**ANTIMICROBIAL SENSITIVITY PATTERN OF URINARY TRACT ISOLATES FROM PARAPLEGIC AND QUADRIPLEGIC PATIENTS FROM NATIONAL ORTHOPAEDIC HOSPITAL, ENUGU.**

**BY**

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**APPROVAL PAGE**

This is to certify that this research work “Antimicrobial sensitivity pattern of urinary tract isolates from Paraplegic and Quadriplegic patients from National Orthopaedic Hospital, Enugu.” by Okoro Benedict Chidiogor in the department of Microbiology has been examined and approved as meeting the requirements for the award of Bachelor of Science (B. Sc.) Degree in Microbiology, Faculty of Natural and Applied Science Godfrey Okoye University, Enugu.

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**DEDICATION**

This research work is dedicated to my late Grandparents. I believe that you people are somewhere better. I will continue to remember and pray for you.

I also dedicate this work to my family especially my parents for their immense support during the course of this work.

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**ABSTRACT**

The Isolation, Identification, and Antimicrobial sensitivity pattern of pathogens isolated from urinary tracts of Paraplegia and Quadriplegia patients at National Orthopaedic Hospital, Enugu was conducted on fifteen urine samples. Urine culture was done using Cysteine Lactose electrolyte deficient agar (CLED), Mac-Conkey agar, and Blood agar and incubated at 37oC for 24 hours. The urine samples were screened for albumin presence using Combi-2 urinalysis dipsticks. Four microorganisms were isolated and identified by means of biochemical testing which included Gram stain, Oxidase test, Sugar fermentation test, IMVIC, motility test, Catalase test, Urease test and Coagulase test. Of the four (4) isolates, two were Gram-positive; one being *Enterococcus* Spp. with cocci shape while the other *Cornyebacterium* Spp. was rod shaped. The other two isolates were Gram-negative members of the *Enterobacteriaceae* family (*Escherichia coli* and *Proteus* Spp.). *Enterococcus* Spp.had the highest prevalence rate of 6 (37.5%); *E. coli* had the second highest frequency of occurrence at 5 (31.25%), *Cornyebacterium* Spp.had a lower rate occurrence at 3 (18.75%), followed by *Proteus* Spp. with the lowest rate of occurrence at 2 (12.25%). Antimicrobial susceptibility assay was carried out on the isolates. *E. coli* and *Enterococcus* Spp.had the highest resistance pattern; *Cornyebacterium* Spp. had a lower resistance pattern while *Proteus* Spp. had the lowest resistance pattern to the antibiotics. The *Enterococci* Spp.was susceptible to drugs like Amikacin, Chloramphenicol and Tetracycline. *E. coli* was susceptible to drugs like Chloramphenicol, Amikacin and Ciprofloxacin. *Cornyebacterium* Spp. was susceptible to Amikacin, Chloramphenicol, Ciprofloxacin and Tetracycline. *Proteus* Spp.was Ceftriaxone, Amikacin, Chloramphenicol, Ciprofloxacin, Sulfamethoxazole trimethroprim and tetracycline. All isolated microorganisms were generally sensitive to Amikacin and Chloramphenicol antibiotics showing thus to be active in-vitro.

**CHAPTER 1**

**1.0 INTRODUCTION**

Drugs are substances that when inhaled, injected, consumed, absorbed, or dissolved under the tongue causes a temporary physiological change in a body. In pharmacology, a pharmaceutical drug also called a medicine is a chemical substance used to treat, cure, present, or diagnose a disease or to promote well-being. Traditionally, drugs has been produced through extraction from medicinal plants, but more recently also by organic synthesis. (Atanasov *et al.*, 2015). The use of medicine may be as preventive medicine that has benefits but does not treat any existing or pre-existing diseases as symptoms. Pharmaceutical drugs are usually categorized into drug classes. A group of drugs well share a similar chemical structure, or have the same mechanism of action, the same related mode of action or target the same illness or related illness (Mahoney and Evans, 2008; WHO, 2003). The Anatomical therapeutic chemical classification system, assigns drugs a unique ATC code, which is an alphanumeric code. Another system of classification is the Biopharmaceutics classification system. These groups of drugs were classified according to their solubility and permeability or absorption properties (Bergstrom *et al*., 2014). Drug resistance is the reduction in the effectiveness of a medication such as an antimicrobial in curing a disease or condition. The term is used in the context of resistance that pathogens or cancers have “acquired”, that is resistance has evolved. Antimicrobial resistance challenges clinical case and drives research. When an organism is resistant to more than one drug, it is said to be multi-drug resistant. The development of antibiotic resistant in particular stems from the drugs targeting only specific bacterial molecules (almost always proteins). Because the drug is also specific, any mutation in these molecules will interface with or negate its destruction effect resulting in antibiotic resistance (Pitman, 2004). Bacteria are capable of not only altering the enzymes targeted by antibiotics, but also by the use of enzymes to modify the antibiotic itself and thus neutralize it. Drug resistant traits are accordingly inherited by subsequent offspring, resulting in a population that is more drug resistant. In domestic environment, drug-resistant strains of organism may arise from seemingly safe activities such as the use of bleach, the use of antibiotics, disinfectants and detergent, soaps etc. Urinary tract infection (UTI) is one of the most common diseases in human societies which occur in women more than men (Al-Badr and Al-Shaikl, 2013; Mody and Juthani, 2014, Zone and Guide, 2017). The urinary tract infection occurrence depends on several factors provide the presence of bacteria (more than 105/ml) in urine (Zone and Guide, 2017). UTI treatment with antibiotics is carried out usually before receiving culture and sensitivity test results. This therapy without drug prescription occasionally leads to antibiotic resistance and treatment failure has result (Zone and Guide, 2017; Gupta *et al*., 2001). Antimicrobial drug resistance is increasing around the world, especially in developing countries (Sadeghabadi *et al*., 2014). According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a threat to modern medicine (WHO, 2014).

Quadriplegic and paraplegic patients are generally patients with spinal cord injuries that have lost the use of all or part of their limbs. Patients with spinal cord injuries (SCI) are prone to repeated UTIs. Symptomatic UTIs continue to prove a significant problem for these patients. They may include fever, foul smelling urine, haematuria (Cardenas and Mayo, 1987). Nosocomial urinary tract infections (UTIs) are often associated with significant morbidity, mortality and health care cost (Saint and Lipsky, 1999; Laupland *et al*., 2005). Patients with spinal cord injury (SCIs) often have indwelling or intermittent urinary catheters and are prone to have asymptomatic bacteria and UTIs. As a result, they frequently receive antimicrobial therapy and have a higher prevalence of antibiotic resistant urinary tract isolates compound to patients without SCI (Girard *et al*., 2006; Salomon *et al*., 2007). Many studies have evaluated patient propensity for development of antibiotic resistance in UTIS. Age, use of a urinary catheter, previously hospitalization and prior antimicrobial use have been identified as common risk factors (Ena *et al*., 1995; De Meuy *et al*., 1999).

**1.1 Aim**

To Isolate, Identify, and test the antimicrobial sensitivity pattern of pathogens isolated from urinary tracts of Paraplegic and Quadriplegic patients.

**1.2 Objectives**

* To Isolate and determine the type of micro-organisms causing urinary tract infection in paraplegia and quadriplegia patients.
* To assay the sensitivity pattern of the isolated microorganisms to antibiotics

**CHAPTER 2**

**2.0 Literature Review**

The development of antibiotics has been a major advancement in patients care. The discovery of antibiotics is considered one of the most remarkable health-related events in the history of medicine (Aminov, 2010; Davies and Davies, 2010).

An antimicrobial is a drug or substance that prevents the growth of microbes or pathogens such as bacteria, fungi, parasites or viruses (Sefton, 2002). The words antibiotic and antimicrobial can often be used interchangeably. The first antimicrobial agent, Salvarsan, was synthesized by Ehrlich and Hata for the treatment of syphilis (Davies and Davies, 2010). This was followed with the discovery of sulfonamides. In 1928, Alexander Fleming discovered penicillin, which was eventually introduced for use in the 1940s (Davies and Davies, 2010; Fleming, 1929). Subscript antimicrobials were developing either from evaluating naturally occurring compounds or by chemically modifying previously discovered antimicrobials (powers, 2004). Given the number of antimicrobials in existence from the 1940s to early 1960s, clinicians were presented with a wide variety of treatment option for their patient (powers 2004).

Antimicrobials (Antibiotics) can be broadly classified based on their chemical structure. The major antimicrobial class are Penicillins, Cephalosporins, Fluoroquinolones, Aminoglycosides, polypeptides or glycopeptides, Tetracyclines, Macrolides, Chloramphenicol, Ansamycins, Lincosamides, Trimethoprim, Fosfomycin, Carbapenems and 5-nitromidazoles (Bryskier, 2005; Mishra and Agrawal, 2012).Antimicrobials can also be classified based on the spectrum of activity against pathogens, into broad and narrow spectrum (Van Saene *et al*., 1998). Broad spectrum antimicrobials are effective against a broad range of Gram-negative and Gram-positive bacteria (Bryskier, 2005; Mishra and Agrawal, 2012). Narrow spectrum antimicrobials are only active against a specific group of pathogens e.g Gram-positive organisms (Mishra and Agrawal, 2012). The discovery and subsequent use of antimicrobials (antibiotics) led to a dramatic reduction in morbidity and mortality due to infectious disease in comparison to the pre-antibiotic era (powers, 2004). However, the use of these miracle drugs has been accompanied by the emergence of pathogens resistant to antimicrobials (Davies and Daavies, 2010). Previously effective antimicrobials against certain pathogens are now no longer effective, which poses significant threat to public health and the possibility of return to the pre-antibiotic era if urgent action is not taken (Cohen, 1992; Davies and Davies, 2010).

**2.1Antimicrobial resistance**

It is the ability of a microbe/bacterium to resist the effect of an antimicrobial agent (Australian commission on safety and Quality in Health care, 2013). As described previously, the words antibiotics and antimicrobial are used synonymously. Antibiotic resistance, in a clear sense, refers to the development of resistant bacteria strains or the ability of bacteria to develop resistance to antibiotics (Tenover, 2006) as opposed to the inclusion of viruses, parasites and fungi. The term AMR (Antimicrobial resistance) although, was chosen because it is most widely used (Robinson *et al*., 2016).

Development of AMR is a naturally occurring process for microbes but is accelerated by the selective pressure resulting from the misuse and overuse of antimicrobials both in humans and animals (World Health Organization, 2014). With the development of each new antimicrobial agent, there has been detection of resistance to the agent following subsequent use (Davies and Davies, 2010; Levy and Marshall, 2004).

Antimicrobials have now become widely used and also misused in both human populations and food-producing animals (World Health Organization, 2014). The increase in use is further driving resistance as the greater the number of antimicrobial resistant pathogens will succeed in the fight for survival (Center for Disease Dynamics, Economics and policy, 2015). There is evidence globally (Center for Disease Dynamics, Economics and policy, 2015; World Health Organization, 2014) showing an increase in resistance of urinary *Escherichia coli* (*E. coli*) isolate to commonly prescribed antimicrobials. There is also strong evidence to support the association between antimicrobial use and this development of resistance in *E.coli* UTI (Bergman *et al*., 2009; Goossens *et al.,* 2005).

Transmission of urinary bacteria i.e. *E. coli* isolates to humans has been identified to occur through environmental exposures including food, animal and travel (Nicolle, 2013; Robinson *et al*., 2016). There is evidence to show wide spread dissemination of antimicrobial resistant urinary *E. coli* clones both within Australia and globally (Johnson *et al*., 2009). Also a population-based surveillance study in Canada reported a significantly increased risk of isolation of urinary *E. coli* isolates with oversea travel, particularly to India, the middle task and Africa (Laupland *et al*., 2008).

**2.2.** **The** **mechanisms for AMR**

The mechanisms for AMR are multifaceted Resistance refers to the naturally occurring resistance of bacteria to antimicrobials (Sefton, 2002). Some bacteria species have intrinsic resistance to one or more antimicrobial classes. When this occurs, the strains of that bacterial species are also resistant to all agents in those antimicrobial groups (Tenover, 2006). In acquired resistance, bacteria that were initially susceptible to an antimicrobial agent become resistant, multiply and spread under the selective pressure following the use of the antimicrobial agent (Tenover, 2006). Acquired resistance can either be genetic or biochemical (Sefton, 2002). Genetic resistance can occur either from mutation or from acquiring resistant genes from other biochemical species. Possible mechanisms for acquiring biochemical resistance by bacteria include production of drug inactivating enzymes, decreased cell permeability, modification of an existing target and acquisition of a target by-pass system (Sefton, 2002). For example, bacteria may modify or change the existing target, which is described as the specific location or side the antimicrobial drug is designed to attach to on the bacterium (Tenover, 2006). They may also produce enzymes that destroy inactive the antimicrobial agent before it has an effect. Bacteria may also alter a protein channel on their cell wall or outer membrane, preventing the antimicrobial drug from entering the bacterial cell wall. Finally, they may use what we know as efflux pumper to expel the antimicrobial agent from the bacteria cell, there by by-passing its target site without it having an effect on the bacteria (Tenover, 2006). Resistant mechanisms exhibited by Gram-negative bacteria like *Escherichia coli* include attraction to target enzymes and plasmid-mediated resistance (Paterson, 2006).

Resistance to specific antimicrobials such as Fluoroquinolones occurs by alteration to the chromosomal gene leading to changes in the target mechanisms or by alteration to the cytoplasmic membrane efflux protein gene, resulting in modification to the permeation mechanism (Dalhoff, 2012). The development and spread of resistance in Entrobacteriaceace threatens to create species that will become resistant to all currently available antimicrobials (Paterson, 2006).

**2.3.** **Urinary tract infection**

Urinary tract infections are one of the most common bacterial infections affecting people in hospitals as well as in the community (Laupland *et al*., 2007). Data from the combined National Ambulatory health care surveys in the USA for 2009-2010 showed that UTI accounted for approximately 9.8 million visits to ambulatory care settings such as primary care, out-patient and emergency department (Centers for Disease control and prevention & National center for Health Statistics, 2015). In the United Kingdom, prevalence of UTI is estimated as 6.0% from 2008 to 2010, although this is based on data from children aged five and below attending general practice clinics (O’Brien *et al*., 2013). Urinary tract infection was said to be the third most common health care – associated infection (HAI) (19% of all HAI’s) in the 2011-2012 point prevalence survey of HAIs in Europe (European Centre for Disease Prevention and Control, 2013). In Beijing, China, UTI was noted to be the second most frequently identified HAI in 2014, accounting for 15% of all HAI (Lui *et al*., 2016.).

Females are most predisposed to UTI as bacteria easily enter the bladder via the short urethra with reported incidence rates being higher than males. For example, in men below 50years of age, the incidence of UTI has been approximated to be 0.0005-0.0008 per a person a year (Seminerio *et al*., 2011) compared with an incidence of 0.5-0.7 per person a year in young women (Hooten *et al*., 1996). Particular groups of people who have a higher risk of developing a UTI include diabetes, pregnant women, the elderly, people with multiple sclerosis, these with underlying urologic anomalies, as well as immune compromised patients such as those with human immune deficiency virus (HIV) and cancer (Foxman and Brown, 2003).

Although an accurate estimate on worldwide incidence or prevalence in UTI is difficult to determine, evidence shows that UTI is an infection which commonly affects females and males, including children and the elderly.

Urinary tract infections pose significant health and economic implication to society. They are a cause of morbidity in the community and also the hospital (Rogers and Peterson, 2011).Urinary tract infection impact considerably on the quality of life of those affected. Recurrent episodes of infection also occur. Recurrent infection may be either re-infection, caused by a new infecting organism, or relapsing infection, caused by the same organism present before therapy. Relapse may occur either because the infecting organism was not completely eradicated from the genitourinary tract by antimicrobial therapy or because re-infection by a persistent colonizing strain in the gut reservoir (Nicolle, 2002). There is a heightened risk of urinary tract infection in patients with a spinal cord injury (SCI). Lower rates occur in those with incomplete injuries. The overall incidence of UTI in SCI is 215 cases per year (Siroky, 2005). In patients practicing clean intermittent catheterization, the mean incidence of UTI is 10.3 cases per 1000 catheter days; after 3 months, the rate is fewer than 2 cases per 1000 catheter days.

UTIS in patients with spinal cord injuries (SCI) develop as a result of neurogenic bladder and the need for catheterization. Pathogenic factors include bladder over-distention, vesicoureteral reflux, high pressure voiding, large post voiding residual volume, stones in the urinary tract and outlet obstruction (Cardenas and Hooten, 1995).

**2.4** **Aetiology of UTI**

Over 80% of UTIs are caused by *Escherichia coli* (Nicolle, 2008; Rogers and Peterson, 2011). This Gram-negative bacterium belongs to the *Enterobacteriaceae* family, which comprises Gram-negative bacteria responsible for important hospital and community acquired infections (Australian Group on Antimicrobial Resistance, 2011). The remaining 20% are caused by other bacteria in the *Enterobacteriaceae* family such as *Klebsiella,* proteins and *Enterobacter* species as well as other pathogens, which include *Staphylococcus saprophyticus*, *Enterococus* species, Group B streptococcus and *Pseudomonas aeruginosa* (Mazzulli, 2012; Ronald, 2012.). Urinary tract infections can be classified based on location of UTI acquisition and provision of healthcare services, into either community-acquired UTI or the broad category of healthcare – associated UTI, which includes hospital-acquired.

Urinary tract infection can be classified anatomically depending on the part of the urinary tract affected. Infection of the lower urinary tract affecting the bladder is referred to as cysticis. Upper urinary tract infection involving the renal tissue is referred to as pyelonephritis (Flores-Mireles *et al*., 2015; Kumar *et al*., 2015; Lichtenberger and Hooten, 2008). Cystitis and pyelonephritis can be further classified clinically into uncomplicated or complicated UTI (Kumar *et al*., 2015).

The clinical grouping of uncomplicated or complicated UTI depends on the host condition (Nielubowicz and Mobley, 2010). Uncomplicated UTI affects otherwise healthy individuals presently, for example, as uncomplicated cystitis and uncomplicated UTI have no evidence of structure abnormalities of the urinary tract (Flores-Mireles *et al*., 2015; Hooten, 2012). Complicated UTI affects people with a structurally and functionally abnormal urinary tract or those with an underlying medical or surgical health issue (Lichtenberger and Hooten, 2008; Neal and Durwood, 2008). Complicated UTI is associated with factors that have an effect on the urinary tract or host defenses such as urinary obstruction, pregnancy, diabetes mellitus and immunosuppression (Flores-Mireles *et al*., 2015). Complicated UTI could present as acute pyelonephritis with intrarenal, perirenal or pararenal abscess and septicemia (Neal and Durwood, 2008; Nielubowicz and Mobley, 2010).

**2.5** **Types of UTI**

In all types of UTI, *E.coli* is the dominating bacterial with species causing up to 85% of all symptomatic UTIS in women. The second most common species is *Staphylococcus saprophyticus*. In patients with reoccurring infection, species such as *Enterococcus faecalis,* *Enterococcus faecrum*, *Klebsiella* Spp, *Proteus* Spp, *Providencia stuartii* and *Morganella morgani*, becoming more common of the UTI organisms. In patients with frequent recurrences or bladder catheters, especially in hospitals and nursing home setting where antibiotics are frequently used in treatment *Pseudomonas aeruginosa*, *Acinitobacter baxmanii*, *Serratia marcescens* and *Stonotrophomonas matlophilia* are important organisms associated. In such patients*, E.coli* accounts for less than 50% of the infections.

Urinary tract infection usually develops in the lower urinary tract (urethra and bladder) and if not properly treated, they ascend to the upper urinary tract (uteters and kidney) and cause severe damage to the kidneys. Other complications caused by UTIs are bladder infections (cystitis), urethra infections (Urethritis), kidney infection (pyelonephritis) and ureter (uretitis)

1. Urethritis is simply an inflammation of the urethra which is a tube that carries urine out of the body. Infection often caused by sexually transmitted infection or due to an injury from an instrument such as urinary catheter or even exposure to an irritating chemical such as antiseptic or spermicide.
2. Cystitis is a bladder infection caused by abnormal growth of bacteria inside the bladder and the most common bacterial infections name them (Chung *et al*., 2010).
3. Ureteritisis infection of the ureters which are tubes connecting the kidney and the bladder. Infection occurs when the ureter to bladder values are not functioning properly and allows urine to reflux from the bladder into the ureters.
4. Pyelonephritis is an infection that affects one or both kidneys. It can happen with infection from above, or if the urine refluxes back to the kidney (Lane and Takhar, 2011).

**2.6 Pathogenesis of urinary tract infection**

Bacteria that cause urinary tract infection usually enter the bladder through the urethra. However, infection also occurs via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra after a bowel movement, which after gaining entrance, organisms such as *E.coli* attaches to the bladder wall and form a biofilm that resist the body’s immune system response (Salvatore *et al*., 2011). Other bacterial characteristics such as motility are also important in the organism pathogenesis of UTIs because it enables the organisms to ascend to the upper urinary tract and obstruct urine flow which may result in Pyelonephritis (Nicolle, 2008). The virulence factors of bacteria play an important role in urinary tract infection. Some organisms particularly uropathogenic *E.coli* (UPEC) which are present within bowel flora can infect the urinary tract by expressing some specific virulence factors that permit adherence and colonization of the lower urinary tract causing urinary tract infections (Litza and Brill, 2010). Adherence of this microorganism depends on three major features; bacteria’s own adhesive mechanism, the receptive features of the urothelium organism and finally the fluid that is present between both surfaces. Adhesive found on the surface of the bacterial membrane are responsible for initial attachment unto urinary tract tissues forming a biofilm. With biofilm formation there is synergism of bacteria with one another to remain viable (Nicolle, 2008). This biofilm form irreversible association with the host cell and present the host’s neutrophils from penetrating its surface (Salvatore *et al*., 2011). Bacteria that have irreversibly attached to a surface usually serve as a means for continued replication and re-growth of other bacteria.

Pathogenesis can also be through ascending or hematogenous route. Ascending route is the most common route of infection in females and is aided by conditions such as pregnancy, urethra, obstruction and instrumentation. Blood borne route (hematogenous route) occurs as a result of bacteremia although it is mostly not common.

**2.7. Long-stay hospitalized and catheterized patients with spinal cord injury (SCI)**

Bacteria develop in at least 10-15 percent of hospitalized patients with individually catheters (Gould *et al*., 2010). Factors associated with an increased risk of catheter associated urinary tract infection include, prolonged catheterization, severe underlying illness, disconnection of the catheter drainage tube and lack of systemic antimicrobial therapy. Bacteria are usually introduced into catheter system at the catheter collecting tube junction or at the drainage bag portal. The organisms then ascend into the bladder within 25 to 72hours causing symptoms of UTI (Pallet and Hand, 2010). This is the major reason why the risk of urinary tract infection is higher in patients with spinal cord injury (SCI), their lack of normal physiological urinal results in neurogenic bladder for most patients this condition cells for the urinary catheter (instrument) to be used on them. Also residual urine in the bladder due to incomplete emptying, renal stones, obstruction of urinary outflow, deregulation of the autonomic nervous system, and an unbalanced bladder evaluation are some of the causes. Uropathogenic *Escherichia coli* (UPEC)is associated with greater than 80% of all uncomplicated UTI, i.e., in the absence of urinary catheter. However, in the presence of a urinary catheter, the spectrum of infecting bacteria species shifts such that UPEC accounts for approximately 50% of catheter associated UTI (CAUTI), and uropathogens less commonly associated with uncomplicated UTI become more prevalent (Flores-Mireles *et al.,* 2014). Indeed, despite the robust inflammatory response associated with urinary catheterization, many bacterial species colonize the urinary catheter and persists within biofilms, which are intrinsically tolerant to host clearance. For example, *Enterococcus faecalis* is associated with approximately 5% of uncomplicated UTI but 15-30% of CAUTI (Maki and Tambyah, 2001). Thus *E. faecalis* UTI is significantly augmented by catheterization.

**2.8 In a study by**

M.A. ISA *et al*., (2013), from the university of Maiduguri on the prevalence of urinary tract infections among children in Maiduguri, Nigeria using Cystein lactose electrolyte deficient agar (CLED). They found different organisms but Escherichia coli was found to be more prevalent while *Proteus* and *Klebsiella* species were the less prevalent species of all other species found were *Staphylococcus aureus*, *Streptococcus faecalis,* *Staphylococcus saprophyticus*.

Oyebola fasugba (2017) from the Australian catholic University found *Escherichia coli* in her review paper work to be the most resistant of all bacteria causing urinary tract infections. Ciprofloxacin was the most used drug hit. In hospital acquired urinary tract infection, *E.coli* was seen to also have higher resistance to drugs like Amoxicillin-Clavulanate, Cefazolin, Gentamicin and Piperacillin-tazabactam when compound to community-acquired UTI.

Annuli John *et al*., (2016) in another study from the Department of Microbiology, University of Calabar carried out a review on the prevalence of UTI amongst adults and found that enterobacteriaceae especially *E.coli* was the most prevalent amongst the organism isolated.

In (2016) Albayrak *et al*., from the Selcuk University School of medicine Turkey carried out in a study on the characteristics of urinary tract infections in patients with spinal cord injuries hospitalized. Urine samples are obtained using a clean catch technique. The samples were inoculated with Cosin methylene blue agar and blood agar with a loop of 1µ. Bacteria identification and antimicrobial susceptibility testing was carried out using an automated vitek system. The study founded that the vast majority of uropathogens organism found are Gram-negative bacteria. *E.coli,* *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. They found that these pathogens which although were susceptible to gentamicin, piperacillin, meropenem, they were resistant ceftriaxone and to some extent ciprofloxacin.

Mansor Khalid *et al*., (2017) of Global journal of health sciences carried out a study on the antibiotics resistance profile of uropathogens isolated from Al Buraimi: Hospital, Oman. Midstream clean catch technique was employed in collection of sample in universal sterile containers. CLED and blood agar was used for incubation and culture. In the Antibiotic susceptibility testing, Disk diffusion method was employed. Muller Hinter agar media was used to evaluate the sensitivity and resistance pattern. Results showed that Gram-negative *E.coli* was found to be the highest prevalent uropathrogen followed by *Klebsiella* species, *pseudomonas*, *A. baumannis, Proteins* Spp, *citrobacter* Spp*, M. morgani and seratia* Spp. In a similar work, the highest antibiotic resistance was noticed in *E.coli* against Nalidixic acid; *E.coli* was found positive for extended spectrum Beta lactamase produces (ESBLs) and showed 100% resistance to Amoxicillin / clavulanic acid, ceftazidim and ceftriaxone.

**CHAPTER 3**

**3.1 Sample Collection**

Fifteen (15) samples of urine were collected into sterile urine containers from the Paraplegic ward of National Orthopaedic Hospital Enugu. The samples were transported to the Microbiology laboratory of Godfrey Okoye University for further analysis.

**3.2 Preparation of Culture**

Cystein lactose electrolyte deficient (CLED) agar, Mac-Conkey agar and Blood agar was prepared according to the manufacturer’s guide and sterilized. The urine samples were inoculated by streak method using a 0.001 L measured wire loop and incubated for 18-24 hours at 37o C. The plates were recovered and the colonies counted. Viable colonies counted above or equivalent to 100 colonies per microliter confirms the presence of Urinary tract infection as per Kass count (Kass, 2002).

**3.3 Urinalysis**

Urine samples were screened for the presence of Albumin (protein) using Combi-2 urinalysis dipstick strips which suggests possible infection.

**3.4 Isolation and Identification of Bacteria**

Sub-cultures of all colonial growth were made from UTI positive primary colonies. Colonies was sub-cultured onto a freshly prepared Blood agar, CLED agar, and Mac-Conkey agar. After incubation and subsequent growth, the colonies were transferred into bijou bottles and kept as stock cultures. Characterization of the pure isolates was performed and it involved colonial characteristics, motility testing. Biochemical tests of Gram reaction, Catalase test. Citrate test, Indole test, Methyl red test, Voges proskeur test, Coagulase test, Oxidase test, Urease test was carried out. Sugar fermentation of Glucose and Mannitol was carried out also. These tests were done to identify the isolates to a generic level as contained in Cheesbrough *et al.,* (2002).

**3.5 Biochemical tests:**

**GRAM STAINING**

* Place slide with heat fixed smear on staining tray.
* Gently flood smear with crystal violet and let stand for 1 minute.
* Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
* Gently flood the smear with gram’s iodine and let stand for 1 minute.
* Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
* Decolourize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5-10 seconds until the alcohol runs almost clear. Be careful not to over- decolourize.
* Immediately rinse with water.
* Gently flood with safranin to counter-stain and let stand for 30 seconds.
* Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
* Blot-dry the slide with paper.
* View the smear using light microscope under oil immersion.

**CATALASE TEST**

* Transfer a small amount of bacterial colony to a surface of clean dry glass slide using a loop or sterile wood stick.
* Place a drop of 3% H2O2 on to the slide and mix.
* Positive: Evolution of oxygen (within 5-10seconds) as evidenced by bubbling.
* Negative: No bubbles or a few scattered bubbles.

**COAGULASE TEST**

* Place a drop of normal saline on each end of a slide or on two or on two separate slides.
* With the loop, emulsify a portion of the isolated colony in each drop to make two thick suspensions.
* Add a drop of human or rabbit plasma to one of the suspensions and mix gently.
* Look for clumping of the organisms within 10 seconds.
* No plasma is added is to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

**CITRATE TEST**

* Inoculate Simmons citrate agar lightly on the slant by touching the top of a needle to a colony that is 18 to 24 hours old.
* Incubate at 350c to 370c for 18 to 24 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.
* Observe the development of blue colour denoting alkalinization.
* Positive: colour change (Prussian blue).
* Negative: no colour change.

**INDOLE TEST**

* Take sterilized test tubes containing 4ml of tryptophan broth.
* Inoculate the tube aseptically by taking the growth from 18 to 24 hours culture.
* Incubate the tube at 370c for 24 to 28 hours.
* Add 0.5 ml of Kovac’s reagent to the broth culture.
* Observe for the presence or absence of ring.
* Positive: Formation of pink or red colour (cherry-red ring).
* Negative: No colour change.

**VOGES PROSKAUER TEST**

* Inoculate the test organism into the VP medium.
* Incubate aerobically at 370c for 24 hours.
* Following 24 hour of incubation, aliquot 2ml of the broth to a clean test tube.
* Incubate the remaining broth for an additional 24 hours.
* Add 6 drops of 5% alpha naphtol and mix well to aerate.
* Add 2 drops of 40% KOH, and mix well to aerate.
* Positive: Pink-red colouration surface within 30minutes (shake the tube vigorously during the 30 minute period).
* Negative: No colour change.

**METHYL-RED TEST**

* Inoculate two test tubes containing VP-MR broth with a pure culture of the organism under investigation.
* Incubate at 35oc for 4 days.
* Add 5 drops of MR indicate solution to the first tube (for VP test Barrit’s reagent to another tube).
* Positive: Red colouration.
* Negative: Yellow colour change.

**GLUCOSE FERMENTATION TEST**

* Peptone water was prepared and distributed into labeled test tubes
* Seven (7) drops of Phenol red solution was added
* Glucose (Carbohydrate source) was added to the peptone water contained in the labeled test tubes
* The prepared test media was sterilized at 121oC for 15 minutes
* The test samples were inoculated into the medium after the sample had cooled
* Sterile Durham tubes were inverted and placed into the tubes
* The test tubes were covered and incubated at 37oC for 18-24 hours
* Change in the colour of the medium from orange/red to yellow (indicated by the change in the colour of the Phenol indicator) indicates a positive result for acid production. No change in the medium colour indicates negative for acid production. Bubbles seen in the inverted Durham tubes indicate a positive result for gas production. No Bubble indicates a negative result.

**MANNITOL FERMENTATION TEST**

* Peptone water was prepared and distributed into labeled test tubes
* Seven (7) drops of Phenol red solution was added
* Mannitol (Carbohydrate source) was added to the peptone water contained in the labeled test tubes
* The prepared test media was sterilized at 121oC for 15 minutes
* The test samples were inoculated into the medium after the sample had cooled
* Sterile Durham tubes were inverted and placed into the tubes
* The test tubes were covered and incubated at 37oC for 18-24 hours
* Change in the colour of the medium from orange/red to yellow (indicated by the change in the colour of the Phenol indicator) indicates a positive result for acid production. No change in the medium colour indicates negative for acid production. Bubbles seen in the inverted Durham tubes indicate a positive result for gas production. No Bubble indicates a negative result.

**OXIDASE TEST**

* Filter papers were divided according to the number of test samples
* Sample colonies were collected and smeared on the designated areas on the filter paper
* Oxidase reagent was prepared by mixing 0.1grams of the reagent with 10ml of distilled water
* The reagent was aseptically introduced to the smears on the filter paper
* A change in colour of the smears from their original colour to a deep blue colour was checked for within the 10-15 seconds after the reagent was placed on the samples. Change in smear colour to blue within 10-15 seconds of the test signifies an oxidase positive microorganism. No colour change signifies oxidase negative.

**UREASE TEST**

* Urea hydrolysis broth medium was sterilized and inoculated with a loop-full of the pure isolated samples
* The tubes were incubated at 35-37o C for 18-24 hours
* Colour changes were observed on all growth medium. If the colour changed from light orange to magenta (pinkish red), the organism is urease positive (produces the enzyme urease). If no colour change was detected, the sample organism is urease negative.

**MOTILITY TEST**

* A semisolid agar medium was prepared (Nutrient agar) and sterilized at 121o C for 15 minutes in a test tube
* Using a straight wire, the samples were collected and inoculated into the agar by a single straight stab down to the center of the tube, about half the depth of the medium. With the wire still in place, the tubes were plugged and covered
* The media were incubated at 37o C overnight
* After incubation, the agar samples were observed for growth in or around the test tube. A spread of growth away from the line of stab indicated motile organisms (Motility positive). Growth only found along the line of stab indicates a motility negative result (Non-motile organisms).

**3.6 ANTIBIOTIC SUSCEPTIBILTY TESTING**

* Muller Hinton agar was prepared according to the manufacturer’s guide and sterilized
* A 0.5 Mac-Farland’s standard was prepared as a measure of turbidity
* 5mls (Five) bottles of normal saline was prepared according to the number of microorganisms isolated and sterilized
* The sample organisms were inoculated into the sterile saline water and compared for turbidity with the 0.5 Mac-Farland’s standard.
* Using sterile cotton swabs, the samples were inoculated by spreading from the saline inoculums onto the agar surface
* Eight (8) antibiotic disks were placed on the agar plate media labeled with the isolated organisms name
* The media plates were inverted and incubated at 37o C for 24 hours
* Zones of inhibition were measured using a millimeter ruler

Antibiotics used in this test include:

* Ceftriaxone (30 µg) of the cephem group of antibiotics
* Meropenem (10µg) of the carbapenem group
* Amikacin (30µg) of the aminoglycoside group
* Tetracycline (30 µg) of the tetracycline group of drugs
* Ciprofloxacin (5 µg) of the quinolones group
* Sulfamethoxazole trimethroprim (25 µg) of the folate pathway inhibitors
* Chloramphenicol (30 µg) of the phenicol group
* Colistine sulphate (25 µg)

**CHAPTER FOUR**

**4.1 RESULT**

**Table 1: Total number of viable bacterial count in Cfu/ml of urine**

|  |  |  |
| --- | --- | --- |
| Samples | Total count | Cfu/ml |
| 1 | 120 | 1.2 x 105 |
| 2 | 110 | 1.1 x 105 |
| 3 | 17 | 1.7 x 104 |
| 4 | 91 | 9.1 x 104 |
| 5 | 50 | 5.0 x 104 |
| 6 | 110 | 1.1 x 105 |
| 7 | 150 | 1.5 x 105 |
| 8 | 125 | 1.25 x 105 |
| 9 | 102 | 1.02 x 105 |
| 10 | 118 | 1.18 x 105 |
| 11 | 108 | 1.08 x 105 |
| 12 | 70 | 7.0 x 104 |
| 13 | 32 | 32 x 104 |
| 14 | 115 | 1.15 x 105 |
| 15 | 130 | 1.3 x 105 |

105 cfu/ml signifies urinary tract infection and can be interpreted as 100 or more colonies per microliter (µl).

**Table 2: Gram reaction and morphology of the bacteria isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| Samples | Gram reaction | Motility | Shape |
| 1,2,4,5,7,10 | + | Non-motile | Cocci shaped |
| 3,6 | - | Motile | Rod shaped |
| 1,7,8,9,10 | - | Motile | Rod shaped |
| 2,5,3 | + | Non-motile | Rod shaped |

Table 2 shows the Gram reaction, motility and cell morphology of the bacterial isolates.

Key: + = Positive - = Negative

**Table 3: Biochemical analysis of the various bacterial isolates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isolates | Cat | Cog | Ind | MR | VP | Cit | Mot | Oxd | Glu | Man | Ure | GS | Probable  Organism |  |
| 1,2,4,5,7,10 | **-** | **-** | **-** | **-** | **+** | **-** | **-** | **-** | **+** (no gas) | **+** | **-** | G.P. cocci | *Enterococcus* Spp. |  |
| 3,6 | **+** | **-** | **-** | **+** | **-** | **+** | **+** | **-** | **+** (gas) | **-** | **+** | G.N. rods | *Proteus* Spp. |  |
| 1,7,8,9,10 | **+** | **-** | **+** | **+** | **-** | **-** | **+** | **-** | **+** (gas) | **+** | **-** | G.N. rods | *Escherichia coli* |  |
| 2,5,3 | **+** | **-** | **-** | **+** | **-** | **-** | **-** | **-** | **-** (no gas) | **-** | **+** | G.P. rods | *Corynebacterium* Spp. |  |

Table 3 above shows the biochemical reactions of all bacteria isolates used in identifying them.

Key: Cat = Catalase test Cog = Coagulase test

Ind = Indole test MR = Methyl red test

VP = Voges Proskaeur Cit = Citrate utilization test

Mot = Motility test Oxd = Oxidase test

Glu = Glucose fermentation test Man = Mannitol fermentation test

Ure = Urease production test GS = Gram stain

**Table 4: Frequency of Bacteria occurrence**

|  |  |  |
| --- | --- | --- |
| Organism | Number of isolates | % Frequency of occurrence |
| *Enterococcus* Spp. | 6 | 37.5 |
| *Proteus* Spp. | 2 | 12.5 |
| *Escherichia coli* | 5 | 31.25 |
| *Cornyebacterium* Spp. | 3 | 18.75 |
| Total | 16 | 100 |

Table 4 shows the frequency of occurrence of bacteria isolates from the urine samples of the patients. *Enterococcus* Spp.has the highest frequency of occurrence followed by *Escherichia coli*. *Cornyebacterium* Spp. had a lower frequency of occurrence followed by *Proteus* Spp. which had the lowest frequency of occurrence*.*

**Table 5: Zones of inhibition (mm) of antibiotic disks on the isolates**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Isolates | Cro (30µg) | Mem (10µg) | Ak (30µg) | C (30µg) | CIP (5µg) | Sxt (25 µg) | Te (30 µg) | Ct (25µg) | Resistance  (%) |
| *Enterococcus* Spp. | 15 mm (R) | -  (R) | 20 mm (S) | 31 mm (S) | -  (R) | -  (R) | 14 mm (I) | -  (R) | 62.5 |
| *Proteus* Spp. | 38 mm (S) | 10 mm (R) | 16 mm (I) | 30 mm (S) | 34 mm (S) | 30 mm (S) | 12 mm (I) | -  (R) | 25 |
| *E. coli* | 11 mm (R) | 17 mm (R) | 20 mm (S) | 28 mm (S) | 24 mm (S) | 10 mm (R) | 7 mm (R) | 13 mm (R) | 62.5 |
| *Cornyebacterium* Spp. | 18 mm (R) | 10 mm (R) | 24 mm (S) | 19 mm (S) | 29mm(S) | -  (R) | 19 mm (S) | 7 mm (R) | 50 |

Table 5 shows the zones of inhibition of the antibiotics.

Key: Cro = Ceftriaxone Mem= Meropenem

Ak = Amikacin C = Chloramphenicol

Cip = Ciprofloxacin Te = Tetracycline

Sxt = Sulfamethoxazole trimethroprim Ct = Colistine sulphate

R = Resistance I = Intermediate

S = Susceptible

Figure 1: Resistance pattern percentage of the isolated microorganisms to antibiotic susceptibility

**Table 6: Clinical and Laboratory Standard Institute guidelines for antimicrobial susceptibility testing (CLSI).**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Antibiotics | E  N  T  E  R  O  B  A  C  T  E  R  I  A  C  E  A  E | S | I | R | E  N  T  E  R  O  C  O  C  C  U  S  S  P  P | S | I | R |
| Ceftriaxone (Cro 30µg) | ≥ 23 | 20-22 | ≤ 19 | ≥ 23 | 20-22 | ≤ 19 |
| Meropenem (Mem 10µg) | ≥ 23 | 20-22 | ≤ 19 | ≥ 23 | 20-22 | ≤ 19 |
| Amikacin (Ak 30µg) | ≥ 17 | 15-16 | ≤ 14 | ≥ 17 | 15-16 | ≤ 14 |
| Tetracycline (Te 30µg) | ≥ 15 | 12-14 | ≤ 11 | ≥ 19 | 15-18 | ≤ 14 |
| Ciprofloxacin (Cip 5µg) | ≥ 21 | 16-20 | ≤ 15 | ≥ 21 | 16-20 | ≤ 15 |
| Sulfamethoxazole trimethroprim (Sxt 25µg) | ≥ 16 | 11-15 | ≤ 10 | ≥ 16 | 11-15 | ≤ 10 |
| Chloramphenicol (C 30µg) | ≥ 18 | 13-17 | ≤ 12 | ≥ 18 | 13-17 | ≤ 12 |
| Colistine sulphate (Ct 25µg) | ≥ 15 | - | ≤ 14 | ≥ 15 | - | ≤ 14 |

(Wayne, PA. (2016). *Performance Standards for Antimicrobial Susceptibility Testing.* Clinical and Laboratory Standard Institute, 26th edition. CLSI supplement M100S)

Table 6 shows the CLSI guidelines for antimicrobial susceptibility testing of various microorganisms. There was no found published guideline for the antimicrobial susceptibility testing of *Corynebacterium urealyticum* but based on the guidelines of similar organisms, susceptibility and resistance patterns were derived.

Key: ≥ = Greater than or equal to

≤ = Less than or equal to

**CHAPTER FIVE**

**5.1 DISCUSSION**

The result from the research showed that there is a high drug resistance to antibiotics used in the treatment of Urinary tract infections by Uropathogens. Unlike similar works of Albayrak *et al.,* (*2014*)who carried out a study on the characteristics of urinary tract infections in patients with Spinal cord injuries (SCI) and found the vast majority of Uropathogens to be *Escherichia coli* and *Klebsiella pneumonia,* this work found the majority of Uropathogens to be *Enterococcus* Spp.as found in the work by Maki and Tambyah, (2001). Based on the percentage frequency of occurrence, *Proteus* Spp. showed the least frequency of occurrence at 12.5%. *Cornyebacterium* Spp. had a percentage frequency of 18.75%. This Gram-positive rod shaped bacteria, although once rare but reoccurring in recent times has been known to cause UTI. It is an opportunistic pathogen in humans, mainly in urinary tract infection (Nieto, 2000). *Cornyebacterium* Spp. has been associated with asymptomatic bacteriuria and rarely, with acute and chronic infections of the urinary tract. *Escherichia coli* had a percentage frequency of occurrence of 31.5%. *E. coli* is known to be a major pathogen that causes urinary tract infections in humans. Mansoor Khalid *et al.,* 2017 carried out a study on antibiotics resistance of Uropathogens found *E. coli* to be the highest prevalent Uropathogen that causes UTI. *Enterococcus* Spp. had the highest prevalence of 37.5%. This prevalence is most likely due to catheterization of the patients who the urine samples were collected from. *Enterococci* are rarely associated with community acquired UTI but play a prominent role in the pathogenesis of catheter associated UTI and are among the predominant pathogens isolated from polymicrobial communities on the surface of indwelling urinary catheters (Dedeic-Ljubovic and Hukic, 2009; Desai PJ *et al.,* 2001; Johnson *et al.,* 1997). From the antibiotic assay carried out using the disk diffusion method. *Enterococcus* Spp.and *Escherichia coli* showed the highest forms of resistance to antibiotics resisting five of eight (5/8) antibiotics used. *Cornyebacterium* Spp. showed resistance to four (4/8) out of eight antibiotics. *Proteus* Spp.had the least resistance pattern. It was able to resist only two (2/8) of the eight antibiotics tested. *Escherichia coli* is been known to be highly resistant to antibiotics. Oyebola Fasugba, (2017) work on *E. coli* showed that the microorganism had high resistance pattern to drugs like Ciprofloxacin, Amoxicillin, Gentamicin, and Piperacillin tazobactam. Werner *et al.,* (2013) reported high resistance of *E. faecalis* to drugs of Cephalosporin, Glycopeptide, Penicillin, and Aminoglycoside. The isolated microorganisms were susceptible to some antibiotics; *Enterococcus* Spp.was susceptible to drugs like Amikacin, Chloramphenicol and Tetracycline. *E. coli* was susceptible to drugs like Chloramphenicol, Amikacin and Ciprofloxacin. *Cornyebacterium* Spp. was susceptible to Amikacin, Chloramphenicol, Ciprofloxacin and Tetracycline. *Proteus* Spp.was Ceftriaxone, Amikacin, Chloramphenicol, Ciprofloxacin, Sulfamethoxazole trimethroprim and tetracycline. All isolated microorganisms were generally sensitive to Amikacin and Chloramphenicol antibiotics.

Although result and degree of resistance vary greatly, this result confirms the increase in the menace of antibiotic drug resistance by microorganisms, specifically those capable of causing UTI in patients suffering from Spinal Cord Injuries (Paraplegia and quadriplegia patients).

**5.2** **CONCLUSION**

All microorganisms isolated showed very high in-vitro sensitivity to Chloramphenicol and Amikacin antibiotics. This shows that although there is a high resistance to antibiotics by Paraplegic and Quadriplegic patients, these drugs can still be effectively used for treatment of Urinary tract infections. Their effectiveness is perhaps due to the fact that these drugs are rarely used and therefore hardly abused during treatment

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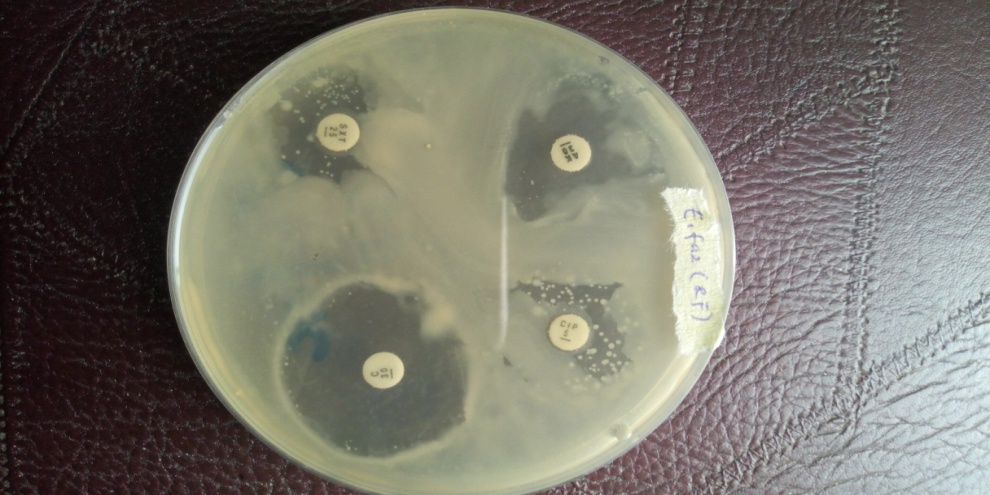
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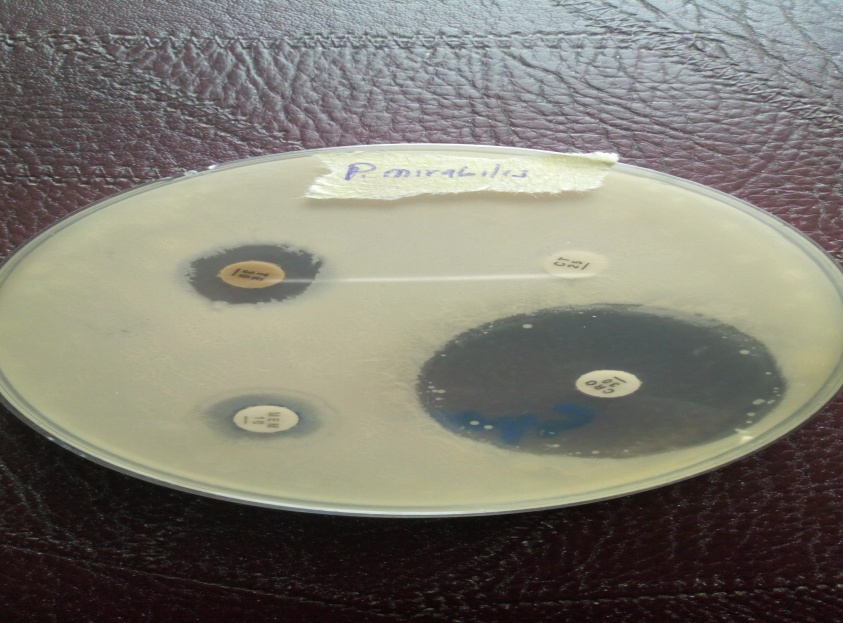
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**APPENDICES**

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