) and type 2 (32%) diabetic patients, followed by methicillin resistant *Staphylococcus aureus* (11.4%) and 12.6%), *Enterobacter* spp (10.3% and 9.7%), *Klebsiella* spp (8.6% and 6.8%), *Staphylococcus aureus* (7.4% and 8.7%), *Pseudomonas aeruginosa* (6.6% and 8.8%), *Proteus mirabilis* (6.0% and 4.9%), *Citrobacter* spp (5.4%) and 4.8%), *Candida* spp (3.7% and 3.9%) *Streptococcus* spp (2.9% and 2.9%), *Enterococcus faecalis* (2.3% and 1.2%), *Staphylococcus saprophyticus* (2.0% and 1.0%) and *Serratia mascescens* (1.4% and 0.4%).

Alemu *et al*, (2012) carried out their study on bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant woman at University of Gondar Teaching Hospital, North West Ethiopia from March 22 to April 30, 2011 using their mid stream urine samples. The overall prevalence of UTI in these pregnant women was 10.4%. The predominant bacterial pathogens were *Escherichia coli* 47.5% followed by coagulase-negative *Staphylococcus* 22.5%, *Staphylococcus aureus* 10% and *Klebsiella pneumoniae* 10%. Significant bacteruria was observed in asymptomatic pregnant women.

The prevalence and predictors of urinary tract infections among children with cerebral palsy in Makurdi, Nigeria, was investigated and were compared to age and sex matched children without cerebral palsy (CP) at Federal Medical Centre Markurdi Nigeria, from Dec 2011 to May 2013. The age range was between 2-15 years with a mean age of 8.63 ± 3.83 years including 30 males and 22 females. UTI was confirmed in 20 (38.5%) C.P children compared to 2 children (3.8%) without CP (P value 0.000). Among CP children, *Escherichia coli* was the commonest organism Isolated in 9 (9/20, 45%). *Streptococcus faecalis* 4(20%) and *Staphylococcus aureus* in 3 (15%) while both *Proteus* spp and *Klebsiella* spp were isolated in 2 children (10.0%) each. *Escherichia coli* was also found in the 2 children without CP (Anigilaje and Bitto, 2013).

In the study conducted by Abaeze and Abasiama (2011), on the prevalence of urinary catheter related infections in Federal Medical Centre, Abeokuta Nigeria between January and July 2010, two hundred patients urine samples collected from female medical wards, Amenity, Obstetrics and Gynecology, children ward and emergency, were cultured and isolated Organisms were characterized and subjected to antimicrobial susceptibility study. Out of the 200 urine samples examined, 82 (41.10%) yielded growth of bacteria with *E.coli* having the highest number of positive cases 29 (35.4%), followed by *Klebsiella pneumoniae* 17 (20.9%), *Pseudomonas aeruginosa* 10 (15.5%), *S. aureus* 13 (12.1%) *Proteus mirabilis* 8 (9.75%) and *C .albicans* 5(6.0%).

In a study by Theodore, (2006), the prevalence and antibiogram of urinary tract infections among prison inmates in Nigeria was carried out using 181 urine samples of prison inmates attending University of Nigeria Teaching Hospital bacteriological laboratory centre from July 30 to August 2005., Of this number, 141 (77%) gave significant bacteriuria. *Escherichia coli* was mostly isolated with a frequency of 47 (33.3%) followed by *Klebsiella pneumoniae* 28 (19.9%), *Staphylococcus aureus* 21(14.9%), *Proteus mirabilis* 21 (14.9%), *Citrobacter freundi* 10(7.1%), *Staphylococcus epidermidis* 7(5.0%), *Streptococcus faecelis* 5 (3.5%) and *Pseudomonas aeruginosa* 2(1.4%).

Several other literatures abound on studies on UTI with bacteria being the major cause of UTI and *E. coli* the leading cause in various groups of people. For general UTI (Ajantha *et al.,* 2011 at Mumbai India; Nandy *et al*., 2007 at Kolkata, India; Lo *et al.,* 2013 at Teaching Hospital of Univeirsty of SaoPaulo; Kolawole *et al.,* 2009 at Delhatu Araf Specialist Hospital, Lafia, Nasarawa State Nigeria; Stanley *et al*., 2013 at University of Port Hacourt Teaching Hospital, PortHarcourt Nigeria; Oladeinde *et al*., 2011 at Okada, Ovia North East Local Government Area of Edo State, Nigeria), in pregnant women (Jalali *et al*., 2014 at Karaj Health Centre, Iran; Hamdam *et al*., 2011 at Khartoum North Hospital, Khartoum, Sudan; Masinde, *et al*., 2009 at Bugando Medical Centre, Tanzania; Moyo *et al*., 2010 at Muhimbili National Hospital, Tanzania; Okonko *et al*., 2010 at Oloyoro Catholic Hospital, Ibadan South West Nigeria; Obiogbolu *et al*., 2009 in Awka Metropolis, South Eastern Nigeria), in females (Rajput *et al*., 2014 at Indore India;

Shaifali *et al*., 2012 at Vivekanada Polyclinic and Institute Medical Sciences, Lucknow), in children (Ojambo 2008 at Mulago Hospital, Kampala, Uganda), in diabetic patients (Ramana and Chudhury 2012 at South India; Saleem and Damel, 2011 at Bangalore City, India; Pragash *et al*., 2014 at rural South India; Longdoh, 2013 at Buca and Limbe Regional Hospital, South West region, Cameroun; Samuel *et al.,* 2014 at Ago-Iwoye, Ogun State, Nigeria; Ophori *et al*., 2010 at Central Hospital, Benin City Nigeria), in catheterized patients (Raji *et al*., 2013 at Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria), in the elderly (Omoregie *et al.,* 2010 at Benin city Nigeria).

2.5.10 Laboratory Diagnosis of UTI

This entails the microscopical examination of a wet film of uncentrifuged urine to determine whether polymorphs (Pus cells) are present in numbers indicative of infection in the urinary tract and the semi-qualitative culture of the urine to determine whether it contains a potentially pathogenic bacterium in numbers sufficient to identify it as the causal infecting organism (significant bacteriuria). The chemotherapy of a proven infection may be guided by in vitro sensitivity tests on the pathogen isolated in culture and the out come of therapy assessed by examination of the urine at the conclusion of treatment. Follow ï up examination of patients who have had urinary tract infection is advisable because a relapse may be clinically silent (Collee *et al.*, 1989).

Significant Bacteriuria

The specimen most easily and therefore most commonly collected is midstream urine (MSU). Proof of a urinary tract infection requires the demonstration that the potential pathogen is present in freshly voided urine in numbers greater than those likely to result from contamination from the uretral meatus and its environs. The observations of Kass (1957) suggested that the number, taken to indicate significant bacteriuria is about 100000/ml ($>10^5$CFU/ml of urine). In true infection, in the absence of prior chemotherapy, the number of the infectious bacteria is likely to be at least as great as this. Accordingly, a quantitative method of culture is adopted to estimate the number of viable bacteria in the specimen. When properly collected, contamination accounts for

less than 10^4 organisms/ml and usually for less than 10^3/ml. Counts due to contamination are variable and the colonies often of diverse species. Specimens from urinary tract infections almost always contain more than 10^4 organisms/ml, usually more than 10^5/ml and often up to 10^8/ml. These high counts, which are fairly constant in serial specimen from the same patient, reflect bacterial multiplication in the urine in vivo and are accepted as indicating significant bacteriuria. The growth obtained in such cases usually represents a single infecting species though some infections with two species e.g *E. coli* and *S. faecalis,* are encountered.

Significant bacteriuria (Count>10^5CFU/ml in a carefully taken and promptly examined sample may sometimes occur in the absence of symptoms and pyuria in patients who subsequently develop symptoms of urinary tract infection e.g. in pregnancy. The detection of such asymptomatic bacteriuria is of value, for there is good evidence of its association with the development of pyelonephritis in some patients.

A rigid adherence to the above guide lines should be avoided and the culture counts should be interpreted in relation to the clinical information about the patient (Maskell, 1982). Thus a few specimens from symptomatic patients with a true infection may contain as few as 10^3 viable bacteria/ml and if the occurrence of contamination can be shown to be minimal, their sensitivities should be tested and their presence reported as probably or possibly significant. Indications that the scanty bacteria are not merely contaminants are

1. The observation that all or almost all the colonies grown from the specimen are of the same species and
2. The finding on microscopy of the presence of pus cells but absence of squamous epithelial cells, which would be indicative of contamination from the perineum and vagina.

When, moreover the specimen has been collected from bladder by suprapubic aspiration, or a freshly inserted urethral catheter, the absence of contamination may be assumed and the presence of even small numbers of bacteria must be regarded as significant (Collee *et al.*, 1989).

Several methods have been devised to access and determine significant bacteriuria. These are semi-quantitative methods. There are several cultural quantitative techniques for determining bacteriuria. These methods include:

a. Pour plate method
b. Surface ï drop method (Miles and Misra)
c. Standard wireloop method
d. Filter paper method
e. Dip-inoculum (slide method)
(Ochei and Kolhatkar, 2000).

The standard loop method is the most popular. This is a semiquantitative method. It is simple and cheap. Holding a calibrated wire loop vertically, dip the loop into a well mixed uncentrifuged urine and inoculate the medium of choice e.g cystein lactose electrolyte deficient (CLED) medium or MacConkey agar. The swarming of *Proteus* is prohibited by CLED and MacConkey agar due to their lack of salt. Incubate the cultures overnight and count the colonies. A single colony is accepted to represent one colony forming unit (CFU). With a standard wire loop of 0.001ml, 100 colonies or more represent significant bacteriuria. Likewise with 0.003ml wire loop, 300 colonies represent signifcnat bacteriuiria (Ochei and Kolhartkar, 2000).

Procedure for Laboratory Examination of Urine (Cheesbrough, 2006).

(1) Description of the appearance of the urine specimen

Report: Colour of Specimen

Whether is clear or cloudy (turbid).

Normal freshly passed urine is clear and pale yellow to yellow depending on concentration.

Note: When left to stand, a cloudiness may develop due to the precipitation of urates in an acid urine or phosphates and carbonates in an alkaline urine. Urates may give the urine a pink ï orange colour.

(2) Examination of urine specimen microscopically

Urine is examined microscopically as a wet preparation to detect:

- Significant pyuria ie WBCôs in excess of 10 cells/UL (10^6/L) of urine
- Red cells

- Casts
- Yeast cells
- *T. vaginalis* motile trophozoites
- *S. haematobium* eggs
- Bacteria (providing urine is freshly collected).

Method of Examination of a Wet Preparation of Urine

i. Aseptically transfer about 10ml of well mixed urine to a labelled conical tube

ii. Centrifuge at 500-1000g for 5 minutes. Pour the supernatant fluid (by completely inverting the tube) into a second container not the original one. This can be used for biochemical test to avoid contaminating the original urine which may need to be cultured (depending on the findings of the microscopical examination).

iii. Remix the sediment by tapping the bottom of the tube. Transfer one drop of the well- mixed sediment to a slide and cover with glass. Do not discard the remaining sediment because this may be needed to prepare Gram smear if WBCôs and, or ,bacteria are seen in the wet preparation

iv. Examine the preparation microscopically using the 10 x and 40 x objective with the condenser irris closed sufficiently to give good contrast.

3. **Urine Chemistry**

Urine chemistry test which are helpful in investigating UTI include:

i. Protein

ii. Nitrite

iii. Leucocyte esterase

There are other biochemical tests to detect other biochemical substances in urine e.g glucose, ketone, bilirubin, urobilinogen, haemoglobin. All these tests can be done using reagent strip tests such as combi 9 test strips.

4. **Culture of the Urine Specimen**

CLED and MacConkey agar are used for culture of urine samples. Other special media may be required for particular organisms.

5. **Biochemical Tests**

For complete identification of colonies, some biochemical tests are required and this is based on the morphology of the suspect organism. This includes IMViC tests, sugar fermentation tests.

6. **Serological test**

   This can also be performed on isolates for further identification.

7. **Antimicrobial Sensitivity Testing**

   Sensitivity tests are carried out on isolates from urine samples with significant bacteriuria particularly from patients with a history of recurring UTI (Cheesbrough, 2006).

2.6 PATHOGENESIS OF STAPHYLOCOCCAL DISEASES

Most staphylococci are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora (Madigan and Martinko, 2005).

Staphylococci, particularly *S. epidermidis*, are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S. aureus* occurs in 20-50% of humans (Brooks *et al.,* 2013).

People who are chronic carriers of *S. aureus* in their nose have an increased risk of skin infections caused by *Staph aureus* (Levinson, 2012). Hand contact is an important mode of transmission of *S. aureus* and hand washing decreases transmission. *Staphylococcus aureus* is also found in the vagina of approximately 5% of woman, which predisposes them to toxic shock syndrome. Additional sources of staphylococcal infection are sheddings from human lesions and fomites such as towels and clothing contaminated by these lesions (Levinson, 2012).

2.7.1 Virulence Factors That Enable Staphylococci To Establish Infection

Antigenic structure of staphylococci contains antigenic polysaccharides and proteins as well as other substances important in cell wall structure: Peptidoglycan, a

polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogeneses of infection: It elicits production of interleukin-I (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorph nuclear Leukocytes, have endotoxin -like activity and activate complement.

Techoic acids, which are polymers of polyribitol phosphate, are cross-linked to the peptidoglycan and can be antigenic. Antiteichoic acid antibodies detectable by gel diffusion many be found in patients with active endocarditis caused by *S. aureus*.

Protein A is a cell wall component of *S. aureus* strain and is a bacterial surface protein that has been characterized among a group of adhesins called microbial surface components recognizing adhesive matrix molecules (MSCRAMMS). Bacterial attachment to host cells is mediated by MSCRAMMS, and these are important virulence factors. Protein A binds to the FC portion of the IgG molecules except IgG_3. The fab portion of the IgG bond to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology, and diagnostic laboratory; for example, protein A with attached IgG molecules directed against specific bacterial antigen agglutinates bacteria that have that antigen (coagglutination). Another important MSCRAMM is clumping factor on the cell wall surface; clumping factor binds non enzymatically to fibrinogen and platelets, yielding aggregation of the bacteria. The remaining MSCRAMMS, too numerous to mention here (See references) play important roles in establishing *S. aureus* colonization and invasion.

Most *S. aureus* strains of clinical importance have polysaccharide capsules which inhibit phagocytosis by polymorphonuclear leukocytes unless specific anitibodies are present. At least, 11 serotypes have been identified, with types 5 and 8 responsible for the majority of infection: These capsule types are targets for a conjugate vaccine. Serologic tests have limited usefulness in identifying Staphylococci (Brooks *et al.*, 2013).

Enzymes and toxins: Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others considered to be toxins,

although they may function as enzymes. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extra chromosomal control; and for others, the mechanism of genetic control is not well defined.

Enzymes

Catalase: *Staphylococcus aureus* produce catalase which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive from the *Streptococcus which are* nagative.

Coagulase and clumping factor: *Staphylococcus aureus* produces coagulase, an enzyme- like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin, together they become enzymatically active and intiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential.

Clumping factor is another example of an MSCRAMM (see earlier) that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase, because clumping factor induces a strong immunogenic response in the host; it has been the focus of vaccine efforts. However, no human vaccine against this factor is available.

Other enzymes: Other enzymes produced by staphylococci include a hyaluronidase, or spreading factor; a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase, proteinases, lipases,and B- lactamase (Brooks *et al.,* 2013).

Toxins

Hemolysins

X- hemolysin is a heterogeneous protein that acts on broad spectrum of eukaryotic cell membranesô The ɓ- toxin degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells. The ŭ-toxin is heterogenous and dissociates into subunits in non conic detergents. It disrupts biologic membranes and may have a role in *S. aureus* diarrhoeal diseases. The ɔ hemolysin is a leucocidin that lyses white blood cells and is composed of two proteins designated S and F. ɔ-Hemolysin can interact with the

two proteins comprising the panton- valentine Leukocidin (PVL) to form six potential two component toxins. All six of these protein toxins are capable of efficiently lysing white blood cells by causing pore formation in the cellular membranes that increase cation permeability. This leads to massive release of inflammatory mediators such as IL-8, Leukotriene and histamine, which are responsible for necrosis and severe inflammation.

Panton – Valentine Leukocidin

This toxin of *S. aureus* has two components and unlike the chromosomally encoded hemolysins above, PVL is encoded on a mobile phage. It can kill white blood cells of human and rabbits. The two components designated as S and F act synergistically on the white blood cell membrane as described for Y- toxin. This toxin is an important virulence factor in CA ï MRSA infections. Both group of hemolysins are regulated by agr.

Exfoliative toxins

These epidermolytic toxins of *S. aureus* are two distinct proteins of the same molecular weight. Exfoliative toxin A is encoded by eta located on a phage and is heat stable (resists boiling for 20 minutes). Exfoliative toxin B is plasmid mediated and heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

Toxic shock syndrome toxin

Most *S. aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin -1 (TSS-1), which is the same as enterotxin F. TSST-1 is the prototypical superantigen. TSST-1 binds to major histocompactibility class (MHC) class II molecules yielding T-cell stimulation, which promotes the protein manifestations of the toxic shock syndrome.The toxin is associated with fever, shock and multi-system involvement, including a desquamative skin rash. The gene for TSST- 1 is found in about 20% of *S. aureus* isolates, including MRSA.

Entero Toxins

There are multiple (A-E,G-J,K-R and U, V) enterotoxins that, similar to TSST -1, are superantigens. Approximately 50% *S. aureus* strains can produce one or more of them. The enterotoxins are heat-stable and resistant to the action of gut enzymes.

Important causes of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25εg of enterotoxin B results in vomiting and diarrhea. The emetic effect of entrotoxin is probably the result of central nervous system stimulation (vomiting centre) after the toxin acts on neural receptors in the gut. The exfoliative toxins, TSST-1 and the entro toxin genes are on a chromosomal element called a pathogencity Island. It interacts with accessory genetic elements- bacteriophages to produce toxins (Brooks *et al.,* 2013).

Resistance of Staphylococci to Antimicrobial Drugs

Staphylococci are variably susceptible to many antimicrobial drugs and rapidly develop resistance to them, which consequently presents difficult therapeutic problems. Resistance is caused by several mechanisms:

ϐ- Lactamase production is common, is under plasmid control and makes the organisms resistant to many penicillins (penicillin G, ampicillin, ticarcillin, piperacillin and similar drugs). The plasmids are transmitted by transduction and perhaps also by conjugation.

Resistance to nafcillin (and to methicillin and oxacillin) is independent of B- lactamase production. Resistance to nafcillin is encoded and regulated by a sequence of genes found in a region of the chromosome called the staphylococcal cassette chromosome mec (SCCmec). Specifically, the mec A gene on this locus encodes a lowï affinity penicillin binding protein (PBP 2a) that is responsible for the resistance. There are 12 different SCC mec types.

In the United States, *S. aureus* and *S. lugdunensis* are considered to be susceptible to vancomycin if the minimum inhibitory concentration (MIC) is 2εg/ml or less; of intermediate susceptibility if the MIC is 4-8 εg/ml; and resistant if the MIC is 16εg/ml or greater. Strains of *S. aureus* with intermediate susceptibility to vancomycin have been isolated in Japan, the United States and several other countries. These are often known as vancomycin-Intermediate *S. aureus* (VISA). They generally have been isolated from

patients with complex infections who have received prolonged vancomycin therapy. Often there has been vancomycin treatment failure. The mechanism of resistance is associated with increased cell wall synthesis and alterations in the cell wall and is not caused by the van genes found in enterococci. *Staphylococcus aureus* strains of intermediate susceptibility to vancomycin usually are nafcillin resistant but generally are susceptible to oxazolidinones and to quinupristin ï dalfopristin.

Since 2002, several isolates of vancomycin- resistant *S. aureus* (VRSA) strains were isolated from patients in the United States. The isolates contained the vanconycin resistance gene van A from enterococci and the nafcillin resistance gene Mec A . Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in *S. aureus* is of major concern world wide.

Plasma-mediated resistance to tetracyclines, erythromycins, aminoglycosides and other drugs is frequent in staphylococci

Tolerance implies that staphylococci are inhibited by a drug but not killed by it- that is there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug. Patients with endocarditis caused by a tolerant *S. aureus* may have a prolonged clinical course compared with patients who have endocarditis caused by a fully susceptible *S. aureus*. Tolerance can at times be attributed to lack of activation of autolytic enzymes in the cell wall (Brooks *et al.*, 2003).

2.7 PREDISPOSING FACTORS TO STAPHYLOCOCCAL INFECTIONS

Disease caused by *S. aureus* is favoured by a heavily contaminated environment (e.g family members with boils) and a compromised immune system. Reduced humoral immunity, including low levels of antibody, complement, or neutrophils, especially predisposes to staphylococcal infections. Diabetes and intravenous drug use predispose to infections by *S. aureus*. Patients with chronic granulomatous diasease (CGD), a disease characterized by a defect in the ability of neutrophils to kill bacteria are especially prone to *S. aureus* infections. *Staphylococcus epidermidis* is found primarily on the human skin and can enter the blood stream at the site of intravenous catheters that penetrate through the skin. *Staphylococcus saprophyticus* is found primarily on the

mucosa of the genital tract in young women and from that site can ascend into the urinary bladder to cause urinary tract infections. (Levison, 2012). Predisposing factors as mentioned earlier for several UTI can also predispose one to *Staph* UTI. This may include use of contraceptive devises such as condoms, diaphrams, diabetes, pregnancy, poor personal care, use of urinary catheters, suppressed immune systems eg due to long usage of antibiotics, cancer, BPH, renal or kidney stones, sexual activity etc.

2.8 PATHOGENESIS OF STAPHYLOCOCCAL UTI

Staphylococcus aureus occurs harmlessly as a commensal parasite on the interior nares and on most areas of skin in 20 to 30 persons of healthy persons (carriers). Other staphylococci called *Staph* albus, are harmless commensal that grow on the whole surface of the skin and in the nostrils and mouth of all persons throughout life but they occasionally act as opportunistic pathogens in persons with defective antimicrobial defenses e.g damaged heart valves or urinary tract anomalies.(Dugid *et al.*, 1978) Thus one can auto-infect one self with *Staphylococcus aureus* from the nose and other staphylococci from other parts of the body and contaminate the urethral opening with hands containing these staphylococcal species or with other fomites such as towel, dirty underwearôs containing them and this can through multiplication, invasiveness and ascending route, reach the urinary bladder to cause UTI. The use of urinary catheters can also push in *Staphylococcus* in the skin into the bladder, thereby causing UTI.

Septicaemia with any of the staphylococcal species due to use of medical devices such as shunts, heart valves, can cause infection in the kidneys (Pyelonephritis). *Staphylococcus aureus* is said to be found in the vagina of approximately 5% of women (Levinson, 2012) and thus can contaminate urethral opening leading to UTI. Sexual activity increases the risk of *S. saprophyticus* UTI because bacteria are displaced from the normal flora of the vagina and perineum into the urethra. Most cases occur within 24 hours of sex (Levinson, 2012). *Staphylococcus saprophyticus* has the capacity to selectively adhere to

human urothelium. The adhesin for *Staph saprophtyticus* is a lactoxamine structure. *Staphylococcus saprophyticus* produces no exotoxins (Levinson, 2010).

Staphylococcus xylosus has been associated with pyelonephritis in humans (Tselenis-Kotsowilis *et al.* 1982), urinary tract infections, bacteremia. Some strains are capable of colonizing surfaces by forming biofilms (Abis encyclopaedia).

Staphylococcus haemolyticus, is part of the skin flora of humans (de Silva *et al.*, 2002) and its largest populations are usually found at the axillae, perineum and inguinal areas. *Staphylococcus haemolyticus* also colonizes prosimians, monkeys and domestic animals (Fischetti *et al.*, 2002. It is a well known opportunistic pathogen and is the second most frequently isolated CoNS, *S. epidermidis* being the first (De Allori *et al.*, 2006).

Infections can be localized or systemic and are often associated with medical devices (Falcone *et al.*, 2006; Poyart *et al.*, 2001; Vile and Stefani, 2006). Like other CoNS, *S. heamolyticus* is often associated with insertion of foreign bodies such as prosthetic valves, cerebrospinal fluid shunts, orthopaedic prosthesis and intra-vascular, urinary and dialysis catheters (Falcone *et al.*, 2006, Poyart *et al.*, 2001, Viale and Stefani, 2006). Human infections include: nature valve endocarditis, Septicaemia, peritonitis, and urinary tract, wound, bone and joint infection (de Silva *et al.*, 2002; Fischetti *et al.*, 2000; de Allori *et al.*, 2006; Flahout *et al.*, 2008). The ability to adhere to medical devices and subsequently form biofilms is a major virulence factor associated with *S. heamolyticus* (de Silver *et al.*, 2002; de Allori *et al.*, 2006; Carca *et al.*, 2005; Fredheim *et al.*, 2009). Biofilm formation increases antibiotic resistance (de Allori *et al.*, 2006; Cerca *et al.*, 2005; Fredheim *et al.*, 2009) and often leads to persistent infections (Consterton *et al.*, 1999; Klingenberg *et al.*, 2007).

Certain strains of *S. haemolyticus* are capable of producing a capsular polysaccharide (cp) (Takeuchi *et al.*, 2005; Flahaut *et al.*, 2008). Capsular polysaccharide is considered a virulence factor because it provides resistance against complement-mediated PMN phagocytosis.

2.9 CLINICAL FEATURES OF STAPHYLOCOCCAL UTI

The clinical features (signs and symptoms) of staphylococcal UTI includes urethritis, cystitis, and pyelonephritis. Patients with UTI caused by *S. saprophyticus* usually present with symptomatic cystitis. Symptoms includes a burning sensation when passing urine, the urge to urinate more often than usual, a ódripping effect after urination, weak bladder, bloated feeling with sharp razor pains in the lower abdomen around the bladder and ovary areas and a razor like pain during inter course. Signs and symptoms of renal involvement are also often registered (Jordan *et al.*, 1980).

Symptoms of pyelonephritis due to *S. xylosus* include fever, pain in the kidney area, dysuria with typical laboratory findings (persistent pyuria) (Tselenis ï Kotsowilis *et al.*, 1982).

2.10 PREVALENCE OF STAPHYLOCOCCAL UTI WORLD WIDE

Staphylococcus aureus have been documented in some literatures as the leading cause of UTI in some studies amongst other urinary bacterial isolates.

Bolaji *et al.* (2013) carried out a study on the incidence of uropathogens from asymptomatic bacteriuric pregnant women in Zaria, Nigeria. Mid stream urine samples of two hundred and fifty (250) women identified as being in the first trimester of pregnancy, atttending antenatal clinics in Sabon- Gari local Government Area Zaria Nigeria were investigated for significant bacteriuria. The result of their study showed that out of the 250 urine samples examined, 146 (58.4%) had bacterial counts greater than 10^5 cfu/ml indicative of asymptomatic bacteriuria. A total of 197 bacterial isolates were obtained with *Staphylococcus* species 46 (23.4%), *Klebsiella* spp 49 (24.9%) *Pseudomonas* spp 18 (9.0%), *Streptococcus* spp 1(0.5%). The isolates were subjected to API kits to confirm their identity which revealed *Staphylococcus aureus* as the predominant among the staphylococcal species encountered 7 (18.0%), *Staph lentus* 3(7.7%), *Staph xylosus* 5 (12.8%), *Staph cohnii* 1(2.6%), *Staph auriculous* 2(5.1%), *Staph epidermidis* 1 (2.6%), *Staph heamolyticus* 1(2.6%), *Micrococcus* spp 4(10.3%), other *Staphylococcus* spp 15 (38.5%).

The study of Amali *et al*. (2008) on urinary tract infection among 213 volunteer female students in the female hostel of University of Agriculture Markurdi , Nigeria, showed that *Staphylococcus aureus* had the highest prevalence in urine 120 (56.3%) followed by *Escherichia coli* 60 (28.2%); *Streptococcus pyogenes* 18 (8.5%). The age group 20-24 years had the highest prevalence.

Omojasola and Omojasola (2001) carried out a study on the pattern of microbial aetiology in urinary tract infection (UTI) among adult subjects in llorin metropolis, Nigeria from June 1999 to June, 2000. A total of 167 patients (84 males and 83 females) aged 18-40 years with symptoms suggestive of UTI (fever, backache, urgency of nictuition), had their mid-stream urine specimens collected for laboratory evaluation. Of this number of urine samples investigated, 139 (83.2%) were positive for UTI. *Staphylococcus aureus* was the most predominant organism encountered 73 (52.5%) followed by *Escherichia* coli 39 (28.1%), *Klebsiella* spp 7 (5.0%), *Pseudomonas aeruginosa* 7 (5.0%), *Proteus* spp 6 (4.3%), faecal streptococci 4 (2.9%) and a mixed culture of *S. aureus* and faecal colifom 3(2.2%). *Staphylococcus aureus* was the main organism recovered from the male subjects (42.4%). Antibiotic sensitivity test results of the *S. aureus* isolates indicates that the most sensitive drug was peflacin (96.6%) followed by gentamycin (89.7%), azithromycin (89.7%), nitrofurantoin (84.8%), ofloxacin (86.2%), erythromycin (72%), chloromphenicol (62.1%) streptomycin (58.7%), tetracycline (34.5%), Cloxacillin (13%), cotrimoxazole (10%) and ampicillin (6.5%).

Urinary tract infection was investigated with respect to its prevalence rate among students of a private university in the western area of Delta state, Nigeria. Two hundred and ninety one freshly voided midstream urine samples from UTI symptomatic and asymptomatic students aged between 15-35 years were used for the study. The study was carried out between June and October 2013. Results of the study showed that amongst uropathogens isolated, *Staphylococcus aureus* was the most abundant (34.8%) followed by *Escherichia coli* (24.4%), *Klebsiella aerogenes* (13.9%), *Candida albicans* (7.8%), coliform organism (6.1%), *Proteus* spp (4.4%), *Enterobacter* spp (4.4%), *Serratia* spp and *Pseudomonas aeruginosa* (1.7%) each while *Providentia* spp had (0.9%). Gram negative bacilli and Gram positive bacteria accounted for 65.2% and 34.8% respectively. Females had higher

occurrence of UTI (64.9%) than males (35.1%) of which the UTI occurred highest in the age group 16-28 years followed by age group 18-27years, 17-33 years, 19-22 years and 22-32 years. *Escherichia coli* and *Staph aureus* occurred most in females of 22 and 23 average ages respectively. More than 50% of microbial strains isolated were sensitive to gentamycin, ofloxacin and tetracycline. More than 60% of the strains were resistant to erythromycin, augumentin, nitrofarantoin. All isolated strains were multi-drug resistant each to 4,5,6,7 and 8 of the selected antibiotics used. (Otajevwo and Eriagbor, 2014).

Lo *et al*. (2013) carried out a cross sectional study in the emergency department of the Teaching Hospital of the University of Sao Paulo from January 1 to December 31, 2010 in patients younger than 15 years old who had clinical suspicion of UTI. Urinary tract infection was confirmed in 137 of the cases collected with catheter and in 154 of the midstream samples, adding to a total of 291 cases with a prevalence of 11.3% of the clinically suspicious cases and 0.4% of all the cases seen in the emergency department. UTI was more prevalent in females with 212 cases (72.6%). *E. coli* was found in (76.6%) of the cases of UTI, followed by *P. mirabilis* (10.3%) and *S. saprophyticus* (4.1%).

In a study on UTI, Pragash *et al*. (2014) investigated on uropathogens among type II diabetic patients and their antimicrobial resistance pattern among rural South Indian population during the period July 2012 to June 2013. Midstream urine specimens obtained by clean catch method were randomly collected from 260 diabetic patients who suffered from urinary tract infection. Results showed that of this number of patients examined, 152 had significant bacteriuria with an overall prevalence of 58%. Females (71%) were shown to be more prone to pathogenic urinary tract infection than males (43%). *Escherichia coli* was the commonest organism isolated (54%) followed by *Klebsiella* (21%), *Staphylococcus aureus* (14%), *Pseudomonas* (12%), coagulase negative *Staphylococcus* CoNS (8%), *Proteus* (4%), *Acinetobacter* and enterococci (17%) each. High level resistance was seen to cotrimoxazole, ciprofloxacin, ceftazidime, and cifipime. Sensitivity of nitrofurantoin to *Pseudomonas* and *Acinetobacter* were not tested as they have intrinsic resistance to that drug. Amikacin was found to be very effective against all the isolates. Most of the isolates were sensitive to imepenem.

Alemu *et al.* (2012) worked on bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women attending antenatal clinic at University of Gondar Teaching Hospital, North West Ethiopia from March 22 to April, 30, 2011. A total of 385 pregnant women were enrolled in the study aged 17-45years. The overall prevalence of UTI was 10.4%. Of all the bacteria isolated, Gram-negative bacteria were more prevalent 27 (67.5%) than Gram positive bacteria 13 (32.5%). The most commonly isolated bacteria were *E.coli* 19 (47.5%) followed by coagulase negative staphylococci 9 (22.5%), *S. aureus* and *K. pneumoniae* 4 (10%) each while *Enterobacter* aerogenes and *Morganella* morgani accounts 2 (5%) each. The result of antimicrobial susceptibility pattern of their isolates showed that most of Gram negative isolates were sensitive to ceftriaxon 26 (96.3%), ciprofloxacin 26 (96.3%), norfloxacin 25 (92.6%), gentamycin 25 (92.6%), amoxicillin-clavulanic acid 16 (59.3%), co-trimoxazole 14 (51.9%) and tetracycline 11(40.7%). However all isolates of Gram negative bacteria were resistant to ampicilline and amoxacillin 27(100%).

Majority of Gram positive were resistant to most of the antibiotics tested than the Gram, negatives. Among the gram positives, 11 (84.6%), 12 (92.3%) and 12 (92.3%) of the isolates were sensitive to ceftriaxon, gentamycin and amoxicillin-clavulanic acid respectively. Coagulase negative staphylococci which were the predominant isolates from Gram positives 9 (69.2%) were resistant to most of the antibiotics tested. The resistance pattern of the isolates were found to be 8 (88.9%) for ampicillin, 7 (77.9%) for co-trimoxazole and tetracycline and 6 (66.7%) for amoxicillin and chloramphenicol. Gentemycin and amoxicillin ï clavulanic were found to be effective against 8 (88.9$) coagulase negative staphylococci.

A total of 514 patients (465 females and 49 males) with signs and symptoms of urinary tract infection (UTI) were screened for urinary tract infection in Okada, a rural community and headquarter of Ovia North East Local Government Area of Edo State. The study was carried out at Igbinedion University Teaching Hospital, Okada from January 2007 to December 2009. The patients were aged 12 to 76 years. Results of their study showed that out of the 514 patients examined, 204 (39.69%) had urinary tract infection. Females had higher percentage of UTI 42.8% versus males 10.2%. The

prevalence of UTI was highest within 21-30 years age group (44.67%) and least within 51-60 years (24.32%) though age did not significantly affect the prevalence of UTI (P=0.253). *Escherichia coli* was the most predominant isolate causing UTI (41.16%) as well as in females followed by *Staphylococcus aureus* (32.35%), *Candida albicans* (12.25%), *Pseudomonas aeruginosa* (2.45%) and *klebsiella* spp (4.41%).

The susceptibility profile of the bacterial isolates showed that ciprofloxacin was the most active antibacterial agent while nalidixic acid, nitrofurantoin, sulphamethoxazole-trimetoprin,, amoxicillin and amoxicillin- clavulanate were poorly active against the bacterial isolates (Oladeinde *et al.,* 2011).

Obiogbolu *et al*. (2009) worked on the incidence of urinary tract infections (UTIs) among pregnant women in Awka metropolis South Eastern Nigeria. The women were aged between 15 to 40 years. Results showed that out of the 100 midstream urine samples collected from these women and examined 54 showed significant bacterial growths. Escherichia coli 20 (37%) was the commonest offending bacterial pathogen isolated. Others were *Klebsiella* spp 11 (21.4%), *Proteus mirabilis* 9 (16.7%), *Pseudomonas aeruginosa* 7 (13%), *Staphylococcus aureus* 4(7.4%) and *Staphylococcus epidermidis* 3 (5.6%).

The prevalence and antibiogram of urinary tract infection was investigated among 181 prison inmates in Nigeria attending University of Nigeria Teaching Hospital bacteriological Centre from July 30th to August 2005. The patients consisted of 115 females and 66 males aged from 7-60 years. Mid stream urine samples were collected from both in and out prison patients and were examined. Results showed that of the total number screened for UTI, 141 (77.9%) gave significant bacteriuria. *Escherichia coli* was mostly isolated with a frequency of 47 (33.3%) followed by *Klebsiella pneumoniae* 28(19.9%), *Staphylococcus aureus* 21 (14.9%), *Proteus mirabilis* 21 (14.9%), *Citrobacter freundi* 10 (7.1%), *Staphylococcus epidermidis* 7 (5.0%), *Streptocooccus faecalis* 5(3.5%) and *Pseudomonas aeruginosa* 2 (1.4%). Invitro antibiotic susceptibility test showed that Gram negative isolates were sensitive to ciprofloxacin, nalidixic acid and gentamycin,

whereas erythromycin and chloramphenicol were active against the Gram-positive isolates. All the Gram negative isolates were sensitive to the quinolone drugs.

2.11 LABORATORY DIAGNOSIS OF STAPHYLOCOCCAL UTI

This involves microscopy, culture and sensitivity (M/C/S). Early morning freshly voided mid stream urine samples are usually used for the investigation. Procedures for laboratory diagnosis of staphylococcal UTI are the same as that for general UTI laboratory diagnosis as stated earlier in this write up. However, the difference is in some culture media that may be used for selective isolation of some stapholoccoccal species and some biochemical tests for identification of staphylococcal species (API tests) (Ochei and Kolhtkar, 2000). Laboratory media that can be used for isolation of staphylococcal species includes cystein lactose electrolyte deficient (CLED) medium or MacConkey agar, Blood agar, Mannitol salt agar.

Biochemical tests used for confirmation of *Staphylococcus* species include catalase test, coagulase test, oxidase test, IMVIC tests, sugar fermentation tests using API test kits, Deoxyribonuclease (Dnase) test. Novobiocin drugs sensitivity test is also used to confirm some staphylococcal isolates which are novobiocin resistant such as *S. saprophyticus*, *S. cohnii* and *S. xylosus* (Marrie *et al*., 1982) . For coagulase test, *S. aureus* is coagulase positive while the other Staphylococcal species are coagulase negative. *Staphylococcus sciuri* is oxidase positive while other Staphylococcal species are oxidase negative.

Catalase test is positive for all *Staphylococcus* species and differentiates them from *Streptococcus* spp which are catalase negative. Dnase test confirms *S. aureus* which is positive for it while other Staphylococcal species are negative. Sugar fermentation tests are used for futher confimatory tests for typing isolates to species level where API test kits are not available. The identification of a particular Staphylococcal species isolates which have significant bacteriuria ($>10^5$cfu/ml) is followed by antibiogram on the isolates so as to aid the clinicians in the treatment of the patient.

2.12 TREATMENT OF S*taph* SPP UTI/DRUG SENSITIVITY PATTERN OF *Staph* URINARY ISOLATES

The work of De and God love (2010), showed that of the 76 *Staph* isolates from urine samples which they examined, 56 were *S. epidermidis* while 20 were *S. aureus.* Of isolates, (51.8%) of *S.epidermidis* were sensitive to ceftazidime followed by ciprofloxacin (46.45%) whereas 45% of the *S.aureus* isolates were sensitive to ceftriaxone followed by cefotaxime and ciprofloxacin (40%).

The work of Egoro *et al.* (2014) showed that 70% of their *Staph aureus* isolates were sensitive to ofloxacin, followed by ciprofloxacin 65%, erythromycin 55%, chloramphenicol 25%, pefloxacin 10% and gentamycin 10% while ampiclox, norfloxacin, clindamycin and streptomycin showed 0% sensitivity pattern.

There is paucity of information on the sensitivity pattern of *S. xylosus* urinary isolates. However, *S. xylosus* is normally sensitive to fleroxacin, methicillin, penicillin, teicoplanin, and tetracycline and resistant to erythromycin and novobiocin. (Wikipedia the free encyclopaedia, 2013).The work of Tselemis-Kothsowilis *et al.* (1982) showed that the *S. xylosus* isolate obtained from the urine sample of the female patient was susceptible to penicillin and resistant to erythromycin (minimum inhibitory concentration, 150µg/ml).The work of Al-Methkhury *et al.* (2008) showed high susceptibility of *S. xylosus* urinary isolates towards ciprofloxacin and high resistance towards erythromycin.

Staphylococcus saprophyticus is usually susceptible to antibiotics commonly prescribed for patients with UTI with the exception of nalidixic acid. Quinolones are commonly used in treatment of *S.saprophyticus* UTI (Wikipedia, 2013). *Staphylococcus saprophyticus* is resistant to novobiocin and this characteristic is used to identify it and distinguish it from *S. epidermidis* which is also coagulase negative but novobiocin sensitive. (Levinson, 2010). Urinary tract infection due to *S. saprophyticus* is usually treated with trimethoprim ï sulfamethoxazole or with a quinolone such as ciprofloxacin (Levinson, 2010).

Staphylococcus epidermidis is highly antibiotic resistant. Most strains produce ß-lactamase but are sensitive to ß-lactamase-resistant drugs such as nafcillin. These are called methicillin- sensitive strains (MSSE). Some strains are methicillin /nafcillin resistant (MRSE) due to altered penicillin- binding proteins. The drug of choice is

vancomycin, to which either rifampicin or an aminoglycoside can be added. Removal of the catheter or other device is often necessary (Levinson, 2010).

Prevalence of virulence factors and antimicrobial susceptibilities of *S. haemolyticus* isolates were studied using 49 *S. haemolyticus* isolates from urinary tract infections (Simago, 2005). All *S. haemolyticus* isolates were sensitive to vancomycin and 79.6% sensitive to clindamycin. Over 70% of the isolates were resistant to chloramphenicol, cotrimoxazole, erythromycin, oxacillin, ampicillin and penicillin G. Multi drug resistance to 5 or more drugs was observed in 69.4% of the isolates (Simago, 2005).

A case study reported a persistent UTI caused by *S. haemolyticus* in a 38yr-old male whose infection was ultimately resolved through the use of the antibiotic trimethoprim-sulfamethoxazole (Gunn and Davis, 1988).

Staphylococcus haemolyticus has the highest level of antibiotics resistance among the CoNS (Fredheim *et al.*, 2009). Various strains are resistant to one or more of the following antibiotics: penicillins, cephalosporins, mecrolides, quinolones, tetracyclines, aminoglycosides, glycopeptides and fosfomycin. Glycopeptides (vancomycin and teicoplanin) resistant strains have begun to emerge (Chiew *et al.*, 2007; Felcone *et al.*, 2006; Takeuchi *et al.*, 2005; Sieradzki *et al.*, 1998).

Most, if not all strains of *S. hominis*, are *hominis* susceptible to penicillin, erythromycin and novobiocin but a divergent strain, *S.hominis* sub sp. *novobiosepticus* (SHN) was found recently which is resistant to novobiocin. It also fails to produce acid aerobically from trehalose and glycosamine. In addition, the 26 strains of this new sub species are resistant to nalidixic acid, penicillin G, oxacillin, kanamycin and streptomycin.

They were also somewhat resistant to methicillin and gentamycin and most strains were resistant to erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole, and ciprofloxacin, as well. In addition, *S. hominis* sub sp. *hominis* is commonly isolated from humans skin but no isolation of SHN from human skin has been reported.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study Area was Enugu State, South East of Nigeria.

3.2 SAMPLING METHOD

3.2.1 **Population sampled**

Patients and pregnant women included in the present study were randomly selected from in- patients and out-patients seen at University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla and Enugu State University Teaching Hospital, (ESUTH) Parklane, Enugu, both in Enugu State, South East Nigeria. The pregnant women patients comprised of pregnant women in their first, second and third trimesters, while the patients were made up of those suffering from debilitating ailments such as diabetes, high blood pressure (HBP), benign prostrate hypertrophy (BPH), prostrate cancer, cardiovascular accident (stroke), kidney stone, surgery cases, suspected UTI cases and other medial conditions.

Apparently healthy subjects included in the study were randomly selected from pupils of two primary schools (International Nursery/ Primary School Trans-Ekulu Enugu and Community Primary School, Amorji-Nike), students of two secondary schools (Girls Secondary School, Abakpa, Nike, Enugu and Announciation Secondary School, Amorji-Nike), students from two tertiary Institutions (University of Nigeria, Enugu Campus, and School of Nursing and Midwifery, Enugu State University Teaching Hospital Enugu), civil servants, traders, housewives and their husbands, artisans, transporters, all residing in Enugu (Tables 3.2 and 3.5). A total of 818 paticipants consisting of 290 males and 528 females were randomly selected and included in this study (Table 3.1). Of the 818 participants studied, 119 were pregnant women, 141were patients with various medical conditions) while 558 were apparently healthy subjects (Table 3.2) . The pregnant women, patients with various medical conditions and apparently healthy subjects were further categorized into various occupational groupings (Table 3.4). The participants were aged between 3 and 87 years. The research work was carried out between 2013 and 2015. Ethical clearances (approvals) were obtained from the Research and Ethics Committees of University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla and Enugu State University Teaching Hospital, (ESUTH), Parklane, Enugu, both in Enugu State, south east of Nigeria. Oral and or written consents were also obtained from the heads of schools and organizations used for the study as well as from the participants or their parents/ guardians before commencement of sample collection. The participants were earlier informed of the date of sample collection.

Table 3.1
Age and Sex Distribution of the Subjects Sampled

n=818

Age group (Years)	No of males sampled	No of females Sampled	Total Sampled
0-10	43	33	76
11-21	50	178	228
22-32	70	174	244
33-43	30	82	112
44-54	41	35	76
55-65	34	18	52
66-76	17	7	24
77-87	5	1	6
Total	**290**	**528**	**818**

Table 3.2

Distribution of the Subjects Sampled According to Health Status

n= 818

Health status	No of males sampled	No of females sampled	Total
Apparently healthy	213	345	558
Pregnant women	-	119	119
Medical problems	77	64	141
Total	**290**	**528**	**818**

Table 3.3

Distribution of the Pregnant Women Sampled

	n= 119
Age group (years)	**Total**
11-21	9
22-32	80
33-43	30
44-54	0
55-65	0
66-76	0

77-87	0
Total	**119**

Table 3.4

Distribution of Subjects According to Occupational Grouping

n=818

Occupational Grouping	**No of males sampled**	**No of females sampled**	**Total**
Students (Tertiary)	42	114	156
Students (secondary)	31	122	153
Pupils (Nurs. &Prim)	61	51	112
Civil servants	67	98	165
Traders/Petitraders/business	44	90	134
House wives	-	32	32
Artisans	16	21	37

Drivers	29	-	29
Total	**290**	**528**	**818**

3.2.2 Sample Collection

Freshly voided early morning midstream urine samples were randomly collected from the patients and subjects included in the study. They were given wide mouth sterile universal bottles with boric acid for collection of their urine samples and were also instructed on how to collect the urine samples. Those on admission who for reason of their condition or ailments could not collect their urine samples unaided and those on urinary catheters were aided by the nurses on duty. Attempts were made to collect urine samples only from patients / subjects who were not on any antibiotic therapy during the period of sample collection or about to prior to that. Questionnaires were administered to the participants, which contained their biodata and details of their ailments. The urine samples were taken to the teaching laboratory of the Department of Medical Laboratory Sciences, University of Nigeria, Enugu Campus for analysis within one hour of collection.

3.3 LABORATORY INVESTIGATIONS

3.3.1 Macroscopic Examination of Urine

On arrival to the laboratory, the urine samples were examined macroscopically for colour and turbidity.

3.3.2 Culture of Urine specimens

The samples were inoculated by streaking with a standard wire loop of 0.003ml onto freshly prepared and well dried MacConkey agar (Batec), blood agar plates and Sabauraud Dextrose agar (oxoid) slant tubes. The MacConkey agar and blood agar plates were incubated at 37^{o}C for 24 hr while the Sabauraud Dextrose agar slant tubes were incubated at 37^{o}Cfor 48 hr. Methods used were according to Ochei and Kolhatkar (2000).

3.3.3 Urine chemistry

Urine chemistry was carried out on the urine samples using dipstick method with Combi. 9Ê . Each urine sample to be investigated was poured into a clean test tube and one strip of Combi 9Ê was dipped into the urine sample and examined for evidence of colour change which depicted the presence of some vital parameters such as sugar, protein blood, ketones, bilirubin, nitrite, ascorbic acid, etc.

3.3.4 Microscopic examination of urine deposits by wet preparation

Microscopy was done on the urine specimens as wet preparation to detect the followings:

i) Pyuria (WBCS in excess of 10 cells/ µl (10^{6}/L) of urine

ii) Red blood cells

iii) Casts

iv) Yeast cells

v) Epithelial cells

vi) Bacterial debris

vii) Presence of motile trophozoites of protozoa eg*Trichomonas vaginalis*

viii) Presence of ova of parasites (*Schistoma haematobium*)

ix) Phosphate and oxalate crystals

Method

This was done by aseptically transferring about 10mls of well mixed urine sample into labelled conical test tube. This was centrifuged at 500 -1000g for 5 minutes. The supernatant fluid was decanted while the deposit was remixed by tapping the bottom of the tube and from this, one drop of the well mixed sediment (deposit) was transferred unto a clean grease free slide and covered with a cover slip.

This preparation was examined microscopically using the 10x and 40 x objective with the condenser iris closed sufficiently to give good contrast.

3.3.5 Macroscopic Examination of Culture plates and Tubes

On completion of the incubation period, the culture plates and tubes were examined for growth and colonies of growth observed were read macroscopically using their colonial morphology which includes colour, size, shape, edge, elevation, consistency, whether haemolytic on blood agar, whether lactose fermenter or not. Samples showing uniform colonial morphology of Ó 10^5cfu/ml of urine were taken as significant for bacteriuria according to Ochei and Kolhatkar (2000). Pure isolates from samples with significant bacteriuria were subjected to Gram staining reaction.

3.3.6 Microscopic Examination of Isolates

Principles of Gram staining reaction

Gram stain reaction is based on the ability of the organisms to resist decolourisation with acetone, alcohol or aniline oil after initial staining with one of the rosaniline basic dyes

and then treating with a mordant. The rosaniline dyes commonly used are crystal violet, methyl violet and gentian violet. Iodine is the mordant used. The primary stain such as crystal violet stains all the bacteria in the smear. Application of a mordant results in the formation of a complex with the primary stain. On addition of a decolourizing agent, some bacteria resist removal of the dye-iodine complex and retain the primary stain. These are called Gram positive bacteria while Gram negative bacteria get decolourized with the decolourizing agent. On addition of a counter stain contrasting in colour with the primary stain, this stains the Gram negative bacteria which were previously decolourized.

Procedure for Gram Staining Reaction

A drop of saline was placed on a clean grease free slide and a colony of the test organism was picked from the plate or tube and emulsified in it to make a smear. The smear was allowed to dry and then fixed with a gentle heat by passing the slide three times over a bunsun flame. The smear was stained with crystal violet for one minute and washed with tap water. Lugols iodine was applied to the smear and left for one minute. This was washed with tap water. The smear was decolourized with acetone for about two seconds and was washed immediately with tap water. The smear was counterstained with neutral red for one minute and washed with tap water. The smear was allowed to dry and examined microscopically.

Results:

Gram positive bacteria: violet

Gram negative bacteria: red

Ochei and Kolhatkar (2000)

3.3.7 Sub-culture onto Nutrient Agar Slope

Pure isolates were transferred onto nutrient agar slopes for use in biochemical and other tests.

3.3.9 Biochemical Tests

3.3.8.1Catalase test ï This helps to differentiate staphylococci from streptococci. **Principle:** The enzyme catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water $2H_2O_2 \longrightarrow 2H_2O + O_2$

When the organisms containing catalase comes in contact with hydrogen peroxide, bubbles of oxygen are given off.

Reagent: Hydrogen peroxide, 3% (10 volume solution).

Method

Two to three drops of H_2O_2 (3%) were placed in a test tube. With a sterile wooden applicator or plastic rod or glass stirrer, some colonies of the organisms were picked and immersed in the H_2O_2 solution. Immediate gas bubbling indicated positive test.

Control

Staphylococcus species positive

Streptococcus species- negative

(ochei and Kolhatkar, 2000)

3.3.8.2 Coagulase Test

This test is used to differentiate *Staph aureus* from other staphylococci.

1. **Principle**: The enzyme coagulase causes plasma to clot by converting fibrinogen to fibrin. Coagulase is produced by *Staphylococcus aureus*. It is considered a pathogenicity test for *S. aureus*.

2. **Bound coagulase**- this converts the fibrinogen directly to fibrin in the absence of the coagulase reacting factors. It is detected by clumping in the slide test. The bound coagulase is associated with the bacterial cells.

Slide test to detect bound coagulase (method)

Two separate drops of saline were placed on a slide. One or two colonies of the organism was emulsified in each of the drops to make thick suspensions. The tip of a straight wire was dipped into the undiluted plasma and the adhering trace of plasma was mixed into one of the bacterial suspensions. Immediate coarse clumping of the mixture was looked for within 5-10 seconds. Clumping indicated positive coagulase test. No plasma was added to the other suspension which served as negative control to the test and differentiated nonspecific granular appearance from true coagulase clumping.

Controls

S.aureus ï Positive

S. epidermidis ï Negative

(Ochei and Kolhatkar, 2000)

3.3.8.3 OXIDASE TEST (CYTOCHROME OXIDASE)

This test is employed to aid in the identification of *Pseudomonas, Neisseria, Vibrio* and other groups.

Principle

The enzyme oxidase will oxidise a redox dye such as tetramethyl paraphenylene diamine dihydrochloride (TMPPDH) to deep purple colour. This enzyme is produced by some aerobic bacteria as part of their respiratory oxidation mechanism.

Reagent

One percent tetramethyl paraphenylene diamine dihychochloride in water. Store in a dark bottle at 4^{o}C or prepare fresh when required; or keep frozen until ready to use.

Method

A loopful of oxidase reagent was added to a filter paper in petri dish. A platinum or plastic loop was used to smear the suspected colony across the wetted filter paper. The filter paper was examined to observe a purple colour appearing across the streak within10 seconds. This indicated positive oxidase test.

Control

Pseudomonas aeruginosa ï positive control

Escherichia coli- negative control

(Ochie and Kolhatkar, 2000).

3.3.8.4 Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium. Indole is then tested for by a colorimetric reaction with P- dimethyl ï amino-benzaldehyde.

Method

The medium (peptone water) was inoculated with the test organism and incubated for 48 hours at 37^{o}C. A period of 96 hours at 37^{o}C was sometimes used for optimum accumulation of indole. 0.5ml Kovacs reagent was added to the medium after incubation. This was shaked gently. A red colour in the alcohol layer indicated a positive reaction (Collee *et al.,*1989).

3.3.8.5 Methyl Red Test

The methyl red test is employed to detect the production of sufficient acid during the fermentation of glucose and the maintenance of conditions such that the pH of an old culture is sustained below a value of about 4.5 as shown by a change in the colour of the methyl red indicator which is added at the end of the period of incubation.

Method

The glucose phosphate peptone water was inoculated lightly from a young agar slope culture and incubated at 37^{o}C for 48 hours. Five drops of the methyl red reagent was added to this broth culture after incubation. This was mixed and read immediately. Positive tests were bright red while negative tests were yellow (Collee *et al.,* 1989).

3.3.8.6 Acetoin Production (Voges-Proskauer) Test

Many bacteria ferment carbohydrates with the production of acetyl methyl carbinol (CH_3 CO. CHOH. CH_3) or its reduction product 2, 3 butylene glycol. (CH_3.CHOH. CHOH.CH_3). The substances can be tested for by a colorimetric reaction between diacetyl (CH_3. CO.CO.CH_3- formed during the test by oxidation of acetyl methyl carbinol or 2, 3 butyleneglycol and a guanidino group under alkaline conditions.

This test is usually done in conjunction with the methyl red test since the production of acetyl methyl carbinol or butylene glycol usually results in insufficient acid accumulating during fermentation to give a methyl-red positive reaction. An organism of the enterobacterial group is usually either methyl red- positive and Voges-Proskauar-negative or methylred- negative and Voges-Proskauerï positive.

Medium (glucose phosphate peptone water as for the methyl red test).

Method I (Oômeara, 1931)

The glucose phosphate peptone water in a tube was inoculated with the test organisms. This was incubated at 37^{o}C for 48 hours only. 0.5ml of OôMeara reagent (40g potassium hydroxide and 0.3g creatine in 100ml distilled water) was added to the medium after incubation. The tube was placed in a 37^{o}c water bath for 4 hours.The tubes was aerated by shaking at intervals. A positive reaction was denoted by the development of an eosin pink colour, usually in 2-5 minutes (Collee *et al.*, 1989)

3.3.8.7 Citrate Utilization Test

This is a test for the ability of an organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen. Koserôs liquid citrate medium or Simonôs citrate agar may be used.

Method

- A saline suspension of the organism to be tested was inoculated into Simmonsô citrate agar in a bottle. This was done by using a straight wire to collect the saline suspension of the organism and this was stabbed into the buttom of the agar and was also streaked on the surface of the slop in a zigzag manner.
- The culture was incubated for 96 hours at $37^{o}C$

Results: Positive test = blue colour and streak of growth

negative test = original green color and no growth (Collee *et al.,* 1989).

3.3.8.8 Preparation of Test Media

Peptone water fermentation test media

Peptone water was reconstituted according to manufacturerôs instruction. Ten ml of Andradeôs indicator was added to 950ml of peptone water in a conical flask and this was sterilized at $121^{o}C$ for 15 minutes. The test compound 10% solution (10g in 100ml of distilled water) was sterilized by boiling at $100^{o}C$ for 20 minutes and 50 ml of this sugar solution was ascetically added to the sterilized peptone water containing Andradeôs indicator. The mixture was swirled and ascetically dispensed in 5ml amounts into sterilized bijou bottles containing Durham tubes.

Method of Inoculation

On cooling, each 5ml sugar solution in bijou bottle was seeded with a speck of the colony of a particular test organism and incubated for 48hours at $37^{o}C$. At the end of the incubation period, the bottles and their contents were examined for the presence of colour changes to pink which indicated acid production and for gas formation in the durham tubes (Collee *et al.,* 1989).

3.3.8.9 Deoxyribonuclease (DNase) plate test

The method is modified from Jeffries (1961)

The DNA agar (oxoid) plate was prepared according to the manufacturers instruction and autoclaved at 121^{o}C for 15 minutes, mixed well and poured in plates. After setting and drying of the plates, each plate was divided into about six sections by drawing lines on its bottom. The material from the colony under test was inoculated by spotting it onto small area in the middle of one of the marked sections of the DNA plate. Other cultures to be tested were also spot ï inoculated onto other sections. The plate was incubated aerobically for 18-24 hours at 37^{o}C. After incubation, the plate was flooded with 1mol/litre (3.6%) hydrochloric acid to precipitate the non-hydrolyzed DNA.The plate was allowed to stand for a few minutes until a white cloudiness was seen in the agar and the plate was carefully examined under strong indirect light against a dark background.

Positive DNase test ï Spot cultures that were surrounded by a clear, uncloudy zone 3mm in radial width from the edge of the colony.

Negative DNase test ï Spot cultures with markedly smaller zones and those with no clearing at all.

3.8.8.10 REF 20 500

Api®Staph

Identification system for staphylococci*, Micrococcus* and related genera.

SUMMARY AND EXPLANATION

API staph is a standardized system for the identification of the genera *Staphylococcus*, *Micrococcus* and *Kocuria*, which uses miniaturized biochemical tests and a specially adapted database. The complete list of those bacteria that it is possible to identify with this system can be found in the identification table at the end of this package insert.

Principle

The API staph strip consists of 20 microtubes containing dehydrated substrates. These microtubes are inoculated with a bacterial suspension, prepared in API Staph medium that reconstitutes the tests. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table and the identification is obtained by referring to the analytical profile index or using the identification software.

Content of the Kit (Kit for 25 tests)

- 25 API staph strips
- 25 incubation boxes
- 25 Ampules of API Staph medium
- 25 result sheets
- 1 packet insert

Composition

Strip: The composition of the API Staph strip is given in the reading table of this package insert

Medium: API staph medium 6ml contains the following:

Year extract 0.5g

Bactopeptone (bovine/ porcine origin) é é .10g

NaCl é ... é é é é é ..5g

Trace elements é é ...10ml

Demineralized wate...qsp 1000ml

pH: 7.0 -7.4

Method for api® staph identification of staphylococci

1. **Preparation of the strip**
   - An incubation box (tray and lid provided) was prepared. About 5 ml of distilled water was distributed into the honey combed wells of the tray to create a humid atmosphere.

- The reference no of the strain of *Staphylococcus* was recorded on the elongated flap of the tray.
- The strip was removed from its packaging and placed in the incubation box.

2 Preparation of the inoculums

- The staphylococcal organism was subcultured onto sheep blood agar or Columbia blood agar which was incubated for 18-24 hr at 37^{o}C. This had previously been confirmed as staphylococci belonging to the Micrococcaccae family by Gram stain, catalase test etc.
- The API staph medium ampule was opened with precautions adhered to
- A homogeneous bacterial suspension with a turbidity equivalent to 0.5 McFarland was prepared from the staphylococcal strain of young culture (18-24 hours old).

3 Inoculation of the strip

- Using a pipette or Psipette, the micortubes were filled with the inoculated API staph medium.
- Anaerobiosis was ensured in the ADH and URE test by filing the cupules with mineral oil to form a convex meniscus
- The incubation box was closed
- Incubation was carried out at 37^{o}C for 18 -24 hours.

4. Reading and Interpretation

Reading the strip:

After the incubation period, reactions were developed by adding I drop of each of the following reagents and then all the reactions were read by referring to the reading table:

- VP test: VPI and VP2 reagents were added and it was left for 10 minutes. A violet ï Pink colour indicated a positive reaction. A pale pink or light pink colour obtained after 10 minutes was considered negative.
- NIT test: NIT 1 and NIT 2 reagents were added and it was left for 10 minutes. A red colour indicated a positive reaction.

- PAL test: ZYMA and ZYMB reagents were added and it was left for 10 minutes. A violet colour indicated a positive reaction.

5. Lysostaphin resistance test
6. Interpretation

Identification was obtained with the numerical profile

- Determination of the numerical profile:

On the result sheet, the tests were separated into groups of 3 and a value of 1, 2 or 4 indicated for each. By adding together the values corresponding to positive reaction within each group, a 7 digit profile number was obtained.

- Identification

This was performed using the database (V4.1)

- With the analytical profile index

The numerical profile was looked up in the list of profiles

- With the api web TM identification software: the 7 digit numerical profile was entered manually via the key board.

Once you have entered all the results on the result sheet into the api webÊ , identification software and press confirm, the Staphylococci was typed to specie and strain with a profile number.

3.3.9 Antimicrobial Drug Sensitivity Testing

Antimicrobial drug sensitivity testing was performed on the isolates from UTI cases with significant bacteriuria. This was performed on both Gram positive and Gram negative isolates. The disc diffusion method Kirby ï Bauers method (Bauer *et al.*, 1966) was adopted in the present study for antimicrobial drug sensitivity testing (Collee *et al.*, 1989, Ochei and Kolhatkar, 2000). In the Kirby Bauer method, the inhibition zone diameters of the test organisms are compared with those of the control strains with reference to the corresponding minimum inhibitory concentrations and interpretative guide lines published by national committee for clinical laboratory standard (NCCLs).

The antimicrobial discs used for drug sensitivity tests in the present study were multi disc (OPTU DISC Nig Ltd).

Method

- A loopful of inoculums was placed in the centre of the nutrient agar plate and was spread all over the surface of the plate with a dry sterile cotton wool swab.
- The surface of the agar was allowed to dry for about 3-5 minutes before adding the antimicrobial discs. The drug used contained ten antimicrobial drug discs held in a circular manner together with a distance of at least 22mm form each other.
- This was placed on the surface of the nutrient agar plate using forceps. The discs were placed at least 14mm from the edge of the plate. Each disc was pressed on the agar using forceps to provide uniform contact with the surface.
- The plate was incubated at 37^{o}C for 16-18 hours.
- An even semi confluent growth (neither too heavy nor too light) was achieved after incubation.

Results

At the end of the incubation, the inhibition zones were measured. The diameter of the inhibition zone was measured from one end to the opposite edge. The zones were measured to the nearest millimeter as complete inhibition of growth appears to the unaided eyes.

Interpretation of result

Interpretative standards provided by NCCLS are susceptible, intermediate and resistant according to the inhibitory zone size for each antimicrobial. The sensitivity test result and corresponding MIC (approx) are determined from the NCCLS table.

Gram positive drug disc (OPTU DISC) used were:

Ciprofloxacin (CPX) 10mcg, Norfloxacin (NB) 10mcg, Gentamycin (CN) 10mcg, Amoxyl (AMX) 20mcg, Streptomycin (S) 30Mcg, Rifampicin (RD) 20mcg, Erythromycim (E) 30mcg, Chloramphemicol (CH) 30mcg, Ampiclox (APX) 20Mcg Levofloxacin (LEV) 20mcg.

Gram Negative Drug Disc

Ofloxacin (Tarivid) 10mcg, Peflacin (PEF) 10mcg, Ciprofloxacin (CPX) 10mcg, Augumentin (AU) 30mcg, Gentamycin (CN) 10mcg, Streptomycin (S) 30mcg, Ceporex (CEP) 10mcg, Nalidixic acid (NA) 30mcg, Septrin (SXT) 30mcg, Ampicillin (PN) 30mcg

3.3.10 Novobiocin Drug Sensitivity Test

Novobiocin sensitivity test was carried out by placing a 5µg novobiocin disc on a plate culture of the test organism. This was incubated at 37^0C for 16 to 18 hours. Method is as usual for disk diffusion test for antibiotic sensitivities. Zone of inhibition of growth was measured to determine sensitivity of the organism to novobiocin or resistance.

Result

A large zone of inhibition of growth e.g. Over 15 mm in diameter around a 7mm disk shows sensitivity to novobiocin by *S. epidermidis* whilst *S. saprophyticus* shows a much smaller zone or grows right up to the disk.

3.4 ANIMAL STUDY

The animal study was designed to assess the pathogenesis and virulence of the three major staphylococcal species implicated in UTI in the present study (*S. aureus, S. xylosus, S. lentus*), in the kidney, bladder and liver following intraperitoneal inoculation of albino Wistar rats. The virulence was assessed using graded doses of the staphylococcal species (0.2, 0.5, 1.0, 0.0 control) ml /kg body weight of the rats in the experiment. The rats were purchased from a private animal house of Mr Maduka Luke Nweke of the department of physiology, University of Nigeria, Enugu Campus (UNEC). They were taken to the animal house of the College of Medicine, UNEC and were allowed to acclimatize for 14 days before inoculation while feeding them daily with grower feed before inoculation. Two strains of *S. aureus* (lab nos 834 and 856Ba), one strain of *S. xylosus* lab no 837 and one strain of *S. lentus* lab no 853 were used for the study. A total of 200 albino wistar rats were used for the study. The animals were placed in 18 cages with the first16 cages (1-16) containing 12 albino Wistar rats each while the last 2 cages (17 and 18) which served as controls contained 4 albino Wistar rats each. Intraperitoneal inoculation of both the saline broth of the staphylococcal tests species as well as the peptone water broth of the staphylococcal test species were conducted on the rats. The rats were weighed before the inoculation. Animals in cages 1, 2,3,15 contained twelve (12) female albinowistar rats each and were inoculated intraperitionally with peptone water broth culture of *S. aureu*s 834, *S. xylosus* 837,*S. aureus* 856Ba and *S.*

lentus 853 respectively. In each of the cages, the rats were grouped into three groups of 4 rats each A,B, C and the groups were inoculated with graded doses of the staphylococcal species A = 0.2ml, B= 0.5ml, C= 1.0ml respectively.

Cages 4,5,6 and 16 contained twelve (12) male albino wistar rats each and were also inoculated intrapertonealy with peptone water broth culture of S. aureus 834, *S. xylosus* 837, *S. aureus* 856Ba and *S. lentus* 853 respectively. In each of the cages, the rats were also grouped into three groups of 4 rats each A,B,C and the groups were inoculated with graded doses of the staphylococcal species A= 0.2ml, B= 0.5ml, C = 1.0ml respectively. Cage 17 contained a group of 4 female rats while 18 contained a group of 4 male rats. Cages 17 and 18 served as control and were not inoculated with any broth culture.

Cages 7,9,11,13 contained female albino Wistar rats while cages 8,10,12,14 contained male albino wistar rats. These eight cages contained 12 albino Wistar rats each and in each cage, the rats were grouped into three groups of 4 rats each A,B,C and these three groups were inoculated intraperitoneally with graded doses of saline broth culture of the test staphylococcal species A= 0.2ml, B= 0.5ml, C = 1.0 ml respectively. Cages 7, 9, 11,13 were inoculated with graded doses of saline broth culture of *S. aureus* 834, *S. xylosus* 837, *S. aureus* 856B and *S. lentus* 853 respectively, cages 8,10,12,14 were also inoculated with graded doses of saline broth culture of *S. aureus* 834, *S. xylosus* 837, *S. aureus* 856 Ba and S. *lentus* 853 respectively.

After inoculation, the animals were returned to their respective cages and were observed for 72 hours while feeding them with their food and water. On completion of the 72 hrs, the animals were brought out of the cages and were weighed and scarificed. The kidney, liver and bladder of these rats were harvested and were put in formalin immediately. They were quickly sent to the histology laboratory of the University of Nigeria Teaching Hospital, Ituku Ozalla Enugu State for tissue processing by Mr. Franklin Achi a laboratory scientist with specialty in histopathology. Later the processed tissue sections on slides were sent for histological analysis.

3.5 STATISTICAL ANALYSIS

All data obtained were analyzed using T-Test, 1-way ANOVA, 2-way ANOVA and Fisherôs exact test (contingency table).

CHAPTER FOUR

RESULTS

Results of the present study showed that out of the 818 patients and subjects examined for urinary tract infection, 307 were positive with a prevalence rate of 37.5% (Table 4.1, Appendix 3). The prevalence rate of staphylococcal UTI in the study was 10.9%

Table 4.1 (Appendix 3) shows the distribution of positive cases for urinary tract infection in the population studied according to age group and sex. The overall prevalence of UTI was 37.5%. Females had higher occurrence for UTI 251(81.8%) than males 56 (18.2%), but the difference in the distribution of positive cases for UTI between the males and

females examined was not statistically significant (P > 0.05, P value = 0.0975 using two-way ANOVA). However, there was statistically significant difference (P < 0.05, P value =0.0001) between the total number of males positive for UTI and that for positive females using Fisher exact test for contingency. The age group 22-32 years had the highest occurrence of UTI 107(34.9%) followed by age group 11-21 years which had 88(28.7%), age group 33-43 years 49(16.0%), age group 44-54 years 23(7.5%), age group 55-65 years 19(6.2%), age group 66-76 years 12(3.9%), age group 0-10 years 8 (2.6%) while the least was age group 77-87 years which had 1(0.3%). The difference in the distribution of positive cases between age groups studied was not statistically significant P > 0.05, P value = 0.4086 using Two way ANOVA)

Figure 1 (Appendix 4) shows the total distribution of positive bacterial isolates in UTI in the population studied. Of the 307 isolates from UTI in the present study, *Staphylococcus* spp were the most abundant organisms encountered 89(29.0%), followed by *Escherichia coli,* 72(23.5%), *Klebsiella* spp 30(9.8%), *Pseudomonas* spp 27(8.8%), *Proteus* spp and *Enterococcus faecalis* 21(6.8%) each, *Streptococcus* spp 19(6.2%), Diptheroids and *Bacillus* spp 8(2.6%) each, *Streptococcus agalactia* 4(1.3%), *Enterobacter* spp and *Micrococcus* spp 3(1.0%) each, while the least were *Lactobacillus* spp and *Candida albicans* which had 1(0.3%)each. The difference between the number of the different organisms isolated from the study was statistically significant (P < 0.05, P value = 0.0001 using one-way ANOVA)

Table 4.1

Distribution of Positive Cases for Urinary Tract Infection in the Population Studied According to Age Group and Sex

n = 818

Age group (Yrs)	No of males sampled	No of females sampled	No of males positive for UTI	%	No of females positive for UTI	%	Total no of M+F sampled	Total no M+F +ve for UTI	%

0-10	43	33	5	62.5%	3	37.5%	76	8	2.6%
11-21	50	178	6	6.8%	82	93.2%	228	88	28.7%
22-32	70	174	12	11.2%	95	88.8%	244	107	34.9%
33-43	30	82	8	16.3%	41	83.7%	112	49	16.0%
44-54	41	35	7	30.4%	16	69.6%	76	23	7.5%
55-65	34	18	10	52.6%	9	47.4%	52	19	6.2%
66-76	17	7	8	66.7%	4	33.3%	24	12	3.9%
77-87	5	1	0	0%	1	100%	6	1	0.3%
Total	**290**	**528**	**56**	**18.2%**	**251**	**81.8%**	**818**	**307**	**37.5%**

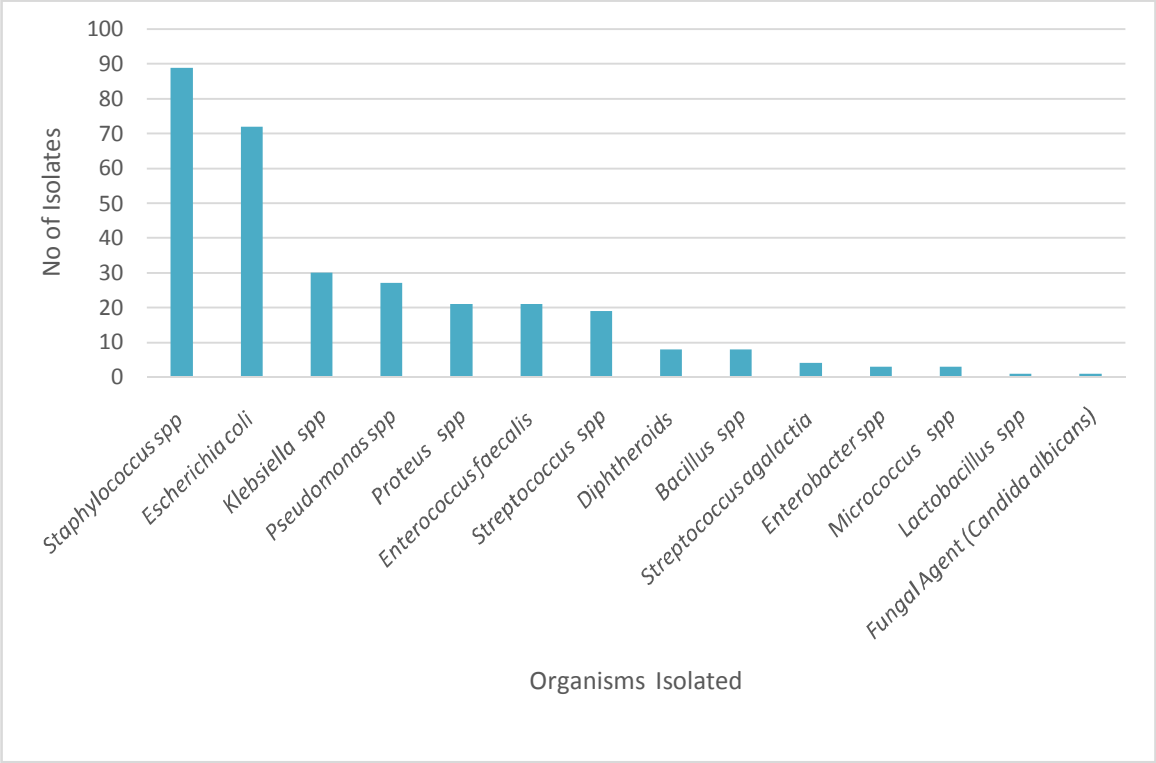

Fig. 1: **Total Distribution of Positive Bacterial Isolates in UTI in the Population Studied**

Table 4.2 (Appendix 5) shows the distribution of positive cases for UTI in the population studied according to age group, sex and bacterial type. Females showed higher occurrence of positive cases for UTI 251(81.8%) than males 56 (18.2%). The age group 22-32 years had the highest occurrence of UTI 107(34.9%) while the least was age group 77-87 which had 1(0.3%). Amongst the isolates, *Staphylococcus* spp showed highest occurrence

89(29.0%) followed by *Escherichia coli* 72(23.3%) while the least were *Lactobacillus* spp and a fungal agent (*Candida albicans*) which had 1(0.3%) each.

Table 4.3 (Appendix 6) shows the distribution of positive cases for Staphylococcal UTI in the population studied according to age group and sex. Of the 818 patients and subjects investigated for UTI, 89 had Staphylococcal UTI with a prevalence rate of 10.9%. Females had higher occurrence of Staphylococcal UTI 69(77.5%) than males who had 20 (22.5%) but the difference in the distribution of positive cases for staphylococcal UTI between males and females examined was not statistically significant (P > 0.05, P value = 0.1224 using two way ANOVA). The difference between the total number of males positive for staphylococcal UTI and that of positive females was statistically significant (P< 0.05, P value = 0.0068 using fisherôs exact test for contingency). The age group 22-32 years had the highest occurrence of Staphylococcal UTI 37(41.6%) followed by the age group 11-21 years 25(28.1%), age group 33-43 12(13.5%), age group 44-54 years 6(6.7%), age group 55-65 years (5.6%), age group 0.-10 years 3(3.4%), age group 66-76 years 1(1.1%) while the least was age group 77 ï 87 which had 0 (0%). The difference in the distribution of positive cases between age groups studied was not statistically significant (P > 0.05, P value = 0.2301 using Two way ANOVA).

Figure 2 (Appendix 7) shows the distribution of *Staphylococcus* species in urinary tract infection in Enugu. Of the 89 Staphylococcal isolates from UTI in the present study, *Staphylococcus aureus* were the most abundant *Staphylococcus* species encountered 40 (44.9%) followed by *Staphylococcus xylosus* 25 (28.1%), *Staphylococcus lentus* 9 (10.1%), *S. capre, S. sciuri, S. haemolyticus* and *S. epidermidis* 3 (3.4%) each, while the least were *S. hominis, S.* capitis and *S. saprophyticus* which had 1(1.1%) each.

The difference between the number of the different *Staphylococcus* spp isolated from the study was statistically significant (P < 0.05, P value = 0.0001 using one way ANOVA).

Table 4.2

Distribution of Positive Cases for UTI in The Population Studied According to Age Group, Sex and Bacterial Type

n =307

Age group (years)	*Staph*	*spp*	*Escherichia*	*coli*	*klebsiella*	*spp*	*Pseudomonas*	*spp*	*Proteus*	*spp*	*Enterococcus*	*faecalis*	*Streptococcus*	*spp*	*Diphtheroids*		*Bacillus*	*spp*	*Streptococcus*	*agalactia*	*Enterobacter*	*spp*	*Micrococcus*	*spp*	*Lactobacllus*	*spp*
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0-10	3	0	1	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
11-21	3	22	1	15	0	8	0	4	0	4	1	6	1	8	0	7	0	3	0	2	0	0	0	2	0	1
22-32	7	30	0	21	2	8	2	9	0	11	0	5	1	1	0	0	0	4	0	2	0	2	0	1	0	0
33-43	2	10	3	14	1	4	1	3	0	3	0	4	1	1	0	1	0	1	0	0	0	0	0	0	0	0
44-54	1	5	2	4	0	3	1	0	0	0	0	3	2	1	0	0	0	0	0	0	1	0	0	0	0	0
55-65	3	2	5	1	0	0	1	3	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
66-76	1	0	3	2	2	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77-87	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	20	69	15	57	5	25	8	19	0	21	1	20	6	13	0	8	0	8	0	4	1	2	0	3	0	1
% Total	35.7	27.5	26.8	**22.7**	**8.9**	**10.0**	**14.3**	**7.6**	0	**8.4**	**1.8**	**8.0**	**10.7**	5.2	0	**3.2**	0	**3.2**	0	**1.6**	**1.8**	0.8	0	1.2	0	0.4
Total M+F	89		72		**30**		**27**		**21**		**21**		19		8		8		4		3		3		1	
% total M+F	29.0		23.5		**9.8**		**8.8**		**6.8**		**6.8**		**6.2**		**2.6**		**2.6**		**1.3**		1		1		**0.3**	

Table 4.3

Distribution of Positive Cases for Staphylococcal UTI in The Population Studied According to Age Group and Sex

n= 818

Age group (Yrs)	No of males sampled	No of males +ve for *Staph* spp UTI	%	No of females sampled	No of females +ve for *Staph* spp UTI	%	Total no of M+F sampled	Total no M+F +ve for *Staph* spp UTI	%
0-10	43	3	100%	33	0	0%	76	3	3.4%
11-21	50	3	12.0%	178	22	88%	228	25	28.1 %
22-32	70	7	18.9%	174	30	81.1%	244	37	41.6%
33-43	30	2	16.7%	82	10	83.3%	112	12	13.5%
44-54	41	1	16.7%	35	5	83.3%	76	6	6.7%
55-65	34	3	60%	18	2	40%	52	5	5.6%
66-76	17	1	100%	7	0	0%	24	1	1.1%
77-87	5	0	0%	1	0	0%	6	0	0%
Total	**290**	**20**	**22.5%**	**528**	**69**	**77.5%**	**818**	**89**	**10.9%**

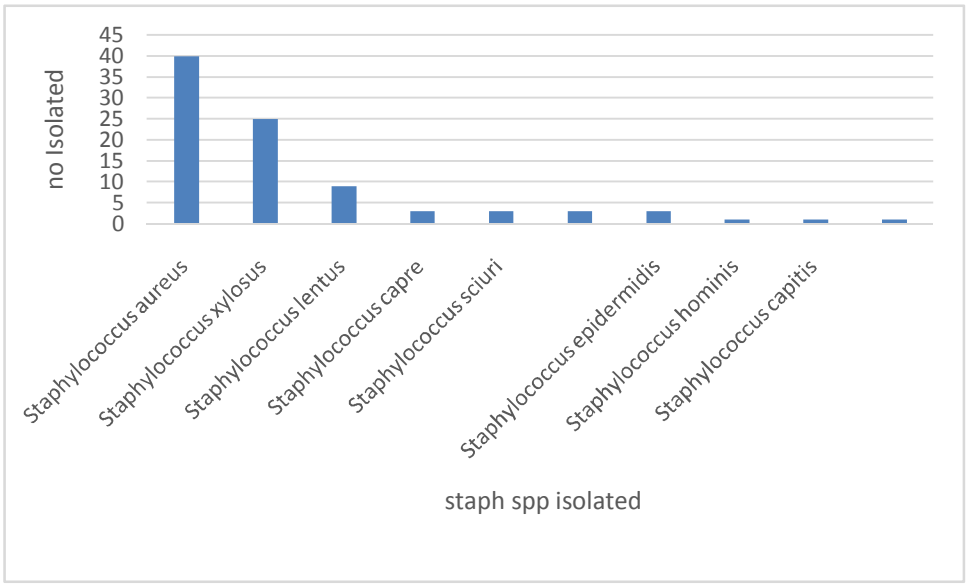

Fig. 2: Distribution of Staphylococcal species in Urinary Tract Infection in Enugu

Table 4.4 (Appendix 8) shows the distribution of positive cases for *Staphylococcal* UTI according to age group and sex. Females had higher occurrence for Staphylococcal UTI 69 (77.5%) than males 20 (22.5%). The age group 22-32 years had the highest occurrence

of Staphylococcal UTI 37(41.6%) followed by age group 11-21 years which had 25 (28.1%) while the least was age group 77-87 years which had 0 (0%). Amongst the isolates, *Staphylococcus aureus* had the highest occurrence for Staphylococcal UTI 40 (44. 9%) while the least were *S.hominis*, *S. capitis* and *S.saprophyticus* which recorded 1(1.1%) each.

Table 4.5 Appendix 9 shows the distribution of positive cases for *Staphylococcus aureus* UTI in the population studied according to age group and sex. Of the 818 patients and subjects examined for UTI in the present study, 40 were positive for *Staphylococcus aureus* UTI with a prevalence rate of 4.9%. Females had higher occurrence of *Staph aureus* UTI 33(82.5%) than males which had 7(17.5%) but the difference in the distribution of positive cases for *Staph aureus* UTI between males and females examined was not statistically significant (P >0.05, P value = 0.1190 using two way ANOVA). However, the difference between the total number of males positive for *Staph aureus* UTI and that of positive females was statistically significant using fisherôs exact test for contingency (P < 0.05, P value = 0.0167). The age group 22-32 had the highest occurrence of *Staph aureus* UTI 18(45.0%), followed by the age group 11-21 years 9 (22.5%), age group 33-43 years 7(17.5%), age group 44-54 years 3(7.5%), age group 55-65 years 2(5%), age group 0-10 years 1(2.5%) while age groups 66-76 and 77-87 years had 0(0%) each. The difference in the ditribution of positive cases for *Staph aureus* UTI between the age groups studied was not statistically significant (P > 0.05, P Value = 0.3246 using two way ANOVA).

Table 4.6 (Appendix 10) shows the distribution of positive cases for *Staphylococcus xylosus* UTI in the population studied according to age group and sex. Of the 818 patients and subjects examined for UTI in the present study, 25 were positive for *Staphylococcus xylosus* UTI with a prevalence rate of 3.1%. Females higher occurrence of *Staph xylosus* UTI 20(80%) than males which had 5 (20%), however the difference in the distribution of positive cases for *Staph xylosus* UTI between males and females examined was not statistically significant (P > 0.05, P value = 0.1443 using two way ANOVA). Contingency of gender between the total number of males positive for *S. xylosus* UTI and that of positive females was also was not significant (P > 0.05, P value = 0.1362).

The age group 11-21 and 22-32 years had the highest occurrence of *S. xylosus* UTI 9 (36%) each, followed by age group 33-43 years which had 3 (12%), age group 55-65 years 2 (8%), age group 44-54 and 66-76 years 1(4%) each while age group 0-10 and 77-87 years were the least 0 (0%) each. The difference in the distribution of *S. xylosus* UTI positive cases between the age groups studied was not statistically significant ($P > 0.05$, P value = 0.3487 two way ANOVA).

Table 4.4

DISTRIBUTION OF POSITIVE CASES FOR *Staphylococcus spp UTI* ACCORDING TO AGE GROUP AND SEX

Age group (years)	No of subjects sampled	*S. aureus*				*S. xylosus*				*S. lentus*				*S. capre*				*S. sciuri*				*S. haemolyticus*				*S. epidermidis*				*S. hominis*				*S. capitis*				*S. saprophyticus*						
		M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%		%	Total No of M	Total No of F-females
0-10	76	1	0	1	33.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	66.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100	0	
11-21	228	1	8	9	36	0	9	9	36	2	2	4	16	0	2	2	8	0	0	0	0	0	0	0	0	0	1	1	4	0	0	0	0	0	0	0	0	0	0	0	0	3	12	22	
22-32	244	2	16	18	48.6	3	6	9	24.3	0	4	4	10.8	0	1	1	2.7	0	1	1	2.7	1	0	1	2.7	1	1	2	5.4	0	1	1	2.7	0	0	0	0	0	0	0	0	7	18.9	90	
33-43	122	1	6	7	58.3	0	3	3	25	1	0	1	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	1	8.3	2	16.7	10	
44-54	78	1	2	3	50	0	1	1	16.7	0	0	0	0	0	0	0	0	0	1	1	16.7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	16.7	0	0	0	1	16.7	5	
55-65	52	1	1	2	40	1	1	2	40	0	0	0	0	0	0	0	0	1	0	1	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	60	2	
66-76	24	0	0	0	0	1	0	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	0	
77-87	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total	818	7	33	40	44.9	5	20	25	28.1	3	6	9	10.1	0	3	3	3.4	1	2	3	3.4	3	0	3	3.4	1	2	3	3.4	0	1	1	1.1	0	1	1	1.1	0	1	1	1.1	20	22.5	69	

Table 4.5

Distribution of Positive Cases for *staphylococcus Aureus* UTI in the Population Studied According to Age Group and Sex

Age group (years)	No of males sampled	No +ve for *Staph aureus* UTI	%	No of females sampled	No +ve for *Staph aureus* UTI	%	Total no of M+F sampled	Total no M+F +ve for *Staph aureus* UTI	%
0-10	43	1	100%	33	0	0%	76	1	2.5%
11-21	50	1	11.1%	178	8	88.9%	228	9	22.5%
22-32	70	2	11.1%	174	16	88.9%	224	18	45.0%
33-43	30	1	14.3%	82	6	85.7%	112	7	17.5%
44-54	41	1	33.3%	35	2	66.7%	76	3	7.5%
55-65	34	1	50%	18	1	50%	52	2	5.0%
66-76	17	0	0%	7	0	0%	24	0	0%
77-87	5	0	0%	1	0	0%	6	0	0%
Total	**290**	**7**	**17.5%**	**528**	**33**	**82.5%**	**818**	**40**	**4.9%**

Table 4.6

Distribution of Positive Cases for *staphylococcus xylosus* UTI in the Population Studied According to Age Group and Sex

Age group (Yrs)	No of males sampled	No +ve for *Staph* xylosus UTI	%	No of females sampled	No +ve for *Staph* xylosus UTI	%	Total no of M+F sampled	Total no M+F +ve for *Staph* xylosus UTI	% prevalance
0-10	43	0	0%	33	0	0%	76	0	0%
11-21	50	0	0%	178	9	100%	228	9	36%
22-32	70	3	33.3%	174	6	66.7%	224	9	36%
33-43	30	0	0%	82	3	100%	112	3	12%
44-54	41	0	0%	35	1	100%	76	1	4%
55-65	34	1	50%	18	1	50%	52	2	8%
66-76	17	1	100%	7	0	0%	24	1	4%
77-87	5	0	0%	1	0	0%	6	0	0%
Total	**290**	**5**	**20%**	**528**	**20**	**80%**	**818**	**25**	**3.1%**

Table 4.7 (Appendix 11) shows the distribution of positive cases for *Staphylococcus lentus* UTI in the population studied according to age group and sex. Of the 818 patients

and subjects examined for UTI in the present study, 9 were positive for *S.lentus* UTI with prevalence rate of 1.1%. Females had higher occurrence of *Staph lentus* UTI 6(66.7%) than males who had 3(33.3%) but the difference in the distribution of positive cases for *S. lentus* UTI between males and females examined was not statistically significant (P > 0.05, P value = 0.5040 using two way ANOVA). The difference in the total number of *S. lentus* positive cases between males and females was also not statistically significant (P > 0.05, P value = 1.0000 using fisherôs exact test for contingency). The age group 11-21 years and 22-32 years had the highest occurrence of *S.lentus* UTI 4(44.4%) each followed by the age group 33-43 years which had 1(11.1%) while the rest of the age groups recorded 0 (0%). The difference in the distribution of *S. xylosus* UTI positive cases between the age groups studied was not statistically significant (P > 0.05, P value = 0.3209 using one way ANOVA).

Table 4.8 shows the prevalence of staphylococcal UTI amongst different occupational groups studied. Out of the total number of each occupational groups sampled and examined for staphylococcal UTI , the prevalence of staphylococcal UTI was highest for traders 25/134 (18.7%) followed by transporters 5/29 (17.2%), house wives 5/32 (15.6%), students tertiary18/156 (11.5%), secondary students 14/153 (9.2%), pupils 9/112 (8.0%), civil servants 11/165(6.7%) while the least were artisans who had 2/37 (5.4%). Variation in the total number of staphylococcal spp UTI positive cases seen between occupational groups studied was statistically significant (P < 0.05, P value = 0.0256 using Fisherôs exact test for contingency).

Table 4.7

Distribution of Positive Cases for *Staphylococcus lentus* UTI in the Population Studied According to Age Group and Sex

Age group (Years)	No of males sampled	No +ve for *Staph lentus* UTI	%	No of females sampled	No +ve for *Staph lentus* UTI	%	Total no of M+F sampled	Total no M+F +ve for *Staph lentus* UTI	%
0-10	43	0	0%	33	0	0%	76	0	0%
11-21	50	2	50%	178	2	50%	228	4	44.4
22-32	70	0	0%	174	4	100%	224	4	44.4%
33-43	30	1	100%	82	0	0%	112	1	11.1%
44-54	41	0	0%	35	0	0%	76	0	0%
55-65	34	0	0%	18	0	0%	52	0	0%
66-76	17	0	0%	7	0	0%	24	0	0%
77-87	5	0	0%	1	0	0%	6	0	0%
Total	**290**	**3**	**33.3%**	**528**	**6**	**66.7%**	**818**	**9**	**1.1%**

Table 4.8

Prevalence of *Staph* spp UTI Amongst Different Occupational Groups Studied

Occp. Groups Studied	**No sampled n=**	**No +ve for UTI**	**% prevalence**
Students (Tertiary)	156	18	11.5%
Students (secondary)	153	14	9.2%
Pupil	112	9	8.0%
Civil servants	165	11	6.7%
Traders	134	25	18.7%
House wives	32	5	15.6%
Artisans	37	2	5.4%
Transporters	29	5	17.2%
Total	**818**	**89**	**10.9%**

Table 4.9 shows the distribution of positive cases for staphylococcal UTI according to occupational groups and types of organisms isolated. Of the 18 positive cases for staphylococcal UTI in the students of tertiary institution investigated, 10 (55.6%) were positive for *S. aureus* UTI followed by *S. xylosus, S.lentus and S. epidermidis* UTI which had 2(11.1%)each, *S.haemolyticus* and *S.hominis* UTI 1(5.6%) while the other staphylococcal spp recorded 0(0%). The distribution of staphylococcal species UTI in the other occupational groups studied can also be seen in table 4.9

Table 4.10 shows the prevalence of staphylococcal UTI amongst the different health conditions studied. Pregnant women had the highest prevalence rate for staphylococcal UTI 24/119 (20.2%) followed by apparently healthy persons who had 60/558 (10.8%) while the least were those with medical conditions who had 5/141(3.5%). Variation in the total number of staphylococcal spp UTI positive cases seen between different health conditions studied was statistically significant ($P < 0.05$, P value = 0.0001 using fisher exact test for contingency).

Table 4.9

Distribution of Positive Cases for *Staphylococcus* UTI According to Occupational Groups and Type of Organisms Isolated

Occupational group studied	No of subject sampled	No +ve for staph spp UTI	S.aureus	%	S. xylosus	%	S. lentus	%	S. capre	%	S. sciuri	%	S. haemolyticus	%	S. epidermidis	%	S. hominis	%	S. caplitis	%	S. saprophyticus	%
Students (Tertiary)	156	18	10	55.6	2	11.1	2	11	0	0.0	0	0.0	1	5.6	2	11.1	1	5.6	0	0.0	0	0.0
Student (Secondary)	153	14	5	35.7	7	50	0	0	2	14.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Pupils (nursery & primary	112	9	2	22.2	1	11.1	3	33	0	0.0	0	0.0	2	22.2	1	11.1	0	0.0	0	0.0	0	0.0
Civil servants	165	11	6	54.5	2	18.2	1	9.1	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	9.1
Traders	134	25	12	48.0	9	36	1	4.0	0	0.0	2	8.0	0	0.0	0	0.0	0	0.0	1	4.0	0	0.0
Housewives	32	5	2	40,0	1	20	2	40.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Artisans	37	2	1	50.0	1	50	0	0	0		0		0		0	0.0	0	0.0	0	0.0	0	0.0
Transport	29	5	2	40.0	2	40.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	**818**	**89**	**40**	**44.9**	**25**	**28.1**	**9**	**10.1**	**3**	**3.4**	**3**	**3.4**	**3**	**3.4**	**1**	**3.4**	**1**	**1.1**	**1**	**1.1**	**1**	**1.1**

Table 4.10

Prevalence of Staphylococcal spp UTI Amongst Different Health Conditions Studied

Health condition	**No sampled**	**No +ve for staph UTI**	**% prevalence**
Apparently healthy	558	60	10.8%
Pregnant cases	119	24	20.2%
Medical conditions	141	5	3.5%
Total	**818**	**89**	**10.9%**

Table 4.11 shows the distribution of positive cases for *Staph* spp UTI according to health status. Of the 60 positive cases for staphylococcal species UTI from apparently healthy cases, 24 (40%) had *S. aureus* UTI followed by *S. xylosus* 19 (31.7%), *S.lentus* 6 (10%) while the least was *S.hominis* which had 0 (0%). Of the 24 positive cases for staphylococcal spp UTI from pregnant women examined, 13 (54.2%) were positive for *S. aureus* UTI, followed by *S. xylosus* 6 (25%) , *S.lentus* 3 (12.5%) while least were *S.sciuri, S.haemolyticus, S. epidermidis, S. capitis* and *S.saprophyticus* which had 0 (0%) each. Out of the 5 positive cases for *Staph* spp UTI from those with medical conditions, 3 (60%) were positive for *S. aureus* UTI, *S.sciuri* 2 (40%) while the rest of the *staphylococcus* spp had 0 (0%) each. Gender variation shows that there was no statistically significant difference ($P > 0.05$, P value = 0.1655 using two way ANOVA) in the distribution of the different staphylococcal spp UTI between positive males and females in apparently healthy cases. However, there was a statistically significant difference between the total number of the various staphylococcal species isolated in apparently healthy cases ($P < 0.05$, P value = 0.0248 using two way ANOVA). Variation in the distribution of staphylococcal spp UTI positive cases between apparently healthy cases, pregnant women and medical conditions studied showed statistically significant difference $P < 0.05$, P value = 0.0035 using two way ANOVA). Also variation in the number of different staphylococcal spp isolated between three health conditions studied showed statistically significant difference ($P < 0.05$, P value = 0.0001 using two way ANOVA).

Table 4.12 shows the distribution of positive cases for staphylococcal spp UTI in the pregnant women studied according to age group and sex. Of the 119 pregnant women examined for UTI, 24 (20.2%) were positive. The age group 22-32 had the highest occurrence of UTI 19(79.2%) followed by the age group 33-43 years 4(16.7%) and lastly age group 11-21 years 1(4.7%).

Table 4.11

Distribution of Positive Cases for *Staph* spp. UTI According to Health Status

Staph organisms Isolated	n = 60 Apparently healthy cases no +ve				n= 24 Pregnant cases no +ve				n=5 Medical Conditions no +ve				n=89 Total	%
	M	F	T	%	M	F	T	%	M	F	T	%		
S. aureus	7	17	24	40%	0	13	13	54.2%	0	3	3	60%	40	44.9%
S. xylosus	5	14	19	31.7%	0	6	6	25%	0	0	0	0%	25	28.1%
S. lentus	3	3	6	10.%	0	3	3	12.5%	0	0	0	0%	9	10.1%
S. capre	0	2	2	3.3%	0	1	1	4.2%	0	0	0	0%	3	3.4%
S. sciuri	1	0	1	1.7%	0	0	0	0%	0	2	2	40%	3	3.4%
S. haemolyticus	3	0	3	5%	0	0	0	0%	0	0	0	0%	3	3.4%
S. epidermidis	1	2	3	5%	0	0	0	0%	0	0	0	0%	3	3.4%
S. hominis	0	0	0	0%	0	1	1	4.2%	0	0	0	0%	1	1.1%
S. capitis	0	1	1	1.7%	0	0	0	0%	0	0	0	0%	1	1.1%
S. saprophyticus	0	1	1	1.7%	0	0	0	0%	0	0	0	0%	1	1.1%
Total	**20**	**40**	**60**	**100%**	**0**	**24**	**24**	**100%**	**0**	**5**	**5**	**100%**	**89**	**10.9%**

Table 4.12

Distribution of Positive cases for Staphylococcal spp UTI in the Pregnant Women Studied According to Age Group

n = 119

Age group (yrs)no of	No of pregnant women sampled	No +ve for Stah UTI	Percentage (%)
11-21	9	1	4.7%
22-32	80	19	79.2%
33-43	30	4	16.7%
Total	**119**	**24**	**20.2%**

Table 4.13 shows the distribution of positive cases for staphylococcal spp UTI in the pregnant women studied according to organisms isolated. Of the 24 staphylococal spp UTI cases encountered in the pregnant women studied, *S. aureus* UTI occurred in 13 (54.2%), followed by *S.xylosus* UTI 6(25%), *S.lentus* UTI 3 (12.5%), *S. capre and S.hominis* UTI 1(4.2%) each while *S. sciuri*, S. *heamolyticus, S. epidermidis*, *S. capitis* and *S. saprophyticus* UTI recorded 0 (0%) each.

Table 4.14 shows the drug sensitivity pattern of Gram positive organisms isolated from the population studied. From the table, *S. aureus* had highest sensitivity to rifampicin and levofloxacin 29/40 (72.5%) each followed by ciprofloxacin 26/40 (65%) while the least were norfloxacin and gentanycin which had 19/40 (47.5%) each. *S. xylosus* had highest sensitivity to rifampicin 18/25 (72%) followed by ciprofloxacin 17/25 (68%) while the least was norfloxacin which had 11/25 (44%). *S. lentus* had highest sensitivity to rifampicin and amoxyl 7/9 (77.8%) each followed by levofloxacin 6/9 (66.7%) while the least were streptomycin and erythromycin which had 4/9 (44.4%).

On the whole, almost all the *Staphylococcus* species and other Gram positive isolates tested had highest sensitivity to rifampicin with the exception of the lone *Staphylococcus saprophyticus* which was resistant to rifampicin and then *Streptococcus* species which was sensitive to levofloxacin and rifampicin with levofloxacin being the most sensitive drug to it. Levofloxacin was the second most sensitive drug to *Staphylococcus* species and other Gram positive isolates while ciprofloxacin came 3rd either as a first line drug or 2nd line drug in degree of sensitivity to them.

TABLE 13

Distribution of Positive cases for Staphylococcal spp UTI in Pregnant Women Studied According to organisms Isolated

n = 24

Staph sp Isolated	Total	%
S. aureus	13	54.2%
S. xylosus	6	25%
S. lentus	3	12.5%
S. capre	1	4. 2%
S. sciuri	0	0%
S. haemolyticus	0	0%
S. epidermidis	0	0%
S. hominis	1	4.2%
S. capitis	0	0%
S. saprophyticus	0	0%
Total	**20**	**20.2%**

TABLE 4.14

DRUG SENSITIVITY PATTERN OF THE DIFFERENT STAPHYLOCOCCAL SPECIES ISOLATED FROM THE POPULATION STUDIED

Organisms Isolated	CPX %	NB %	CN %	AMX %	S %	RD %	E %	CH %	APX %	LEV %
S. aureus	26/40 (65%)	19/40 (47.5%)	19/40 (47.5%)	21/40 (52.5%)	24/40 (60%)	29/40 (72.5%)	24/40 (60%)	22/40 (55%)	24/40 (60%)	29/40 (72.5%)
S. xylosus	17/25(68%)	11/25(44%)	12/25(48%)	12/25(48%)	14/25(56%)	18/25(72 %)	12/25(48%)	12/25(48%)	13/25(52%)	14/25(56%)
S. lentus	5/9 (55.6%)	5/9 (55.6%)	5/9 (55.6%)	7/9 (77.8%)	4/9 (44.4%)	7/9 (77.8%)	4/9(44.4%)	5/9 (55.6%)	5/9 (55.6%)	6/9 (66.7%)
S. capre	3/3 (100%)	3/3 (100%)	3/3 (100%)	2/3 (66.7%)	2/3 (66.7%)	3/3 (100%)	2/3 (66.7%)	3/3 (100%)	3/3 (100%)	3/3 (100%)
S. sciuri	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	1/3 (33.3%)	1/3 (33.3%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)
S. haemolyticus	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7%)	2/3 (66.7)	2/3 (66.7%)	2/3 (66.7%)
S. epidermidis	3/3 (100%)	2/3 (66.7%)	2/3 (66.7%)	0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100/%)	2/3 (66.7%)	3/3 (100%)	3/3 (100%)
S. hominis	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
S. capitis	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)
S. saprophyticus	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)

CPX - Ciproflox
AMX - Amoxyl
E - Erythromycin
LEV - Levofloxacin
NB - Norfloxacin
S - Streptomycin
CH - Chloramphenicol
CN - Gentamycin
RD - Rifampicin
APX - Ampiclox

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Animal study designed to assess the pathogenesis and virulence of *Staphylococcus aureus, Staphylococcus xylosus* and *Staphylococcus lentus* on the kidney, bladder (urinary system) and the liver following intraperitonal inoculation of albino wistar rats with these *Staphylococcus* species isolates produced some tissue alterations. The Virulence was assesed using graded doses of the *Staphylococcus* species (0.2,0.5,1.0, 0.0 control) ml /kg body weight of the rats in experiments A, B,C,D respectively after 72 hrs of the inoculation. D served as control and was not inoculated.

In experiments A, B, C and D of group I inoculated with *Staphylococcus* aureus, the histology of the liver parenchyma remained intact [Plate 1(54L)]. There was occasional mild infiltration of the inflammatory cells [Plate 2 (65L)] at the peri-portal and pericentral areas of the liver. The kidney demonstrated a higher degree of toxigenic modification. There were presence of tubular casts in varying degree, tubular erosions [Plate 3 (56K)] and glomerular oedema [Plate 4 (59k)] in high doses of the inoculums at the kidney.

The extent of cellular damage increased with more concentration of the inoculum. One remarkable feature of *Staphylococcus aureus* is the tubular casts characterizing all values of the *Staphylococcus aureus* inoculums in kidney tubules [Plate 5 (5K)]

The bladder upon inoculation with infective doses of 0.2-1.0 ml/kg body weight of *Staph aureus* showed mild effect on the Mulsculartone. The transitional epithelia of the bladder remained intact [Plate 6 (69B)]. Infiltration of inflammatory cells which was in proportion of the infective doses of the inoculum was noted.

In group II model of assessment of pathogenesis and virulence of *Staphylococcus xylosus* after 72 hours of inoculation, the experiments E, F, G produced a relative less degree of cell modification/alteration and damage. Experiment H which comprises of peptone water only, served as control and as such produced no adverse effect. There was no visible alternation of the liver parenchyma. All hepatocytes of the rats liver including those of the control rats were normal [Plate 7 (56L)]. There were tubular casts [Plate 8 (45K)] in the kidney tubules. The glomerulus was mildly distorted, with mild infiltration of inflammatory cells. Patchy erosion of glomerulus was also noted. Capsular oedema

was noted in rats inoculated with high doses of *S. xylosus*. Tubular oedema with haemorrhagic patches was also seen in the stroma [Plate 9 (39k)].

In the bladder, the infective dose of *S. xylosus* inoculums did not affect the *musculature* of the bladder. There were inflammatory cells in the mucosae and connective tissues of the bladder [Plate 10 (59B)]. The transitional epithelia were intact and well defined Plate 11(58B)].

Group III model of experimentation using infective doses of *Staphylococcus lentus* had similar effects in the liver sections of rats inoculated. The liver showed no toxigenic effect. The central cannals were intact, radiated round by the liver sinusoids. All doses (0.2-1.0)ml/kg body weight of the infective doses of *S. lentus* produced into glomerular oedema, inflammatory infiltration and tubular casts in proportion to the inoculated dose [Plate 12 (85K)]. The bladder did not manifest erosion with *inflammatory* cells.

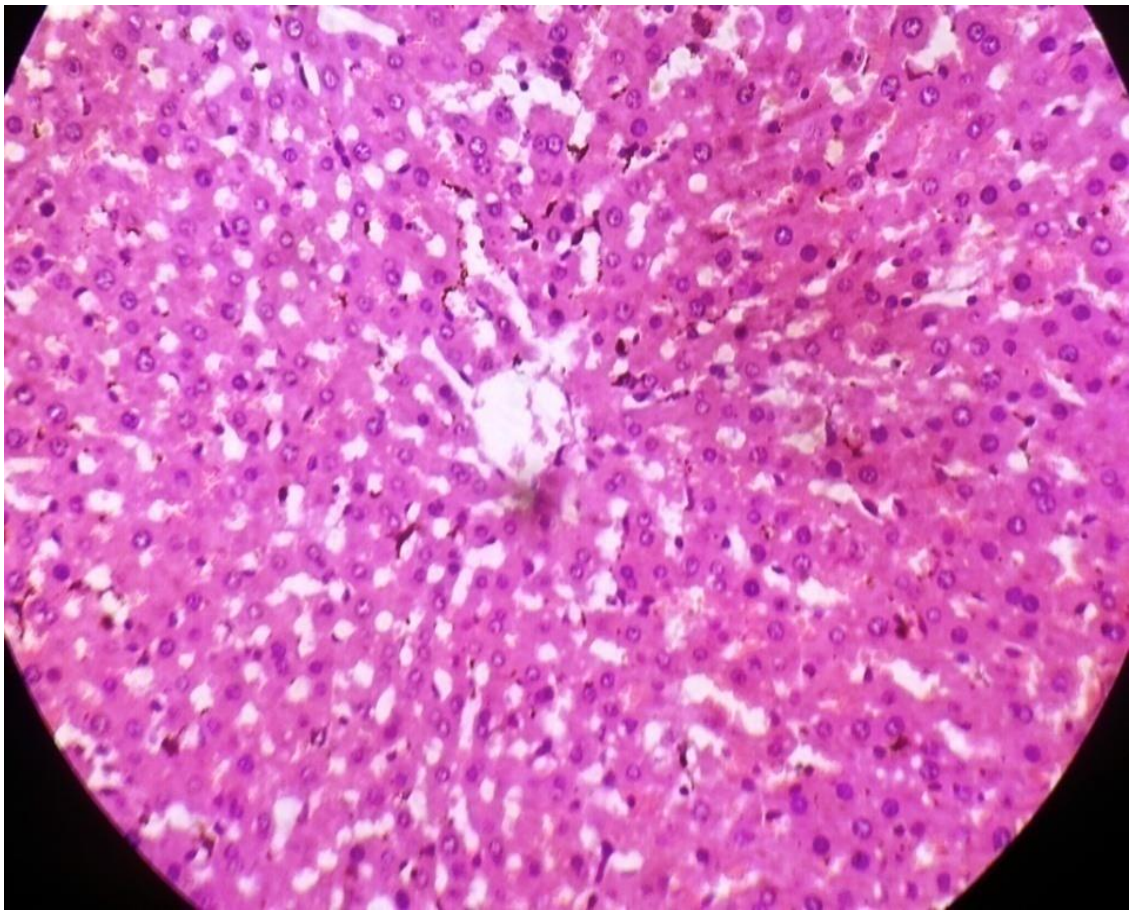

Plate 1 (54L) intact liver parenchyma

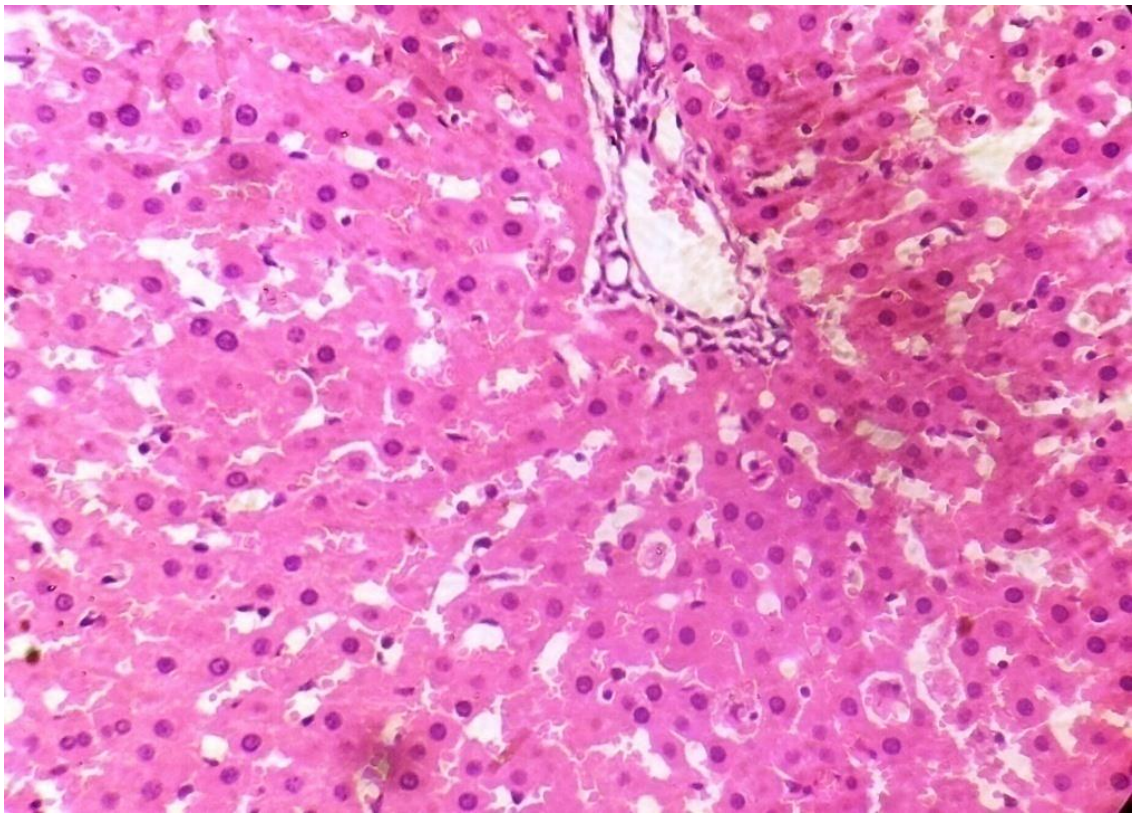

Plate 2: (65L) Mild infiltration of the Inflammatory cells at periportal and pericentral areas of the liver

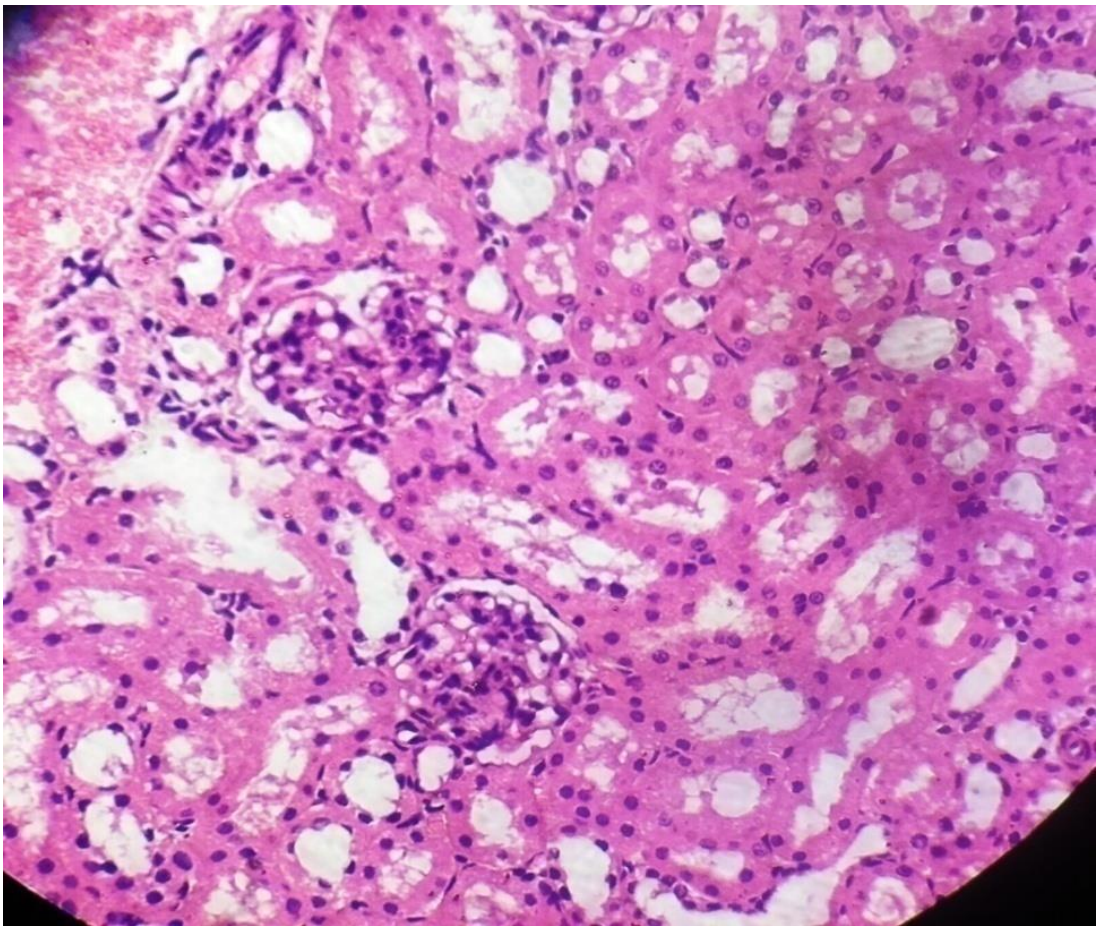

Plate 3 (56K) Higher degree of toxigenic modification with presence of tubular cast in varying degree, tubular erosion in the kidney

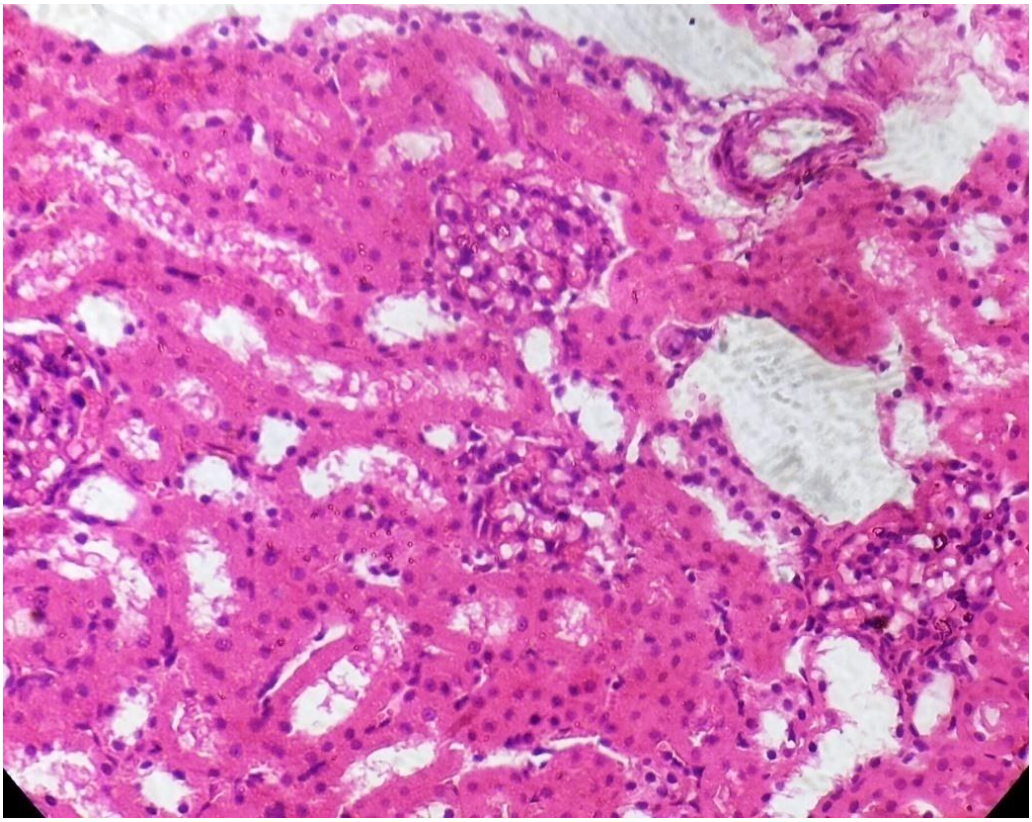

Plate 4: (59K) The Glomerular Oedema in high doses of the Inoculum of *S. arueus* at the kidney

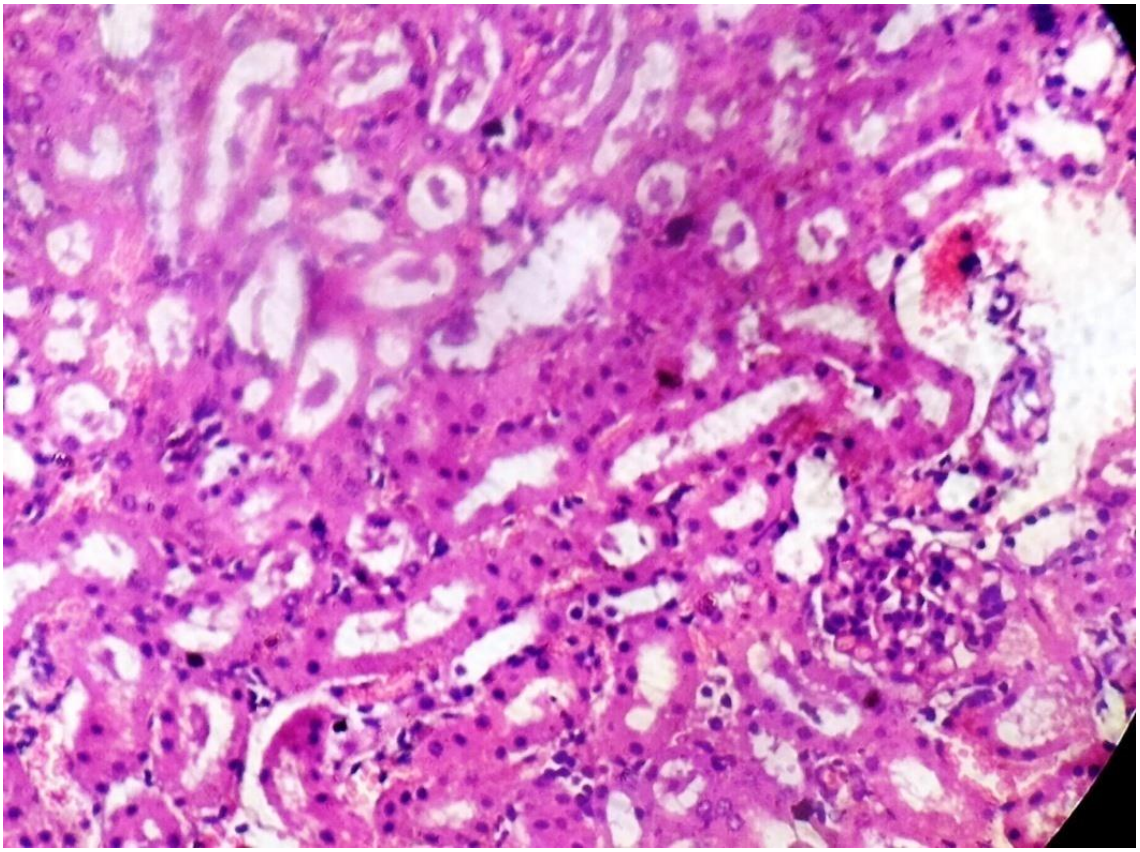

Plate 5: (5K) Tubular cast at all values of *S*. *aureus* inoculums in the kidney tubules

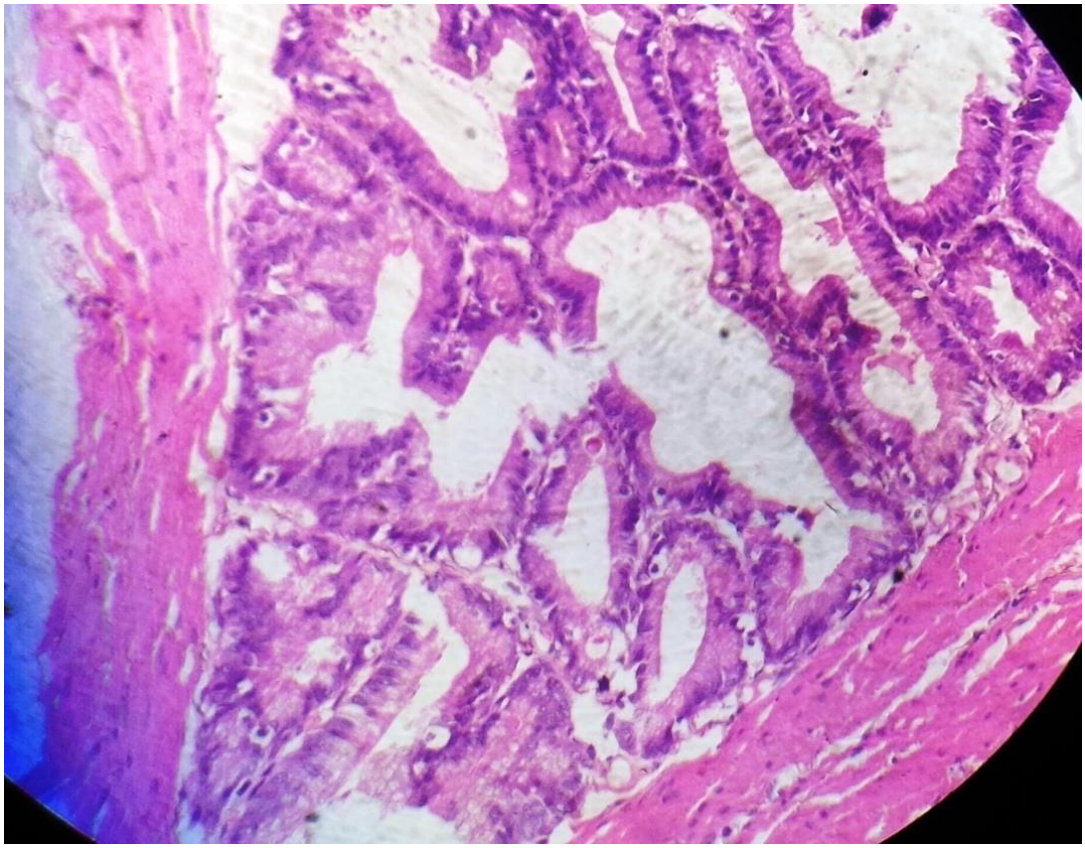

Plate 6: (69B) Mild effect by S. *aureus* on the musculartone the transitional epithelia of the bladder remained intact. Also shows infiltration of inflammatory cells

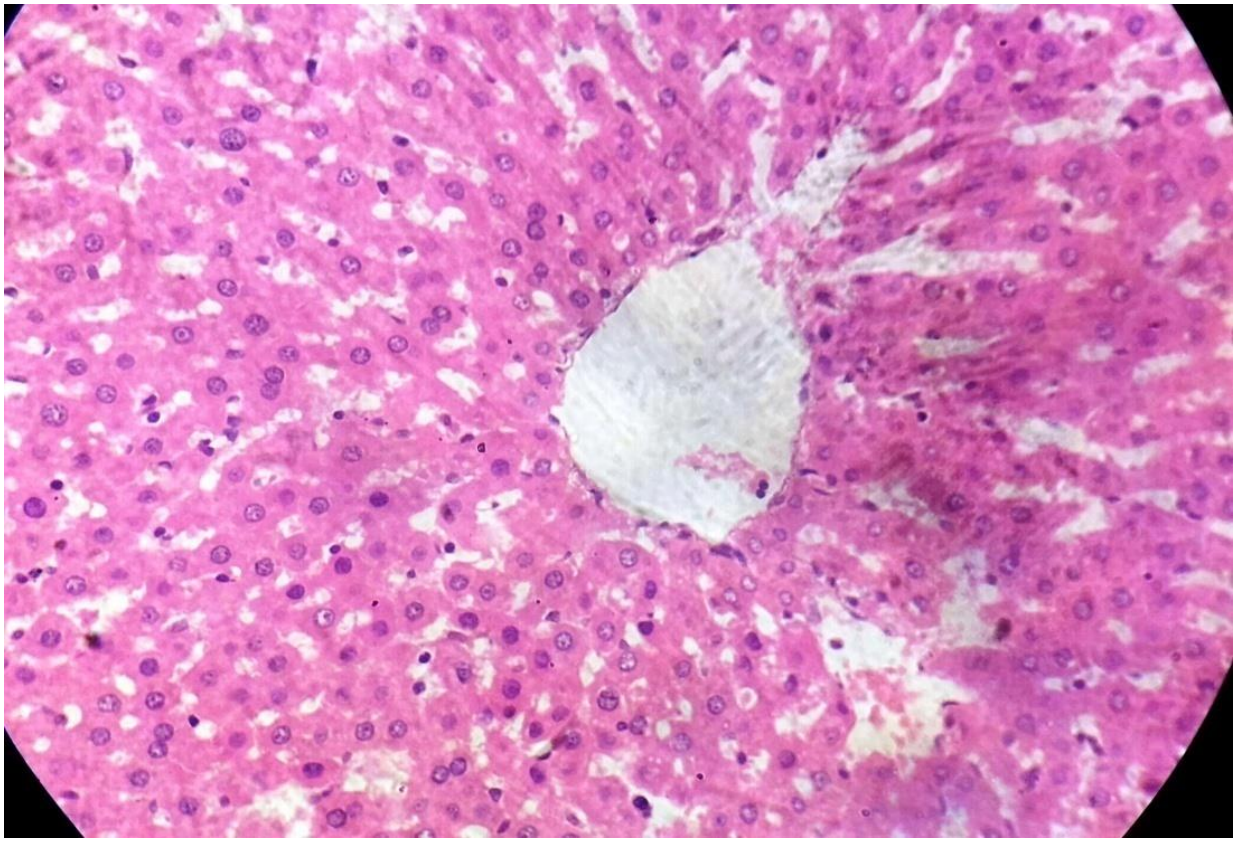

Plate 7: (56L) No visible of alteration of the liver parancyma

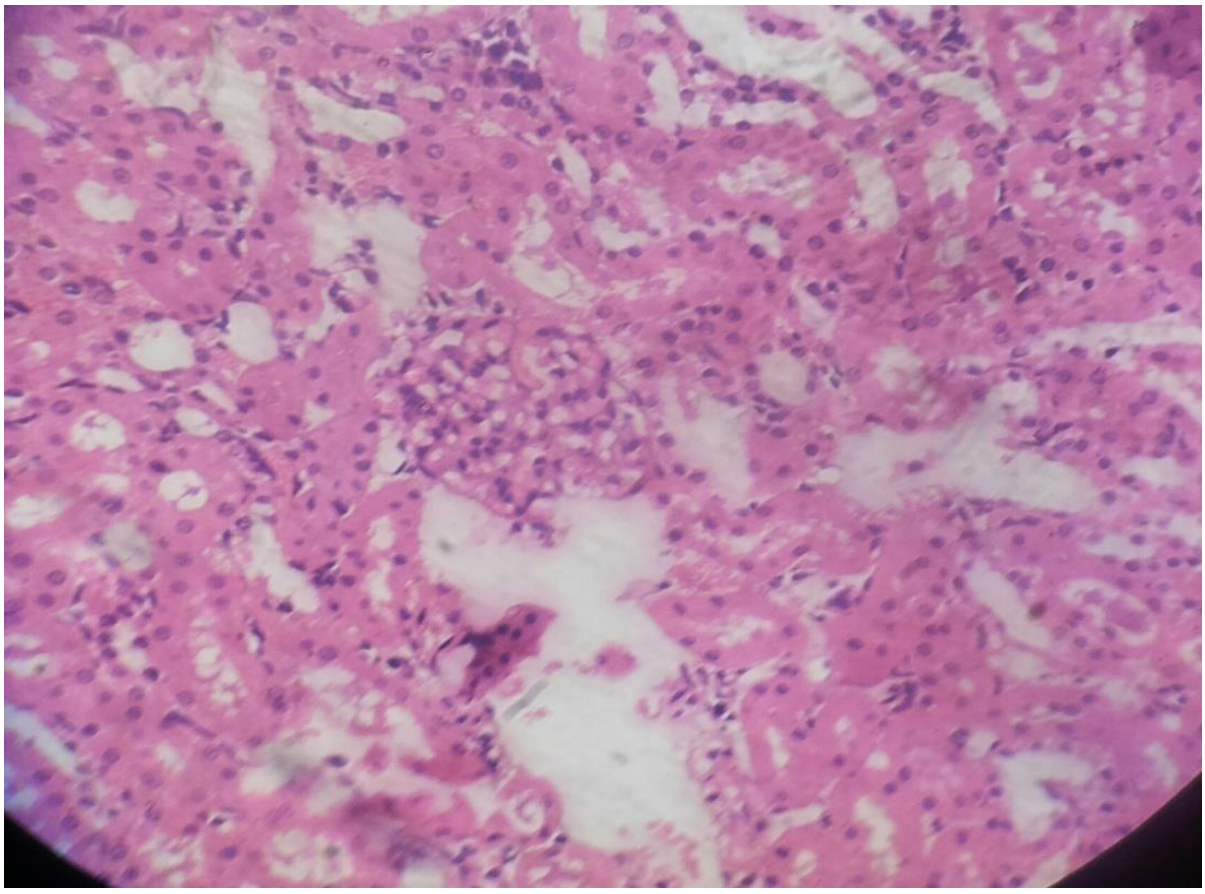

PLATE 8: (45K) 1 Tubular casts in the Kidney Tubules by *S. xylosus*

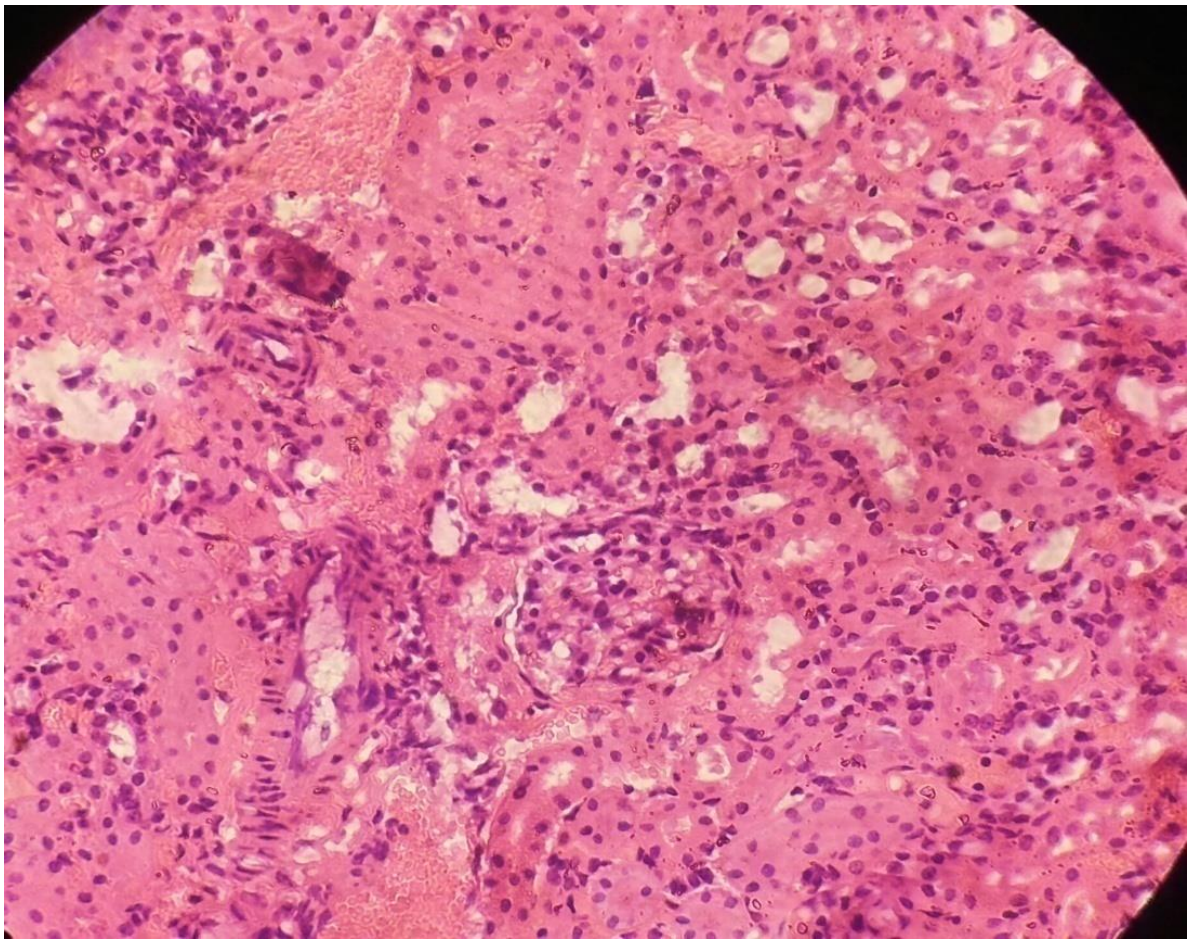

PLATE 9: (39K) Mild distortion of glomerulus, mild infiltration of inflammatory cells, patchy erosion of glomerulus, capsular oedema in with high doses of S. *xylosus*, tubular oedema with haemorrhagic patches in the stroma

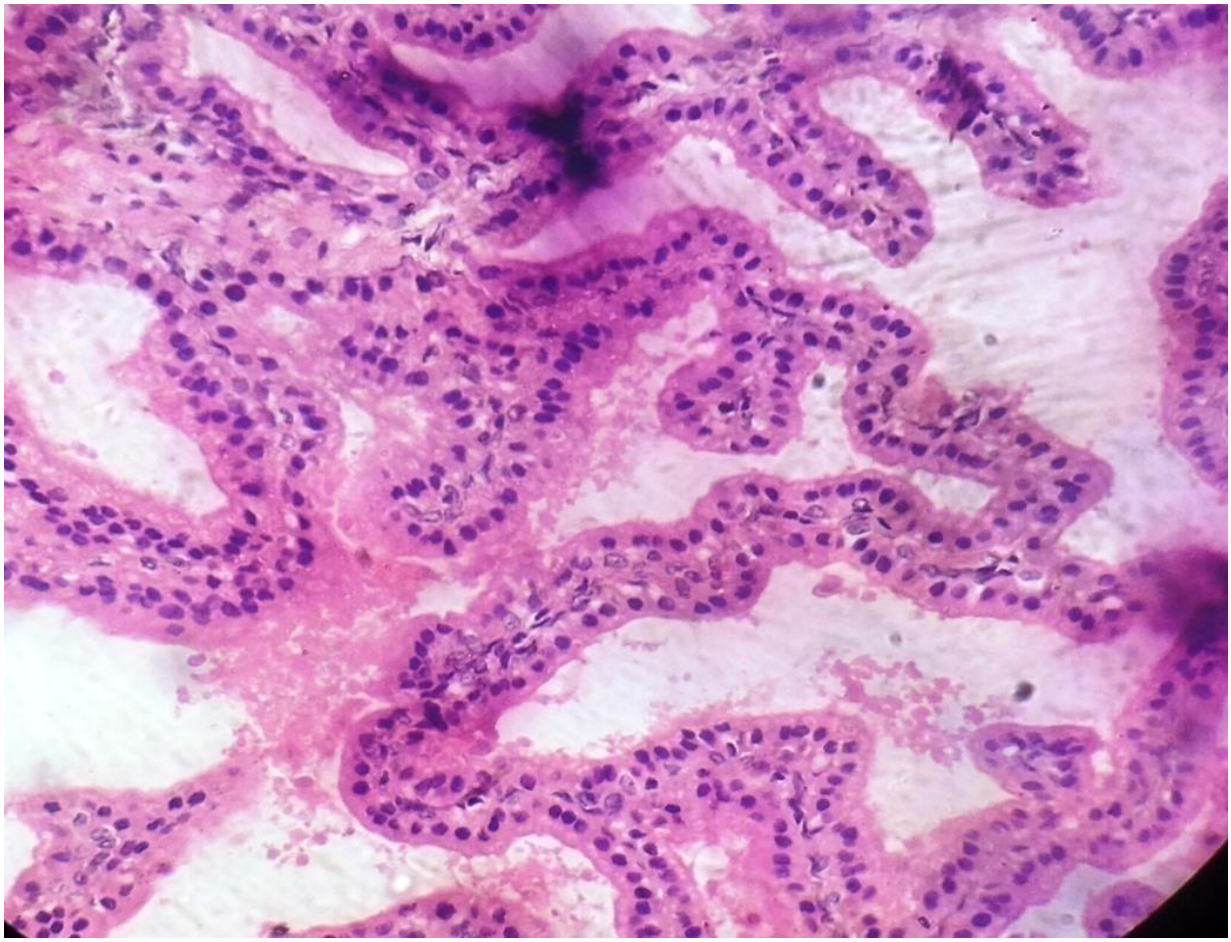

PLATE 10: (59B) Inflammatory cells in the mucosae and connective tissues of the Bladder

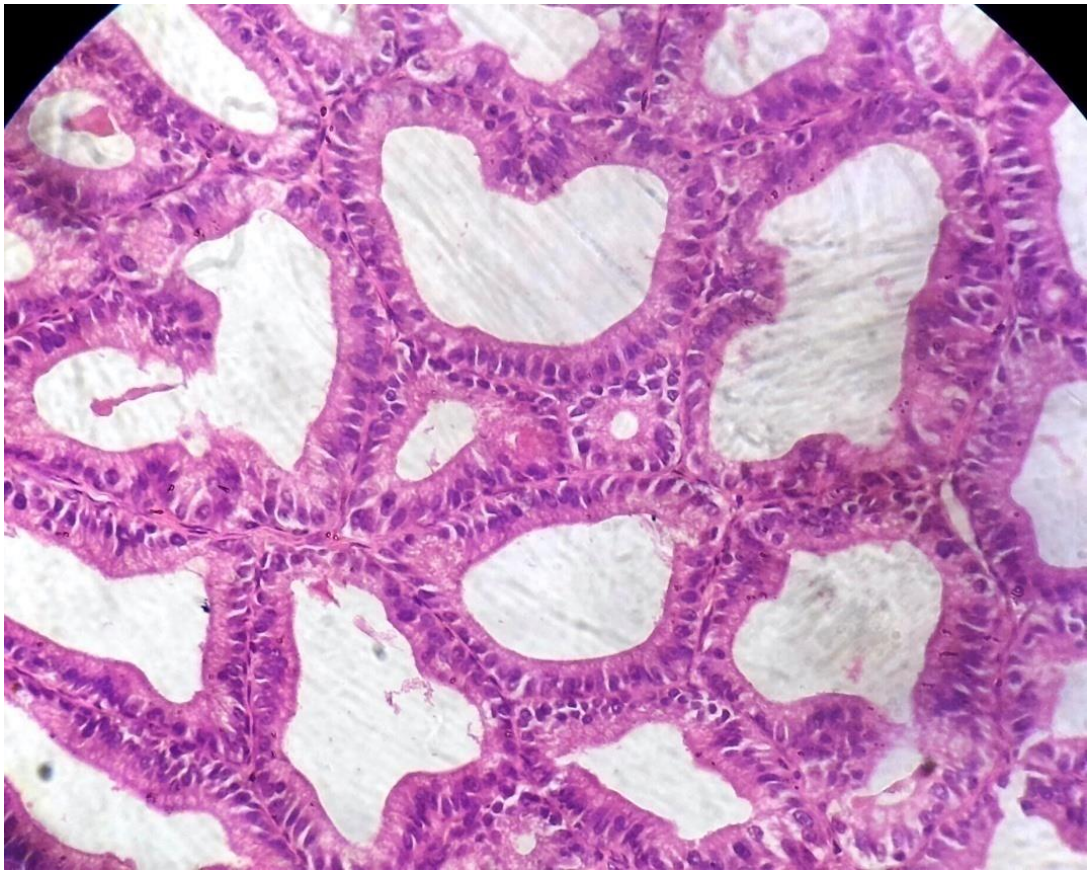

Plate 11: (59B) Intact Transitional Epithelia

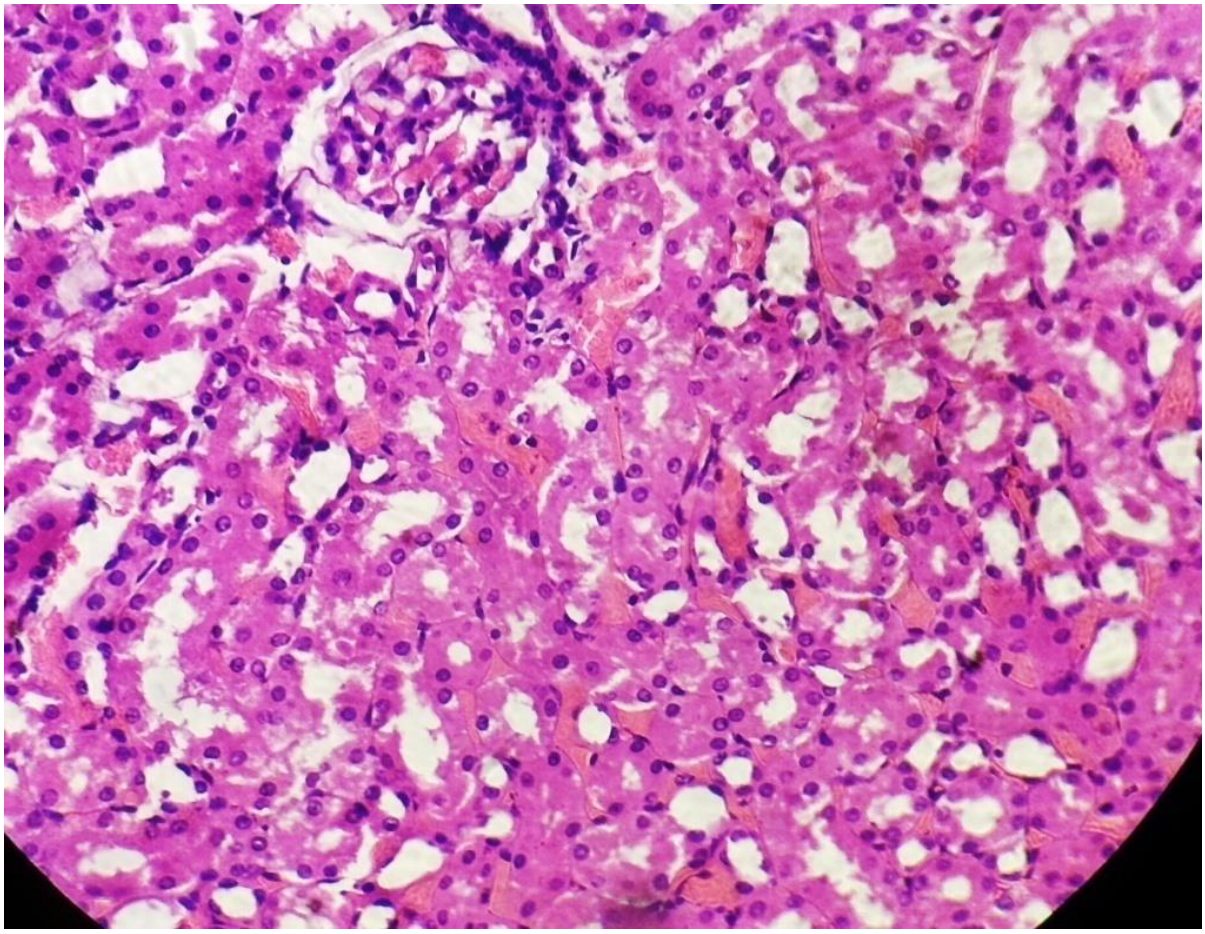

PLATE 12: (85K) Infiltration and Tubular casts in the kidney by *S . auerus*


PLATE 13
SHOWING CULTURE OF
Staphylococcus aureus no 834
AFTER 24HRS INCUBATION

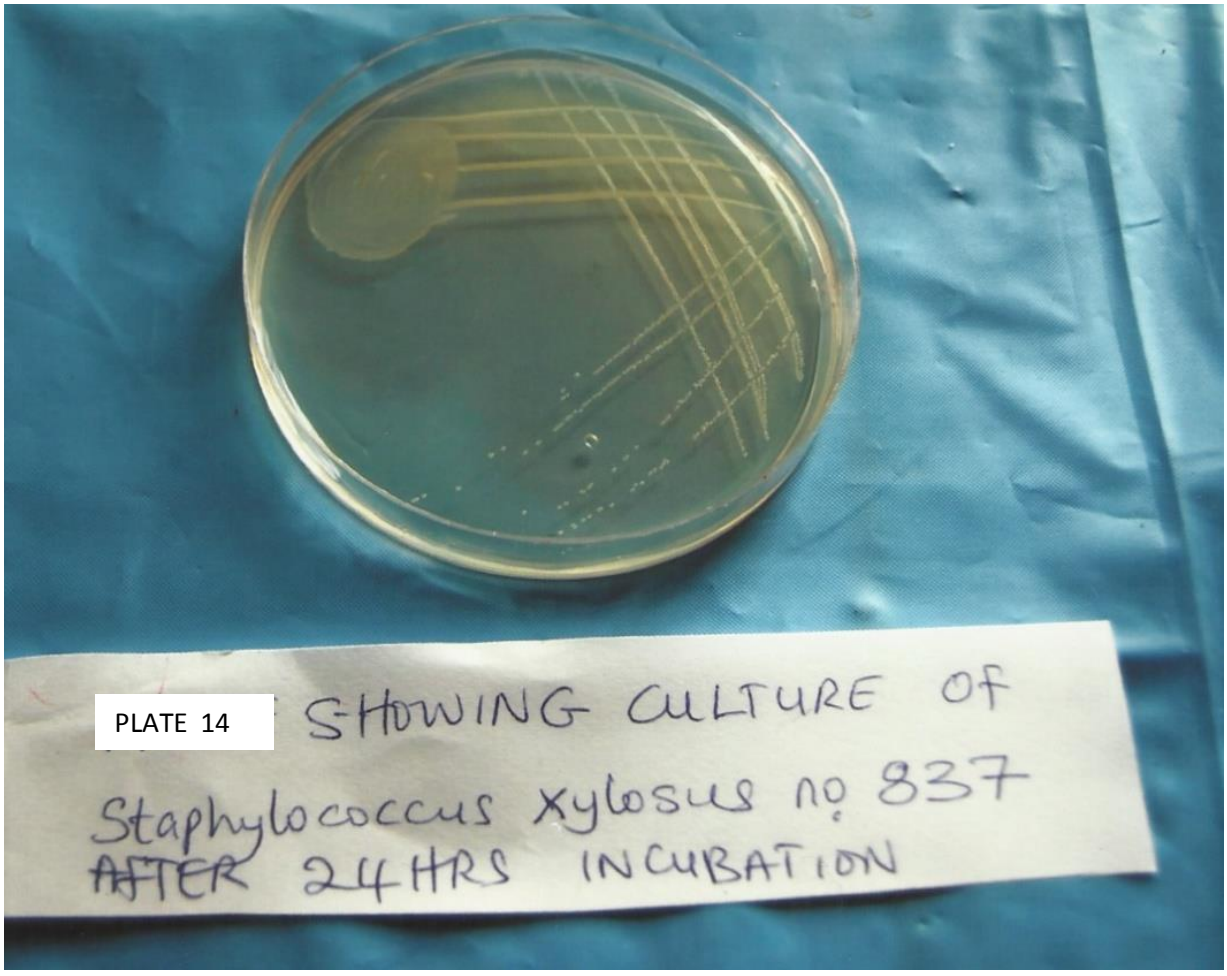
PLATE 14
SHOWING CULTURE OF
Staphylococcus xylosus no 837
AFTER 24HRS INCUBATION


SHOWING CULTURE OF
Staphylococcus (entus no 853Ba
(OLD CULTURE)

PLATE 15:


PLATE 16
SHOWING CULTURE OF
Staphylococcus aureus no 856Ba
AFTER 24 HRS INCUBATION

Plate 17: Nutrient agar plate with drug discs (Sensitivity Test) showing clearance of the Organisms by the drugs.

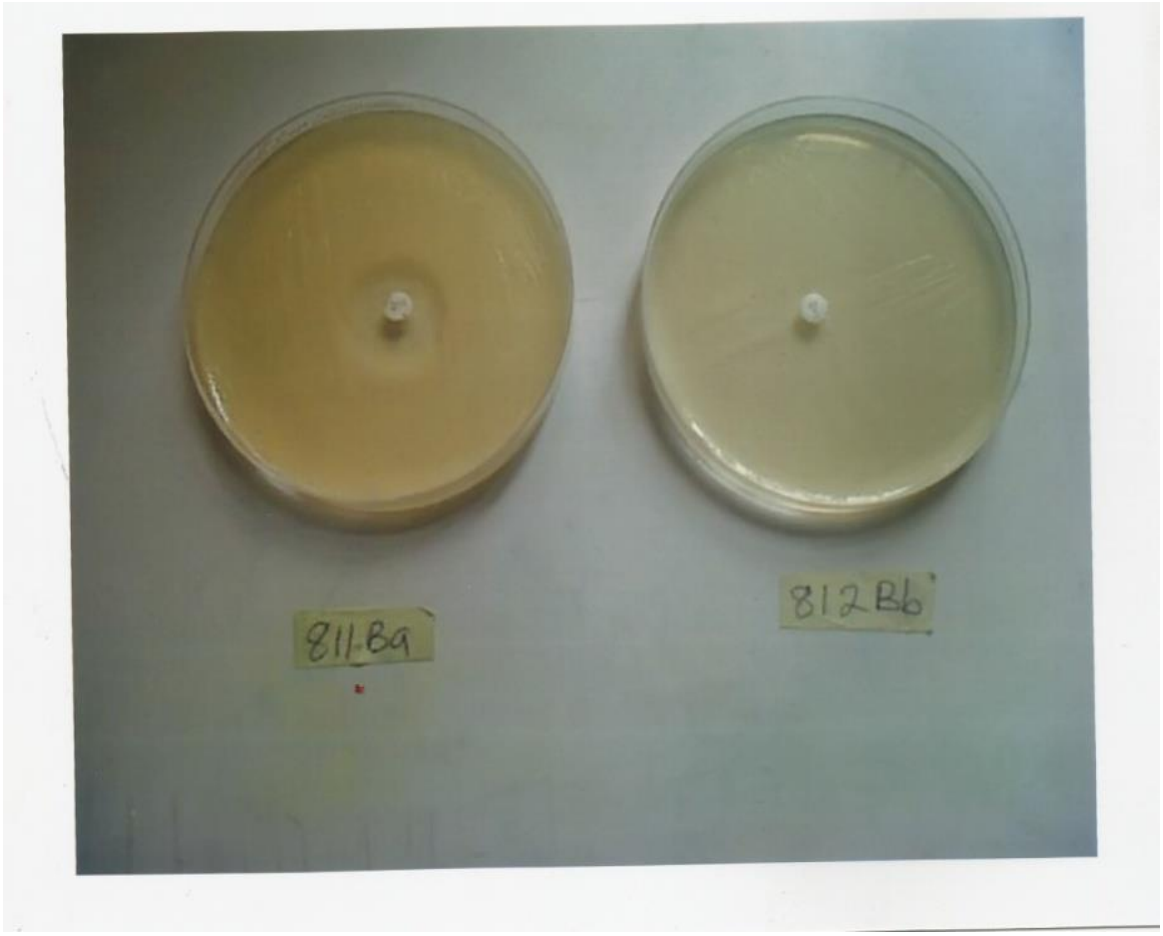

Plate 18: Novobiocin drug senstivity test on nutrient agar plate culture.. Plate on the right shows negative test while plate on the left shows positive test with zone clearance around the Novobiocin Drug Disc. (oxoid) used for Sensitivity Test in this Study.

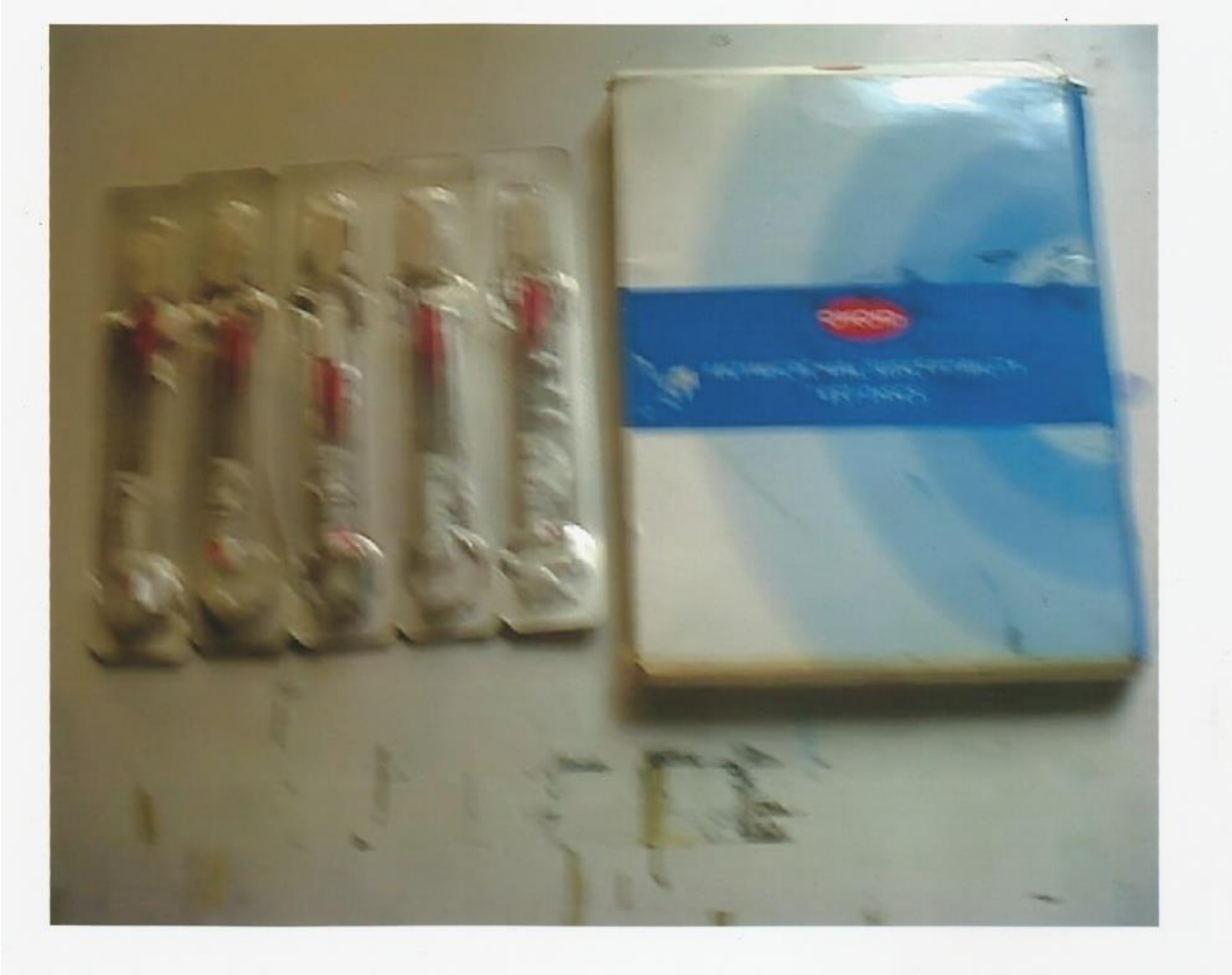

Plate 19: Novobiocin drug disc (oxoid) used for the sensitivity test in this study**.**

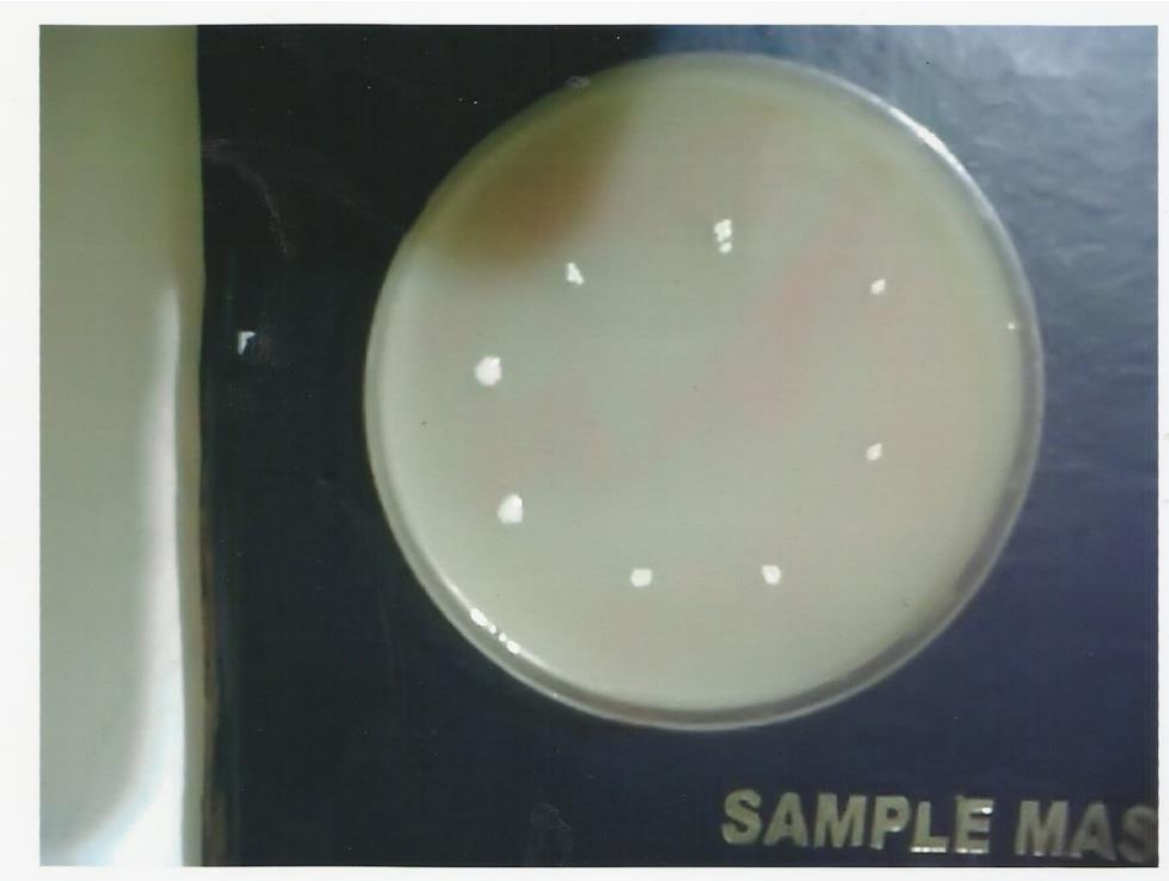

Plate 20: DNASE agar plate showing spot cultures that were not surrounded by clear uncloudy zones (negative DNASE test)

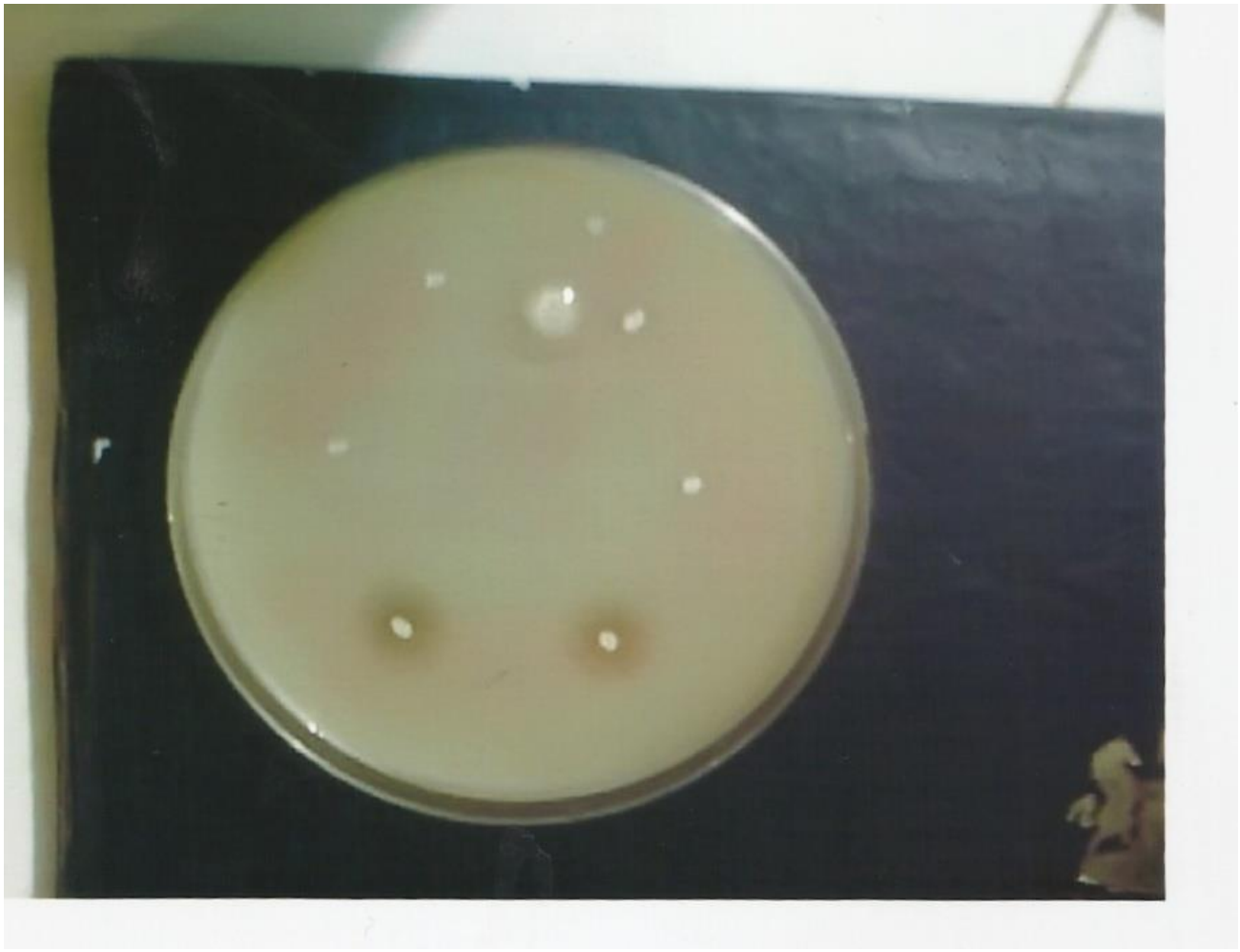

Plate 21: DNASE agar plate showing spot cultures that were surrounded by clear uncloudy zones (postive DNASE test)

Plate 22 : S. *aureus*, S. *xylosus*, S. *lentus* and a second strain of S. *aureus* in saline broth for animal inoculation

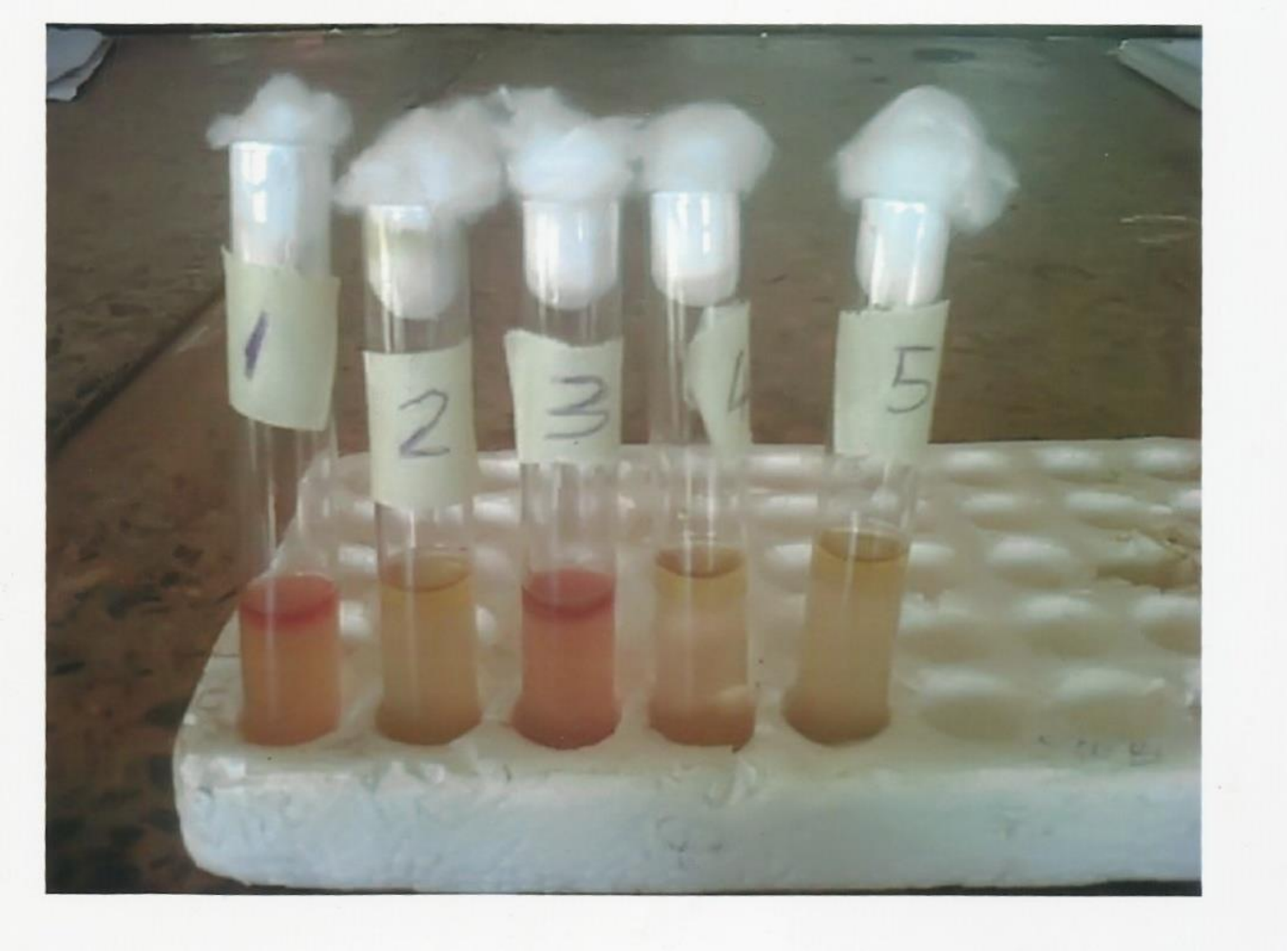

PLATE 23: Indole test showing positive and negative results**.**

PLATE 24: Methyl red test showing postive and neagtive results. **.**

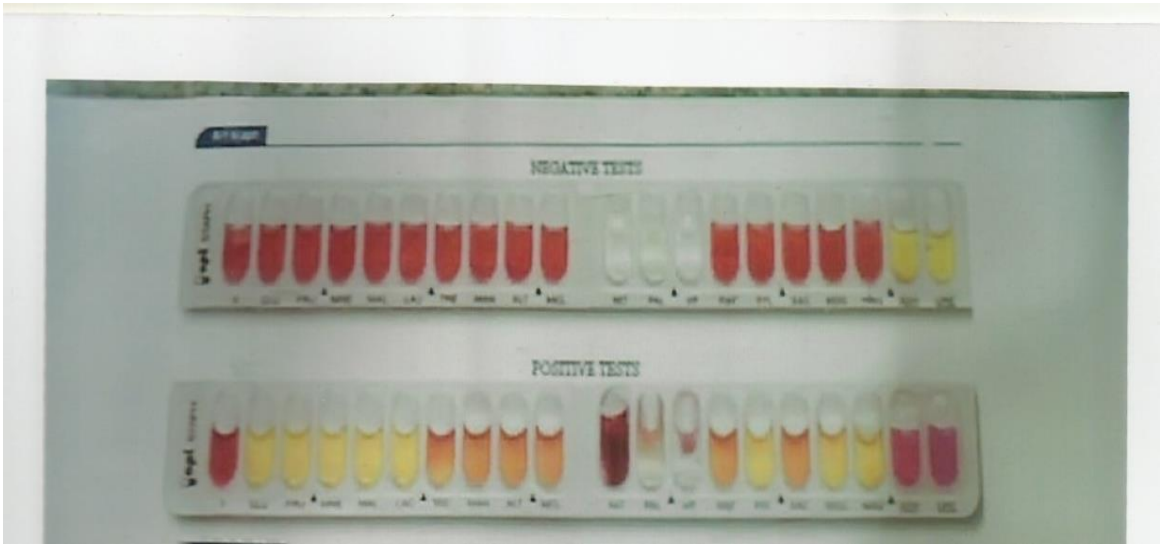

Plate 25: API® Staph control for positive and negative results

Plate 26: API® Staph biochemical test result for *Staphylococcus aureus* (Lab No. 834)

PLATE 27: API® Staph biochemical test result for *Staphylococcus xylosus* (LAB No. 837)

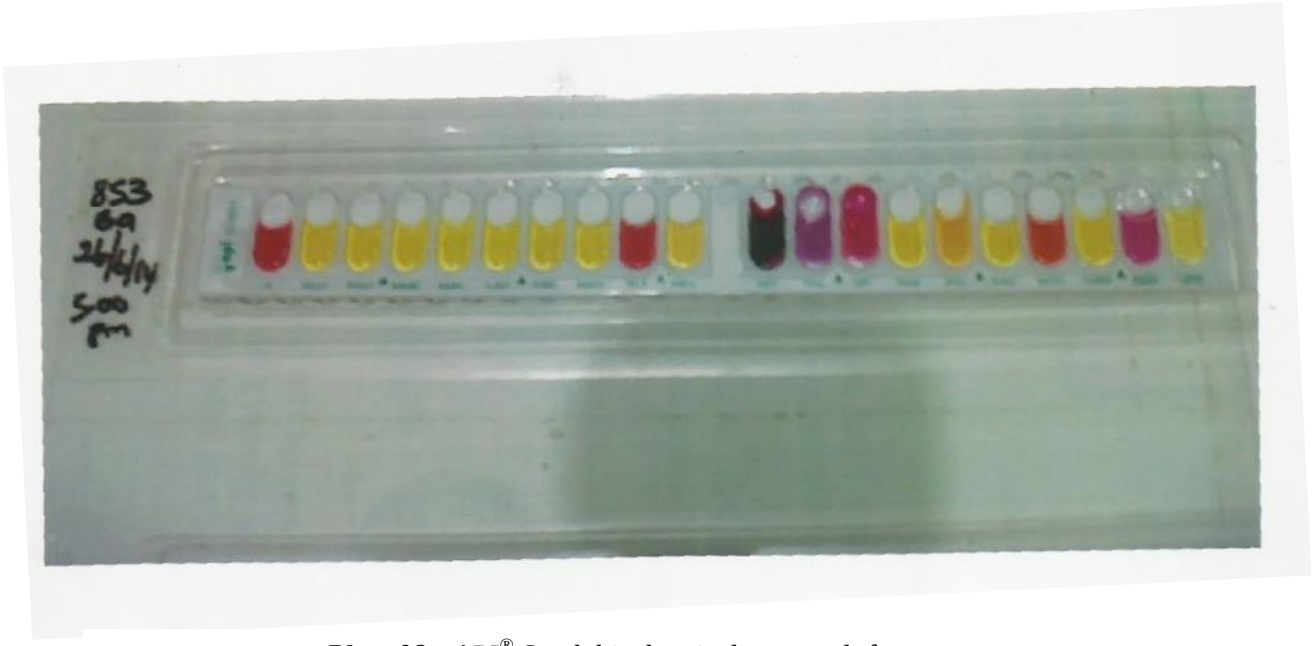

Plate 28: API® Staph biochemical test result for *Staphylococcus lentus* (Lab No. 853Ba

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The prevalence of UTI in the present study was 37.5%. Many studies elsewhere on UTI have shown higher or lower prevelance rates for UTI; Lo *et al.* (2013) at Sao Paulo Brazil on pediatrics patients aged up to 15 years, Ojambo (2012) in Kampala, Uganda on children 2 months to 12 years with fever, Orhue (2014) in Benin Nigeria on patients with symptoms of UTI, all recorded lower prevalence for UTI 11.3%, 14.6% and 14.58% respectively than the present study. The works of Oladiende *et al.* (2011) on the rural community (Okada town) in Edo State Nigeria, Anigilage and Bitto (2013) among children with cerebral paulsy (cp) aged between 2 and 15 years in Markudi Nigeria both had similar prevlances for UTI 39.69% and 38.5% respectively compared to the result of the present study. However, the works of Kolawole *et al.* (2010) on patients attending Dalhatu Specialist Hospital, Lafia, Nasarawa State, Nigeria, Amaeze *et al*. (2013) on patients with suspected cases of UTI (symptomatic) aged 18-43 years at Abuja,Nigeria, Omojasola and Omojasola (2010) on adult patients with symptoms suggesting UTI aged 18-40 years in Ilorin Nigeria, all had higher prevalence rates for UTI 60%, 57.78% and 83.2% respectively than the present study.

The differences in the prevalence rates obtained from UTI from these studies when compared to the results of the present study could be attributed to various factors such as differences in age groups, occupation, geographical location, cultural habits and practices, personal and environmental hygiene levels, socio-economic status of the different populations they studied as well as the health status of the participants in these studies. Some groups studied by these authors were children, some adult population; some were patients with diverse medical conditions including suspected cases of UTI (with signs and symptoms of UTI) while some were rural communities with diverse occupations. The present study population included persons of the age group 3-87 years (apparently healthy individuals, pregnant women and those with diverse medical conditions) coming from

different occupational groups which included pupils, students, traders, transporters,artisans, house wives, civil servants.

Sex preponderance of general UTI in the present study supports females 81.8% than males 18.2%. This agrees with previous works (Oladeinde *et al*., (2011) at Okada town in Edo State, Nigeria 42.8% in females versus 10.2% in males; Orhue (2014) 61.9% in females versus 38.1% in males. The difference between the total number of positive males and positive females for UTI in the present study was statistically significant ($P < 0.05$) using Fisherôs exact test for contingency. The higher occurrence of UTI in women than in men could be attributed to shorter urethral length in women which increases the chance for asending infection into the urinary tract and in girls the moist periurethral and vaginal areas promote growth of uropathogens (Chang and ShortLiffe, 2006).

Age group 22-32 years had highest occurrence for general UTI in the present study 107 (34.9%) followed by age group 11-21 years which had 88 (28.7%), age group 33-43 49 (16.0%) while the least was age group 77-87 which had 1(0.3%). This age group difference was however not statistically significnat ($P > 0.05$). The higher occurences of UTI in the age groups 22-32, 11-21 and 33 - 43 years falls into the sexually active and reproductive years (Chang and ShortLiffe *et al*. (2006) hence the result obtained.

Staphylococcal UTI recorded the highest frequency of occurrence for UTI 29.0% than other organisms isolated in the present study followed by *Escherichia coli* 23.5% and then other organisms. This result is not in agreement with many results of previous studies on UTI where *E. coli* was most often reported as the most prevalent organism isolated from UTI [Pragash *et al.,* (2014) in India; Ajanta *et al.,* (2011) in India; Sowmya and Lakshmidevi (2013) in Mysore karnatata; Kolawole *et al*., (2009) in Nasarawa State Nigeria; Amaeze *et al.,* (2013) in Gwagwalada, Abuja, Nigeria; Samuel *et al.,* (2014) in Ago-Iwoye, Ogun State, Nigeria; Stanley *et al.,* (2013) in Port-Harcourt, Nigeria; Oladeinde *et al.* (2011) in Okada town Edo State, Nigeria; Obiogbolu *et al.*(2009) in Awka, Anambra State Nigeria] and a host of others. However, few studies have documented *Staphylococcus* species especially *S. aureus* as the most frequent organism isolated in their work on UTI which is in agreement with the result of the present study [Bolaji *et al*. (2013) in Zaria Nigeria; Amali *et al.* (2008) in Markurdi, Benue, State

Nigeria; Omojasola and Omojasola, (2001) in Ilorin, Nigeria and Alo *et al.*(2010) in Ebonyi State, Nigeria]. The highest frequency of occurence of *Staphylococcius* spp amongst the isolates in the present study (29.0%) could be as a result of the fact that most *Staphylococcus* species are normal flora of the nose, skin, vagina and could easily infect the opening of the urethra and ascend to cause UTI and may also be attributed to low level of personal hygiene in the present study area.

In the present work, females had higher occurrence of staphylococcusal UTI (77.5 %%) than males (22.5%). Shorter urethral length in females than in males, pregnancy, use of diaphragm, wiping from back to front, sex, predispose women more to UTI than men. The difference in the total number of males positive for staphylococcal UTI and that of positive females was statistically significant ($P < 0.05$, P value =0.0068 using Fisherôs exact test for contingency.

The age group 22-32 years had the highest occurrence for staphylococcal UTI in the present work 37 (41.6%) followed by age group 11-21, 25 (28.1%) and age group 33-43 years 12 (13.5%). These are sexually active age groups and reproductive years and since sex and pregnancy are predisposing factors to UTI, it is therefore not surprising that the current results were obtained. However, age group differences was not statititcally significant $P > 0.05$, P value = 0.2301 using two way ANOVA.

Of the 89 staphylococcal UTI cases encountered in the present work, *S. aureus* UTI had the highest frequency with 40 (44.9%) followed by *S. xylosus* 25 (28.1%), *S. lentus* 9 (10.1%), *S. capre, S. sciuri, S. haemolyticus, S. epidermidis* 3 (3.4%) each while *S.hominis, S. capitis* and *S. saprophyticus* had1 (1.1%) each, and the difference between the number of the various staphylococcal isolates was statistically significant $P < 0.05$ P value = 0.0001 using one ANOVA. The work of Bolaji *et al.* (2013) showed that frequency of *Staph* isolates in their work were *S. aureus* 7 (18.0%), *S. xylosus* 5(12.8*%) S. lentus* 3 (7.7%), S. *auriculous* 2 (5.1%), *S. epidermidis*, *S. haemolyticus* and *S. cohnii* 1 (2.6%) each. The work of Bolaji *et al*. (2013) and present work have similarities since in both, *S. aureus* had the highest frequency of occurrence in staphylococcal UTI followed by *S. xylosus*, *S.lentus*, then *S. haemolyticus* and *S. epidermidis* which had same frequencies. The highest frequency of occurrence of S*taphylococcus aureus* in

staphylococcal UTI in both works could be explained since *S. aureus is* the commonest of the *Staphylococcus* spp both as a normal flora of the skin, nose and vagina as well as in the environment compared to the other *Staph* spp. Also *S. aureus* could have more pathogenic armories that enable it to be more invasive and pathogenic than other *Staph* species. Other works elsewhere had either a higher or lower frequency of occurrence for *S. aureus* in UTI compared to the present work which was 44.9%. Higher frequency of occurrence for *S. aureus* UTI came from Omojasola and Omojasola (2001) in Ilorin Nigeria 52.5%. Other works have recorded lower frequency of occurrence than the present study for *S. aureus* UTI; Pragash *et al*. (2014) 14% in India among diabetics, Samuel *et al*. (2014) 10% in Ogun State Nigeria among diabetics; Okonko *et al*. (2010) in Ibadan Nigeria among pregnant women; Hamdan *et al.* (2011) 39.3% in Sudan among pregnant women; Stanley *et al*. (2013) 33.3% in psychotic patients at PortHarcount Nigeria.The different frequencies of occurrence obtained from other areas which were either higher or lower than that of the present study area could be as a result of differences in location, level of personal and environmental hygiene, educational, socio-economic status and level of exposure to health eductaion between the different study areas and between those areas and the present study area.

For the distribution of *S. aureus* UTI in the population studied, the age group 22-32 had the highest occurrence of *S. aureus* UTI 45% followed by to age group 11-21 years 22.5%. For *S. xylosus,* the age group 11-21 and 22-32 had the highest occurrence of UTI (36%) each while for *S. lentus* the age group 11-21 years and 22-32 years also had the highest frequency of occurrence 44.4%) each. These two age groups are the most sexually active age groups and sex predisposes to UTI, hence their being the highest in occurrence of UTI. The difference in the distribution of positive cases for *Staph aureus*, *Staph xylosus* and *Staph lentus* UTI between the age groups studied was not statistically significant ($P > 0.05$, P value = 0.3246; $P > 0.05$, P value = 0.3487 and $P > 0.05$, P value = 0.3209 respectively using two way ANOVA)

Females had higher occurrence of UTI in *Staph aureus* UTI 33 (82.5%) than males 7 (17.5%) as well as in *Staph xylosus* UTI 20 (80%) for females vs 5 (20%) for males and *Staph lentus* UTI 6 (66.7%) for females vs 3 (33.3%) for males. However the difference

between the total number of positive males and positive females for *S.aureus* UTI was statistically significance (P < 0.05, P value = 0.0167) using Fisherôs exact test for contingency) but was not statistically significant (P > 0.05, P value = 0.1362) for *S. xylosus* UTI and (P > 0.05, P value = 1.0000) for *S.lentus* UTI using Fishers exact test for contingency. Age group differences for positive cases for *S. aureus, S. xylosus* and *S. lentus* UTI was not statistically significant (P > 0.05) respectively.

The prevalence of *Staphylococcus* spp UTI in menopausal women 13.6% was slightly higher than that from the premenopausal women 13.0% supporting the statements of Vorvick (2013) and Lights and Boskey (2012) that menopause is a risk factor for UTI. The difference between the total number of positive cases for staphylococcal UTI seen in premenopausal group and that seen in menoposual groups studied was not statistically significant (P > 0.05, P value = 0.8396) using Fisherôs exact test). There was no statistically significant difference in the distribution of positive cases for staphylococcal UTI between premenopausal and menopasual group studied (P > 0.05, P value = 01706) using two way ANOVA. In both groups, *S. aureus* UTI recorded the highest frequency of occurrence amongst the other staphylococcal UTI (50%) for menopausal and 47.5% for premenopausal) with menopausal rate for *S. aureus* UTI being slightly higher than premenopausal rate but the difference in the number of the various staphylococal species isolated in UTI between premenopausal and menopausal groups showed no statistically significant difference (P > 0.05, P value = 0.0805). Also the difference between the number of the various *Staphylococacus* species in premenopausal compared to that seen in menopausal group was not statistically significant. (P > 0.05, P value = 0.2365). The age group 33-43 years and 44-54 years had the highest occurrence for staphylococcal UTI in the menopausal group (37.5%) each while the age group 22-32 years had the highest for premenopausal group 49.2%. Age group difference in the number of positive cases for staphylococcal UTI between premenopausal and menopausal groups studied showed no statistically signficant difference (P > 0.005, P value = 0.5700). These age groups in the two groups (menopausal and premenopausal) are the peak of sexually active age groups which could predispose to UTI hence the results obtained.

Traders/peti-traders had the highest prevalence rate for staphylococcal UTI 18.7% followed by transporters 17.2%, housewives 15.6% while the least were artisans who had 5.4%. Variation in the total number of staphylococcal spp UTI positive cases between occupational groups studied was statistically significant ($P < 0.05$, P value = 0.0256) using Fisherôs exact for contingency. The difference in the number of the various staphylococcal isolates between occupational groups studied was statistically significant ($P < 0.05$, P value = 0.005. The difference between total of positive cases for the various occupations studied was also statistically significant ($P <0.05$, P value = 0.0494) using one way ANOVA. The highest prevalence for general UTI and staphylococcal UTI by traders could be attributed to the fact traders stay all day in the market where there are only commercial toilets and a great number of them use these very few toilet facilities that are very unkempt which predispose them to UTI.

Amongst the three major groupings for this study, pregnant women had the highest prevalence rate for staphylococcal UTI (20.2%) followed by apparently healthy persons (10.8) while the least were those with medical conditions (3.5%).Variation in the total number of staphylococcal spp UTI positive cases seen between the different health conditions studied (apparently healthy, pregnant women and medical condition) was statistically significnant ($P < 0.05$, P value = 0.0001 using Fisherôs exact test for contingency). The highest prevalence rate recorded for staphylococcal UTI by the pregnant group is not surprising since pregnancy is a predisposing factor to UTI (Ferede *et al.,* 2012; Jalilah *et al.*, 2014; Okonko *et al.*, 2010, Hamdam *et al.*, 2011). Due to general anatomical and hormonal changes, pregnant women are susceptible to UTI (Dafnis and Sabatini, 1992). Urinary stasis during pregnancy is a major cause of UTI in pregnancy (Olowu, 1996). Pregnancy increases the risk of UTI amongst other factors due to the pressure of gravid uterus on the ureters causing stasis of urine flow and is also attributed to the humoral and immunological changes during normal pregnancy (Ramzan *et al.,* 2004). The physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosurea which encourages bacterial growth in the urine (Lucas and Cunningharm, 1993).

In the pregnant population studied for UTI, staphylococcal UTI had the highest overall frequency of occurrence among other organisms isolated 38.1%. The highest frequency of occurrence of *Staph* UTI in pregnant women studied could be as for the same reason given for general UTI.

The prevalence of staphylococcal UTI in pregnant women in the present study was 20.2%. Ferede *et al.* (2012) in their study in Ethiopia recorded a prevalence of 5% for staphylococcal species in UTI (CNS 6/200, *Staph aureus* 4/200 total 10/200), Moyo *et al.* (2010) in their study at Muhimbili, Tanzania recorded a prevalence of 6.5 for staphylococcal UTI (CNS 7/200, *Staph aureus* 6/200, total = 13/200). These prevalences recorded by Ferede *et al*. (2012) and Moyo *et al.* (2010) are much lower than that of the present work 20.2%. Difference in environment, social habits of the community, standard of personal hygiene and educational levels between the various study areas could be attributed to the different prevalence rates for staphylococcal UTI.

The age group 22-32 years had the highest occurrence for staphylococcal UTI 79.2% in pregnant women studied followed by the age groups 33-43, 16.7% while the least as age group 11-21, 4.7%. Sexual activity and pregnancy are risk factors for UTI and age group 22-32 years is the most sexually active age group, hence the highest prevalence obtained.

Gender varation shows that there was no statistically significnant difference ($P > 0.05$, P value = 0.1655 using two-way ANOVA) in the distribution of staphylococcal UTI between positive males and females in apparently healthy cases. However, there was a statistically significant diference between the number of various *Staphylococus* spp isolated in apparently healthy cases ($P < 0.05$, P value = 0.0248 using two-way ANOVA). Variation in the number of different staphylococcus spp isolated between healthy conditions studied showed statistically significant difference ($P < 0.05$, P value = 0.0001 using two-way ANOVA). Non-hospitalized patients/ subjects had a prevalance rate of 11.5% for staphylococcal UTI while hospitalized patients had 0%. Thus, staphylococcal UTI seems to be a community acquired infection from the present study and not a nosocomial infection.

The present study has shown that *S. aureus* had the highest frequency of occurence amongst the staphylococcal UTI in the whole population studied 44.9% followed by *S. xylosus* 28.1%, *S. lentus* 10.1%, *S. capre, S. sciuri, S. haemolyticus* and *S. epidermidis* 3.4% each while the least were *S. hominis, S. capitis* and *S. saprophyticus* which had 1.1% each. In the pregnant women studied, *S. aureus* also had the highest frequency of occurrence amongst the *Staphylococcus* spp UTI 54.2% followed by *S. xylosus* 25%, *S. lentus* 12.5%, *S. capre* and *S. hominis* 4.2% while *S. sciuri*, *S. heamolyticus, S. epidermidis*, *S. capitis* and *S. saprophyticus* had 0%. In both the general population and the pregnant women studied *S. aureus* UTI had the highest frequency, followed by *S. xylosus* UTI, and next was *S. lentus* before the rest of the *Staphylococcus* species which had very low results. Hence these three were the major *Staphylococcus* species responsible for the greater number of staphylococcal UTI in the present work.

Result of the drug sensitivity pattern carried out on the urinary isolates showed that *Staphylococcus aureus* had highest sensitivity to rifampicin and levofloxacin 72.5% in the present study followed by ciprofloxacin 65%, streptomycin, erythromycin and ampiclox 60% each while other drugs used followed. *Staphylococcus xylosus* showed highest sensitivity to rifampicin 72% followed by ciprofloxacin 68%, levofloxacin and streptomycin 56% each, ampiclox 52%, erythromycin 48% while other drugs used in the present study followed. *Staphylococcus lentus* showed highest sensitivity to rifampicin and amoxyl 77.8% in the present work followed by levofloxacin 66.7%, ciprofloxacin 55.6% and then others.

In summary, all the staphylococcal UTI isolates from present study with the exception of the lone *S. saprophyticus* showed highest sensitivity to rifampicin followed by levofloxacin while ciprofloxacin came third. Pathological studies on the three commonest *Staphylococcus* species encountered in the present study revealed varying degrees of toxicity effects for the kidney, liver, and badder with *S. aureus* showing a higher degree of toxigenicity especially with the kidney and effects were more significant than when compared with *S. xylosus* and *S. lentus* with effects generally increasing with increased dosing.

5.2 CONCLUSION

The present work has shown a prevalence of 37.5% for urinary tract infection generally and 10.9% for staphylococcal urinary tract infection amongst the patients and subjects studied. Females had higher occurrence of general UTI and staphylococcal UTI then male counterparts with the age 22-32 years showing the highest occurrence of general UTI as well as for staphylococcal UTI which difference was statistically significant using Fishers exact test ($P < 0.05$). Age distribution for both general UTI and staphylococcal UTI showed no statistically significant difference ($p > 0.05$) using two-way ANOVA. *Staphylococcus aureus* had the highest occurrence followed by *S. xylosus*, *S. lentus, S. capre*, *S. sciuri*, *S. heamolyticus*, *S. epidermidis*, *S. hominis*, *S. capitis* while the least was *S. saprophyticus* which difference was statistically significant ($P < 0.05$).

Pregnant women had highest prevalence for staphylococcal UTI amongst the three different categories studied (the apparently healthy group, pregnant women and those with different medical conditions e.g Diabetes mellitus hypertension etc)

Traders had the highest prevalence for UTI amongst the different occupational groups studied and there was a statistically significant variation in the number of positive staphylococcal UTI cases according to occupational grouping using Fishers exact test ($P < 0.05$) .

A further grouping of the female population into menopausal and premenopausal showed that 8 (13.6%) of the 59 menopausal women and 61 (13%) of the 469 pre-menopausal women had staphylococcal UTI. Of the 10 *Staphylococcus* species encountered, S. *aureus*, *S. xylosus*, *S. sciuri* and *S. capitis* were isolated in positive cases for UTI from menopausal women with *S. aureus* again ranking highest followed by *S. xylosus*; while *S. lentus* had no positive cases. Nine of the *Staphylococcus* species encountered were isolated from premenopausal women except for S. *heamolyticus*. The five cases from the 141 different medical conditions showed the involvement of *S. aureus* in diabetes, hypertension and schistosomiasis while S. *scuri* was involved in malaria and body pain.

On the whole, most staphylococcal isolates and other Gram positive isolates from the present study showed highest sensitivity to rifampicin followed by levofloxacin and thirdly ciprofloxacin.

Animal models revealed more toxigenic modifications on the kidney with tubular erosions, glomerular oedema with varying degrees according to dosing. *Staphylococcus aureus* had a remarkable feature cutting across all doses of tubular casts. This effect is less marked in *S. xylosus* and least in *S. lentus*. For liver and bladder, there were less marked effects for the 3 species again in varying degrees with occasional inflammatory cell at the periportal / pericentral areas while the bladder at the ID_{50} didnôt affect muscular tone and the transitory epithelial cells remained largely intact. Generally, effects were milder in the *xylosus* and *lentus* species.

5.2.1 RECOMMENDATIONS

1. Staphylococcal UTI should be investigated during any request for urine culture in our hospitals and must be typed down to species.
2. Sensitivity test must be carried out on positive staphylococcal UTI cases in our hospitals once significant bacteriuria is confirmed.
3. Pregnant women should be screened periodically before delivery for staphylococcal UTI to avoid complications.
4. Hospitalized patients should be screened for UTI generally in order to rule out nosocomial UTI.
5. Traders and persons in various occupations and the public at large are advised to visit their doctors for screening for UTI so as to rule out asymptomatic UTI which if not treated could progress to symptomatic UTI with complications like pyelonephitis and kidney failure.
6. Symptomatic cases of UTI should be treated immediately to avoid complications.
7. Public health education on ways of prevention of UTI such as improvement on personal and environmental hygiene, correct practice of sexual intercourse, should be carried out to the public on social media like television, radio, internet, through public seminars to students in schools and to occupational groups in their meetings.
8. Government should ensure proper sanitary and environmental health conditions in our hospitals and government establishment so as to reduce contamination of our environment and probably decrease the number of these organisms.

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APPENDIX 1

DISTRIBUTION OF CANDIDATES WITH DIFFERENTMEDICAL CONDITIONS

Medical Conditions	**No of males sampled**	**No of females sampled**	**Total**
Diabetics	8	10	18
Hypertensive	6	7	13
Kidney stone	2	2	4
Diabetes/HBP/BPH	1	0	1
Diabetes/BPH	1	0	1
Diabetes /HBP	3	5	8
Prostate cancer	2	0	2
BPH	20	0	20
CVA (Stroke)	0	3	3
R/O UTI	3	4	7
Surgical cases	20	6	26
Other ailments	11	27	38
Total	**77**	**64**	**141**

APPENDIX 2

DISTRIBUTION OF CANDIDATE WITH DIFFERENT MEDICAL CONDITIONS ACCORDING TO WHETHER INPATIENTS OR OUT PATIENTS

Medical Condition	In patients			Out patients			Grand total	%
	M	F	T	M	F	T	in/out patients	
Diabetics	1	5	6	7	5	12	18	6.9%
Hypertensive	1	0	1	5	7	12	13	5.0%
Kidney stone	0	0	0	2	2	4	4	1.5%
Diab/HBP/BPH	0	0	0	1	0	1	1	0.4%
Diab/BPH	0	0	0	1	0	1	1	0.4%
Diab/HBP	1	1	2	2	4	6	8	3.1%
Prostate cancer	0	0	0	2	0	2	2	0.8%
BPH	0	0	0	20	0	20	20	7.7%
CVA	0	2	2	0	1	1	3	1.2%
R/out UTI	0	0	0	3	4	7	7	2.7%
Surgical cases	20	6	26	0	0	0	26	10.0%
Other ailments	1	3	4	10	24	34	38	14.6%
Total	**24**	**17**	**41**	**53**	**47**	**95**	**141**	**100%**

Key

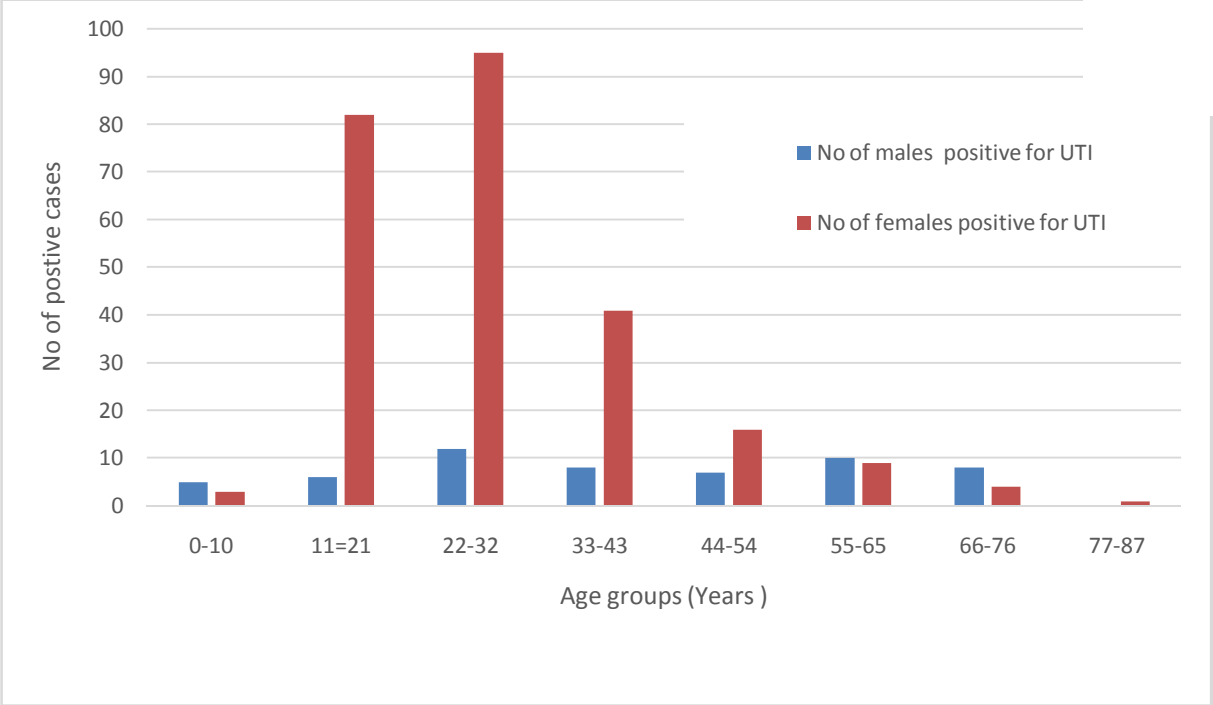
Diab =Diabetes

HBP =High Blood Pressure

BPH =Benign Prostate Hypertrophy

R/Out =Rule Out

APPENDIX 3



DISTRIBUTION OF THE POSITIVE CASES FOR URINARY TRACT INFECTION IN THE POPULATION STUDIED ACCORDING TO THE AGE GROUP AND SEX

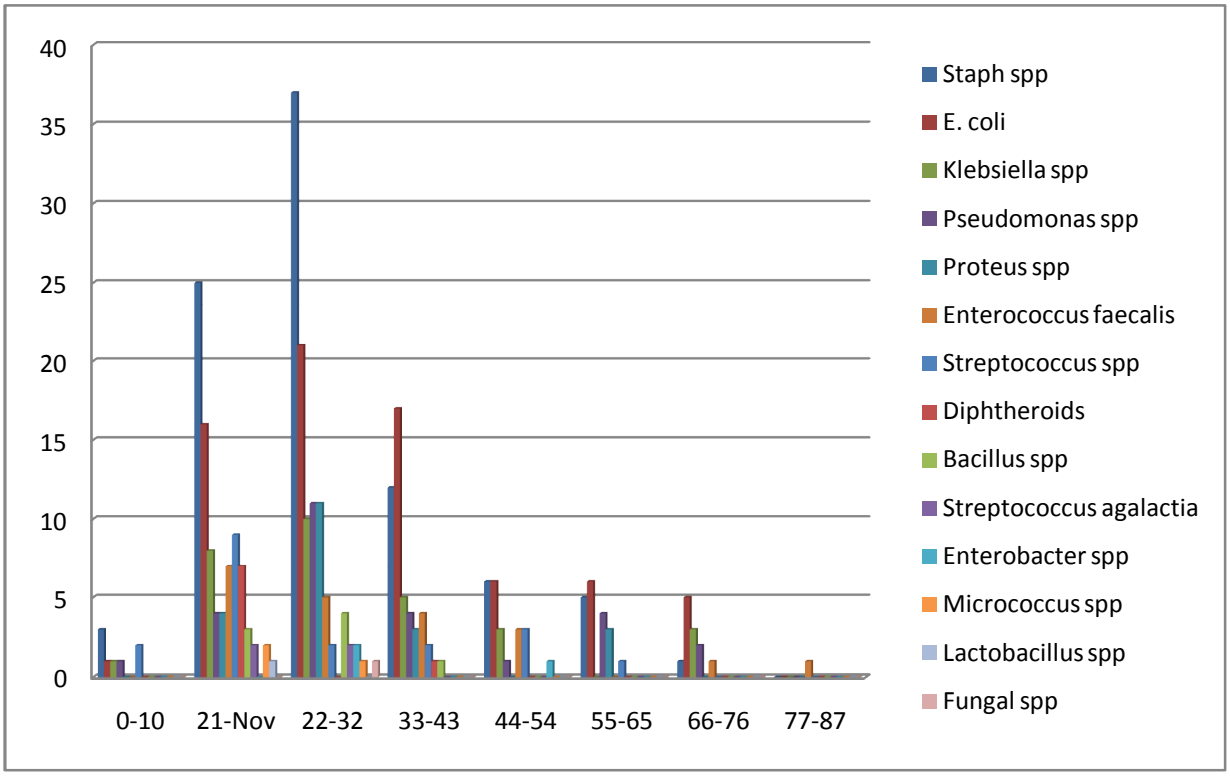
APPENDIX 4

TOTAL DISTRIBUTION OF POSITIVE BACTERIA ISOLATES IN URINA TRACT INFECTION IN THE POPULATION STUDIED

n=307

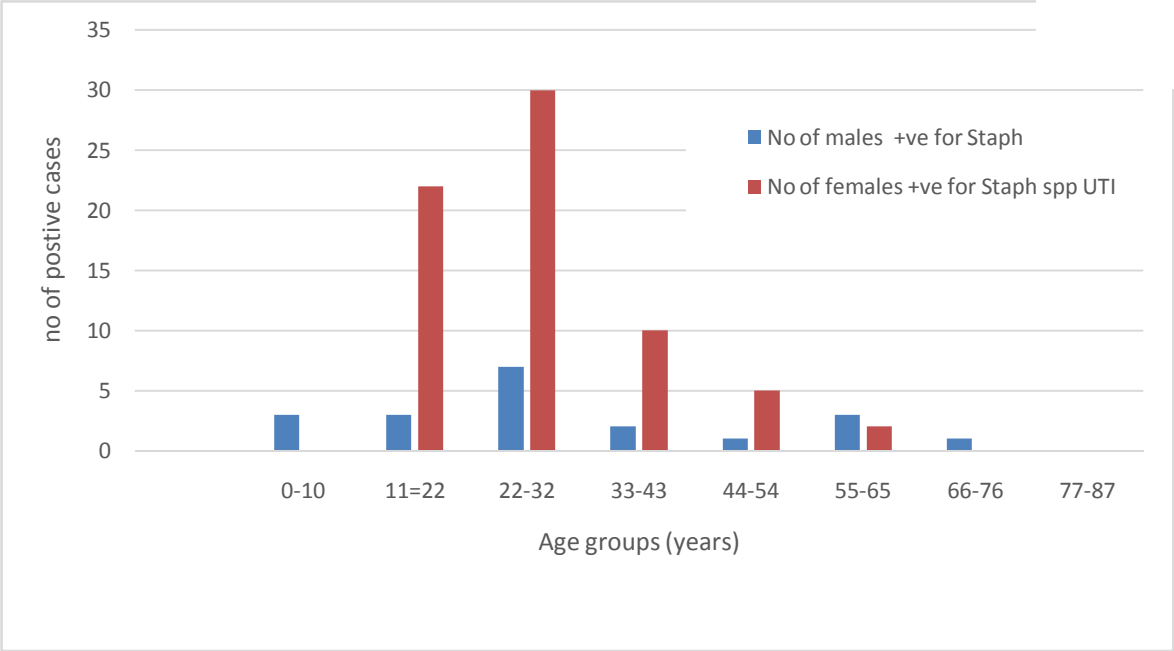
Organisms isolated	No organisms isolated	%
Staphylococcus spp	89	29.0%
Escherichia coli	72	23.5%
Klebsiella spp	30	9.8%
Pseudomonas spp	27	8.8%
Proteus spp	21	6.8%
Enterococcus faecalis	21	6.8%
Streptococcus spp	19	6.2%
Diphtheroids	8	2.6%
Bacillus spp	8	2.6%
Streptococcus agalactia	4	1.3%
Enterobacter spp	3	1.0%
Micrococcus spp	3	1.0%
Lactobacillus spp	1	0.3%
Fungal Agent *(Candida albicans)*	1	0.3%
Total	**307**	**100%**

APPENDIX 5



DISTRIBUTION OF POSITIVE CASES FOR UTI IN THE POPULATION STUDIED ACCORDING TO AGE GROUP, SEX AND BACTERIAL TYPE

APPENDIX 6



DISTRIBUTION OF THE POSITIVE CASES FOR STAPHYLOCOCCAL UTI IN THE POPULATION STUDIED ACCORNF TO THE AGE GROUP AND SEX

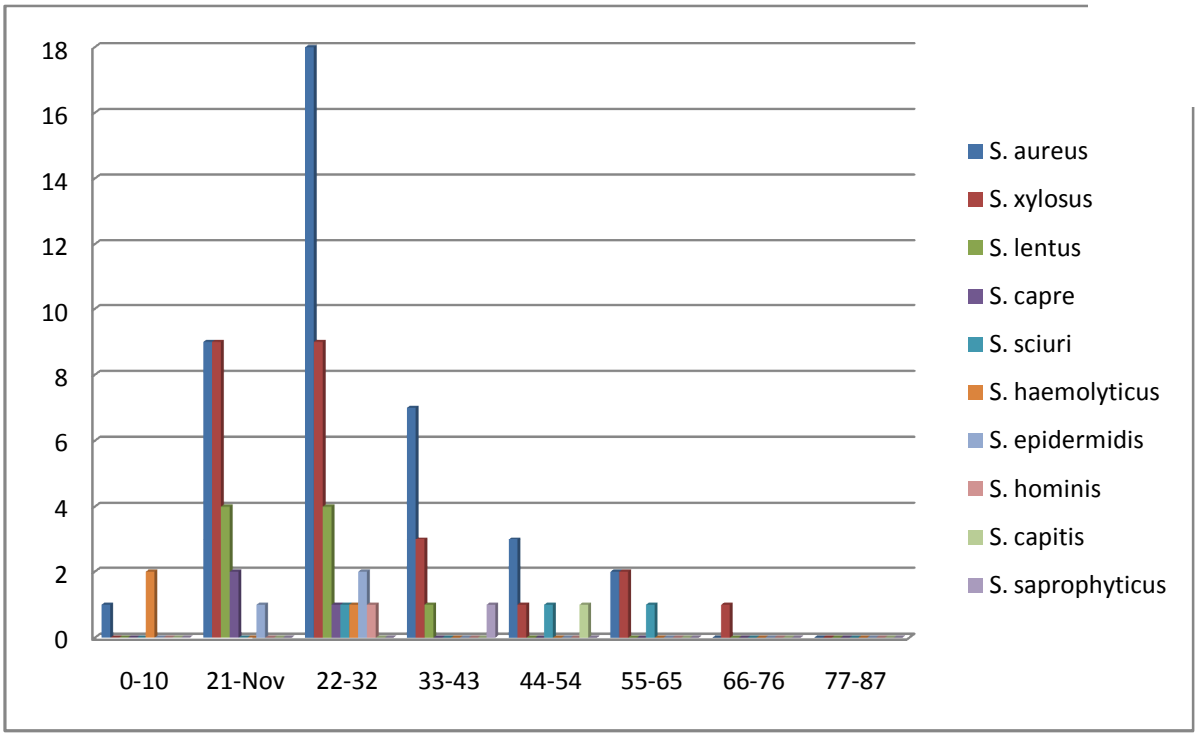
APPENDIX 7

DISTRIBUTION OF *Staphylococcus spp.* IN URINARY TRACT INECTION IN ENUGU

n= 89

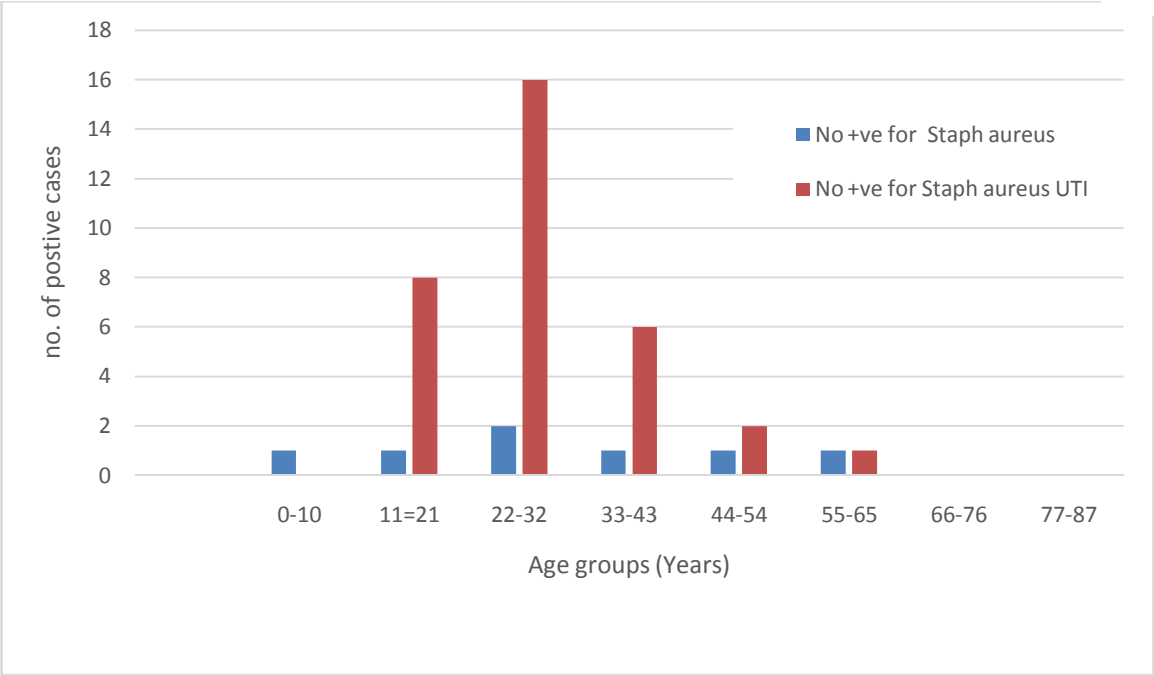
Staphylococcus Species **Isolated**	**No Isolated**	**%**
Staphylococcus aureus	40	44.9%
Staphylococcus xylosus	25	28.1%
Staphylococcus lentus	9	10.1%
Staphylococcus capre	3	3.4%
Staphylococcus sciuri	3	3.4%
Staphylococcus haemolyticus	3	3.4%
Staphylococcus epidermidis	3	3.4%
Staphylococcus hominis	1	1.1%
Staphylococcus capitis	1	1.1%
Staphylococcus saprophyticus	1	1.1%
Total	**8 9**	**10.9%**

APPENDIX 8



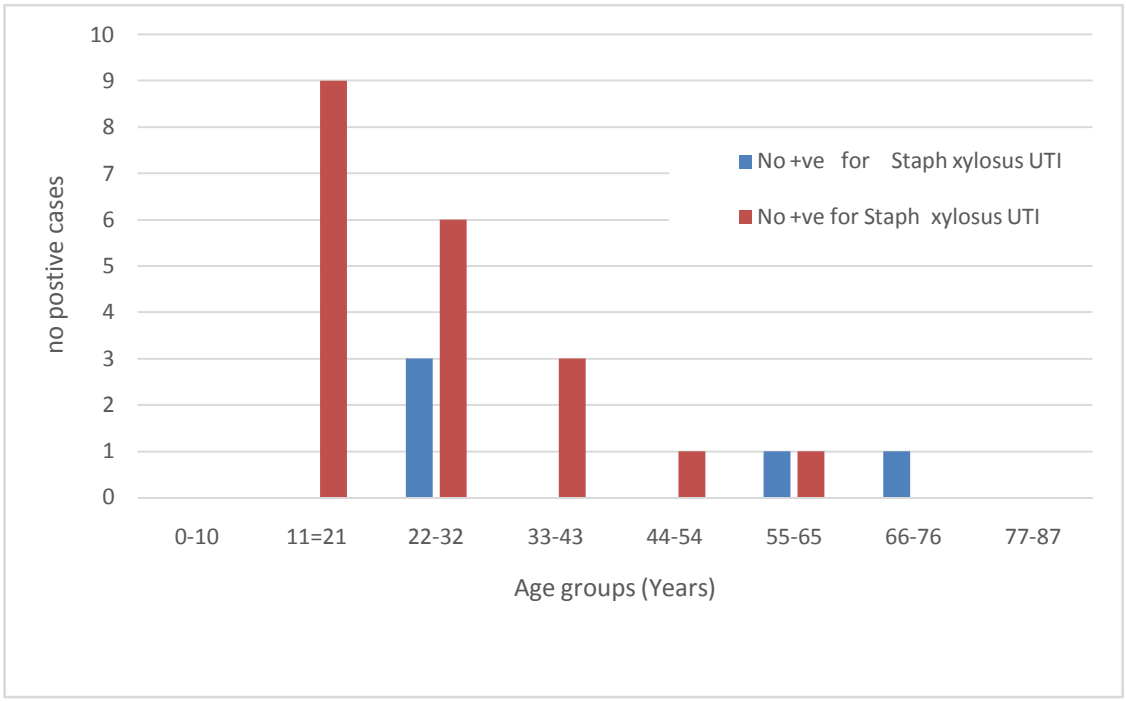
DISTRIBUTION OF POSITIVE CASES OF STAPHYLOCOCCAL SPP UTI ACCORDING TO AGE GROUP AND SEX.

APPENDIX 9



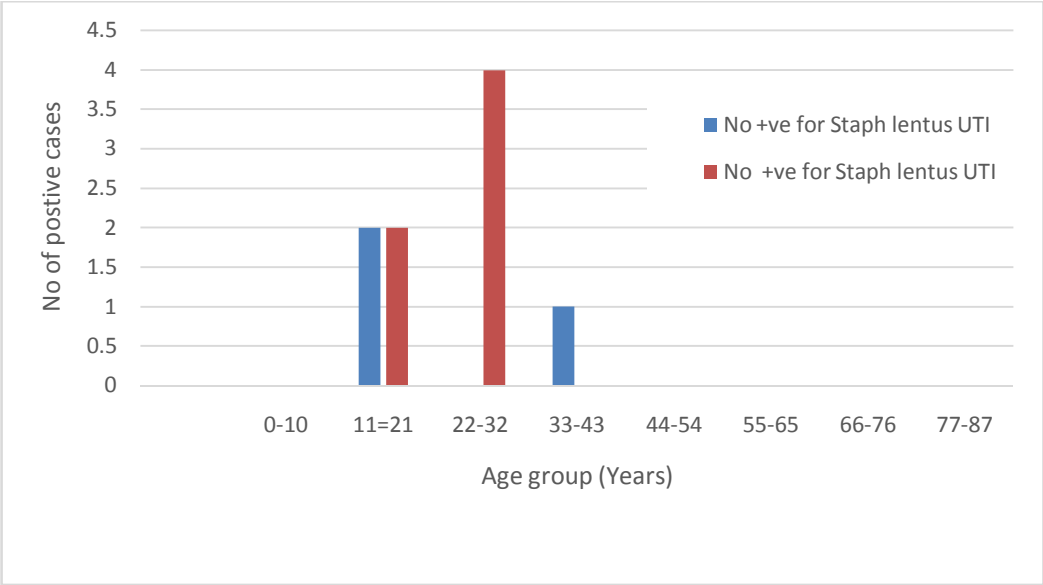
DISTRIBUTION OF POSITIVE CASES FOR *Staphylococcus aureus* UTI THE POPULATION STUDIED ACCORDING TO THE AGE GROUP AND SEX

APPENDIX 10



DISTRIBUTION OF POSITIVE CASES FOR *Staphylococcus xylosus* UTI THE POPULATION STUDIED ACCORDING TO THE AGE GROUP AND SEX

APPENDIX 11



DISTRIBUTION OF POSITIVE CASES FOR *Staphylococcus lentus* UTI THE POPULATION STUDIED ACCORDING TO THE AGE GROUP AND SEX

APPENDIX

Indole test

Medium

Peptone (brand containing sufficient tryptophan) é 20g

Sodium chloride, NaCl é ..5g

Distilled water é 1 litre

Adjust the PH to 7.4 dispense and sterilize by autoclaving at 121^{o}C for 15 min.

Kovac's reagent

Amyl or Isoamyl alcohol é 150ml

P-Dimethyl ï amino-benzaldehydeé .10g

Conc. Hydrochloric acid. HCL é .50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator, shake gently before use.

Methyl Red Test

Medium (glucose phosphate peptone water)

Peptone é é 5g

Dipotassium hydrogen phosphate, K_2HPO 4 é ..5g

water é é .1 Litre

Dissolve the peptone and phosphate, adjust the Ph to 7.6 filter, dispense in 5ml amounts and sterilize at 121^{oC} for 15min. Sterilize the glucose solution by filteration and add 0.25ml to each tube (final concentration 0.5%)

Methyl red indicator solution

Methyl red é 0.1g

Ethanol é ..300 ml

Distilled water é .200 ml

Citrate utilization test

Simmons medium

Simmons citrate medium is a modification of Koserôs medium with agar and an indicator added.

Kosers mediumé é 1 litre

Agar é é é é 20g

Bromothymol blue, 0.2%..... 40ml

Dispense, autoclave at 121^{o}C for 15min and allow to set as slopes

Deoxyribonuclease (DNase) plate test

The method is modified from Jeffries (1961)

DNA agar (Oxoid DNase agar) was used and contains the followings per litre

Tryptose é é 20g

Deoxyribonuclease é é .2g

Sodium chloride é é 5g

Agar powder é é .12g

pH é é 7.3

Reagent and material required but not provided (these were bought separately)

Mineral oil (ref. 70100)

Reagents: VP1 + VP2 (Ref. 70422)

NIT1 + NIT 2 (Ref. 70442)

Zym A (Ref. 70494)

Zym B (ref. 70493)

Mcfarland standard (Ref. 70900)

API staph analytical profile index (ref. 20590) or api web TM identification software (ref. 40011) (consult Biomerieux)

Material: - Pipettes or PSipettes

- Ampule rack
- Ampule protector
- General microbiology laboratory equipment

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 21 data tables and 0 info tables:

- **2way ANOVA of Variation of UTI:Tabular results**
- **Contingency of Gender Variation of UTI**
- **1way ANOVA of Variation of UTI Causative Isolates:Tabular results**
- **2way ANOVA of Staphylococcus spp:Tabular results**
- **Contingency of Gender Variation of Staph spp UTI**
- **1way ANOVA of Variation of Staph spp in Staph UTI:Tabular results**
- **2way ANOVA of S. aureus UTI:Tabular results**
- **Contingency of Gender Variation of S. aureus UTI**
- **2way ANOVA of S. xylosus UTI:Tabular results**
- **Contingency of Gender Variation of S. xylosus UTI**
- **2way ANOVA of S. lentus UTI:Tabular results**
- **Contingency of Gender Variation of S. lentus UTI**
- **Contingency of Variation according to Menopause stage**
- **Contingency of Variation of Staph UTI btw Occupations**
- **Contingency of Variation of Staph spp UTI in different Health Conditions**
- **2way ANOVA of Gender Variation of Staph spp UTI in Apparently Healthy:Tabular results**
- **2way ANOVA of Health status Variation of Staph. spp UTI:Tabular results**
- **Contingency of Variation in Symptom based Staph UTI**
- **2way ANOVA of Symtom based Staph UTI:Tabular results**
- **Contingency of Variation of Staph UTI in Pregnancy**
- **Contingency of Variation in Symptom based Staph UTI in Pregnancy**

2way ANOVA of Variation of UTI: Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Variation of UTI			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	19.19	0.0975		
Age Group	44.05	0.4086		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Age Group	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Gender	1	2377	2377	3.655
Age Group	7	5456	779.4	1.199
Residual	7	4552	650.3	
Number of missing values	0			
Bonferroni posttests				
Male vs Female				
Age Group	Male	Female	Difference	95% CI of diff.
0-10	5.000	3.000	-2.000	-141.0 to 137.0
11-21	6.000	82.00	76.00	-63.03 to 215.0
22-32	12.00	95.00	83.00	-56.03 to 222.0
33-43	8.000	41.00	33.00	-106.0 to 172.0
44-54	7.000	16.00	9.000	-130.0 to 148.0
55-65	10.00	9.000	-1.000	-140.0 to 138.0
66-76	8.000	4.000	-4.000	-143.0 to 135.0
77-87	0.0	1.000	1.000	-138.0 to 140.0
Age Group	Difference	t	P value	Summary
0-10	-2.000	0.05546	P > 0.05	ns
11-21	76.00	2.107	P > 0.05	ns
22-32	83.00	2.302	P > 0.05	ns
33-43	33.00	0.9151	P > 0.05	ns
44-54	9.000	0.2496	P > 0.05	ns
55-65	-1.000	0.02773	P > 0.05	ns
66-76	-4.000	0.1109	P > 0.05	ns
77-87	1.000	0.02773	P > 0.05	ns

Contingency of Gender Variation of UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 2		
Fisher's exact test			
P value	< 0.0001		
P value summary	***		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	Yes		
Strength of association			
Relative Risk	0.4062		
95% confidence interval	0.3158 to 0.5225		
Difference between proportions			
Fraction of top, bottom row in left column	0.1931, 0.4754		
Difference between fractions	0.2823		
95% confidence interval of difference	0.2129 to 0.3517		
Data analyzed	Pos	Neg	Total
Male	56	234	290
Female	251	277	528
Total	307	511	818

1way ANOVA of Variation of UTI Causative Isolates:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 3				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	14				
F	6.679				
R square	0.4698				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	145.7				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1176	13	90.47		
Residual (within columns)	1327	98	13.54		
Total	2503	111	Significant?	Summ	95% CI of diff
	Mean		P < 0.05?	ary	-4.223 to 8.473
Tukey's Multiple Comparison Test	Diff.	q	No	ns	1.027 to 13.72
Staphylococcus spp vs E. coli	2.125	1.633	Yes	**	1.402 to 14.10
Staphylococcus spp vs Klebsiella spp	7.375	5.668	Yes	**	2.152 to 14.85
Staphylococcus spp vs Pseudomonas sp	7.750	5.956	Yes	**	2.152 to 14.85
Staphylococcus spp vs Proteus spp	8.500	6.533	Yes	**	2.402 to 15.10
Staphylococcus spp vs E. faecalis	8.500	6.533	Yes	***	3.777 to 16.47
Staphylococcus spp vs Streptococcus spp	8.750	6.725	Yes	***	3.777 to 16.47
Staphylococcus spp vs Diphtheroids	10.13	7.781	Yes	***	4.277 to 16.97
Staphylococcus spp vs Bacillus spp	10.13	7.781	Yes	***	4.402 to 17.10
Staphylococcus spp vs S. agalactia	10.63	8.166	Yes	***	4.402 to 17.10
Staphylococcus spp vs Enterobacter sp	10.75	8.262	Yes	***	4.652 to 17.35
Staphylococcus spp vs Micrococcus sp	10.75	8.262	Yes	***	4.652 to 17.35
Staphylococcus spp vs Lactobacillus sp	11.00	8.454	Yes	***	-1.098 to 11.60
Staphylococcus spp vs C. albicans	11.00	8.454	No	ns	-0.7230 to 11.97
E. coli vs Klebsiella spp	5.250	4.035	No	ns	0.02696 to
E. coli vs Pseudomonas spp	5.625	4.323	Yes	*	12.72
E. coli vs Proteus spp	6.375	4.899	Yes	*	0.02696 to
E. coli vs E. faecalis	6.375	4.899	Yes	*	12.72
E. coli vs Streptococcus spp	6.625	5.092	Yes	**	0.2770 to 12.97
E. coli vs Diphtheroids	8.000	6.148	Yes	**	1.652 to 14.35
E. coli vs Bacillus spp	8.000	6.148	Yes	**	1.652 to 14.35

E. coli vs Streptococcus agalactia	8.500	6.533	Yes	***	2.152 to 14.85
E. coli vs Enterobacter spp	8.625	6.629	Yes	***	2.277 to 14.97
E. coli vs Micrococcus spp	8.625	6.629	Yes	***	2.277 to 14.97
E. coli vs Lactobacillus spp	8.875	6.821	Yes	***	2.527 to 15.22
E. coli vs Candida albicans	8.875	6.821	No	ns	2.527 to 15.22
Klebsiella spp vs Pseudomonas spp	0.3750	0.2882	No	ns	-5.973 to 6.723
Klebsiella spp vs Proteus spp	1.125	0.8646	No	ns	-5.223 to 7.473
Klebsiella spp vs E. faecalis	1.125	0.8646	No	ns	-5.223 to 7.473
Klebsiella spp vs Streptococcus spp	1.375	1.057	No	ns	-4.973 to 7.723
Klebsiella spp vs Diphtheroids	2.750	2.113	No	ns	-3.598 to 9.098
Klebsiella spp vs Bacillus spp	2.750	2.113	No	ns	-3.598 to 9.098
Klebsiella spp vs S.agalactia	3.250	2.498	No	ns	-3.098 to 9.598
Klebsiella spp vs Enterobacter spp	3.375	2.594	No	ns	-2.973 to 9.723
Klebsiella spp vs Micrococcus spp	3.375	2.594	No	ns	-2.973 to 9.723
Klebsiella spp vs Lactobacillus spp	3.625	2.786	No	ns	-2.723 to 9.973
Klebsiella spp vs Candida albicans	3.625	2.786	No	ns	-2.723 to 9.973
Pseudomonas spp vs Proteus spp	0.7500	0.5764	No	ns	-5.598 to 7.098
Pseudomonas spp vs E. faecalis	0.7500	0.5764	No	ns	-5.598 to 7.098
Pseudomonas spp vs Streptococcus s	1.000	0.7685	No	ns	-5.348 to 7.348
Pseudomonas spp vs Diphtheroids	2.375	1.825	No	ns	-3.973 to 8.723
Pseudomonas spp vs Bacillus spp	2.375	1.825	No	ns	-3.973 to 8.723
Pseudomonas spp vs S. agalactia	2.875	2.210	No	ns	-3.473 to 9.223
Pseudomonas spp vs Enterobacter spp	3.000	2.306	No	ns	-3.348 to 9.348
Pseudomonas spp vs Micrococcus spp	3.000	2.306	No	ns	-3.348 to 9.348
Pseudomonas spp vs Lactobacillus spp	3.250	2.498	No	ns	-3.098 to 9.598
Pseudomonas spp vs C.albicans	3.250	2.498	No	ns	-3.098 to 9.598
Proteus spp vs E. faecalis	0.0	0.0	No	ns	-6.348 to 6.348
Proteus spp vs Streptococcus spp	0.2500	0.1921	No	ns	-6.098 to 6.598
Proteus spp vs Diphtheroids	1.625	1.249	No	ns	-4.723 to 7.973
Proteus spp vs Bacillus spp	1.625	1.249	No	ns	-4.723 to 7.973
Proteus spp vs S. agalactia	2.125	1.633	No	ns	-4.223 to 8.473
Proteus spp vs Enterobacter spp	2.250	1.729	No	ns	-4.098 to 8.598
Proteus spp vs Micrococcus spp	2.250	1.729	No	ns	-4.098 to 8.598
Proteus spp vs Lactobacillus spp	2.500	1.921	No	ns	-3.848 to 8.848
Proteus spp vs Candida albicans	2.500	1.921	No	ns	-3.848 to 8.848
E. faecalis vs Streptococcus spp	0.2500	0.1921	No	ns	-6.098 to 6.598
E. faecalis vs Diphtheroids	1.625	1.249	No	ns	-4.723 to 7.973
E. faecalis vs Bacillus spp	1.625	1.249	No	ns	-4.723 to 7.973
E. faecalis vs S.agalactia	2.125	1.633	No	ns	-4.223 to 8.473
E. faecalis vs Enterobacter spp	2.250	1.729	No	ns	-4.098 to 8.598
E. faecalis vs Micrococcus spp	2.250	1.729	No	ns	-4.098 to 8.598
E. faecalis vs Lactobacillus spp	2.500	1.921	No	ns	-3.848 to 8.848
E. faecalis vs Candida albicans	2.500	1.921	No	ns	-3.848 to 8.848
Streptococcus spp vs Diphtheroids	1.375	1.057	No	ns	-4.973 to 7.723
Streptococcus spp vs Bacillus spp	1.375	1.057	No	ns	-4.973 to 7.723
Streptococcus spp vs S. agalactia	1.875	1.441	No	ns	-4.473 to 8.223

Streptococcus spp vs Enterobacter spp	2.000	1.537	No	ns	-4.348 to 8.348
Streptococcus spp vs Micrococcus	2.000	1.537	No	ns	-4.348 to 8.348
Streptococcus spp vs Lactobacillus	2.250	1.729	No	ns	-4.098 to 8.598
Streptococcus spp vs C.albicans	2.250	1.729	No	ns	-4.098 to 8.598
Diphtheroids vs Bacillus spp	0.0	0.0	No	ns	-6.348 to 6.348
Diphtheroids vs S. agalactia	0.5000	0.3843	No	ns	-5.848 to 6.848
Diphtheroids vs Enterobacter spp	0.6250	0.4803	No	ns	-5.723 to 6.973
Diphtheroids vs Micrococcus spp	0.6250	0.4803	No	ns	-5.723 to 6.973
Diphtheroids vs Lactobacillus spp	0.8750	0.6725	No	ns	-5.473 to 7.223
Diphtheroids vs Candida albicans	0.8750	0.6725	No	ns	-5.473 to 7.223
Bacillus spp vs S.agalactia	0.5000	0.3843	No	ns	-5.848 to 6.848
Bacillus spp vs Enterobacter spp	0.6250	0.4803	No	ns	-5.723 to 6.973
Bacillus spp vs Micrococcus spp	0.6250	0.4803	No	ns	-5.723 to 6.973
Bacillus spp vs Lactobacillus spp	0.8750	0.6725	No	ns	-5.473 to 7.223
Bacillus spp vs Candida albicans	0.8750	0.6725	No	ns	-5.473 to 7.223
S. agalactia vs Enterobacter spp	0.1250	0.09607	No	ns	-6.223 to 6.473
S. agalactia vs Micrococcus spp	0.1250	0.09607	No	ns	-6.223 to 6.473
S. agalactia vs Lactobacillus spp	0.3750	0.2882	No	ns	-5.973 to 6.723
S. agalactia vs Candida albicans	0.3750	0.2882	No	ns	-5.973 to 6.723
Enterobacter spp vs Micrococcus spp	0.0	0.0	No	ns	-6.348 to 6.348
Enterobacter spp vs Lactobacillus spp	0.2500	0.1921	No	ns	-6.098 to 6.598
Enterobacter spp vs Candida albicans	0.2500	0.1921	No	ns	-6.098 to 6.598
Micrococcus spp vs Lactobacillus spp	0.2500	0.1921	No	ns	-6.098 to 6.598
Micrococcus spp vs Candida albicans	0.2500	0.1921	No	ns	-6.098 to 6.598
Lactobacillus spp vs Candida albicans	0.0	0.0			-6.348 to 6.348

2way ANOVA of Staphylococcus spp:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 4			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	13.64	0.1224		

Age Group	55.41	0.2301		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Age Group	ns	No		
			Mean	F
Source of Variation	Df	Sum-of-	square	3.086
Gender	1	squares	150.1	1.790
Age Group	7	150.1	87.06	
Residual	7	609.4	48.63	
		340.4		
Number of missing values	0			
Bonferroni posttests				
Male vs Female				
Age Group	Male	Female	Difference	95% CI of diff.
0-10	3.000	0.0	-3.000	-41.02 to 35.02
11-21	3.000	22.00	19.00	-19.02 to 57.02
22-32	7.000	30.00	23.00	-15.02 to 61.02
33-43	2.000	10.00	8.000	-30.02 to 46.02
44-54	1.000	5.000	4.000	-34.02 to 42.02
55-65	3.000	2.000	-1.000	-39.02 to 37.02
66-76	1.000	0.0	-1.000	-39.02 to 37.02
77-87	0.0	0.0	0.0	-38.02 to 38.02
Age Group	Difference	t	P value	Summary
0-10	-3.000	0.3042	P > 0.05	ns
11-21	19.00	1.927	P > 0.05	ns
22-32	23.00	2.332	P > 0.05	ns
33-43	8.000	0.8112	P > 0.05	ns
44-54	4.000	0.4056	P > 0.05	ns
55-65	-1.000	0.1014	P > 0.05	ns
66-76	-1.000	0.1014	P > 0.05	ns
77-87	0.0	0.0	P > 0.05	ns

Contingency of Gender Variation of Staph spp UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 5		
Fisher's exact test			
P value	0.0068		

P value summary	**		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	Yes		
Strength of association			
Relative Risk	0.5277		
95% confidence interval	0.3276 to 0.8501		
Difference between proportions			
Fraction of top, bottom row in left column	0.06897, 0.1307		
Difference between fractions	0.06172		
95% confidence interval of difference	0.01710 to 0.1063		
Data analyzed	Pos	Neg	Total
Male	20	270	290
Female	69	459	528
Total	89	729	818

1way ANOVA of Variation of Staph spp in Staph UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Variation of Staph spp in Staph UTI				
One-way analysis of variance					
P value					
P value summary	< 0.0001				
Are means signif. different? (P < 0.05)	***				
Number of groups	Yes				
F	10				
R square	7.126				
	0.4781				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value	95.52				
P value summary	< 0.0001				
Do the variances differ signif. (P < 0.05)	***				
	Yes				
ANOVA Table					
Treatment (between columns)	SS	df	MS		
Residual (within columns)	194.1	9	21.57		
Total	211.9	70	3.027		
	406.0	79			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant ? P < 0.05?	Summary	95% CI of diff
S. aureus vs S. xylosus	1.875	3.048	No	ns	-0.9739 to 4.724
S. aureus vs S. lentus	3.875	6.300	Yes	**	1.026 to 6.724
S. aureus vs S. capre	4.625	7.519	Yes	***	1.776 to 7.474
S. aureus vs S. sciuri	4.625	7.519	Yes	***	1.776 to 7.474
S. aureus vsS. heamolytus	4.625	7.519	Yes	***	1.776 to 7.474
S. aureus vsS.epidermidis	4.625	7.519	Yes	***	1.776 to 7.474
S. aureus vs S. hominis	4.875	7.926	Yes	***	2.026 to 7.724
S. aureus vs S. capitis	4.875	7.926	Yes	***	2.026 to 7.724
S.aureus vs S. saprophyticus	4.875	7.926	Yes	***	2.026 to 7.724
S. xylosus vs S. lentus	2.000	3.252	Yes	***	-0.8489 to 4.849
S. xylosus vs S. capre	2.750	4.471	No	ns	-0.09894 to 5.599
S. xylosus vs S. sciuri	2.750	4.471	No	ns	-0.09894 to 5.599
S. xylosus vs S.heamolytus	2.750	4.471	No	ns	-0.09894 to 5.599
S. xylosus vs S.epidermidis	2.750	4.471	No	ns	-0.09894 to 5.599
S. xylosus vsS. hominis	3.000	4.877	No	ns	0.1511 to 5.849
S. xylosus vs S. capitis	3.000	4.877	Yes	*	0.1511 to 5.849

S. xylosus vs S. aprophyticus	3.000	4.877	Yes	*	0.1511 to 5.849
S. lentus vs S. capre	0.7500	1.219	Yes	*	-2.099 to 3.599
S. lentus vs S. sciuri	0.7500	1.219	No	ns	-2.099 to 3.599
S. lentus vs S. heamolytus	0.7500	1.219	No	ns	-2.099 to 3.599
S. lentus vs S.epidermidis	0.7500	1.219	No	ns	-2.099 to 3.599
S. lentus vs S. hominis	1.000	1.626	No	ns	-1.849 to 3.849
S. lentus vs S. capitis	1.000	1.626	No	ns	-1.849 to 3.849
S. lentus vs S. saprophyticus	1.000	1.626	No	ns	-1.849 to 3.849
S. capre vs S. sciuri	0.0	0.0	No	ns	-2.849 to 2.849
S. capre vs S. heamolytus	0.0	0.0	No	ns	-2.849 to 2.849
S. capre vs S.epidermidis	0.0	0.0	No	ns	-2.849 to 2.849
S. capre vs S. hominis	0.2500	0.4064	No	ns	-2.599 to 3.099
S. capre vs S. capitis	0.2500	0.4064	No	ns	-2.599 to 3.099
S. capre vs S. saprophyticus	0.2500	0.4064	No	ns	-2.599 to 3.099
S. sciuri vs S. heamolytus	0.0	0.0	No	ns	-2.849 to 2.849
S. sciuri vs S.epidermidis	0.0	0.0	No	ns	-2.849 to 2.849
S. sciuri vs S. hominis	0.2500	0.4064	No	ns	-2.599 to 3.099
S. sciuri vs S. capitis	0.2500	0.4064	No	ns	-2.599 to 3.099
S. sciuri vs S. saprophyticus	0.2500	0.4064	No	ns	-2.599 to 3.099
S.heamolytus vs S.epidermidis	0.0	0.0	No	ns	-2.849 to 2.849
S.heamolytus vs S. hominis	0.2500	0.4064	No	ns	-2.599 to 3.099
S.heamolytus vs S. capitis	0.2500	0.4064	No	ns	-2.599 to 3.099
S. heamolytus vs S. saprophyticus	0.2500	0.4064	No	ns	-2.599 to 3.099
S.epidermidis vs S. hominis	0.2500	0.4064	No	ns	-2.599 to 3.099
S.epidermidis vs S. capitis	0.2500	0.4064	No	ns	-2.599 to 3.099
S.epidermidis vs S. saprophyticus	0.2500	0.4064	No	ns	-2.599 to 3.099
S. hominis vs S. capitis	0.0	0.0	No	ns	-2.849 to 2.849
S hominis vs S.saprophyticus	0.0	0.0	No	ns	-2.849 to 2.849
S.capitis vs S.saprophyticus	0.0	0.0	No	ns	-2.849 to 2.849

2way ANOVA of S. aureus UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	S. aureus UTI			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	15.65	0.1190		
Age Group	49.63	0.3246		
Source of Variation	P value summary	Significant?		
Gender	ns	No		

Age Group	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Gender	1	42.25	42.25	3.155
Age Group	7	134.0	19.14	1.429
Residual	7	93.75	13.39	
Number of missing values	0			
Bonferroni posttests				
Male vs Female				
Age Group	Male	Female	Difference	95% CI of diff.
0-10	1.000	0.0	-1.000	-20.95 to 18.95
11-21	1.000	8.000	7.000	-12.95 to 26.95
22-32	2.000	16.00	14.00	-5.953 to 33.95
33-43	1.000	6.000	5.000	-14.95 to 24.95
44-54	1.000	2.000	1.000	-18.95 to 20.95
55-65	1.000	1.000	0.0	-19.95 to 19.95
66-76	0.0	0.0	0.0	-19.95 to 19.95
77-87	0.0	0.0	0.0	-19.95 to 19.95
Age Group	Difference	t	P value	Summary
0-10	-1.000	0.1932	P > 0.05	ns
11-21	7.000	1.353	P > 0.05	ns
22-32	14.00	2.705	P > 0.05	ns
33-43	5.000	0.9661	P > 0.05	ns
44-54	1.000	0.1932	P > 0.05	ns
55-65	0.0	0.0	P > 0.05	ns
66-76	0.0	0.0	P > 0.05	ns
77-87	0.0	0.0	P > 0.05	ns

Contingency of Gender Variation of S. aureus UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 8		
Fisher's exact test			
P value	0.0167		
P value summary	*		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	Yes		

Strength of association			
Relative Risk	0.3862		
95% confidence interval	0.1730 to 0.8622		
Difference between proportions			
Fraction of top, bottom row in left column	0.02414, 0.0625		
Difference between fractions	0.03836		
95% confidence interval of difference	0.007460 to 0.06926		
Data analyzed	Pos	Neg	Total
Male	7	283	290
Female	33	495	528
Total	40	778	818

2way ANOVA of S. xylosus UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 9			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	14.07	0.1443		
Age Group	49.47	0.3487		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Age Group	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Gender	1	14.06	14.06	2.702
Age Group	7	49.44	7.063	1.357
Residual	7	36.44	5.205	
Number of missing values	0			
Bonferroni posttests				
Male vs Female				
Age Group	Male	Female	Difference	95% CI of diff.
0-10	0.0	0.0	0.0	-12.44 to 12.44
11-21	0.0	9.000	9.000	-3.439 to 21.44
22-32	3.000	6.000	3.000	-9.439 to 15.44

33-43	0.0	3.000	3.000	-9.439 to 15.44
44-54	0.0	1.000	1.000	-11.44 to 13.44
55-65	1.000	1.000	0.0	-12.44 to 12.44
66-76	1.000	0.0	-1.000	-13.44 to 11.44
77-87	0.0	0.0	0.0	-12.44 to 12.44
Age Group	Difference	t	P value	Summary
0-10	0.0	0.0	P > 0.05	ns
11-21	9.000	2.789	P > 0.05	ns
22-32	3.000	0.9298	P > 0.05	ns
33-43	3.000	0.9298	P > 0.05	ns
44-54	1.000	0.3099	P > 0.05	ns
55-65	0.0	0.0	P > 0.05	ns
66-76	-1.000	0.3099	P > 0.05	ns
77-87	0.0	0.0	P > 0.05	ns

Contingency of Gender Variation of S. xylosus UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 10		
Fisher's exact test			
P value	0.1362		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	0.4552		
95% confidence interval	0.1726 to 1.200		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Pos	Neg	Total
Male	5	285	290
Female	20	508	528
Total	25	793	818

2way ANOVA of S. lentus UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 11			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	2.82	0.5040		
Age Group	57.37	0.3209		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Age Group	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Gender	1	0.5625	0.5625	0.4961
Age Group	7	11.44	1.634	1.441
Residual	7	7.938	1.134	
Number of missing values	0			
Bonferroni posttests				
Male vs Female	Male	Female	Difference	95% CI of diff.
Age Group	0.0	0.0	0.0	-5.806 to 5.806
0-10	2.000	2.000	0.0	-5.806 to 5.806
11-21	0.0	4.000	4.000	-1.806 to 9.806
22-32	1.000	0.0	-1.000	-6.806 to 4.806
33-43	0.0	0.0	0.0	-5.806 to 5.806
44-54	0.0	0.0	0.0	-5.806 to 5.806
55-65	0.0	0.0	0.0	-5.806 to 5.806
66-76	0.0	0.0	0.0	-5.806 to 5.806
77-87				
Age Group	Difference	t	P value	Summary
0-10	0.0	0.0	P > 0.05	ns
11-21	0.0	0.0	P > 0.05	ns
22-32	4.000	2.656	P > 0.05	ns
33-43	-1.000	0.6640	P > 0.05	ns
44-54	0.0	0.0	P > 0.05	ns
55-65	0.0	0.0	P > 0.05	ns
66-76	0.0	0.0	P > 0.05	ns
77-87	0.0	0.0	P > 0.05	ns

Contingency of Gender Variation of S. lentus UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 12		
Fisher's exact test			
P value	1.0000		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	0.9103		
95% confidence interval	0.2293 to 3.614		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Pos	Neg	Total
Male	3	287	290
Female	6	522	528
Total	9	809	818

Contingency of Variation according to Menopause stage

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 13		
Fisher's exact test			
P value	0.8396		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		

Strength of association			
Relative Risk	1.043		
95% confidence interval	0.5252 to 2.069		
Difference between proportions			
Fraction of top, bottom row in left column	0.1356, 0.1301		
Difference between fractions	0.005529		
95% confidence interval of difference	-0.08575 to 0.09681		
Data analyzed	Pos	Neg	Total
Menopausal	8	51	59
Pre-menopausal	61	408	469
Total	69	459	528

Contingency of Variation of Staph UTI btw Occupations

	Data Set-A
Table Analyzed	Data 14
Chi-square	
Chi-square, df	15.95, 7
P value	0.0256
P value summary	*
One- or two-sided	NA
Statistically significant? (alpha<0.05)	Yes
Data analyzed	
Number of rows	8
Number of columns	2

Contingency of Variation of Staph spp UTI in different Health Conditions

	Data Set-A
Table Analyzed	Data 15
Chi-square	
Chi-square, df	18.42, 2
P value	0.0001
P value summary	***
One- or two-sided	NA
Statistically significant? (alpha<0.05)	Yes
Data analyzed	
Number of rows	3

Number of columns	2

2way ANOVA of Gender Variation of Staph spp UTI in Apparently Healthy:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 16			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	4.78	0.1655		
Bacteria(Staph spp)	76.32	0.0248		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Bacteria(Staph spp)	*	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Gender	1	20.00	20.00	2.278
Bacteria(Staph spp)	9	319.0	35.44	4.038
Residual	9	79.00	8.778	
Number of missing values	0			
Bonferroni posttests				
Male vs Female				
Bacteria(Staph spp)	Male	Female	Difference	95% CI of diff.
S. aureus	7.000	17.00	10.00	-5.459 to 25.46
S. xylosus	5.000	14.00	9.000	-6.459 to 24.46
S. lentus	3.000	3.000	0.0	-15.46 to 15.46
S. capre	0.0	2.000	2.000	-13.46 to 17.46
S. sciuri	1.000	0.0	-1.000	-16.46 to 14.46
S. haemolyticus	3.000	0.0	-3.000	-18.46 to 12.46
S. epidermidis	1.000	2.000	1.000	-14.46 to 16.46
S. hominis	0.0	0.0	0.0	-15.46 to 15.46
S. capitis	0.0	1.000	1.000	-14.46 to 16.46
S. saprophyticus	0.0	1.000	1.000	-14.46 to 16.46
Bacteria(Staph spp)	Difference	t	P value	Summary
S. aureus	10.00	2.387	P > 0.05	ns
S. xylosus	9.000	2.148	P > 0.05	ns
S. lentus	0.0	0.0	P > 0.05	ns

S. capre	2.000	0.4773	P > 0.05	ns
S. sciuri	-1.000	0.2387	P > 0.05	ns
S. haemolyticus	-3.000	0.7160	P > 0.05	ns
S. epidermidis	1.000	0.2387	P > 0.05	ns
S. hominis	0.0	0.0	P > 0.05	ns
S. capitis	1.000	0.2387	P > 0.05	ns
S. saprophyticus	1.000	0.2387	P > 0.05	ns

2way ANOVA of Health status Variation of Staph. spp UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 17			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	25.74	0.0998		
Medical Status	12.03	0.0035		
Bacteria(Staph spp)	50.22	0.0001		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Medical Status	**	Yes		
Bacteria(Staph spp)	***	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	18	172.1	9.559	1.812
Medical Status	2	80.43	40.22	7.624
Bacteria(Staph spp)	9	335.8	37.31	7.072
Residual	20	105.5	5.275	
Number of missing values	10			
Bonferroni posttests				
Apparently Healthy vs Pregnant	Apparently			
Bacteria(Staph spp)	Healthy	Pregnant	Difference	95% CI of diff.
S. aureus	12.00	13.00	1.000	-9.212 to 11.21
S. xylosus	9.500	6.000	-3.500	-13.71 to 6.712
S. lentus	3.000	3.000	0.0	-10.21 to 10.21
S. capre	1.000	1.000	0.0	-10.21 to 10.21
S. sciuri	0.5000	0.0	-0.5000	-10.71 to 9.712
S. haemolyticus	1.500	0.0	-1.500	-11.71 to 8.712

S. epidermidis	1.500	0.0	-1.500	-11.71 to 8.712
S. hominis	0.0	1.000	1.000	-9.212 to 11.21
S. capitis	0.5000	0.0	-0.5000	-10.71 to 9.712
S. saprophyticus	0.5000	0.0	-0.5000	-10.71 to 9.712
Bacteria(Staph spp)	Difference	t	P value	Summary
S. aureus	1.000	0.3555	P > 0.05	ns
S. xylosus	-3.500	1.244	P > 0.05	ns
S. lentus	0.0	0.0	P > 0.05	ns
S. capre	0.0	0.0	P > 0.05	ns
S. sciuri	-0.5000	0.1778	P > 0.05	ns
S. haemolyticus	-1.500	0.5333	P > 0.05	ns
S. epidermidis	-1.500	0.5333	P > 0.05	ns
S. hominis	1.000	0.3555	P > 0.05	ns
S. capitis	-0.5000	0.1778	P > 0.05	ns
S. saprophyticus	-0.5000	0.1778	P > 0.05	ns
Apparently Healthy vs Medical Condition(Unhealthy)	Apparently Healthy	Medical Condition(Unhealthy)	Difference	95% CI of diff.
S. aureus	12.00	1.500	-10.50	-18.84 to -2.162
S. xylosus	9.500	0.0	-9.500	-17.84 to -1.162
S. lentus	3.000	0.0	-3.000	-11.34 to 5.338
S. capre	1.000	0.0	-1.000	-9.338 to 7.338
S. sciuri	0.5000	1.000	0.5000	-7.838 to 8.838
S. haemolyticus	1.500	0.0	-1.500	-9.838 to 6.838
S. epidermidis	1.500	0.0	-1.500	-9.838 to 6.838
S. hominis	0.0	0.0	0.0	-8.338 to 8.338
S. capitis	0.5000	0.0	-0.5000	-8.838 to 7.838
S. saprophyticus	0.5000	0.0	-0.5000	-8.838 to 7.838
Bacteria(Staph spp)	Difference	t	P value	Summary
S. aureus	-10.50	4.572	P<0.01	**
S. xylosus	-9.500	4.136	P<0.01	**
S. lentus	-3.000	1.306	P > 0.05	ns
S. capre	-1.000	0.4354	P > 0.05	ns
S. sciuri	0.5000	0.2177	P > 0.05	ns
S. haemolyticus	-1.500	0.6531	P > 0.05	ns
S. epidermidis	-1.500	0.6531	P > 0.05	ns
S. hominis	0.0	0.0	P > 0.05	ns
S. capitis	-0.5000	0.2177	P > 0.05	ns
S. saprophyticus	-0.5000	0.2177	P > 0.05	ns
Pregnant vs Medical Condition(Unhealthy)	Pregnant	Medical Condition(Unhealthy)	Difference	95% CI of diff.
Bacteria(Staph spp)				

S. aureus	13.00	1.500	-11.50	-21.71 to -1.288
S. xylosus	6.000	0.0	-6.000	-16.21 to 4.212
S. lentus	3.000	0.0	-3.000	-13.21 to 7.212
S. capre	1.000	0.0	-1.000	-11.21 to 9.212
S. sciuri	0.0	1.000	1.000	-9.212 to 11.21
S. haemolyticus	0.0	0.0	0.0	-10.21 to 10.21
S. epidermidis	0.0	0.0	0.0	-10.21 to 10.21
S. hominis	1.000	0.0	-1.000	-11.21 to 9.212
S. capitis	0.0	0.0	0.0	-10.21 to 10.21
S. saprophyticus	0.0	0.0	0.0	-10.21 to 10.21
Bacteria(Staph spp)	Difference	t	P value	Summary
S. aureus	-11.50	4.088	P<0.01	**
S. xylosus	-6.000	2.133	P > 0.05	ns
S. lentus	-3.000	1.067	P > 0.05	ns
S. capre	-1.000	0.3555	P > 0.05	ns
S. sciuri	1.000	0.3555	P > 0.05	ns
S. haemolyticus	0.0	0.0	P > 0.05	ns
S. epidermidis	0.0	0.0	P > 0.05	ns
S. hominis	-1.000	0.3555	P > 0.05	ns
S. capitis	0.0	0.0	P > 0.05	ns
S. saprophyticus	0.0	0.0	P > 0.05	ns

Contingency of Variation in Symptom based Staph UTI

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 18		
Fisher's exact test			
P value	0.0773		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	1.484		
95% confidence interval	0.9694 to 2.271		i
Difference between proportions			
Fraction of top, bottom row in left column	0.1461, 0.09844		
Difference between fractions	0.04763		
95% confidence interval of difference	-0.004100 to 0.09936		

Data analyzed	Pos	Neg	Total
Symtomatic	26	152	178
Non-Symptomatic	63	577	640
Total	89	729	818

2way ANOVA of Symtom based Staph UTI:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 19			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	7.79	0.8637		
Symptom	6.35	0.1332		
Age Group	45.25	0.0576		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Symptom	ns	No		
Age Group	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	7	52.47	7.496	0.4385
Symptom	1	42.78	42.78	2.503
Age Group	7	304.7	43.53	2.547
Residual	16	273.5	17.09	
Number of missing values	0			
Bonferroni posttests				
Symptomatic vs Non-Symptomatic		Non-		
Age Group	Symptomatic	Symptomatic	Difference	95% CI of diff.
0-10	0.0	1.500	1.500	-11.51 to 14.51
11-21	3.000	9.500	6.500	-6.506 to 19.51
22-32	6.500	12.00	5.500	-7.506 to 18.51
33-43	1.000	5.000	4.000	-9.006 to 17.01
44-54	0.5000	2.500	2.000	-11.01 to 15.01
55-65	1.500	1.000	-0.5000	-13.51 to 12.51
66-76	0.5000	0.0	-0.5000	-13.51 to 12.51

77-87	0.0	0.0	0.0	-13.01 to 13.01
Age Group	Difference	t	P value	Summary
0-10	1.500	0.3628	P > 0.05	ns
11-21	6.500	1.572	P > 0.05	ns
22-32	5.500	1.330	P > 0.05	ns
33-43	4.000	0.9675	P > 0.05	ns
44-54	2.000	0.4837	P > 0.05	ns
55-65	-0.5000	0.1209	P > 0.05	ns
66-76	-0.5000	0.1209	P > 0.05	ns
77-87	0.0	0.0	P > 0.05	ns

Contingency of Variation of Staph UTI in Pregnancy

	Data Set-A
Table Analyzed	Data 20
Chi-square	
Chi-square, df	1.966, 2
P value	0.3741
P value summary	ns
One- or two-sided	NA
Statistically significant? (alpha<0.05)	No
Data analyzed	
Number of rows	3
Number of columns	2

Contingency of Variation in Symptom based Staph UTI in Pregnancy

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 21		
Fisher's exact test			
P value	0.3470		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			

Relative Risk			
95% confidence interval	1.411 0.6887 to 2.890		
Difference between proportions			
Fraction of top, bottom row in left column			
Difference between fractions	0.2500, 0.1772		
95% confidence interval of difference	0.07278 -0.07987 to 0.2254		
Data analyzed			
Symtomatic	Pos	Neg	Total
Non-Symptomatic	10	30	40
Total	14	65	79
	24	95	119

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Our Ref: UNTH/CSA.329/VOL.5

Date: 13TH Sept., 2012

NHREC/05/01/2008B-FWA00002458-1RB00002323

ETHICAL CLEARA NCE CERTIFICATE

TOPIC: EPIDEMIOLOGY AND PATHOPHYSIOLOGICAL DETERMINANTS OF STAPHYLOCOCUS SAPROPHYTICUS URINARY TRACT INFECTION.

BY: **ONYEBUEKE EBERE ADAEZE**

FOR: A DISSERTATION FOR A DOCTORATE DEGREE (Ph. D) IN MEDICAL LABORATORY SCIENCES OF THE FACULTY OF HEALTH SCIENCES AND TECHNOLOGY OF THE COLLEGE OF MEDICINE, UNIVERSITY OF NIGERIA.

This research project on the above topic was reviewed and approved by the University of Nigeria Teaching Hospital Health Research Ethics Committee.

This certificate is valid for **one year** from date of issue.

Prof. R.E. Umeh
Chairman, Health Research Ethics Committee

17/10/12
Date

UNIVERSITY OF NIGERIA TEACHING HOSPITAL

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Our Ref. UNTH/CSA.329/Vol.6

Date 20th July, 2010

NHREC/05/01/2008B

ETHICAL CLEARANCE CERTIFICATE

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PROF. R. E. UMEH
Chairman, Health Research Ethics Committee.

Date: 22/07/2010

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CHAIRMAN MEDICAL ADVISORY COMMITTEE

Our Ref: ....

Date 16TH APRIL, 2010

TO WHOM IT MAY CONCERN

ETHICAL PERMISSION FOR RESEARCH INVESTIGATION

Permission is hereby given to ONYEBUEKE EBERE A.

to conduct the research ON EPIDEMIOLOGY AND PATHOPHYSIOLOGICAL DETERMINANTS OF Staphylococcus saprophyticus URINARY TRACT INFECTION

in the ESUT Teaching Hospital Parklane, Enugu bearing in mind all the ethical implications.

16/4/10

Dr. C. J. G. Orjiake
(Chief Consultant Physician and
Enugu State Chairman, Ethical Committee)

INFORMED CONSENT FORM

INTRODUCTION:

Dear subject (volunteer), the study that we wish you to participate in is titled **"STUDIES ON EPIDEMIOLOGY AND PATHOPHYSIOLOGICAL DETERMINANTS OF *Staphylococcus saprophyticus* URINARY TRACT INFECTION".**

The research wishes to find out the prevalence of the bacterial organism *Staphylococcus saprophyticus* in our environment using a study population which we wish that you will be part of.

The bacterial organism in question causes urinary tract infection which could lead to ill health such as urethritis (inflammation of the urethra), cystitis (infection of the bladder), pyelonephritis (infection of the kidney), urosepsis (infection of the blood) with their complications which could be very serious such as endocarditis, still birth, death.

The study wishes to also find out the epidemiology (mode of infection, predisposing factors, general incidence) and pathophysiological determinants (how it cause ill health) of the bacterial organism in urinary tract infections so as to enable the researcher recommend control and possible eradication measures.

The volunteer is expected to collect from the researcher a sterile urine bottle and use this to collect early morning midstream urine to be submitted within 30minutes of collection to the researcher to enable laboratory studies to be performed on it.

The collection of the urine sample is simple and will pose no problem to the subject (volunteer).

Voluntary nature of participation

The participation in the research is voluntary and the subject can withdraw from the study at any time he or she wishes without any repercussion or victimization.

Study procedure

The patient/subjects shall be instructed on how to collect early morning midstream urine by the researcher or the nurses on duty using the sterile urine bottles. The urine samples shall be collected between 6.00am and 6.30am in the morning by the volunteer or by the aid of nurses/doctors on duty for those who are incapacitated to collect by themselves and this shall be submitted to the researcher within 30minutes of collection to enable quick laboratory investigation to be carried out on them within 1hour of collection. The collection of the urine sample shall be done only once except in the case of missing sample or sample that poured away mistakenly or sample that was not properly collected. Question for the patient/subject shall be answered either orally and / or by the use of questionnaire. Urine microscopy and culture shall be

carried out on all urine samples collected from volunteers while sensitivity shall be carried out on samples positive for bacterial infection.

Risks

No risk is anticipated in the collection of the urine samples. Patients/subjects shall benefit by receiving results of the urine microscopy, culture and sensitivity that shall be performed on their samples.

Confidentiality

All results obtained from the study subjects (volunteers) shall be treated with high confidentiality.

Feed back

For contact, the researcher is Mrs. E.A Onyebueke, a P.hD student of the Department of Medical Laboratory Sciences, University of Nigeria, Enugu Campus. Phone number-08033500885

Response

Result of the investigation shall be put in envelopes sealed and delivered to the subjects through the office of the Chief Medical Laboratory Scientist in charge of the Medical Microbiology Laboratory of the hospital where the sample was collected.

In the case of subject gotten from home to home canvassing and other institutions (control group), the researcher shall personally deliver their results to them.

Thank you.

....

Name and signature of subject Name and signature of researcher

Date ..... Date .....

.....

Name and signature of witness

Date .....

QUESTIONNAIRE

1. Name:.....
2. Age:.....
3. Sex:.....
4. Home Address:.....
5. Office Address/School Address:.....
6. Marital Status: Single ☐ Married ☐ Widow ☐ Widower ☐
   Separated ☐ Divorced ☐
7. Student: Yes ☐ No ☐
8. If yes, what level?.....
9. Occupation:.....
10. Hospital Attending:.....
11. Are you on admission: Yes ☐ No ☐
12. If yes, for how long:.....
13. Clinical diagnosis of illness:.....
14. Duration of illness:.....
15. Pregnant: Yes ☐ No ☐
16. Gestation period if pregnant: 0-3months ☐ 4-6 months ☐
    7-9 months ☐
17. Parity: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
    Above 6 state the number ☐
18. Are you on any family planning method? Yes ☐ No ☐
19. If yes, which one: Condom ☐ Spermicide ☐ Diaphram ☐
    Any other specify:.....
20. Are you on drug: Yes ☐ No ☐
21. If yes, which one:.....
22. Are you on antibiotic therapy: Yes ☐ No ☐
23. If yes, state length of use:.....

24. Are you on steroid therapy: Yes ☐ No ☐

25. If yes, state length of use:.....

26. Do you have kidney stone: Yes ☐ No ☐

27. How often do you have sex: Once a week ☐ Twice in a week ☐
Three times a week ☐ More than three times a week ☐

28. When last did you have sex: A day ago ☐ 2-3 days ago ☐
4-7 days ago ☐ >1 week ago ☐ > 2 weeks ago ☐
Not sure ☐

29. Are you still menstruating: Yes ☐ No ☐

30. If no, when did it stop: Last year ☐ 2-3 years ago ☐
4-5 years ago ☐ > 5 years ago ☐

31. If yes, when was your last menstrual date prior to sample collection:
.....

32. Do you clean after each urination: Yes ☐ No ☐

33. If yes, how:.....

34 How often do you urinate during the day from 6.00am to 10.00pm:
Once ☐ Twice ☐ Three times ☐ Four times ☐ Five times ☐
> Five times ☐

35 How often do you urinate during the night from 10.00pm to 6.00am:
Once ☐ Twice ☐ Three times ☐ > 3 times ☐

36 Do you experience urgency when you want to urinate: Yes ☐ No ☐

37 Do you have burning sensation when passing urine: Yes ☐ No ☐

38 Do you have dripping effect after urination: Yes ☐ No ☐

39 Do you have bloated fleeing: Yes ☐ No ☐

40. Any other sign? Specify:.....

41. Do you have any abnormal feelings during sex: Yes ☐ No ☐

42. If yes, please specify:.....

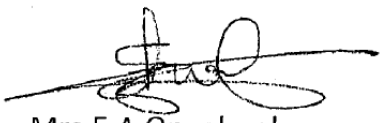
INFORMED CONSENT

Dear Parent/Guardian, I am a Ph.D Student and lecturer in the Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology College of Medicine UNEC.

I am carrying out my Ph.D research work titled "Studies on Epidemology and Pathophysiological determinants of *Staphylococcus saprophyticus* Urinary tract infection". The research entails the collection of urine samples from different groups of patients with varied illnesses and from persons without any apparent illness who shall serve as control samples. This includes both children of primary and secondary school age. The urine samples from these patients/subjects shall be examined in the laboratory for evidence of Urinary Tract Infection with this bacterial organism *Staphylococcus saprophyticus* and any other bacterial organism and the tests shall include: **urine microscopy, culture and sensitivity.**

The collection of the urine samples shall pose no problem to the pupils/students. Results of the laboratory investigations shall be delivered to the parents/guardians of the volunteers through the Principal/Headmaster or Headmistress of their school.

I therefore wish to solicit your approval to allow your child/ward to participate in this laboratory tests as it will reveal the health status of your child/ward to you and give you the opportunity to treat the child/ward if any urinary tract infection is encountered. Please sign the space provided if you approve of your child's/ward's participation. Please note that this laboratory test is free. Thank you.



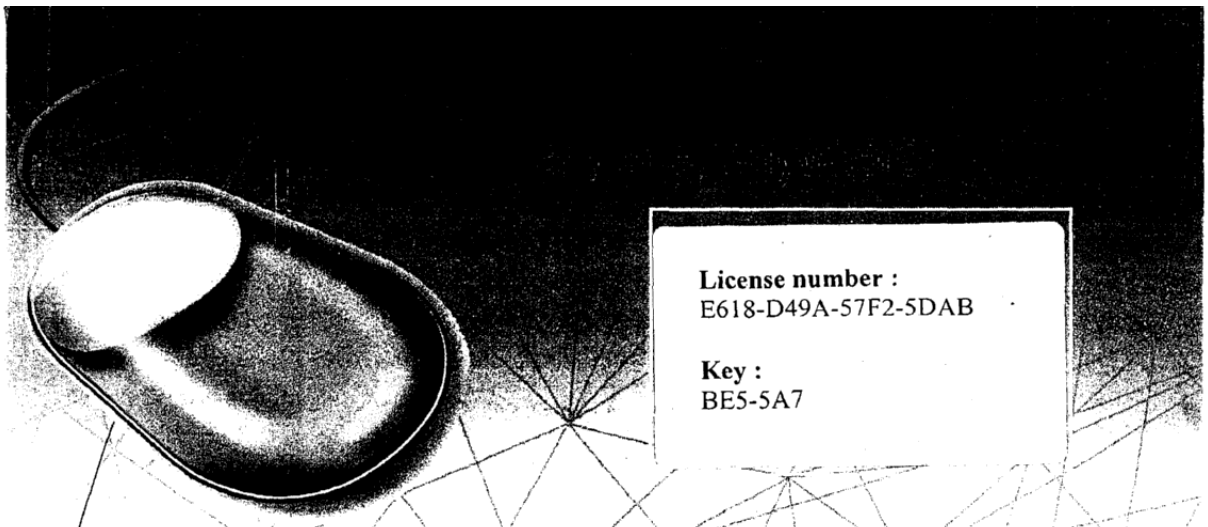
...../.....

Name of parent/guardian and signature

Mrs E.A Onyebueke

Lecturer/Researcher

08033500885



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Key :
BE5-5A7

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Prima di sottoscrivere la licenza e di registrarvi, verificare i diritti di accesso ad Internet presso il vostro amministratore di rete e/o il vostro provider di Internet

Estimado(a) Cliente,

No seguimento da sua encomenda, enviamos-lhe o seu Número de Licença e a sua Chave para poder subscrever uma licença de utilização do programa **apiweb** no endereço seguinte: https://apiweb.biomerieux.com

Antes de subscrever a licença e de se registar, verifique por favor os seus direitos de acesso à Internet junto do seu Administrador de rede e/ou do seu fornecedor de acesso de Internet.

Sem outro assunto, e com os nossos melhores cumprimentos, subscrevemo-nos,

Atenciosamente,

Σε συνέχεια της παραγγελίας σας, παρακαλούμε όπως βρείτε τον Αριθμό Άδειας σας και τον Κωδικό, που επιτρέπει την εγγραφή στην άδεια χρήσης **apiweb**, στην παρακάτω διεύθυνση: https://apiweb.biomerieux.com
Πριν την εγγραφή της άδειας, ελέγξτε τα δικαιώματά σας πρόσβασης στο Διαδίκτυο με τον υπεύθυνο δικτύου σας ή/και τον προμηθευτή σας πρόσβασης Διαδικτύου.

THE RESEARCHER ONYEBUEKE EBERE .A. TEACHING PREGNANT WOMEN HOW TO COLLECT EARLY MORNING MIDSREAM URINE SAMPLES AT ENUGU STATE TEACHING HOSPITAL (ESUTH) ENUGU

THE RESEARCHER ONYEBUEKE EBERE .A. EXTREME RIGHT WITH NURSES AT ANTENATAL CLINIC, ENUGU STATE TEACHING HOSPITAL (ESUTH) ENUGU

THE RESEARCH ONYEBUEKE E.A (LEFT) AND PASTOR CHRIS IREOBA HEAD OF EASTERN NIGERIA MEDICAL CENTRE LABORATORY

THE RESEARCHER ONYEBUEKE E.A SEECOND FROM THE EXREME LEFT WITH STAFF OF EASTERN NIGERIA MEDICAL CENTRE LABORATORY

THE RESEARCHER ONYEBUEKE POURING SABAURAUD DEXTROSE AGAR INTO TUBE FOR THIS STUDY AT PROF. N.F ONYEMELUKWE'S LABORATORY

THE RESEARCHER ONYEBUEKE A. REGISTERING URINE SAMPLES RECEIVED FOR THIS STUDYAT PROF. N.F ONYEMELUKWE'S LABORATORY

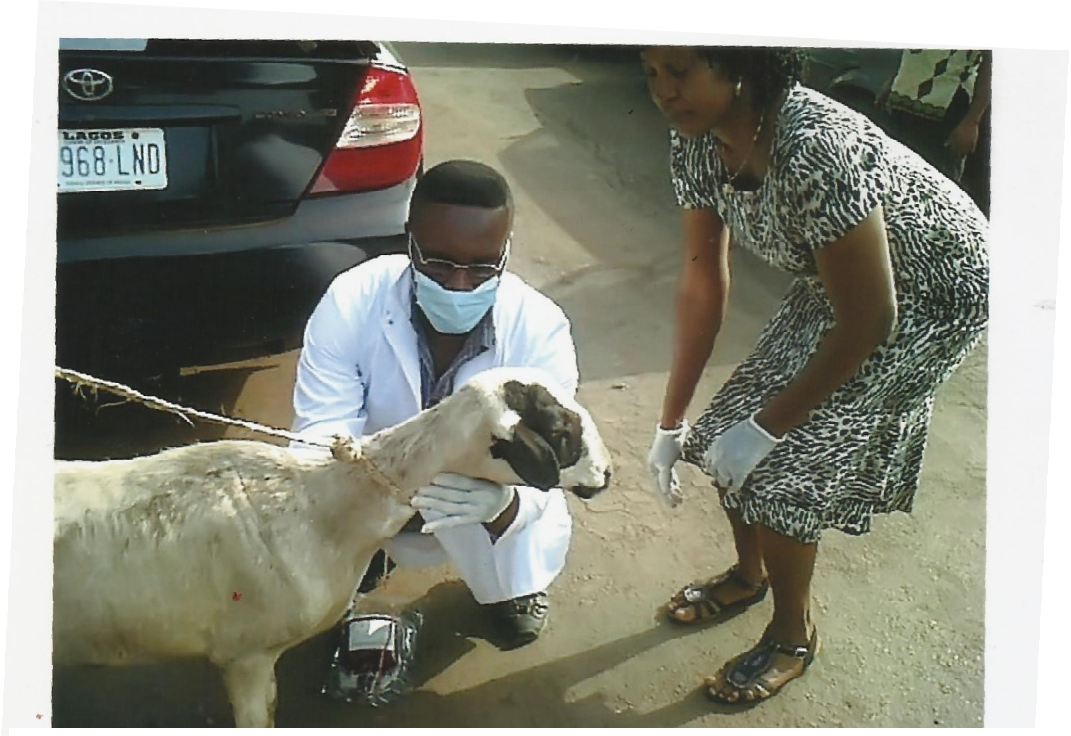
THE RESEARCHER ONYEBUEKE EBERE A. (RIGHT) WITH OGOCHUKWU EGBO STAFF OF PROF. N.F. ONYEMEELUKWES LABORATORY

THE RESEARCHER ONYEBUEKE EBERE A. DOING NOVOBLOCIN SENSITIVITY TEST FOR THIS STUDY AT PROF. N.F. ONYEMEELUKWES LABORATORY

THE RESEARCHER ONYEBUEKE PUTTING UP SUBCULTURE ON NUTRIENT AGAR PLATE FOR THIS STUDY AT EASTERN NIGERIA MEDICAL CENTRE ENUGU

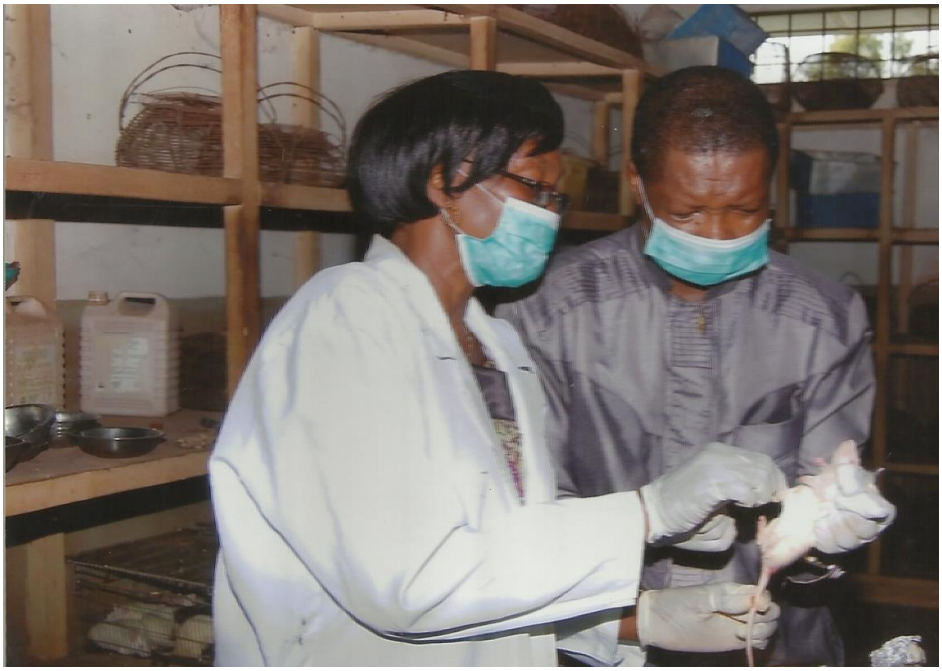
THE RESEARCHER ONYEBUEKE DOING CATALASE TEST FOR THIS STUDY AT PROF. N.F ONYEMELUKWE'S LABORATORY

THE RESEARCHER ONYEBUEKE EBERE A.PERFORMING API® STAPH BIOCHEMICAL TESTS FOR THIS STUDY AT SAFETY MOLECULAR LABORATORY RIDEWAY ROAD ENUGU



THE RESEARCHER ONYEBUEKE EBERE. A. RIGHT WITH DR. A.C. ONUBA (VETERINARY DOCTOR) DURING COLLECTION OF SHEEP BLOOD AT OLD ARTISIAN MARKET ENUGU

THE RESEARCHER ONYEBUEKE EBERE A. (RIGHT) WITH DR. P.U ACHUKWU (LEFT) DURING INTRA-PERITONEAL INOCULATION OF THE ALBINO WISTAR RATS

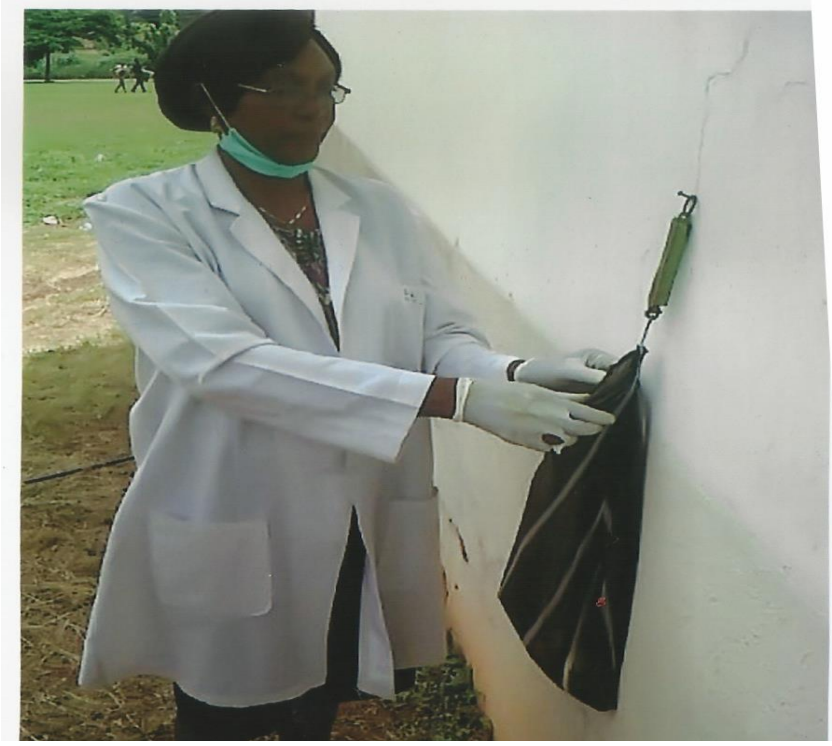


THE RESEARCHER ONYEBUEKE EBERE A. (LEFT) WITH DR. P.U ACHUKWU (RIGHT) DURING INTRA-PERITONEAL INOCULATION OF THE ALBINO WISTAR RATS

THE RESEARCHER ONYEBUEKE EBERE A. (LEFT) WITH DR. P.U ACHUKWU (RIGHT) DURING INTRA-PERITONEAL INOCULATION OF THE ALBINO WISTAR RATS

THE RESEARCHER ONYEBUEKE EBERE A. (LEFT) WITH POST-GRADUATE STUDENTS AND DR. P.U ACHUKWU DURING DISSECTION OF ALBINO WISTAR RATS

THE RESEARCHER ONYEBUEKE EBERE A. (RIGHT) WITH DR. SILAS UFELLE DURING DISECTION OF THE ALBINO WISTAR RATS AT ANIMAL HOUSE UNEC.



THE RESEARCHER ONYEBUEKE EBERE. A. WEIGHING ALBINO WISTAR RATS AT ANIMAL HOUSE UNEC