PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF GRAM NEGATIVE BACTERIA IN THE URINE OF CARITAS UNIVERSITY STUDENTS

BY

EGBOLUCHE GODSWILL .N.

MB/2008/415

A PROJECT PRESENTED TO THE DEPARTMENT OF MICROBIOLOGY/BIOTECHNOLOGY, FACULTY OF NATURAL SCIENCE, CARITAS UNIVERSITYAMORJI NIKE, ENUGU STATE.

AUGUST 2012.

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC) DEGREE IN MICROBIOLOGY/BIOTECHNOLOGY

SUPERVISOR, Dr. NWAZIRI

AUGUST 2012.

CERTIFICATION

This is to certify that this project was written by Egboluche Godswill .N. with registration number MB/2008/415 of the Department of Microbiology/Biotechnology, Faculty of Natural Science Caritas University Amorji-nike Enugu state. This work has been read and approved as meeting the requirements for the award of Bachelor of Science (B.sc) degree in Microbiology/Biotechnology.

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DEDICATION

This is project is heartily dedicated to the Almighty God, Creator of Heaven and Earth for his immeasurable grace and his faithfulness during the project work and academic pursuit.

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ABSTRACT.

In order to access the prevalence and sensitivity pattern of urinary pathogens, 60 midstream urine samples from students of Caritas University were investigated using cultural methods. Samples were examined microscopically and cultured in blood agar and Macckonkey agar. Disk diffusion method was used for antibiotic testing. Of the 60 urine samples 48 yielded significant growth with a prevalence rate of 80%. It was observed that females were more infected than the males with a prevalence rate of 56.70% and 43.30% respectively under the ages of 18-25yrs. *Escherichia coli* was the most predominant. The isolates were very sensitive to Gentamycin, Nitrofurantoin and Ofloxacin which were the (most sensitive) and the most resistant were Tetracycline, Cortrimozol, Cephalexin and Ampicillin. Therefore, Nitrofurantoin, Gentamycin, Ofloxacin were strongly recommended for the treatment of UTI as indicated in the study.

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CHAPTER ONE

INTRODUCTION

Gram negative bacteria are bacteria that do not retain their crystal violet dye in the gram staining protocol. They are differentiated by their cell wall structure. The following characteristics are displayed by gram negative bacteria as follows

- Cytoplasmic membrane
- Thin peptidoglycan layer(much thinner than gram positive)
- Outer membrane containing lipopolysaccharide outside the peptidoglycan layer
- Porin exists in the outer membrane, which acts like pores
- There is a space between the layer of peptidoglycan and the secondary cell membrane, called the periplasmic space
- If present, flagella have four (4) supporting rings instead of two
- No techoic acid or lipopolysaccharide

Some examples of gram negative bacteria include; *Escherichia coli, Salmonella species, Pseudomonas species, Klebsiella species, Proteus species, Helicobacter species, Mosoxella species, Cyanobacteria species, Spirochetes species.*

They also constitute a serious problem in urinary tract infections in many parts of the world. Appropriate antimicrobial treatments are often critical to decreasing morbidity and mortality among hospitalized patients having the infections caused by the pathogens. Gram negative bacteria are non-spore forming bacilli that grow rapidly on ordinary laboratory media under both aerobic and anaerobic conditions. It has been estimated that symptomatic urinary tract infects (UTI) occurs in as many as 7million visits to emergency units and 100,000 hospitalised annually. UTI has been the most common hospital acquired infections, accounting for as many as 35% of nosocomial infection. It is the second most common cause of bacteraemia in hospitalised patients (Nacem, 2000). UTI is known to occur in all populations but has a particular impact on females of all ages and males at two extremes of life, immuno-compromised patients and anyone with function or structural abnormalities of the urinary and excretory system.

UTI is known to be the microbial invasion of any of the tissues of the urinary tract reaching from the renal cortex to the urethrameatus (Nicolle, 2000). It is also known to be the presence in two consecutive urine samples of greater than 100rods (10^5) organisms per ml of a single bacterial strain in the urinary tract. UTI can be categorized in ascending or descending. Infections which are confined to the urethral or the bladder are ascending and referred to as uretitis or cystitis respectively. On the other hand, the pathogens spread from one or other infected body site to the kidney down along the ureter to the bladder. Such descending UTI cause severe kidney infection, a condition called pyelonephritis (Parsons, 1958). This is potentially more serious; infections to the urethra are called urethritis and to the prostrate gland are called prostitis. This classification is the presence or absence of symptoms, reoccurrence or absence or presence of complicating factors which are host factors facilitating establishment and maintenance of bacteraemia or worsening the prognosis of UTI`s engaging the kidney.

Majority of pathogens are gram negative species with predominance of members of *Enterobacteriace* (Neu, 1992). *Escherichia coli* accounts for majority of urinary tract infections in young women but other gram negative

rods of different genera such as *proteus* species and *pseudomonas aeruginosa* an aerobic gram negative rod is also troublesome. As a urinary tract pathogens because of its resistance to antimicrobial medicine make it difficult to treat successfully (Nester et al. 1998).

Antibiotics are used for the control of bacterial infections in human. Generally, gram negative bacteria are sensitive to many antimicrobial agents but strains from different patients and carriers differ in the pattern and degrees of sensitivity to different drugs. Increasing antimicrobials resistance in bacterial pathogen is a worldwide concern. The prevalence of antimicrobial resistance among urinary tract infectious agents is also increasing (Mathai et al. 2001 : Karaloswsky et al. 2001) and its treatment has become more complicated due to increasing resistance and empirical therapy leading to treatment failures of most associated with gram negative bacteria (Blondeau et al. 1999). The present study investigated the pattern of gram negative uropathogens and their antimicrobial resistance pattern among the clinical isolates to the commercially available antibiotics that are often prescribed in urinary tract infectious cases

1.1 Aims and objectives

- To find out the prevalence of gram negative organisms in the urinary tract among caritas university students.
- To investigate their antibiotic sensitivity pattern to enable formulation of drugs for urinary tract infection in our community.
- To determine the age and sex prevalence.
- To determine the prevalence of bacterial strains and their antimicrobial susceptibility in urine.
- To find the pathogenic bacteria commonly responsible with UTI and susceptibility patterns this will help the clinicians to choose the right empirical treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 MICROORGANISMS FOUND IN URINE AND THEIR ETIOLOGY

The etiology of is dependent on four factors but bacterial species are the most common are more dominating thereby causing up to 80-85% of all symptomatic UTI in women . There are also factors which enhance the invasion of organisms in the urine. They include sex, age, hospitalization and obstruction in urinary tract. Females which are however believed to be affected more affected more than males have a shorter urether and wider urethra (Archarya, 2011). The anatomical relationships of the females urether and vagina makes the bacteria been massed up the ureter into the bladder during pregnancy and child birth

2.1.1. BACTERIA

Bacteria are subjectively quantified in the urine as few moderate and many can be detected in unstained urine sediments when insufficient in quantity. The rod-

shaped bacteria and chains of cocci are often readily identifiable. The most commonly organism responsible for UTI is the *Escherichia coli* which causes up to 80- 85% of UTI infections. (Takahashi et al. 2006). Following *E. coli* is *Staphylococcus spp* especially *staphylococcus saprophyticus* which is sometimes called *micrococcus* and cause infection in young women of sexually active age (Mandell et al. 2005). Other organism which causes UTI includes *Enterococcus faecalis, Enterococcus feacium, Klebsiella spp, Proteus spp, Pseudomonas spp, Enterobacter, Providence, Morganella spp* etc. which occurs in patients with recurrent infections. In some patients with very frequent recurrences or bladder catheters especially in hospitals and nursing home settings where antibiotics are frequently used involves these organism; *Acinetobacter spp, Serretia spp, Citrobacter spp etc. Cornyebacterium urealyticum* has recognised as an important nosocomial pathogen (Serano et al. 1996).

Findings of *Proteus spp* may indicate that a paitent has renal calculi as these organism grow in alkaline environment. Anaerobic pathogens are rarely pathogens in the urinary tract. *Mycoplasma* also causes UTI and detected in the genital infections of infants. These infants become colonized with genital

mycoplasma through birth canal since infants born by caeserian sections have mycoplasma than those delivered vaginally. The *mycoplasma* include *mycoplasma hominis* and *ureaplasma uriticum* are common inhabitants of human genital urinary tract and the rate of colonization with these organisms vary greatly among different age groups about 30% of new born infants girls showed vaginal colonization with *ureaplasma uriticum* and small percentage of boys showed less colonization

2.1.2. VIRUSES

Most times viruses infect the urinary tract during measles, mumps, etc, occurrence. Epithelial cells containing viral inclusions appear in the urine in measles cytoglomerulous infections, variecella and other common infections. Fatal cases of intestinal nephritis associated with mumps increases the infections in urine (Williams et al. 1992). Adeno virus type ii has been isolated from urine of children with acute haemoharrgicystisis who showed serologic evidence of infection by this agent (Lohr, 1991). This means that viruses might be the cause of childhood illness associated with dysuria and pyuria

2.1.2. FUNGI

Yeast and yeast like forms have been known to associate with UTI (Nicolle, 2000). Pathogenic species of *Candida* in which the predominating is *Candida albicans* affect the urinary tracts (Nicolle, 2000). Opportunistic infection of *candida spp* and other yeast are the essential causes of complications in immunocopromised paitients (Lohr, 1991). Some of the factors which enhance the growth of these infections are malnutrition, corticosteroids, antibiotic therapy and debilitating diseases (Lohr, 1991). It has been also observed that vaginal candidiasis prevails more in female children with protein energy malnutrition. Sometimes, urinary tract colonization by candida in healthy or normal patients particularly when associate with long term catheters drainage tend to be much more and in the absence of obstructor is rarely progressive (Komaroff, 2000). Molds are rarely known to cause UTI; however, species of mucor has been reported to produce prostatics, epididymal and renal infections in both young and old.

2.1.4 . PROTOZOA

Protozoan infections of the urinary tract area sexually transmitted disease in man and woman. *Trichomonas vaginalis*, protozoan is a flagellated protozoan and causes trichomoniasis in female, it causes urethritis and protitis and sometimes may be asymptomatic in both sex (Gender et al. 1993). When the bladder is affected, dysuria sometimes may be severe and acute. The occurance of trichomoniasis is much higher in women than men affecting about 20% of females during reproduction years. It is reported that trichomoniasis causes about 12-15% of non-gonococci urethritis in men.

2.2.1. ENTRY OF BACTERIA INTO THE URINARY TRACT

Most bacteria causes UTI, but the most common is *E.coli* which is responsible or about 80-85% of UTI(Ortho women's report, 2002 according to Azubike et al (1994), urinary tract infections are categorised as ascending or descending routes(Azubike et al.1994). Three years later, Hooton Stamm (1997) stated that infections occurs when microorganism usually bacteria from digestive tract cling to the opening of the urethra and begin to multiply. Four routes have been purposed the ascending routes, from the urethra to bladder, then by the urether

to kidney, the haematogenous routes, with seeding of the urether to the kidney during the course of bacteraemia; intestine to kidney by way of lymphatic and direct infections. These bacteria may be present in the vaginal and rectal areas

2.2.2. ROUTES OF BACTERIA INFECTION

It is known that infections from the ascending route affect mostly the kidney which emerges to the urethra and peurethral tissues into the bladder and then enter into the ureter finally into renal pelvis, several factors can be dispose the urinary tract to infection. Any abnormality of the urinary tract that obstructs the flow of urine sets the pace for infection to occur (Bachellor et al. 1997). Quoting Fogazzi (2004) and his colleague a few bacteria that manage to invade the urinary defence and enter the bladder can multiply to high levels during this time causes infection. An enlarged prostrate gland can also slow the flow of urine, thus raising the risk of infection. Another commonly known cause of UTI is the use of diaphragm for contraception in which the diaphragm may press on the neck of the bladders, preventing it from emulsifying completely and leaving a pool of stagnant urine for bacteria may also enter when the diaghram is left for longer time than required. Catheters are also known to be a

tube that is placed in the bladders to drain off urine when a patient is unconscious or deeply ill from surgery. The route of the entry of infection is the connection that is linked to the outside and this makes it easier for pathogens to reach the bladder (Noran, 1996). Pregnant women are seen to be more prone to UTI due to homornal changes and the movement of the urinary tract during child birth (Miller et al. 1995) wearing of tight under wears like tight pants, bike riding, spray perfumes causes irritation to the genital area and may be associated with bladder infection. Lack of fluids also promotes the risk of UTI as the individual does not have a frequent irration.

The normal urinary tract is sterile but gets infected with normal flora by overcoming the natural defence of the normal sterile urinary tract, thus acting as opportunistic pathogens (Mckerow et al. 1984)

2.2.3. SYMPTOMS OF UTI

An individual might be infected without having on the symptoms showing up, while most have the symptoms. Based on the records, the most common clinical symptoms associated with UTI that brings medical attention are those referable to the urinary includes;

Dysuria – its early symptoms, may be burning or pain on the tip of the penis (for men), itching or in pain during urination, discomfort in the lower abdomen and a frequent urge to urinate may arise (Stamm, 1997)

The clinical preservation associated with acute pynerhitis is familiar and include recurrent regions and fever, nausea and vomiting etc. the clinical signs associated with it is divided into two categories. Those related to infection and they are related to degree and location of injury within the kidney, consequently the infectious aspect of the disease may be minor. Although intermittent episodes of full bi-non pyelone nephritis may occur, these are the exceptions. More common is asymptomatic symptoms referable to lower urinary vague complaints or flank or abdominal discomfort and intermittent low grade fever (Pinson et al. 2006)

2.2.4. DIAGNOSIS

The standard for Diagnosis is the detection and identification of the causative pathogens in urine (schmiemann et al. 2010). The information obtained from medical history of the patient is essential also the sign and symptom of the infection at the moment leads to a proper diagnosis.

The minimum level of bacteria ranges between $10^3 – 10^5$ pure colonies forming units/ml of urines (Akinyemi et al. 1992). This allows for the organism to be collected in large quantities from the patients. Carefulness is also applied during the collection of the specimen so as to avoid external contamination. After the clean catch urine is collected in a sterile container, it is sent to laboratory where the urine test is done using cysteine lactose Electrolyte deficient Agar (Cled) and Blood Agar (BA) (Jawetez et al. 1999) is carried out, in microscopy is also done along with the gram stain technique.

2.2.5. TREATMENT

The reason for treating UTI is to prevent the reoccurrence of systemic sepsis to relieve symptoms and to eradicate the uropathological bacterial strains from faecal and vaginal reservoirs. The use of antimicrobial testing is to observe and verify the drugs that are sensitive and resistant to each of the particular organism in question. Those drugs includes Amoxicillin, Cephalexin, Gentamycin, Nalixidic acid etc. bladder infections can be treated by the delivery of effective concentrations of those antibiotics into the body.

Unfortunately, in recent times resistance of organisms to some antibiotics has increased and it has been observed that frequent used of these antibiotics have caused an obvious increase in the development of resistance, third generation Cephalosporin's with aminoglycosides, Quoninoles are used to treat acute UTI. Trimethoprim and nitrofuration for used from prophylaxis in the treatment of relapses or chronic UTI (Takahashi et al. 1995).

2.2.5.1. AIMS IN THE TREATMENT OF UTI.

There are two predominant aims in the treatment of treating UTIs (Complicated or uncomplicated)

- Rapid and effective response to therapy and prevention of recurrence in the individual patient treated
- Prevention of emergence of resistance to chemotherapy in the microbial environment or least prevention of further increase of resistance.

2.2.5.2. FUTURE STRATEGIES IN THE TREATMENT OF BACTERIA/UTIS

The current research goals comprise the following targets.

- Known substances are: approved in term of higher bioavailability's, longer half-life, better PK/PD performance release formulation (i.e. extended/gastric release formulation, liposomal formulation)

- Known substances are evaluated for the indications (i.e. UTI).

- New derivatives of known substance classes are developed in order to enlarge the bacterial spectrum, improve bioavailability, and improve antimicrobial action (i.e. younger generation substances).

- New substances classes which should have new molecular targets are developed.

- New strategies to improve susceptibility of bacteria are developed (i.e. efflux-pump initiators)

- New strategies to slow down the emergence of antimicrobial resistance are developed

- Alternative antimicrobial substances are under discovery (e.g. bacteriophages, bacteriophage enzymes)

- New compounds for vaccination in uncomplicated and possible as well as in complicated UTI are developed

2.2.6. PREVENTION AND CONTROL

The urinary system is structured in a way that helps to ward of infection. The following are preventive measures of UTI which includes:

1. Constant drinking of water eliminates live bacteria from patching on the walls of the bladder and this can increase irritation
2. Consumption of cranberries, blueberries and vitamins help to eliminate infections
3. Wiping of the vagina immediately after urination is also advised
4. Contraceptive methods other than a diaphragm and spermiades is also encouraged
5. The catheter closed and its removal as soon as possible is also advised
6. Abstinence from sex without contraceptive like condoms and abstinence from wearing tight wears and clothes helps to ward off these organisms

2.3. ANTIMICROBIAL RESISTANCE

Antimicrobial resistance is the resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive. Resistant organisms includes bacteria, viruses, and some parasites are able to withstand attack by antimicrobial medicines, such as antibiotics, antiviral and antimalarial, so that standard treatments become effective and infections persist may spread to others. Antimicrobial resistance is a consequence use particularly these of antimicrobial medicines and develops when a microorganism mutates or acquires a resistance gene. Gene for drugs resistance may be present on bacterial chromosome, plasma, transporon and intergron. Because they are often found on motile neck genetic element they can freely exchange between bacteria. Spontaneous mutation in the bacteria chromosomes although they do not occur very often (with exception of M.tuberculosis), can make bacteria drug resistance. Usually such mutation result in a change in the drug target; therefore the antibiotic can not bind an inhibit growth if a patients fails to take prescribe antibiotics as directed, resistance mutants survive and flourish because of the competitive advantage over non resistant strain.

Antibiotic promotes development of antibiotic resistance bacteria. Antibiotic resistance occur in some way that reduces or eliminate the effectiveness of drugs, chemical or other agents designed to curve or prevent infection, the bacteria survive and continue to multiply causing more harm (CDC, 2004). Part of the problem is that bacteria and other orgs that causes infection are remarkably resilient and can develop ways to survive drugs meant to kill or weaken them (FOA, 2004). Drug resistance infections increase risk of death and often associate with prolonged hospital stay, and sometimes complicate (Lewis, 1995). When an antibiotic attack a group of bacteria cell that are highly susceptible, it causes the death of the bacteria, resistance bacteria can make their into people through food chain, finding a home at the intestinal tract after the product is eaten unless antibiotic resistance problem are defected as the emerge and actions are taken to control them, the world control the forced with previously treatable disease that have again become untreatable as in the day before antibiotics were developed (FOA, 2004). If drugs are retaining their effect on the pathogens, they have to be used more responsibly society can accept some increase n fraction of resistant bacteria when a disease need to treat the rise is unacceptable when antibiotic need is not essential.

2.3.1 MECHANISM OF DRUG RESISTANCE

Bacteria can resist the effect of antimicrobials through a variety of mechanisms. In some cases this resistance is innate but in many other it is acquired.

2.3.1.1 DRUG- INACTIVATING ENZYME.

Some organisms produce enzyme that chemically modifies a specific drug in such way as to render it ineffective. Remember that bacteria that synthesize the enzyme penicillinase are resistant to the bactericidal effect of penicillin another example the enzyme chloramphenicol acetyl tranferase chemically alters the antibiotic chloramphenicol.

2.3.1.2 ALTERATION IN THE TARGET MOLECULE.

An antimicrobial drug generally recognizes and binds to a specific target molecule in a bacterium interfering with the function. Minor structural changes in the target, which results from mutation, can prevent the drug from binding. For e.g. alteration in the penicillin binding proteins prevents B-lactam drugs from binding to them, similarly a change in the ribosomal RNA, this target

from the microlids prevents these drugs from interfering with ribosomal function.

2.3.1.3 DECREASE UPTAKE OF THE DRUGS

The porin protein in the outer membrane of gram negative bacteria selectively permit small hydropholic molecule to enter a cell. Alteration in these proteins can therefore alter permeability and prevent certain drugs from entering the cells. By excluding entry of a drug, an organism avoids its effect.

2.3.1.4 INCREASED ELIMINATION OF THE DRUGS.

The system that bacteria used to transport detrimental compound out of a cell are called efflux pump. Alteration that results in the increased expression of the pumps can increase the overall capacity of an organism to eliminate a drug, thus enabling the organism to resist higher concentration of the drug. In addition, structural charges might influence the arrays of drug that can be actively pumped out resistance that develops by this mechanism is particularly worrisome because it potentially enables an organism to become resistant to several drugs simultaneously.

2.3.2. CONDITIONS INFLUENCING THE EFFECTIVENESS OF ANTIMICROBIAL DRUGS.

Destruction of microorganism and inhibition of microbial growth are not simple matter because the efficiency of an antimicrobial agent is affected by at least five (5) factors namely.

- population size
- population composition
- concentration and intensity of an antimicrobial agents
- duration of exposure
- Temperature

2.3.2.1 POPULATION SIZE

Because an equally fraction of a microbial population is killed during each internal, a larger population acquires a longer time to die than smaller ones.

2.3.2.2 POPULATION COMPOSITION

The effectiveness of an agent varies greatly with the nature of the organism being treated because microorganism differs markedly in susceptibility because spores are much more resistant to most antimicrobial agent than are vegetative from the younger cell are usually more readily destroyed than mature organisms. Some species are able to withstand adverse condition better than others, for instance, mycobacterium tuberculosis, which causes tuberculosis is much more resistant to antimicrobial agents than most other bacteria.

2.3.2.3 CONCENTRATION AND INTENSITY OF ANTIMICROBIAL AGENT

Often, but not always, the more concentration a chemical agent or intense a physical agent the more rapidly microorganism are destroyed. However, agent effectiveness usually is not directly released to concentration or intensity. Over a short range, a small increase in concentration leads to exponential rise in effectiveness beyond certain point, increase may not raise the killing rate much at all, sometimes an agent is more effective at low concentration .For example 70% ethanol is more bacterial than 95% ethanol before the activity is enhanced by the activity of water.

2.3.2.4 DURATION OF EXPOSURE.

The longer the population is exposed to an antimicrobial agent the more organisms are killed to achieve sterilization exposure should be low, enough to reduce the probability of survival.

2.3.2.5 TEMPERATURE

An increase in temperature at which a chemical test often enhances its activity. Frequently at lower concentration of disinfectant or sterilizing agent can be used at a higher temperature.

2.3.3 ACTION OF ANTIMICROBIAL DRUGS

Antimicrobial drugs are either bactericidal or bacteriostatic, which means that actually kill the pathogens or bacteriostatic, which means they slow or prevent reproduction. In either case, the defences of the host will also be needed. The following ways which antimicrobials drugs acts against microorganisms they include:

2.3.3.1 INHIBITION OF CELL SYNTHESIS

Since this attacks the cell wall these drugs have little effect on host cells which do not contain peptidoglycan. Penicillin bacitracin, and vancomycine acts in this way;

Inhibition of protein synthesis

Since ribosomes of prokaryotic cells are slightly different from eukaryotes they can be used as target

Chloramphenicol, erythromycin, streptomycin and tetracycline's act in this way.

2.3.3.2 INHIBITION OF CELL MEMBRANE

This is a mode of action of both some antibacterial and some antifungals. Antifungals are able to work mostly against fungus cell membranes because they contain ergosterol instead of cholesterol. However, this antibiotics are potentially quite toxic to the host. E.g. are the polymixins and antifungals, such as amphotericin B, miconazole and ketoconazole.

2.3.3.3 INHIBITION OF NUCLEIC ACID SYNTHESIS

Selective toxicity varies but these interfere with DNA replication and transcription. Riframpin and the quinolone are typical examples

2.3.3.4 INHIBITION OF ESSENTIAL METABOLITES

The sufals and trimethoprim work this way. They interfere with interfere with the pathway by which bacteria synthesize folic acid by a different pathway, these drugs have less effect on human cells

CHAPTER THREE

MATERIALS AND METHODS

Freshly voided mid-stream urine samples were received between July 2012 and August 2012 and a total of 60 samples were collected from both male and female students of Caritas University.

3.1 SAMPLE COLLECTION

On the urine sample were indicated the name, age, sex, room number, state of origin including the date and time of collection. Only students who had not been on antibiotic preceding one week of sample collection were enrolled. The reasons for requesting urine microscopy/culture/sensitivity, is to identify or indicate urinary tract infection associated with gram negative organisms. 15-20ml of clean catch mid-stream urine samples were collected after clear instructions on collections including cleaning the genitalia before voiding of urine.

Early morning mid-stream urine samples were collected using sterile made mouthed containers with screw cap tops. Each of the samples were observed

with the naked eyes to ascertain if the urine was clear or cloudy (turbid) and the colour of the urine was also observed.

The urine was mixed thoroughly by rotating the container clockwise and anticlockwise directions. A loopful of the urine was collected by inserting the flamed and allowed to cool wire loop vertically into the urine. The loopful urine was streaked peripherally over the surface of the Macckonkey agar. Before the streaking, these media were divided into four segments so as to lower the cost of the materials. All plates were incubated at 37^0c aerobically for 24hrs. The plates were examined macroscopically. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per millimetre of urine. A significant bacterial count was taken as any count equal or in excess of 10,000 cfu ml. Representative of growing colonies were picked with repeated streaking. Resulting pure cultures obtained were used for biochemical test aimed at identifying the bacteria isolates and its microscopy was also carried out.

3.1.2 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial sensitivity was tested for each isolated organism using the disk diffusion method of each isolated organism using the disk diffusion method of Kirby-Baurer as described by the National Committee for Clinical Laboratory Standard(Clinical Laboratory Standard Institute 2001).the multi disc contains the following antibiotic for gram negative organisms and they are Ampicillin(Amp), Gentamycin(GEN), Nitrofurantoin (NIT), Contrimozole(COT), Ofloxacin(OFX), Cefuroxime(CER), Tetracycline(TET) and Cephalexin(CEPH)

A disc of blotting paper was impregnated with a known volume and appropriate concentration of an antimicrobial and it was placed in a plate of sensitvity testing agar that was uniformly inoculated with test organism. The antimicrobial diffused from the disc to the medium and the growth of the organism was inhibited at a distance from the disc that was associated to the sensitivity of the organism. Strains that were sensitive to the antimicrobial were inhibited at a distance from the disc where as resistant strains had smaller zones of inhibition. Zones of inhibitions from 8-12mm signifies sensitivity while and below 8mm were regarded as resistance.

3.1.3 URINALYSIS TEST

After initial inoculation on Macckonkey and Blood Agar a media-test, combi-9 impregnated with test strips for urine parameters was dipped into each urine specimen. The reactions were examined visually immediately after allowing excess urine to drip off comparing the colours produced against the colour shade used for charting and estimating the reactions. The parameters tested were Ph, Leucocytes, Ketones, Protein, Glucose, Bilirubin, Urinobilnogen, Nitrate and BSlood.

3.2. GRAM STAINING

A flamed wire loop was used to pick a colony from a plate and a thin film smear was made on a clean grease free slide. The film was allowed to dry and was heat fixed by waving over flame of a Bunsen burner. It was then covered with crystal violet reagent for one (1) minute. The slide was placed on a rack over a sink and rinse in a slowly running tap for 5seconds

The film was flooded with iodine solution for 1 minute rinsed slowly running water for 5 seconds. It was decolourized with alcohol reagent slowly until no

more dye runs out. The smear was covered with Saffranin reagent for 30 seconds and rinsed in slowly running water. It was then air dried before viewing under the microscope. The stained slide was viewed with oil immersion lens x100 of the microscope. Gram positive bacteria appeared purple while grain negative cells appeared pink or red.

Results:

Gram positive – purple

Gram negative – pink or red.

3.3. BIOCHEMICAL TESTS.

This is used for the identification of the exact organism present in the urine specimens. The biochemical test used are;

- Catalase test.
- Coagulase test.
- Motility test
- Methyl red test.

- Indole test.
- Urease test.
- Citrate utilisation test.

3.3.1 CATALASE TEST

This test was used to demonstrate which of the isolation could produce the enzyme catalase that release oxygen from hydrogen peroxide (H_2O_2). This test is usually used as an acid to differentiate staphylococci from streptococci and to differentiate other catalase positive organisms from catalase negative (Barker 1976).

METHOD

- Two millimetre of hydrogen peroxide (H_2O_2) solution was poured into a test tube.
- With a sterile applicator stock a colony of the test organism was picked and immersed in the hydrogen peroxide (H_2O_2) solution and the tube was observed for bubbles indicating

Active bubbles – positive catalase test.

No bubbles –negative catalase test.

3.3.2 COAGULASE TEST:

This was used to determine the organism which is capable of producing the enzyme coagulase. The coagulase enzyme causes plasma to clot by converting fibrinogen to fibrin. Bound coagulase converts fibrinogen directing to fibrin without requiring a coagulase reacting factor. It can be detected by clumping of bacterial cells in the slide test (Cheesbrough, 2004).

METHOD

- A drop of distilled water was placed on two separate clean grease free glass slides.
- A colony of the test organism was emulsified in each of the drops to make 2 thick suspensions.
- A loopful of plasma was added to one the suspension, and mixed gently.

3.3.3 MOTILITY TEST

This test was used to determine which of the isolates was motile. Motility test is usually used to differentiate motile organisms from non-motile ones. For this test the hanging drop technique was carried out described by Kirk et al. (1975).

METHOD

- A little Vaseline jelly's was rubbed around the cavity of a hanging drop slide.
- A drop of peptone water containing the pure culture was placed on a cover slip.
- The hanging drop slide with a ring of Vaseline at the centre was then placed over the drop of peptone slide which was quickly inverted and viewed under the microscope using oil immersion objective lens.

3.3.4 METHYL RED TEST

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually

used as an acid in the identification and differentiation of the *Enterobacteriacae* (Barker, 1976). This test was carried out as described by Kirk et al (1975).

The alkali oxidizes the acetyl methyl carbonyl (acetonic) diacetyl which gives the pink colour

3.3.5 UREASE TEST

This test was used to demonstrate the ability of isolate to produce the enzyme urease which splits urea forming ammonia the test is usually used to differentiate organisms like *proteus* from non-urease positive organisms (Barkers & Breach 1976). The methods used was described by Speck (1976)

METHOD

A loop full of the isolate was used to inoculate a tube of urea agar. The tubes were incubated at 37°C a change in colour from yellow to red confirm the presence of urease

RESULTS

Red colour- positive urease test

No red colour – negative urease test

3.3.6 INDOLE TEST

It is used to differentiate the *Enterbacteriacea* such as *Escherichia coli* which is negative. Indole positive bacteria break down the amino acid tryptophan with the release of indole

METHOD

I. The test organism was inoculated into peptone water containing tryptophan

II. The broth was incubates at 35°C for 48hrs

III. Indole was tested by adding 0.5ml kovac's reagent and shaking. Indole positive which form a red colour layer when kovac's reagent was added after 10mins(Kovac's reagent is 4- p- dimethylaminobenzaldehyde)

RESULT

Red surface layer – positive indole test

No red surface – negative indole test

3.3.7 CITRATE UTILISATION TEST

This test was used to identify which of the isolates can utilise citrate as the sole source of carbon for metabolism. The test is usually used to as an acid in the differentiation of organisms of *Enterobacteriacae* and most other genera (Baker and Beach 1976). The medium used for this test was the Simon's citrate agar

METHOD

Slant tubes of Simon citrate agar were incubated with young culture of isolates, the inoculation was done by stabbing the medium on the tube using sterile straight tubes of buffered glucose. Peptones broth were tightly inoculated with isolates. The tube was incubated at 37°C for not less than 48 hours. About 5 drops of methyl red reagent was added into 5ml of the culture. The production of a bright red colour immediately on the addition of the reagent showed a positive result.

RESULT

Bright red colour- positive citrate test

No bright red colour-negative citrate test

CHAPTER FOUR

RESULT

A total of 60 samples were collected from Caritas University students were investigated. Out of the 60 samples, 34 samples were females while 26 samples were males with age range of 18-25yrs. Out of the 60 samples, 48 samples were positive, 21 were female while 22 were males. The remaining samples that showed no significant bacterial growth were discarded. Out of 26 samples from males 21 were positive with prevalence rate of 43.3% while 34 samples from females gave 27 were positive with prevalence rate of 56.7% as shown in table 1.Therefore, the prevalence rate of positive cases for male and females were 43.7% and 56.3% respectively.

E.coli had the highest prevalence rate of 50% followed by *Klebsiella spp* 14.6%, *pseudomonas spp* 10.4%, *Proteus spp* 6.3% and the other gram positive organisms put together had 18.8% as shown in table 2.

It was also observed that Gentamycin, Nitrofurantoin and Ofloxacin were the most sensitive antibiotics in the study while tetracycline Cephalexin,

Ampicillin and Cotrimozole gave poor sensitivity or resistance. As shown in table 3 from this measures above. Gentamycin , Nitrofurantoin and Ofloxacin proved the best antibiotics against gram negative bacteria.

Table 1: Shows Sex Distribution of cases and their Prevalence rate (18-25yrs)

Sex	Total case	Positive cases	% Positive cases
Male	26	21	48.3
Female	34	27	56.3
Total	60	48	100.00

Table 2: Shows the bacteria distribution of positive cases without prevalence rate.

Bacteria isolate	Positive cases	% Positive cases
E.coli.	24	30.0
Klebsiella spp	2	14.6
Pseudomonas spp	5	10.4
Proteus spp	3	6.3
Others (gram positives)	9	18.8
Total	48	100.00

Calculation of prevalence as shown in the Table 2 above.

E. coli- $\frac{24}{48}\text{x}\frac{100}{1}\% = 50\%$

Klebsiella spp- $\frac{7}{24}\text{x}\frac{100}{1}\% = 14.58\%$

Proteus spp- $\frac{3}{48}\text{x}\frac{100}{1}\% = 6.25\%$

Others (Gram +ve) - $\frac{9}{48}\text{x}\frac{100}{1}\% = 18.75\%$

Overall prevalence for positive cases is $\frac{48}{60}\text{x}100 = 80\%$

Table 3. Shows the sensitivity pattern of bacteria isolated and their antimicrobial resistance

Bacteria isolates	Antibiotics							
	AMP	GENT	NIT	COT	OFX	CER	CEPH	TET
E.coli	6mm	10mm	9mm	4mm	10mm	2mm	0mm	4mm
Kleb spp	10mm	9mm	11mm	5mm	11mm	10mm	8mm	11mm
Pseudo spp	0mm	3mm	9mm	6mm	8mm	1mm	4mm	2mm
Proteus spp	7mm	7mm	4mm	5mm	11mm	2mm	5mm	3mm
Others								
(Gram +ve)	8mm	10mm	6>8mm	8mm	9mm	4mm	6mm	5mm

Key

0-8mm. shows antibiotics resistivity

8-12mm shows antibiotic sensitivity.

Amp: Ampicillin

Gent: Gentamycin

Nit: Nitrofurantoin

Cot: Cotrimozole

OFX: Ofloxacine

CER: Cerfuroxime

CEPH: Cephalexin

TET: Tetracycline.

CHAPTER 5

DISCUSSION, CONCLUSION, RECOMMENDATION.

5.1. DISCUSSION.

The overall prevalence of UTI in this study was 80% and females were significantly more affected than males. Previous reports (Aimel et al. 1973; Robert et al. 1993) of studies carried out in different parts of the world indicates higher incident among females than males, this may be explained by the fact that females pass short urethra. Also the spread of normal flora in faecal materials from the anus to the vagina from where the bladder could be infected as a result of poor anal cleaning could be responsible for the observed result in female urine sample.

The prevalence of UTI in Caritas University which is found to be 80% is quite alarming . This figure is higher than the prevalence rate of 51.35% recorded by Savi et al. (2011) and 60% obtained in another study in the north central region of Nigeria. This calls for caution among the female students in Caritas

University. The high rate maybe due the increase in female with poor hygienic practice and also indiscriminate sexual behaviour among the female students.

E.coli was the commonest organism isolated, this is in conformity with the previous work done by Handeau et al. 2011. The least resistance by the bacterial isolates to antimicrobial agents was observed to be Tetracycline, Cotrimozole, Ampicillin, Cephalexin as seen from this study. The factors contributing to those resistance may be due to indiscriminate abuse of antibiotic by students. Other factors may include poor quality of drugs, poor storage and exposed drug (Okeke et al. 1999) etc. the reduction of antibiotics prescription and dispensation have been associated with reduced antibiotic resistance (Schiemann et al. 2010).

5.2. CONCLUSION

This study has revealed that UTI among female students is a very difficult health problem which must be properly addressed. This has also revealed that the most causative organisms of UTI in this university, community among the female students are the gram negative organisms which were shown to be sensitive to the following drugs. Gentamycin, Ofloxacin and Nitrofurantoin. It could be suggested that in the face of clear UTI symptoms and in the absence of physician or clinician of these three drug abuse (Gentamycin, Ofloxacin and Nitrofurantoin) could be procured and used and with an experienced doctor is seen for confirmation.

5.3. RECOMMENDATION

This is the appropriate time to investigate infection control measures which are lacking in the most health care institutions, firm, industries etc. There is also need to establish the sense and control surveillance agencies. There is need for interaction between physicians and microbiology department because there have been cases where by the antibiotics tested are different from those frequently prescribed since the laboratories use only the susceptibility diffusion disc available to them.

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APPENDIX I

MATERIALS

MacConkey agar

This medium is best prepared from ready to use dehydrated powder, available for most suppliers of culture media.

Contents: peptone, lactose, bile salts, sodium chloride, neutral red agar.

PREPARATION:

1 prepare as instructed by manufacturer's instructions sterilize by autoclaving at 121^{o}c for 15 minutes

2 When the medium has cooled to 50-55^{o}c, mix well and dispense aseptically in sterile Petri dishes. Date the medium and give it a batch number.

3 Store the plates at 2-8 preferably in plastic bags to prevent loss of moisture.

4 Shelf-life: up to 4weeks providing there is no change in the appearance of the medium to suggest contamination or an alteration of pH

MEDIA PREPARATION

NUTRIENT AGAR (NA)

The medium was used for the enumeration of bacterial cells and also to maintain pure cultures.

PREPARATION

The preparation involves measuring twenty-eight grams (28g) of the powder on a weighing balance and suspending it into 1 litre of distilled water (or equivalent w/v for lower volumes of distilled water). This was boiled over a Bunsen burner to dissolve completely and subsequently sterilized by autoclaving at 121^{o}c for 15minutes. After cooling to about 47^{o}C, the sterile molten medium was distributed i.e. about 20ml each into sterile Petri dish.

COMPOSITION:

The medium composed of the following

Beef extract 3.0g/l

Peptone 5.0g/l

Sodium Chloride 8.0g/m

PH 7.3 $\pm$ 0.2

Batch number 110/24/147

Expiring date 2014/05

The medium was used to enrich and develop the innocular that were used to inoculate the agar plates. It was also used to maintain cultures for some biochemical tests.

Fig.1 oxidase test

Fig.2 urease test

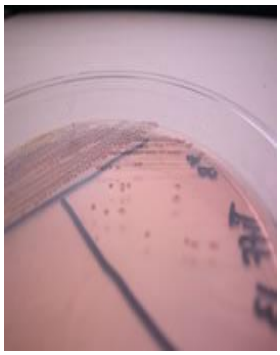

Fig.3 macConkey culture plate

Fig. 4 Catalase test test

Fig.5 indole test

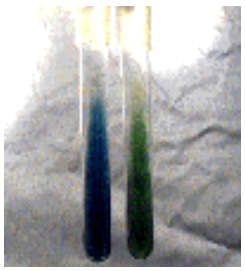

Fig.6 simmons citrate

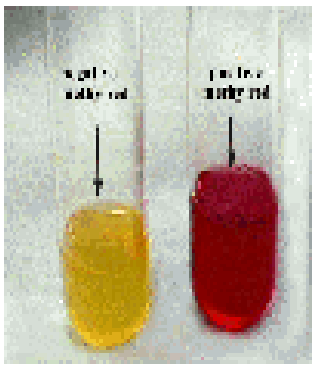

Fig.7 methyl red test

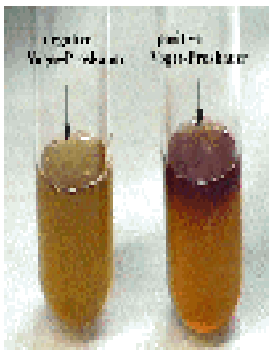

Fig.8 vp test

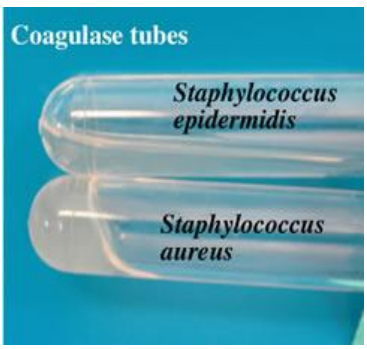

Fig.9 coagulase test

APPENDIX II

Biochemical test results:

Blood Agar.	Nutrients Agar	Mac Agar	Cat	Cit	Coa	ind	m.r	mot	oxi	V.p	bacisolate
Large grey Colonies	Mucoid Vicious White colonies	mucoid pink colonies	+	+	-	-	-	-	-	-	+	*Kleb spp*
Fishy Odour Produced	creamy colonies round smooth colour surface swampy growth	colonies	+	-	+	+	+	+	+	-	*Proteus* spp
Mucoid Colonies smooth surface	milky colonies	smooth pink colonies	+	-	-	+	+	+	-	-	*E.coli*
Large flat Colonies	creamy white colonies with Red surface	pale yellow	+	-	-	+	+	+	-	-	*Pseudo spp*