ISOLATION AND IDENTIFICATION OF BACTERIA FROM URINE OF MALE UNDERGRADUATES IN UNIVERSITY OF BENIN

BY

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CERTIFICATION

This is to certify that this project work was carried out by FESTUS AKHERE ARIOHUODION in the Department of Microbiology, Faculty of Life Sciences, and University of Benin, Benin City under my supervision.

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APPROVAL

I certify that this work has been accepted in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) in the Department of Microbiology, University of Benin, Benin City.

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DEDICATION

This project is dedicated to Almighty God and my supervisor PROF. (MRS.) O.I ENABULELE

ACKNOWLEGDEMENT

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ABSTRACT

This present study focused on isolation and identification of bacteria isolated from urine sample of undergraduate students at the University of Benin, Benin City. A total of fifteen (15) urine samples were collected and transported to the laboratory for bacteriological analysis. The total bacteria count ranged from 6.2×10^5 CFU/ml - 8.0×10^5 CFU/ml. Bacterial species isolated includes *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Proteus mirabilis*. *E. coli* was the most predominant isolate accounting for about (55%) followed by *S. aureus* (25%), *S. saprohyticus* (15%) and *P. mirabilis* (5%). The antibiotic susceptibility to antibacterial agent revealed that of *S*. aureus all the isolates were (100%) resistant to Zinnacef and Cephalexin while they were (100%) sensitive to ciprofloxacin. S. saprohyticus 15(100%) were resistant to cephalexin and (100%) sensitive to ciprofloxacin. E. coli and P. mirabilis susceptibility test revealed that 5(5%) of *P. mirabilis* showed resistance to Cephalexin and Septrin and was sensitive to Ciprofloxacin, Amoxicillin and Augmentin. however, 55(100%) of *E. coli* were resistant to cephalexin, moderate sensitivity to septrin and (100%) sensitivity to ciprofloxacin. The isolates were also tested for their ability to produce gelatinize. All bacterial isolates produced gelatinize. The ability of the bacterial isolates obtained to produce gelatinize is of a serious medical importance as this may have contributed to the multi-drug resistance of the isolates and as such, further research should be carried out to discover new antibiotics effective against these organisms.

CHAPTER ONE

INTRODUCTION

Urine is a liquid by product of metabolism in humans and many other animals. About 91-96% of urine consists of water. It contains organic compounds such as proteins, urea, creatinine, uric acid, trace amounts of hormones, enzymes, carbohydrates, fatty acids, pigments, mucins and inorganic such as sodium(Na), potassium(K), chloride(CI), magnesium (Mg), calcium(Ca), ammonium (NH_3) and sulphates (SO_4) (Cartmell *et al*., 2015). Healthy urine is not toxic however it is not sterile, in the urethra epithelial cells lining, the urethra is colonized by facultative anaerobic rods and cocci (Madigan and Brock, 2009). Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. Up to 60% of women have at least one symptomatic UTI during their lifetime. Around 10% of women in the United States have one or more episodes of symptomatic UTIs each year. Young, sexually active women 18-24 years of age have the highest incidence of UTIs. About 25% of these women have spontaneous resolution of symptoms, and an equal number become infected (Sobel 2014). The prevalence of UTIs in men is significantly lower than in women, occurring primarily in men with urologic structural abnormalities and in older adult men. UTI is classified into disease categories according to the site of infection: cystitis (the bladder), pyelonephritis (the kidney), and bacteriuria (the urine). Colonization of urine in the absence of clinical symptoms is called asymptomatic bacteriuria (ABU). ABU occurs in up to 6% of healthy individuals and 20% of elderly individuals. ABU strains generally do not cause symptoms, and most patients with ABU do not need treatment. Furthermore, colonizing ABU strains may actually help to prevent infection by other more virulent bacteria (Darouiche *et al*, 2001).

Escherichia coli is responsible for more than 80% of all UTIs and causes both ABU and symptomatie UTI (Svanborg and Godaly, 1997). *Escherichia coli* strain 83972 is the most common organism associated with asymptomatic bacteriuria (ABU) in contrast to uropathogenic *E.coli* (UPEC) which causes symptomatic urinary tract infections (UTI). *Escherichia coli* dominate as the causative agent in all patient groups. *Enterococeus faecalis*, *Klebsiella pneumonia* and *Proteus mirabilis* are also common.

1.1 AIMS AND OBJECTIVES

The objectives of this study were to;

i. To isolate and identify bacterial isolates in the urine of male undergraduate students
ii. determined the susceptibility of the bacterial to antibacterial agents
iii. determined the gelatinase production of the bacteria from urine

CHAPTER TWO

LITERATURE REVIEW

2.1 URINE

Urine, a typically sterile liquid by-product of the body, is secreted by the kidneys through a process called urination and excreted through the urethra. Urine is often used as a diagnostic feature for many disease conditions. These may be based on either physical or chemical components that may give insight to processes within the body, often through urinalysis, a common clinical analysis of urine. Physical characteristics that can be applied to urine include color, turbidity (transparency), smell (odor), pH (acidity - alkalinity) and density. Many of these characteristics are notable and identifiable by vision alone, but some require laboratory testing. Color: Typically yellow-amber, but varies according to recent diet and the concentration of the urine. Drinking more water generally tends to reduce the concentration of urine, and therefore causes it to have a lighter color. Dark urine may indicate dehydration. Red urine indicates red blood cells within the urine, a sign of kidney damage and disease. Smell: The smell of urine may provide health information. For example, urine of diabetics may have a sweet or fruity odor due to the presence of ketones (organic molecules of a particular structure) or glucose. Generally fresh urine has a mild smell but aged urine has a stronger odor similar to that of ammonia. The pH of normal urine is generally in the range 4.6 - 8, with a typical average being around 6.0. Much of the variation occurs due to diet. For example, high protein diets result in more acidic urine, but vegetarian diets generally result in more alkaline urine (both within the typical range of 4.6 - 8).Density: Density is also known as "specific gravity." This is the ratio of the weight of a volume of a substance compared with the weight of the same volume of distilled water. The density of normal urine ranges from 0.001 to 0.035.Turbidity: The turbidity of the urine sample is gauged subjectively and reported as clear, slightly cloudy, cloudy, opaque or floceulent. Normally, fresh urine is either clear or very slightly cloudy. Excess turbidity results from the presence of suspended particles in the urine, the cause of which can usually be determined by the results of the microscopic urine sediment examination. Common causes of abnormal turbidity include: increased cells, urinary tract infections or obstructions. Abnormalities in any of these of physical characteristics may indicate disease or metabolic imbalances. These problems may seem superficial or minor on their own, but can actually be the symptoms for more scrious discases, such as diabetes mellitus, or a damaged glomerulus. Urine is an aqueous solution of greater than 95% water, with a minimum of these remaining constituents, in order of decreasing concentration:Urea 9.3 g/L.Chloride 1.87 g/L.Sodium 1.17 g/L.Potassium 0.750 g/L.Creatinine 0.670 g/L.Other dissolved ions, inorganic and organic compounds (proteins, hormones, metabolites). Urine is sterile until it reaches the urethra, where epithelial cells lining the urethra are colonized by facultatively anaerobic gram-negative rods and cocci. Urea is essentially a processed form of ammonia that is non-toxic to mammals, unlike ammonia, which can be highly toxic. It is processed from ammonia and carbon dioxide in the liver.

2.2 URINARY TRACT

The urinary tract, from the kidneys to the urethral meatus, is normally sterile and resistant to bacterial colonization despite frequent contamination of the distal urethra with colonic bacteria. The major defense against UTI is complete emptying of the bladder during urination. Other mechanisms that maintain the tract's sterility include urine acidity, the vesicoureteral valve, and various immunologic and mucosal barriers.

2.3 URINARY TRACT INFECTION

About 95% of UTIs occur when bacteria ascend the urethra to the bladder and, in the case of pyelonephritis, ascend the ureter to the kidney. According to the CDC, UTIs are the most common bacterial infection requiring medical care, resulting in 8.6 million ambulatory care visits in 2007, 23% of which occurred in the ED (CDC 2011). Over 10.8 million patients in the United States visited the ED for the treatment of UTIs between 2006 and 2009 and 1.8 million patients (16.7%) were admitted to acute care hospitals (Sammon 2014). The economic burden of using the ED for the treatment of UTIs is estimated at $2 billion annually. In addition, UTIs rank as the No. 1 infection that leads to an antibiotic prescription after a physician's visit (Abbo 2014). Catheter-associated UTIs (CA-UTIs) are the most common type of health care-associated infections reported to the National Healthcare Safety Network, making up two-thirds of hospital-acquired UTIs (CDC 2017). The symptoms of UTIs are generally mild, and inappropriate use of antibiotics can lead to antibiotic resistance; therefore, it is important to establish the appropriate criteria for treatment using narrow-spectrum antibiotics for the optimal duration.

2.4 EPIDEMIOLOGY

Up to 60% of women have at least one symptomatic UTI during their lifetime. Around 10% of women in the United States have one or more episodes of symptomatic UTIs each year. Young, sexually active women 18-24 years of age have the highest incidence of UTIs. About 25% of these women have spontaneous resolution of symptoms, and an equal number become infected (Sobel 2014). The prevalence of UTIs in men is significantly lower than in women, occurring primarily in men with urologic structural abnormalities and in older adult men.

2.5 PATHOPHYSIOLOGY

Lower UTIs, also known as cystitis, are significantly more prevalent in women than in men. This is primarily because of anatomic differences, including shorter urethral length and moist periurethral environment in women. Urinary tract infections typically start with periurethral contamination by a uropathogen residing in the gut, followed by colonization of the urethra and, finally, migration by the flagella and pili of the pathogen to the bladder or kidney. Bacterial adherence to the uroepithelium is key in the pathogenesis of UTI. Infections occur when bacterial virulence mechanisms overcome efficient host defense mechanisms. Upper UTIs, also known as pyelonephritis, develop when uropathogens ascend to the kidneys by the ureters. Infections can occur when bacteria bind to a urinary catheter, a kidney, or a bladder stone or when they are retained in the urinary tract by a physical obstruction. In severe cases of pyelonephritis, the affected kidney may be enlarged, with raised abscesses on the surface. Staphylococcus aureus bacteremia or endocarditis can lead to hematogenous seeding of the bacteria to the kidneys, causing suppurative necrosis or abscess formation within the renal parenchyma (Sobel 2014). In contrast, gram-negative bacilli rarely cause kidney infection by the hematogenous route. According to an experimental model of pyelonephritis, the main renal abnormality reported is the inability to maximally concentrate the urine (Sobel 2014). This concentration defect occurs early in the infection and is rapidly reversible with antibiotic therapy. An obstruction can lead to progressive destruction of the affected kidney and subsequent renal insufficiency.

2.6 PREDISPOSING FACTORS

In the non-pregnant adult woman with a normal urinary tract, bacteriuria infrequently progresses to symptomatic cystitis or pyelonephritis. The urethra is usually colonized with bacteria, and sexual intercourse can force bacteria into the male bladder. Furthermore, spermicides increase colonization of the vagina with uropathogens and adherence of Escherichia coli to vaginal epithelial cells. Patients with structural abnormalities develop UTIs largely from obstruction of the urine flow. Urinary stasis increases susceptibility to infection. Men of any age and pregnant women are susceptible to lesions that result in obstruction.

2.7 CYSTITIS

Cystitis is the term used to describe a bladder inflammation that is very often due to an infection which is usually of bacterial origin. Examples of such micro-organisms include Escherichia coli (70-95% of all cases) and *Klebsiella pneumonia*. A bladder infection may become a serious health problem if not treated, as the infection will otherwise spread to the kidneys. This may result in renal failure or pyelonephritis (Beers *et al*., 2006). Due to complications of cystitis, mortality rates can be as high as 1% in men and 3% in women (Bavaro *et al*, 2015). Non-infective episodes of cystitis are rare and they may be due to radiation therapy, certain medicines such ascyclophosphamide, this is thought to be due to the metabolites that are excreted in the urine. Effects appear to be related to the dose of medication taken and to the duration of therapy ,the long-term use of a catheter and hypersensitivity to certain chemicals that can be found in spermicidal gels, feminine hygiene sprays and shower gels or bath foams. Interstitial cystitis (IC), also known as painful bladder syndrome, should not be confused with cystitis. IC is a chronic bladder inflammation that is not bacterial in origin. It affects both sexes and known causes are sexual intercourse, mental and/or physical stress and menses in women. IC may occur in association with other conditions, such as sinusitis, hay fever, fibromyalgia,migraines and food allergies. Treatment does not involve antibiotics but requires personalized detailed patient education. Patients must be well informed on potential trigger factors. This will help patients enjoy long periods of remission and a better quality of life.

2.7.1 Risk factors

Cystitis commonly occurs in males; about 20% of women, sooner or later, develop a UTI

(Gupta *et al*, 2011). This is mainly due to women having a shorter urethra than men. Women aged 18-30 years are very prone to getting cystitis. Sexually active females are at a greater risk of developing cystitis as sexual intercourse can result in bacteria being pushed into the urethra. Hormonal changes that occur in pregnancy and the use of diaphragms also attribute to an increased risk of cystitis. Altered hormonal levels in postmenopausal women and a bladder or uterine prolapse may cause incomplete bladder emptying are also associated with cystitis (Bavaro et al, 2015). Cystitis may also arise as a complication of another illness, an example being diabetes. On the other hand, cystitis is quite rare in younger men and children. Whenever a man presents with symptoms pertaining to cystitis he should be immediately referred to a doctor as the symptoms may be indicative of an underlying pathology, such as stones in the bladder or an enlarged prostate (Alan, 2002). Children are very susceptible to kidney and bladder damage as a result of a urinary tract infection. A UTI in children may be an indication of structural abnormalities within the urinary tract and it hence merits further investigations by a urologist.

2.7.2 Symptoms

The onset is usually sudden, typically with frequency, urgency, and burning or painful voiding of small volumes of urine. Nocturia, with suprapubic pain and often low back pain, is common. The urine is often turbid, and microscopic (or rarely gross) hematuria can occur. A low-grade fever may develop. Pneumaturia (passage of air in the urine) can occur when infection results from a vesicoenteric or vesicovaginal fistula or from emphysematous cystitis.

2.7.3 Diagnosis

Various diagnostic studies confirm cystitis. These include a urine dipstick test, urinalysis and a bacterial culture. Imaging studies are not indicated in the routine evaluation of cystitis (Gupta, *et* a.l, 2011). A dipstick test is usually sufficient to diagnose an episode of cystitis.

2.7.4 Treatment

Pharmacotherapy in cystitis aims to provide symptomatie relief to the Patients eradicate the infection, prevent complications and early treatment is recommended to reduce the risk of complications.

2.8 PYELONEPHRITIS

Pyelonephritis is a type of urinary tract infection (UTI) that affects one or both kidneys.

2.8.1 Causes

Pyelonephritis is caused by a bacterium or virus infecting the kidneys. Though many bacteria and viruses can cause pyelonephritis, the bacterium Escherichia coli is often the cause. Bacteria and viruses can move to the kidneys from the bladder or can be carried through the bloodstream from other parts of the body. A UTI in the bladder that does not move to the kidneys is called cystitis.

2.8.2 Risk factors

People most at risk for pyelonephritis are those who have a bladder infection and those with a structural, or anatomic, problem in the urinary tract. Urine normally flows only in one direction from the kidneys to the bladder. However, the flow of urine may be blocked in people with a structural defect of the urinary tract, a kidney stone, or an enlarged prostate the walnut-shaped gland in men that surrounds the urethra at the neck of the bladder and supplies fluid that goes into semen. Urine can also back up, or reflux, into one or both kidneys. This problem, which is called vesicoureteral reflux (VUR), happens when the valve mechanism that normally prevents backward flow of urine is not working properly. VUR is most commonly diagnosed during childhood. Pregnant women and people with diabetes or a weakened immune system are also at increased risk of pyelonephritis.

2.8.3 Symptoms

In acute pyelonephritis, symptoms may be the same as those of cystitis. One third of patients have frequency and dysuria. However, with pyelonephritis, symptoms typically include chills, fever, flank pain, colicky abdominal pain, nausea, and vomiting. If abdominal rigidity is absent or slight, a tender, enlarged kidney is sometimes palpable. Costovertebral angle percussion tenderness is generally present on the infected side. In urinary tract infection in children,

Symptoms often are meager are less characteristic.

2.8.4 Diagnosis

The tests used to diagnose pyelonephritis depend on the patient's age, gender, and response to treatment and include the following:

Urinalysis. Urinalysis is testing of a urine sample. The urine sample is collected in a special Container in a health care provider's office or commercial facility and can be tested in the same location or sent to a lab for analysis. The presence of white blood cells and bacteria in the urine indicate infection.

Urine culture. A urine culture is performed by placing part of a urine sample in a tube or dish With a substance that encourages any bacteria present to grow. The urine sample is collected in a Special container in a health care provider's office or commercial facility and sent to a lab for Culture. Once the bacteria have multiplied, which usually takes 1 to 3 days, they can be Identified. The health care provider can then determine the best treatment.

2.8.5 Treatment

Pyelonephritis is treated with antibiotics, which may need to be taken for several weeks. While a Urine sample is sent to a lab for culture, the health care provider may begin treatment with an antibiotic that fights the most common types of bacteria. Once culture results are known and the bacteria is clearly identified, the health eare provider may switch the antibiotic to one that more effectively targets the bacteria. Antibioties may be given through a vein, orally, or both. Urinary tract obstructions are often treated with surgery. Severely ill patients may be hospitalized and limited to bed rest until they can take the fluids and medications they need on their own. Fluids and medications may be given intravenously during this time. In adults, repeat urine cultures should be performed after treatment has ended to make sure the infection does not recur. If a repeat test shows infection, another 14-day course of antibiotics is prescribed; if infection recurs again, antibiotics are prescribed for 6 weeks.

2.9 URETHRITIS

Urethritis is inflammation of the urethra and is a lower urinary tract infection. The urethra is a fibro-muscular tube that urine exits the body through, and semen in males. Its more common in men than in women. Urethritis is characterized as gonococcal or nongonococcal.

2.9.1 Etiology

Inflammation of the urethra is most frequently an infectious etiology, with STIs being the most common cause. Sexually transmitted urethritis has two classifications: gonococcal urethritis (GCU) following infection with Neisseria gonorrhea or nongonococcal urethritis (NGU). Other etiologic agents associated with urethritis include: Neisseria gonorrhea is the leading cause of urethritis. Neisseria gonorrhea is a gram-negative diplococci bacteria transmitted through sexual intercourse. Patients are commonly co-infected with Chlamydia trachomatis. The incubation period is 2-5 days. Chlamydia trachomatis is the most common nongonococcal cause of urethritis and is transmittable through sexual intercourse. Chlamydia trachomatis is a small gram-negative obligate intracellular parasite. It is commonly co-infected with Mycoplasma genitalium and Neisseria gonorrhea. The incubation period is usually 7-14 days. Mycoplasma genitalium can cause recurrent or persistent urethritis and is commonly the causative agent in men with nongonococcal urethritis. This organism is small self-replicating bacteria lacking a cell wall. This organism can be difficult to detect given its slow-growing nature.

2.9.2 Symptoms

Symptoms of urethritis can include: Clear or mucous-like fluid from the penis or vagina People with urethritis can have redness or swelling at the tip of the penis. Pain or burning feeling when urinating Itching or irritation in the urethra - the tube that urine passes through Other than the usual symptoms of urethritis, adults or children may also experience: Abdominal pain Unwillingness to urinate Loss of bladder control It is also possible to have urethritis and not have any symptoms.

2.9.3 Treatment

The first step in treating UTIs is to classify the type of infection, such as acute uncomplicated

cystitis or pyelonephritis, acute complicated cystitis or pyelonephritis, CA-UTI, asymptomatic bacteriuria (ASB), or prostatitis (Coyle 2017). The Infectious Diseases Society of America (IDSA) recommends that empiric regimens for uncomplicated UTIs be guided by the local susceptibility, particularly to E. coli. They recommend considerin trimethoprim/ sulfamethoxazole if the local resistance rate is less than 20% and fluoroquinolones if the resistance rate is less than 10% (Gupta 2011). The empiric regimen for complicated UTIs should also be guided by local susceptibility trends of uropathogens, and definitive regimens should be tailored according to susceptibility results, when available (Sobel 2014). Collateral damage should be considered when deciding Non treatment for uncomplicated UTIs (Gupta 2011). Collateral damage refers to ecological adverse effects, including the selection of drug-resistant organisms from antibiotic use, particularly when broad-spectrum cephalosporins and fluoroquinolones are used to treat UTIs. Broad-spectrum cephalosporins have been associated with subsequent infections caused by vancomycin-resistant enterococci, ESBL-producing K. pneumoniae, ß-lactam-resistant A. baumannii, and Clostridium difficile infection. Prior use of fluoroquinolones has been linked to subsequent colonization or infections with methicillin-resistant S. aureus or fluoroquinolone-resistant P. aeruginosa (Paterson 2004). The preserved in vitro susceptibility of E. coli to nitrofurantoin and fosfomycin suggests that they cause limited collateral damage, perhaps because of their minimal effects on bowel flora. Antibiotics with a lower potential for collateral damage are preferred for uncomplicated cystitis because the infection is often self-limiting, even without treatment, and the risk of progression to tissue invasion or sepsis is minimal. In fact, studies have shown that 25%-42% of women with uncomplicated cystitis achieved clinical cure even though they did not receive antibiotic treatment or received an inactive antibiotic (Hooton 2012).

2.10 ASYMPTOMATIC BACTERURIA

The presence of bacteria in the urine of an asymptomatic patient is known as asymptomatic bacteriuria.

2.10.1 Epidemiology

Asymptomatic bacteriuria is common, with varying prevalence by age, sex, sexual activity, and the presence of genitourinary abnormalities In healthy women, the prevalence of bacteriuria increases with age, from about I percent in males five to 14 years of age to more than 20 percent in women at least 80 years of age living in the community (Nicolle 2003). Escherichia coli is the most common organism isolated from patients with asymptomatic bacteriuria. Infecting organisms are diverse and include Enterobacteriaceae, Pseudomonas aeruginosa, Enterococcus species, and group B streptococcus. Organisms isolated in patients with asymptomatic bacteriuria will be influenced by patient variables: healthy persons will likely have E. coli, whereas a nursing home resident with a catheter is more likely to have multi-drug-resistant polymierobic flora e.g P. aeruginosa. Enterococcus species and gram-negative bacilli are common in men (Warren *et al*, 1982).

2.10.2 Diagnosis

The presence of a significant quantity of bacteria in a urine specimen properly collected from a person without symptoms or signs of a UTI characterizes asymptomatic bacteriuria (Rubin et al,1992). Quantitative criteria for identifying Significant bacteriuria in an asymptomatic person are either at least 100,000 colony-forming units (CFUs) per mL of urine in a voided midstream clean-catch specimen or at least 100 CFUs per mL of urine from a catheterized Specimen (Warren et al, 1982).

2.10.3 TREATMENT

Asymptomatic bacteruria doesn't present symptoms so there is no need for treatment

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Fifteen (15) urine samples were aseptically obtained from three male undergraduates across five (5) faculty in University of Benin using sterile universal bottles and immediately transported to the Laboratory at the Department of Microbiology, University of Benin for bacteriological analysis. All the samples were analyzed within 24hours of collection.

3.3 PREPARATION OF MEDIA

All media were prepared according to manufacturer's instruction. The media used in this study

Include Nutrient agar and MacConkey Agar. Molten Nutrient and MacConkey Agar were cooled to 40°C and poured aseptically into each petri dish.

3.3 Serial dilution

Three sterile test tubes were labeled A, B and C with the aid of a sterile pipette, 9ml of sterile water was introduced into each test tube. 1ml of urine sample was introduced into test tube A and serially diluted to test tube C. 1ml was plated from test tube B and C. It was then incubated at 37^0 C for 24hours.

3.4 Standardization of the Isolates

MacFarland standard (0.5) was prepared by mixing 0.05ml of 1% barium chloride ($BaCl_2$) with 9.95ml of 1% Sulfuric acid(H_2SO_2) to form barium sulphate suspension. The turbid solution (McFarland standard) formed was transferred into a test tube for comparison with different bacterial inoculums suspension (Cheesbrough, 2006)

3.5 Antibiotics susceptibility test

Kirby-bauer disc diffusion technique was used as to determine the antibacterial activity of isolated bacteria. 20ml Mueller Hinton agar plates were prepared following the manufacturer's instructions. 1ml aliquot of each test organism suspension (standardized) was transferred onto the well-dried Mueller Hinton agar plates and was spread evenly following slow rotation of the plates and excess was decanted. The plates were allowed to dry, with the aid of sterile forcep, antibiotic disc were impregnated in the well-dried Mueller Hinton agar plates. The antibiotics disk contains Ceftazidime(30g), cefuroxime (30g), Gentamicine (10g), ofloxacine(5g), augmentin (30g), nitrofurantoin(300g) ciprofloxacin (5g). The plates were incubated for 24h. At 37°C. The resultant visible zones of inhibition were measured in millimetres (mm). Zones were interpreted using the NCCL. (Cheesbrough, 2002).

3.6 Gelatinase production test

Pure culture of the bacteria isolated were grown on the surface of Nutrient agar enrich with 2% gelatin and incubated at 37 degree Celsius for 72hrs. A clear zone around the inoculum spot indicated a breakdown of gelatin by the enzyme gelatinase.

CHAPTER FOUR

RESULTS

4.1 Result of the total bacteria count from urine sample

A total of fifteen (15) students across five faculty participated in this study comprising of male undergraduates. They were aged between 18 and 26. The samples collected from the individuals were all urine samples. The results obtained are represented below. Table 1 shows the total bacteria count of the urine samples from male undergraduates students within the age of twenty-five to seventeen. The count ranged from 6.2×10^5 CFU/ml - 8.2×10^5 CFU/ml. individual with the age twenty-five has the highest bacteria load and the least bacteria load was age twenty-one.

Table 1: Total bacteria count of urine across five faculty

Faculty	1	2	3
A	$8.0 \times 10^5 \pm 0.03$	$7.5 \times 10^5 \pm 0.03$	$7.8 \times 10^5 \pm 0.03$
B	$7.6 \times 10^5 \pm 0.03$	$7.5 \times 10^5 \pm 0.04$	$6.3 \times 10^5 \pm 0.05$
C	$7.6 \times 10^5 \pm 0.04$	$8.2 \times 10^5 \pm 0.02$	$7.8 \times 10^5 \pm 0.03$
D	$6.2 \times 10^5 \pm 0.05$	$6.5 \times 10^5 \pm 0.05$	$6.3 \times 10^5 \pm 0.05$
E	$7.2 \times 10^5 \pm 0.03$	$7.6 \times 10^5 \pm 0.03$	$8.2 \times 10^5 \pm 0.03$

Key: A – Faculty of Life Science

B – Faculty of Education

C – Faculty of Agricultural Science

D – Faculty of Management Science

E – Faculty of Art

1 – Urine sample

2 – Urine sample

3 – Urine sample

4.2 Gelatinase production test of the bacterial isolated from urine

The results presented in the table below showed the total bacteria isolated from urine and the virulence factor (Gelatinase production) of the isolates. All percentage of bacteria isolated in table 2 were positive to gelatinase. The broken-down of gelatin by this organism, reveal how deadly this organism could be if gained entrance into the system.

Table 2: Gelatinase activity of bacterial isolates

Isolates	n (%)	Gelatinase production
S. aureus	25	+
E. coli	55	+
S.saprophyticus	15	+
Proteus sp.	5	+

4.3 Haemolysin formation for bacterial isolates from urine

The result presented in table 3 shows the haemolytic capability of bacterial isolates from urine of undergraduates students. It can be deduced from the table that among twenty-five (25) *S. aureus* only fifteen (15) produced haemolysin while forty *E. coli* strain and all *Proteus* sp. produced ß-haemolysin but *S.saprophyticus* didn't produced haemolysin. This table further shows that *S.saprophyticus* is the only non-pathogenic bacteria isolated from urine. On the other hand, *E. coli*, *S. aureus*, and *Proteus* sp. are pathogenic isolates. A Clear zones around the area of inoculation is indicative of β-haemolysin formation.

Table 3: Haemolysin activity of urine isolates

Urine	Total no of isolates	No. haemolytic isolates	Type of haemolysin	Percentage (%)
S. aureus	25	15	ß-haemolysin	60
E. coli	55	40	ß-haemolysin	72.7
S.saprophyticus	15	0	γ-haemolysin	0
Proteus sp.	5	5	ß-haemolysin	100

4.4 Antibacterial susceptibility pattern to antibacterial agents

The results presented in table 3 shows the sensitivity of the bacterial isolated from urine across five faculty. Gram positive and Gram negative isolates all showed (100%) sensitivity towards ciprofloxacin.

Table 4: Antibacterial Susceptibility pattern of bacterial isolates from urine

Isolates	n (%)			Antibacterial agent		
		CPX	AM	SXT	PEF	S
S. aureus	25(25)	0(0.0)	18(72)	20(80)	18(72)	20(80)
E. coli	55(55)	0(0.0)	0(0.0)	30(54)	25(47)	6(11)
S.saprophyticus	15(15)	0(0.0)	10(67)	8(53)	11(73)	3(60)
Proteus sp.	5(5)	0(0.0)	1(17)	1(10)	3(60)	0(0.0)
Total						

KEY:

CPX – Ciprofloxacin

AM – Amoxicillin

S – Septrin

PEF – Perfloxacin

SXT – Sulfamethoxazole

CHAPTER FIVE

5.0 Discussion

Escherichia coli, Staphylococcus aureus and *Proteus mirabilis* were isolated from the urine of male undergraduate in the University of Benin. The prevalence of the bacteria isolated from the urine of certain male undergraduate students were 55(55%) *Escherichia coli*, 25(25%) *Staphylococcus aureus*, 15(15%) *Staphylococcus saprohyticus* and 5(5%) *Proteus mirabilis*. In a similar study by Ezediala *et al*., (2015) who studied the isolation, identification and antibiotics susceptibility of urine samples of males living in Chukwuemeka Odumegwu Ojukwu University Uli Campus, Anambra State, Nigeria. Reported that the most predominant isolate were *E. coli* (56.38%), *S. saprohyticus* (20.21%), *S. aureus* (11.70 %), *K. pneumonia* (7.45 %), and *P. mirabilis* 4.26%). The result is also in consonance with the findings of Poonam and Ulka (2003). Who reported a high occurrence of *E.coli* in urine analysis. Odoki *et al* (2019) also reported in their research that the most prevalent isolate was *Escherichia coli*. The studies of Mehr *et al*. (2004). According to Priyardharsini *et al* (2014), the most common isolate was *E. coli*, (31.25%) followed by *Staphylococus aureus* (16.66%), *Klebsiella pneumonia* (14.50%), *Psudomonas aeruginosa* (12.50%) and *Streptococcus* sp. The prevalence of *Escherichia coli* may be due to infection as well as the shortness of the male urethra (Foxman, 2003). *S. saprophyticus* accounted for about (15%) of the isolated organisms, This does not agree with the work of Nadia *et al*.(2004) who reported that among bacteria isolated from urine samples, Staphylococcus saprophyticus was the least (1.5%). The bacteriological analysis of urine based on the study field and age revealed that out of the five (5) urine samples collected from the male students, 23(23%) samples were significant for UTI (10' CFU/ml), 19(19%) samples were suspected for UTI (10%. 10' CU/ml) and 39(39%) samples were not significant for UTI (below 10- CFU/ml). This study noted that urine samples collected from students from medical fields were least significant to

UTI. This could be due to these students being exposed and thoroughly taught the risk factors associated with the cases of UTI, and they decided to put them into practice. Bloomberg et al. (2012) highlighted that education on the risk factors associated with UTI, is one of the major ways of reducing cases of UTI. The occurrence of UTI among the students could be attributed to the poor sanitary conditions of the environment due to over congestion of the hostel. Similar conclusions were drawn by different researchers (Bloomberg *et al*., 2005; Obirikwurang *et al*, 2012). The study showed that urine samples collected from students between 21 to 24 years were most significant for UTI. The highest cases of UTI among students between 21 to 24 years could be attributed to the fact that most males in this group are young highly sexually active males, changes of sexual partners and use of spermicides (Bint and Hill, 1994). The relative levels of hemolytic activity among the bacterial isolates were determined using sheep blood agar. Less than 24hrs culture bacterial isolates in nutrient broths were streak on freshly prepared sheep blood agar and incubated overnight at ambient temperature. Erythrocyte lysis showed that hemolysis has occurred. Result from the work indicates that haemolytic strains of *E. coli* is higher in urine samples than other bacterial isolated. 60% of *E. coli* isolated from urine of undergraduates students were β – haemolytic where a clear zone was shown around the region of inoculation, while 40% of *E. coli* isolated from urine of undergraduates students were non haemolytic. This shows that a higher percentage of haemolytic strains of *E. coli*, this is similar to the findings of Aghemwenhio *et al.* (2017). The non haemolytic strain of *E. coli* are less to say that they are not pathogenic and the reason for this could be they lack gene that code for hemolysin.. It can be deduced from table 3 that among twenty-five (25) *S. aureus* only fifteen (15) produced haemolysin while forty *E. coli* strain and all *Proteus* sp. produced ß-haem olysin but *S.saprophyticus* didn't produced haemolysin. The table further shows that *S.saprophyticus* is the only non-pathogenic bacteria isolated from urine. On the other hand, *E. coli*, *S. aureus*, and *Proteus* sp. are pathogenic isolates. A Clear zones around the area of inoculation is indicative of β-haemolysin formation. The antibiotic susceptibility for S. aureus revealed that all the isolates were (100%) resistant to Cephalexin but were sensitive to Perfloxacin, Amoxicillin while (100%) were sensitive to ciprofloxacin. 5(5%) of P. mirabilis showed high resistance to Cephalexin, and Septrin and was sensitive to Ciprofloxacin and Amoxicillin however, 55(100%) of E. coli were resistant to cephalexin and most sensitive to septrin and ciprofloxacin. Ciprofloxacin was proved to be the most effective against the Gram positive and Gram negative isolates studied in this work. This agrees with the findings of Geofrey *et al*. (2013) in which the isolated Gram positive and Gram negative isolates all showed (100%) sensitivity towards ciprofloxacin. This could be attributed to the wider spectrum of activity of this antibiotic (Arora and Arora, 2008). The resistance exhibited by the isolates against some of the conventional antibiotics could be attributed to the ability of these organisms to acquire mechanisms which might be genetic or acquired features, which allow them to resist the action of the antibiotics. Similar conclusions were drawn by other researchers (Deizell and Laferre, 2000; Bockitwetan el al. 2012). Antibiotics resistance has increased worldwide leading to failures in the treatment of human infectious disease (Afroz et al., 2013). Resistance to antibiotics could be as a result of enzymatic inactivation or modification of antimicrobial agents, impermeability of the cell wall or cell membrane, expulsion of the drugs through the efflux pump or alteration of the target receptors (Prescott et al. 2002). The bacteria isolated from this study showed that *S. aureus*, *E. coli*, and *P. mirabilis* were strong gelatinase producers.

5.1 conclusion

In conclusion, this study has shown that pathogenic and multi-drug resistant isolates were obtained from the urine samples used for this study. Microbial resistance is a widespread phenomenon which constitutes an ever present health hazard to the successful eradication of infectious diseases. The presence of Gram negative rods could be indicative of a possible urinary tract infection and medical attention should be sought to determine the extent of infection. The ability of the bacterial isoloates obtained to form slime layer is of serious medical importance as this may have contributed to the multi-drug resistance of the isolates and as such, further research should be carried out to discover new antibiotics effective against these organisms

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APPENDIX I

Table 1: Cultural characteristics, Morphological characteristics and Biochemical characteristics Of bacterial isolates from urine of male undergraduate

Cultural characteristics				
Colour	cream	Cream	Cream	Cream
Shape	Irregular	Round	Circular	Circular
Elevation	Flat	Raise	Raise	Raise
Margin	Undulate	Smooth	Entire	Entire
Size	Large	medium	medium	Large
Morphological characteristics				
KOH	+ve	-ve	-ve	-ve
Gram stain	-ve	+ve	+ve	+ve
Cell mophology	Rod	Cocci	Cocci	Rod
Cellarrangement	Singly	Cluster	Cluster	Singly
Biochemical characteristics				
Catalase	+	+	+	+
Indole	+	+	+	-
Oxidase	-	-	-	-
Voges-proskauer	Variable	-	-	-
Spore forming		-	-	-
Glucose	+	+	+	+
Lactose	+	+	+	-
Sucrose	+	+	+	-
Mannitol	+	+	+	-
H2S production	-	-	+	+
Identity	E.coli	*S. aureus*	*S.saprophyticus*	*Proteus* sp.

APPENDIX II

PREPARATION OF MEDIA

Nutrient Agar28g of powdered nutrient agar were dissolved in 1000ml of deionized water allowed to soak for 10minutes and then sterilized with an antoclaving for 15minutes at 121^{0}C allowed to cool and pour into petri dishes.

Eosin Methylene Blue

37.5g of powdered EMB agar was dissolved in 1000ml of deionized water. Allow to soak for 10minute, swirl to mix and sterilize by autoclaving at 121^{0}C for 15minutes. Allow to cool at 47^{0}C and pour into petri dishes.

Peptone water / Broth

3.8g of powdered peptone water was dissolved in 280ml of distilled water allow to soak for 10minutes and 5ml was dispensed into 5 labelled sterile test-tubes then sterilized by autoclaving for 15minutes at 121^{0}C

Thio sulphate citrate Bile Salt(TCBS)

88g of powdered TCBS cholera medium agar was dissolved in 1000ml of deionized water, allow to soak for 10minutes, swirl to mix the bring to the boil and cool to 45^{0}C and pour into petri dishes

Sammon citrate Agar

24g of powdered SCA was dissolved in 1000ml of deionized water, soak for 10minutes, and swirl to mix. Dispense into test tubes by adding 5ml and sterilized by autoclaving at 121^{0}C for 15minutes. The medium is set as a slope ensuring that the slant is over a butt about 3cm deep.

Procedure for sub culturing

Pure isolates were obtained by selecting discrete colonies and having them subcultured onto petri dishes containing freshly prepared NA media. The bacteria isolates were also transferred by streak method onto free plates respectively.

MORPHOLOGICAL IDENTIFICATION

Gram Staining

1. A thin smear was prepared on clean glass allowed to air dried and then flame it.
2. The smear was stained with crystal violet for 60 seconds.
3. Rapidly wash off the stain with clean water for 5 seconds.
4. Tip off all the water and cover the smear with lugol's iodine for 60 seconds and washed off under slowly running tap.
5. Decolourized using 90% ehanol and washed immediately with clean water.
6. The smear was covered with safranine reagent for 30 seconds then washed off the stain slowly under running tap.
7. The slide was blot dry using paper towel.
8. The strained cell were examined microscopically with oil immersion using only 100 objective lens.
9. Gram positive cells stain purple while gram negative cells stain pink or red.

BIOCHEMICAL TEST

Sugar Fermentation(Glucose)

The smear solution were 1% of glucose. The sugar glucose was prepared and sterilized with nutrient agar at 121^0C for 15minutes. Phenol red was used as indicator for acid production. The colony was inoculated on the nutrient agar containing the glucose. The presence of dark color shows the organism can ferment glucose (Cheesbrough, 2004).

Oxidase test

Procedure: place a piece of filter paper in a clean petri dish and add 2 0r 3 drops of freshly prepared oxidase reagent. Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper. Positive colonies turn bluish – purple (Cheesbrough, 2004).

Catalase test

Procedure: 1ml hydrogen peroxide solution was poured in a test tube. A sterile glass rod was used to collect or remove several colonies of the test organism and immersed. In the hydrogen peroxide solution, the test tubes was observed for immediate bubbling of gases which indicate a positive reaction (Cheesbrough, 2004).

Citrate utilization

Procedure: prepare slopes of the medium in bijou bottles as recommended by the manufacturer(store at 2-8^0C). Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.Incubate at 35^0C for 48hours, look for a bright blue color in the medium (Cheesbrough, 2004).

Coagulase test

Procedure: place a drop of distilled water on each and of a slide or on two separate slides. Emulsify a colony of the test organism (previously checked by gram staining) in each of the drops to make two three suspensions.Note: colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar. Add a loopful of plasma to one of the suspensions and mix gently, look for clumping of the organisms within 10 seconds (Cheesbrough, 2004).

Methyl red test

Procedure: the PH of a culture was sustained below a value of 4.5 as shown by the change in color of the methyl red, indicator which was added at the end of the period of incubation. Cultures were inoculated into 5ml of peptone water medium and incubated at 37^0C for 3days. 3drops of methyl red indicator was then added (Cheesbrough, 2004).