ISOLATION AND IDENTIFICATION OF *Candida* SPECIES FOUND IN HUMAN URINE SAMPLES (FEMALE)

BY

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BENIN CITY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES)

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CERTIFICATION

This is to certify that this project work was carried out by OMOBUDE OSARIEMEN EDITH (Miss) with matriculation number LSC1606012 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This report is dedicated to the Almighty and ever living God, for giving me the grace and strength to carry out this project work and to my parents Mr & Mrs Omobude and lovely brothers Mr Felix, Mr Kelly and Mr bright Omobude for their total support and motivation to complete this work.

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May God bless you all.

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ABSTRACT

Candida species are reportedly the most common human fungal pathogens. The incidence of urinary tract infections (UTIs) caused by *Candida* species has increased in recent decades. However, such infections rarely occur in the absence of any predisposing factors. The development of *Candida* species infection depends on several factors such as age, sex, and immunity of the host-pathogen relationship. Regarding this, the purpose of this study was to determine the prevalence of *candida* species in urine of female. The current study was conducted on fifty (50) urine samples collected from female students within the age of 17 to 24 residing in university of Benin using a sterile universal container. Each of the collected samples was diluted serially and incubated in petri dish containing Sabourad Dextrose Agar (SDA) at 37^0C for 72hours. After subculturing, *Candida* species were diagnosed differentially using the germ tube test, colony staining, sugar fermentation test and microscopic morphological examination. Result shows that, the color of a urine sample is not an indicating factor of the presence of candida (infection) as candida growth were significantly present in all color types. According to the results, 40%, 20%, 17.1%, 5.7%, 5.7%, 5.7% and 5.7% of the isolates were identified as *Candida albicans, Candida tropicalis, Candida guilliermondii, Candida krusei, Candida parasiliosis, Candida pseudotropicalis and Cadida glabrata,* respectively. Our result showed no significant correlation between age and prevalence of Candida in the urine. Based on the obtained results, *Candida albicans* species was the most prevalent Candida species. Hence, *Candida. albicans* are the main course of urinary tract infections in female.

CHAPTER ONE

1.0 INTRODUCTION

Urine is a bodily waste product that is released by the kidney and contains various metabolic wastes, particularly urea and other nitrogenous substances that the kidneys filter from the blood. The urethra allows for the body's excretion of urine, which is stored in the urinary bladder. (Baig, 2011). The main cause of fungal urinary tract infections (FUTI) is a type of Candida. For this type of clinical diagnosis, the urine can be a crucial specimen. Pathogenic organisms called fungi can also result in widespread infections that affect the kidneys. Other substances, such as Cryptococcus spp., can also result in urinary tract infections in addition to Candida. Infections can also be brought on by non-yeast fungi, such as various Aspergillus, Mucorales, Blastomyces, and Histoplasma family members, especially in people with impaired immune systems (Kauffman, 2014). The diagnosis of urinary tract infections depends on the microscopy and culture-based detection and identification of Candida in urine samples (UTI). Leukocyturia is a crucial indicator of a urinary tract infection (UTI), which can be brought on by either bacteria or fungi (Reilly et al., 2013; Fogazzi, 2010). It is also a symptom of the organism's immunological response to a UTI. One of the most prevalent illnesses in both outpatients and hospitalized patients are urinary tract infections (UTIs) (Rashedmarandi et al., 2008). Urinary tract infections (UTIs) caused by Candida species are becoming more widespread and have become the most prevalent clinical finding, especially in hospitalized patients (Manisha et al., 2011). Nearly 10%–15% of nosocomial UTIs are caused by Candida species (Lundstrom and Sobel, 2001; Kauffman et al., 2000). Candidaturia is the term for when there are species of Candida in the urine. According to Colombo and Guimaraes (2007), candiduria is the isolation of Candida species in the urine of both symptomatic and asymptomatic patients. The Candida genus of yeasts can be found in a variety of environments, including food, water, soil, and the microbiota of both

humans and animals. The vaginal microflora, urethra, and lungs are all predominantly populated by commensal microbes that live in the digestive tract (Valle et al., 2003). However, these same yeasts have the potential to turn pathogenic and are therefore regarded as opportunistic if there is an imbalance in their relationship with the host. The molecular profile of yeasts that colonize the host is identical to that observed in instances of infection, which could also lead to candidemia and subsequently candiduria (Dignani et al., 2003; Mendonça et al., 2013). This imbalance may be caused by impairment of the host defense mechanisms (extremes of age, underlying disease, immunosuppression), or disruption of anatomical barriers (burns, catheters, or invasive surgeries). C. albicans, a member of the Candida genus, is the pathogen that has been isolated from the majority of clinical samples (Kalantar et al., 2015). However, in an international surveillance study, *C. glabrata* was introduced as the predominant species (Salehi *et al.,* 2016). Other Candida species isolated from UTIs include *C. tropicalis*, *C. krusei*, *C. guilliermondii*, *C. kefir*, and *C. parapsilosis* (Adrian *et al.,* 2006; Tan and Chlebicki, 2016). A few studies with small sample size have addressed the epidemiology and risk factors of candiduria, as well as species distribution in this disease. The isolation of Candida spp. from urine cultures may indicate colonization or urinary tract infection (candiduria), but it may also be a sign of severe systemic candidiasis or candidemia (Huang *et al.,* 2013; Colombo and Guimarães, 2007). Candida spp. can reach the urinary tract via the ascending route, from the urethra to the bladder, or by hematogenous spread, as Candida spp. is filtered by the kidneys and excreted in the urine. The ability to form biofilm and the production of hydrolytic enzymes, which disrupt cell membranes, facilitate the spread of yeasts in the host, causing infection (Freitas *et al.,* 2014; Sardi *et al.,* 2010). Lesions in the renal pelvis, tubules, and ureters, and formation of a “fungus ball”, which blocks and causes injury to the urinary system, are the main complications

associated with candiduria, while development of candidemia is unusual (Fisher *et al.,* 2011, Sobel *et al*., 2011). The source of candidiasis in humans is mostly endogenous, as Candida spp. are commensals in the digestive tract of a vast range of healthy people. Some conditions allow these commensal yeasts to become opportunistic, resulting in candidiasis in different site of the body.

1.1 AIM

The aim of this study was to isolate and identify Candida species from human urine samples (female).

OBJECTIVES

The specific objectives of this study were to:

1. collect urine samples from females.
2. isolate and stain Candida species.
3. identify using microscopic examination, sugar test and germ tube test.
4. determine the prevalence of Candida species.

CHAPTER TWO

2.0 LITERATURE REVIEW

Candida species, which are found in the human gastrointestinal system, vagina, oral cavity, skin, as well as mucosal surfaces, are a component of the natural human flora (Sardi *et al*., 2013). The genus Candida contains over 100 distinct species, although only a small number of them may cause disease in humans. Candida spp. are microbiota in healthy humans, but in immune-compromised circumstances, they can end up causing infections in humans (kucukates *et al*., 2016). In patients with impaired immune systems, Candida spp. can also be opportunist as well as life-threatening systemic illnesses as well as chronic mucocutaneous diseases. This has been true for the past ten years (Ozer *et al*., 2016). Human fungal infections that are Candida species may result in genitourinary candidiasis, that affects either sexes equally, includes oral candidiasis, candiduria, as well as candidiasis of the digestive system, vulvovaginal candidiasis, balanoposthitis and balanitis in males (Udayalaxmi *et al*., 2014). has a number of virulence characteristics that promote pathogenicities, including adhesion to a variety of tissues including inanimate sites, the creation of biofilms, phenotypic switching, dimorphism, and the synthesis of hydrolytic enzymes (Alenzi, 2016). Both bladder and urethra are the primary sites of disease with in lower urinary tract (Aslan and Gulmez, 2016). According to Kauffman (2014), candiduria is the presence of Candida species with in urine. Vulvovaginal candidiasis has been the second-most common disease of a feminine genital system (Thomas and Tracy, 2015). Candida spp., especially *C. albicans, C. glabrata, and C. tropicalis,* include the most frequent pathogens in nosocomial UTIs. C. albicans also one of the most widely distributed species among all Candida spp (Jamil *et al*., 2016). Compared to men, women are more likely to contract Candida species (Mahmoudabadi *et al*., 2012). Each of them uses a distinct class of antifungal to either slow down the development of or completely eradicate various fungal diseases. Since they

are few in quantity but a great number of antibiotics can be employed to combat mycotic diseases, antifungal medicines make up a significant portion of the therapy for UTI (Kolaczkowska and Kolaczkowski, 2016). Order to have an impact on the fungal cell membrane, the classes of presently offered medications that correspond to group polyn but also azole break the fungal cell wall (Ellis, 2002). *C. tropicalis* and *C. pararapsilosis* are both typically sensitive to azoles, notwithstanding the rise in Candida spp. that are resistance to antifungal medications that has only lately been noted globally. Fluconazole in particular makes *C. glabrata* and *C. krusei* inherently very resistant to antifungal medications (Jamil *et al.,* 2017).

2.1.1 Candida cystitis

A lower UTI symptom called *Candida cystitis* is brought on by Candida. Symptomatic candiduria could occasionally be brought on by *Candida cystitis*. The bladder may become infected by Candida species. Candida species, commonly referred to as a symptomatic lower UTI, can develop Candida cystitis since the urinary bladder is typically sterile. Symptomatic candiduria can occasionally be brought on by *Candida cystitis*. Incontinence, dysuria (painful or tough urination, which involves scorching, tingling, or stinging of a urethra as well as meatus accompanied to voiding), in additionally hematuria are signs of *Candida cystitis* (urinating with blood in them) (Achkar and Fries, 2010).

2.1.2 Candida pyelonephritis

Serious nosocomial upper urinary tract infections (UTIs), such as candidemia and sepsis, could result from candid pyelonephritis (inflammation spreads all through the system whenever chemicals enter the bloodstream to combat a disease.). This can start a domino effect that disturbs several organ systems, leading to their failure but rather, in rare instances, death. The

much more typical primary signs of candida pyelonephritis include fever as well as candiduria. (Behzadi and Behzadi, 2008).

2.2 Urine formation

In addition to other metabolic wastes like urea as well as other nitrogenous substances that now the kidneys remove first from bloodstream, urine is a bodily residue generated by the kidney. The urethra allows for the body's excretion of urine, which is collected in the urinary bladder. (Atif,2011) The kidney would be the organ in responsible for producing urine. The two independent kidneys that make up a human being's anatomy are typically found towards the left and right of a spine with in retroperitoneal area. Given that they only make up 0.5percent of the entire body weight, the kidneys are remarkably highly vascular organs, absorbing an average of 20–25% of the cardiac output (Rose and Post, 2001). The nephron is just the kidney's primary filtration organ. A glomerulus, a set of tubules, as well as a collecting duct make up the nephron. Although the precise number of nephrons varies from person to person, the overall number is fixed at birth. Fresh nephron development as well as nephron replacement are not possible following birth. In general, there are one million nephrons per kidney (Floege *et al*.,2010). Throughout a network of arteries, serum is fed towards the nephron before passing in via the capillary bed as well as across the efferent arteriole towards the glomerular capillaries. Fluid is forced to cross the semipermeable glomerular membrane first from blood segment into the urine space (Bowman's space) by the pressure that is hydrostatic with in capillary bed. The water, ions, and tiny molecules in this ultrafiltrate are quickly filtered thru the membrane in addition to having a similar osmolality to plasma. Proteins as well as erythrocytes, which are bigger molecules, are typically blocked from getting into the filtration membrane. The endothelial cells

of a renal capillaries, the basement membrane, as well as the epithelial cells lining the urine area make up this filtration membrane or barrier. Therefore, the presence of protein or erythrocyte in the urine may indicate that this barrier is broken (Haraldsson *et al*., 2008).

The ultrafiltrate will subsequently go via a succession of tubules as well as a collecting duct in Bowman's space. This refinement procedure enables the reabsorption of water as well as solutes by the ultrafiltrate as well as the release of extra byproducts. The maintenance of life also depends on this refinement process. The kidney filters about 180 liters of fluid per day in a normal 70 kilogram person. lack of the capability to uptake solutes as well as water from the filtrate, the living could never survive. Since the blood's electrolyte, mineral, as well as pH equilibrium are maintained whereas only 1-2 liters of urine are typically removed from the body daily, the kidneys are indeed very effective at about this method. The proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, as well as the collecting duct are the four main tubular sections of the nephron that influence the final proportion as well as quantity of the urine. Reabsorption as well as excretion are made possible by a specific set of channels in addition to the transporters found in each specific tubule section. Sodium is primarily recycled back into these sections. Theoretically excreting 180 liters of water each day would lead to a lack of 25,500 mmol of sodium each day, which is inappropriate for life. More than 99percent of this sodium can be recycled back by the renal tubules thanks to their effective reabsorption method, resulting in a daily sodium excretion rate of only 100 mmol. There are further instances of the kidney's effectiveness in reabsorption. The kidney's utility man, the proximal convoluted tubule reabsorbs that much water as well as solute compared to any other portion of the nephron. Within that section, between 55 and 65 percent of the entire ultrafiltrate is recycled back. In this section, 90percent of the bicarbonate, 65percent of the sodium, and 55percent of the chloride are

recycled back along with the majority of the purified glucose as well as amino acids. The osmolality of the ultrafiltrate exiting the proximal tubule is virtually identical to that of the ultrafiltrate entering because solutes/ions as well as water are both reabsorbed with in proximal convoluted tubule. Or, to put it differently, in this section, urine is not concentrated nor diluted (Floege *et al*., 2010). A descending limb as well as an ascending limb make up the Henle loop. Water is generally permeable but somewhat impervious to solutes along the descending limb. Since the ascending limb is not permeable to water, the nephron's dilution segment starts here since the extraction of solutes, not water, lowers the concentration of ultrafiltrate. 25 percent of the filtered sodium as well as chloride can be recycled back in this region of the nephron thanks to the Na+K+2Cl symporter upon that apical membrane of a thick ascending limb. The loop diuretics class of diuretics have their action site in this transporter. Bartter syndrome is caused by inherited or acquired failure of this transporter. Clinical signs of this condition include hypokalemia, metabolic alkalosis, as well as hypercalciuria, which are comparable to that seen in individuals who have been administered a loop diuretic (Mehta and Jim, 2017). The distal tubule, which continues the nephron's eluting portion, is comparatively impermeable to water. In this section of the nephron, an extra 5–10 % of filtered sodium and chloride can be reabsorbed thanks to the Na+Cl symporter on the apical membrane of the distal convoluted tubule (Rose and Post, 2001). The thiazide diuretic class of diuretics has its site of action in this transporter. Gitelman syndrome is caused by inherited or acquired failure of this transporter. Clinical signs of this condition include hypokalemia, metabolic alkalosis, and hypocalciuria, which are comparable to those seen in individuals who have been treated thiazide diuretics. The collecting duct fine-tunes acid and potassium excretion as well as salt reabsorption. 1–3 percent more of the filtered sodium load can be reabsorbed thanks to the epithelial sodium channel (ENaC) on the apical

membrane of the main cell in the collecting duct. Aldosterone mineralocorticoid upregulates this channel (Rose and Post, 2001). Spironolactone and eplerenone are examples of mineralocorticoid receptor antagonists that cause a downregulation of ENaC and sodium diuresis. Liddle syndrome, a condition with symptoms like those of a strong aldosterone state (increased sodium reabsorption, hypervolemia, hypertensive, hypokalemia), is brought on by a hereditary or acquired triggering mutation of ENaC. (Mumford *et al*., 2019). A clinical condition known as sodium wasting, hypovolemia, and hyperkalemia, which are symptoms of a low aldosterone state, is caused by an inactivating mutation (Chang *et al*., 1996). In the lack of antidiuretic hormone, the collecting duct is impervious to water (ADH). Aquaporin channels are used to make the collecting duct water permeable when ADH is present. According to the needs of the individual, the urine might be further diluted or concentrated in the collecting duct. Hyperosmolality as well as effective circulatory volume depletion are the two main factors that stimulate ADH release (Tetti *et al*., 2018). Urine leaves the nephron's collecting duct and joins a converging network of tubules with additional collecting ducts. The minor calyces and major calyces are formed by the union of these ducts, and they eventually converge in the renal pelvis. The ureter then transports the urine into the bladder as it keeps flowing from the renal pelvis. The bladder stores urine from both kidneys until micturition (urination) takes place.

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2.3 Method of Preservation of Urine Specimen

a. Physical Method;

It involves Refrigeration and Freezing.

b. Chemical Method;

It involves the Use of chemical preservatives such as Thymol**,** Toluene**,** Formaldehyde**,** Hydrochloric acid (HCl)**,** Chloroform**,** Boric acid. (Kassa *et al.,* 2002).

Candida urinary tract infection predisposing factors

- Diabetes mellitus
- Renal transplantation
- Age extremes
- Urinary tract instrumentation
- Female sex
- Prolonged hospitalization
- Urinary tract congenital abnormalities
- Intensive care unit admission
- Urinary tract structural abnormalities
- Broad-spectrum antibiotics
- Indwelling urinary tract devices
- Bladder dysfunction

2.4.1 *Candida albicans*

As a diploid dimorphic fungus, *Candida albicans* is the most common cause of fungal nosocomial UTIs and systemic candidiasis globally. The most well harmful characteristics of the dimorphic fungus *Candida albicans* is shape flexibility, as in the ability to move between yeast and filamentous forms. Additionally, a number of characteristics of Candia albicans, including adhesion, invasion, releasing hydrolytic enzymes, stereotropism (thigmotropism), and biofilm

formation, are unquestionably regarded as harmful processes. The method of fungal colonization as well as infection is determined by the morphology of *Candida albicans*. The three types of *Candida albicans* are the loose septate pseudohyphae, which have an elongated ellipsoid look of the hyphae divisions, the septate truehyphae, as well as the single-celled budding yeast with an ovoid shape. According to documented accounts, both yeast cells and real hyphae contributed significantly to UTI candidiasis. *Candida albicans*' pseudohyphal form is also referred as the fungus' switch structure. Hence, the life span of *Candida albicans* has a clear morphological evolutionary basis. (2010) Achkar and Fries.

Use of antibiotics is a major factor in the development of candiduria because they decrease endogenous bacterial flora, particularly inside the stomach as well as reduced genital tract as well as superficial region around the urethral meatus, which might also facilitate the invasion of Candida species. It could reduce the host's resistance towards Candida disease by reducing phagocyte activity as well as antibody generation. In each of its life phases, comprising commensalism and pathogenesis, Candida albican adheres to other *Candida albicans* cells, host cells, or inanimate surfaces using a particular class of proteins known as adhesins (Guler *et al.,* 2006).

2.4.2 Candida glabrata

Due to the absence of pseudohyphae, *Candida glabrata* was included with the Torulopsis genus. in 1978, to be exact. With the exception of their overall size, that can be quite small, *Candida glabrata* colonies on Sabouraud Dextrose Agar (SDA) are almost unrecognizable from that of other Candida species. They are shiny, smooth, as well as creamy in coloration. Compared to *Candida albicans* (4-6 m), *Candida tropicalis* (4-8 m), and Candida parapsilosis (2.5-4 m),

Candida glabrata cells (1-4 m) are considerably smaller. *Candida glabrata* ferments as well as integrates solely glucose with regard towards the chromogenic medium CHROMagar, where its colonies show up as white, pink, or purple. *Candida glabrata* is haploid, as well as many flaws in the breeding system have also been found (Brunke and hube, 2013). Despite its inability to produce filaments, *Candida glabrata* may colonize both host tissues in addition to abiotic surfaces, in which it grows as a multilayered biofilm structure (Iregui *et al*., 2005). The presence of certain proteins known as adhesins on a Candida species' cell wall is a significant factor associated with that capability.

2.4.3 Candida krusei

Candida krusei, one of the clinically relevant Candida species, are often prolonged and resemble "long grain rice," a property they share with Candida kejyr (formerly Candida pseudotropicalis). *Candida krusei* is 2-2-56 x 4.3-1 5.2 pm in size and has a wide range in the both length as well as width. The ideal temperature range for *Candida krusei* is around 43 and 45 °C. Even though the most of health related Candida spp. requires biotin for development and others have other vitamin needs, only *Candida krusei* could thrive in vitamin-free conditions. *Candida krusei* only takes up and ferments glucose out of a variety of different polysaccharides. *Candida krusei* could be the sole species of the clinically important Candida spp. that grows on Sabouraud's dextrose agar as growing colonies with either a matt or a rough whitish yellow surface, in contrast to the convex colonies of the numerous Candida spp. This characteristic, along with the fact that it seems to be "long grain rice" underneath a microscope, helps in the precise identification of a species (Samaranayake and MacFarlane, 1990). It has been demonstrated that *Candida krusei* produces a wide variety of fatty acids as metabolites as well as aetoin when grown in culture

conditions containing lactose. It also produces a variety of short-chain carboxylic acids if it is grown in saliva that has been supplemented using glucose. (Gunasekaran, 1980).

2.4.4 Candida tropicalis

A vegetative cell of *Candida tropicalis* ranges in form from circular to oval as well as measures between 2 and 10 micrometers. Various media allow *Candida tropicalis* to grow successfully. The Sabouraud's agar, which includes peptone as well as sugar, is a frequently utilized medium. It's sufficient for species identification, but it comes with the drawback of encouraging mycelial growth and inhibiting conidia production. The cornmeal agar is another often used media that aids in the development of conidia. Other forms of growth media include potato-glucose, potato-carrot, tomato juice, lima bean, and the others. The ideal growth temperature is around 25 as well as 35 °C (77 and 95 °F), and adding sugar or fat to the medium will promote growth. *Candida tropicalis* colonies are white, butyrous, smooth, and have a fringed border. *Candida tropicalis* generates blastoconidia during budding, an asexual method of reproduction. Blastoconidia could stretch in morphology as their population grows, giving rise to structures known as pseudohyphae. In 2010 (kothavade *et al*.)

2.5 Sabouraud Dextrose Agar

Fundamentals and Interpretation Sabouraud Dextrose Agar is a variation of the mixture published by Sabouraud (Wehr and Frank, 2004) for the culture of fungi (yeasts, moulds), especially helpful for a fungi linked to skin illnesses (Carlier's modification; Jorgensen *et al*., 2015). The method is also utilized to detect microbial contamination in clinical samples, cosmetics, as well as food (Salfinger and Tortorello, 2015). Peptone from fungi supplies nitrogenous chemicals. Dextrose acts as a source of fuel. Low pH and high dextrose content

encourage the growth of mushrooms while avoiding contaminating germs in samples in the test (Murray *et al*., 2003). Particular specimen Clinical samples include food, beauty products, and skin scrapings. In sterile examinations of pharmaceutical and beauty items, Sabouraud Dextrose Agar is a traditional media for the cultivation, isolation, and identification of yeasts and molds. Additionally, it is employed in the precise identification of *Candida albicans* in pharmaceuticals.

2.5.1 Composition of sabouraud Dextrose Agar

The composition can be adjusted in order to obtain optimal performance. For 1000ml of water.

Dextrose…. 40.0g

Pancreatic digest of animal tissues 5.0g

Pancreatic digest of casein 5.0g

Bacteriological agar..... 15.0g

(Murray *et al.,* 2003).

Lactophenol cotton blue

The most popular approach is this one. Since the architecture of the fungal cell walls are preserved by lactic acid, the slides could be permanently fixed (Stevens, 2002). The much more popular staining agent for examining yeasts and molds is lactophenol cotton blue (LPCB), that can be utilized as either a mounting fluid for wet mounts as well as a stain (Khubnani *et al*., 1998). Making it is simple. The mixture consists of three ingredients: cotton blue, that discolors the chitin found in fungi, lactic acid, that retains fungus structures, and phenol, which again will kill any living things. Fungi turn blue when lactophenol cotton blue is added, enabling it to see

and examine them. Aniline blue as well as lactofuchsin stains are additional options that may be employed as well as they both operate on the exact principles as that of the LPCB stain. If used appropriately as well as left on for a few weeks, the lactofuchsin stain can keep the hyphae's shape as well as configuration. The procedures for using lactophenol cotton blue are as follow;

1. mix the specimen whether a skin scraping, fluid exudate or tissue with two drops of 10% KOH on a clean slide.
2. add one, or at most two drops of the lactophenol cotton blue mountant/stain to the slide
3. press a cover slip gently to make a thin mount avoiding air bubbles. Gentle warming can also aid in clearing the mount
4. examine the prepared slide under low power (x10) with reduced lighting. Switch to high power (x40) to check for the presence of suspected fungal structures.

2.6 Sugar fermentation

To ascertain an organism's capacity to ferment glucose while producing gas as well as acid, a sugar fermentation test was done. Typically, peptone water medium with 1percent in terms fermentable sugar as well as 0.01percentage phenol red is used to make sugar signal broth. Every test tube typically receives approximately ten milliliters of sugar broth. Durham tube, which might catch the gas if generated, was properly inverted. The test tubes were autoclaved, filled with the a loopful of a test organisms' 24-hour-old culture, and afterwards cultured for 2–7 days at 36–1 °C while being monitored every day for acid as well as gas generation. Acid generation is detected by yellow coloration, whereas gas production is demonstrated by medium displacement with in Durham tube (Fawole and Oso, 2004).

2.7 Germ Tube Test

A screening test called the "germ tube test" is used to distinguish *Candida albicans* from those other yeast. Reynolds and Braude published the first study on germ tube (GT) production in 1956. Candida generates germ tubes after 3 hours of development at 37°C in human or sheep blood. These germ tubes is shown on wet KOH films as filamentous outgrowths spreading from yeast cells. Both Candida dubliniensis and *Candida albicans* are present. While cultured on a proteinaceous medium, 95–97% of *Candida albicans* isolates produce germ tubes. (2011) Sudbery The fundamental idea behind the germ tube test is that enhanced protein as well as ribonucleic acid biosynthesis occurs during the creation of the germ tube. Tryptic soy broth as well as fetal bovine serum, which are necessary ingredients for protein synthesis, are found in Germ Tube Solutions. To ensure a stable, this is lyophilized. One amongst *Candida albicans*' virulence factors is indeed the germ tube. This quick test presumes the presence of *Candida albicans*. (Sudberys**.** 2002)

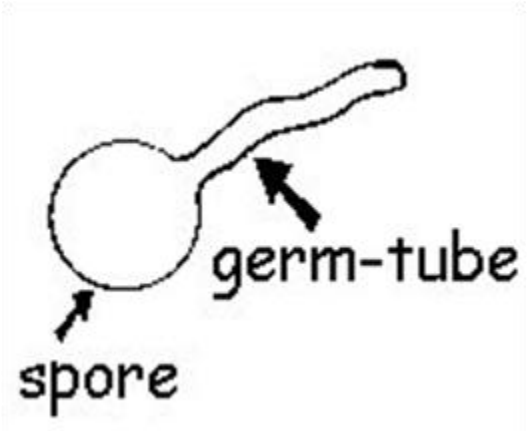

Figure 1*: Candida albican* germ tube test

(Sudbery, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study area

The study was carried out in Benin city, Edo state. Urine samples were gotten from the university of Benin female hostels in ugbowo campus.

3.2 Sample collection

A total of fifty (50) samples were collected from female hostel in the university of Benin. Queen Idia hostel (hall 1) and Tinubu Hall (hall 2) were the locations. The urine were collected as early as 8am, a mid stream urine was used, this is done by collecting the urine from the middle part of the first morning urine, it is important for urine culture. A universal bottle was used in the urine collection, the universal bottle was labeled before handing over to our donors. The urine was collected aseptically from the donors while wearing spandex hand gloves to avoid contamination. All specimen was transported immediately to the laboratory and culturing within the 2hours of collection. The urine was cultured inside a sabouraud dextrose agar, lactophonel cotton blue was use for staining after subculturing. Sugar test was done using peptone powder, sugars, water and bromothymol blue as indicator. Germ tube was done using human serum. Physical examination was carried out on the urine such as recording the colour of the urine and Age of the donor.

3.3 preparation of culture media and culturing

The media used in this study was sabouraud dextrose agar, the media was used for isolation and subculturing. 65g of sabouraud dextrose agar was weighed and dissolved in 1000ml of water, distilled water was prepared by pouring 9ml of water into bottles and sterililzing. It was sterilized using an autoclave for 15mins at 121psi. After sterilization we allowed the agar to cool for 45^{o}C, 5ml of ciproflaxin was added to the molten agar and was mixed properly by rocking the flask on the working bench. Serial dilution was done and 1ml was poured into the labeled sterile petridish. The agar was poured into the petridish containing the 1ml of diluted urine and was mixed thoroughly and allowed to solidify. It was inverted and incubated for 72hrs at 37^{o}C and growth was observed.

3.4 subculture.

The petridish that was cultured and was observed for growth, the plate that had viable growth were counted and recorded. It was also subcultured to get pure colony. Sabouraud dextrose agar was prepared and sterilized, it was allowed to cool and was poured into petridish then allowed to solidify. A sterilized inoculating loop was used to pick a loopful of inoculum and was streaked in the new labeled agar plate and incubated for 24hrs at 37^{o}C. The plates were observed for growth.

3.5 Staining

Microscopic slides were washed and degrease, a loopful of water was placed on the slide, a loopful of inoculum was placed on the slide and smeared, it was allowed to air dry, the inoculum was fixed by passing it through the flame and the slide was stain with lactophenol cotton blue for 1min and was rinsed with water and the slide was allowed to air dry . The slide was viewed under the microscope using x40 magnification and the shapes were recorded.

3.6 Sugar preparation

The sugar used was glucose, maltose, lactose, galatose and sucrose. 2g of peptone was measured, 1g of sugar (glucose) was added and mixed in 100ml of water, 2ml of bromothymol blue was added as an indicator for colour change. This step was repeated for the other sugars maltose, lactose, galatose and sucrose.

The prepared sugar were dispense into separate labeled plain container and steriled, after sterilization it was allowed to cool. A loopful of inoculum was placed inside the plain container containing the sugars separately, it was incubated for 24hrs at 37^{o}C and observed for colour change. If there is a colour change from blue to yellow it means there was acid production(positive), from blue to green it means there was no acid production(negative).

3.7 Germ tube test for identification of *Candida albicans*

Colony inoculums was inoculated into 0.5 ml of human serum in a test tube and incubated at 37^{o}C for 2-4 hours. After incubation, a loop-full of culture was placed on a glass slide, overlaid with a cover-slip and examined microscopically for the presence or absence of germ tubes. Formation of germ tubes was seen as long tube like projections extending from the yeast cells with no constriction or septa at the point of attachment to the yeast cells. The germ tube is an indicative of *Candida*. *Albican.*

CHAPTER FOUR

RESULT

Table 1: Physical appearance of urine and the culturing result

Sample	Ages	Colour	Culturing result
f1	19	Clear	-
f2	20	Light Yellow	+
f3	20	Light Yellow	+
f4	18	Clear	+
f5	19	Amber	+
f6	21	Amber	+
f7	18	Light Yellow	+
f8	19	Light Yellow	+
f9	19	Light Yellow	+
f10	17	Amber	+
f11	16	Light Yellow	-
f12	21	Clear	-
f13	17	Clear	-
f14	20	Clear	-
f15	20	Clear	+
f16	23	Light Yellow	+
f17	24	Light Yellow	-
f18	21	Clear	+
f19	23	Clear	+
f20	21	Light Yellow	-
f21	17	Clear	+
f22	23	Clear	+
f23	22	Amber	+
f24	19	Light Yellow	+
f25	18	Clear	-
f26	20	Clear	+
f27	21	Light Yellow	+
f28	22	Clear	+
f29	23	Clear	+
f30	21	Clear	-
f31	22	Amber	+
f32	20	Clear	+
f33	20	Clear	+
f34	20	Amber	+
f35	22	Clear	+
f36	21	Amber	-
f37	23	Light Yellow	-
f38	21	Amber	-
f39	22	Light Yellow	+
f40	18	Light Yellow	+
f41	19	Amber	+
f42	24	Amber	+
f43	22	Clear	+

f44	22	Light Yellow	+
f45	21	Amber	+
f46	20	Light Yellow	+
f47	23	Clear	-
f48	21	Light Yellow	+
f49	22	Light Yellow	-
f50	18	Light Yellow	-

- + = presence of fungi, - = absence of fungi

From table 1 above it was observed that the participants of this study where within the age of 16 to 24 and majority of the collected urine sample appeared either clear of light yellow. 40% of the total collected samples were Clear in appearance, 38% were Light yellow while 22% were Amber in appearance. The result above further showed that although fungi growth was observed in all types of colours, Amber colour has the highest occurrence of fungi growth with 81.8% occurrences followed by Light yellow with 68.4% occurrence with Clear coloured urine have the least percentage occurrence of fungi growth 65%.

Table 2: Morphology of isolate and their respective count

Sample	Age	Total viable fungi	Shape

		count (cfu/ml)	
f2	20	1.5×10^3	Oval
f3	20	8×10^3	Oval
f4	18	5.1×10^3	Circular
f5	19	1×10^1	Oval
f6	21	TNC	Circular
f7	18	4.6×10^3	Oval
f8	19	4×10^3	Circular
f9	19	4.1×10^3	Oval
f10	17	1.6×10^4	Oval
f15	20	2×10^1	Circular
f16	23	8×10^2	Oval
f18	21	1×10^1	Oval
f19	23	2×10^1	Oval
f21	17	4.6×10^2	Oval
f22	23	1.16×10^3	Oval
f23	22	2×10^1	Oval
f24	19	1.6×10^3	Circular
f26	20	1.0×10^2	Oval
f27	21	1.6×10^2	Oval
f28	22	9×10^3	Oval
f29	23	7×10^3	Oval
f31	22	7×10^1	Circular
f32	20	1.2×10^4	Oval
f33	20	2.4×10^3	Oval
f34	20	6×10^1	Circular
f35	22	3.4×10^4	Oval
f39	22	1.38×10^4	Circular
f40	18	1×10^1	Oval
f41	19	1.1×10^4	Circular
f42	24	1.2×10^2	Oval
f43	22	2.2×10^2	Circular
f44	22	4.3×10^2	Oval
f45	21	8×10^1	Oval
f46	20	1.32×10^4	Circular
f48	21	7.8×10^2	Oval

The morphology of the isolated fungi species is displayed in table 2 above. The result showed that the isolated species are either circular or oval in shape. However, majority of the isolated fungi species are Oval in shape with a percentage value of 68.6% while those circular in appearance were 31.4%. It was further observed that sample with fungi count greater than a

bench mark of 5.1 x 10^3 cfu/ml were from subject of 20 years and above except for sample f4 which was slightly above the bench mark with a value of 5.1 x 10^3 cfu/ml and from a subject of 18 years of age.

Table 3: Result of Sugar Test and Germ Tube Test for the Identification of Candida Species

Sample	Galactose	Maltose	Lactose	Sucrose	Glucose	GTT result	Candida species
f2	+	-	-	+	+	-	*Candida guilliermondii*
f3	+	+	-	-	+	+	*Candida albican*
f4	+	-	-	+	+	-	*Candida guilliermodii*
f5	+	+	-	+	+	-	*Candida tropicalis*
f6	+	-	-	+	+	-	*Candida guilliermondii*
f7	+	-	-	+	+	-	*Candida guilliermondii*
f8	+	+	-	+	+	-	*Candida tropicalis*
f9	+	+	-	-	+	+	*Candida albican*
f10	+	+	-	-	+	+	*Candida albican*
f15	+		-	-	+	-	*Candida guilliermondii*
f16	+	+	-	-	+	+	*Candida albican*
f18	+	+	-	-	+	+	*Candida albican*
f19	+	+	-	-	+	+	*Candida albican*
f21	+	+	-	-	+	+	*Candida albican*
f22	+	+	-	-	+	+	*Candida albican*
f23	+	+	-	-	+	+	*Candida albican*
f24	+	-	+	+	+	-	*Candida pseudotropicals*
f26	+	-	-	-	+	-	*Candida Parasililosis*
f27	+	+	-	-	+	+	*Candida albican*
f28	+	+	-	-	+	+	*Candida albican*
f29	-	-	-	-	+	-	*Candida krusei*
f31	-	+	+	+	+	-	*Candida tropicalis*
f32	+	+	-	-	+	+	*Candida albican*
f33	+	+	-	+	+	-	*Candida tropicalis*
f34	-	+	+	+	+	-	*Candida tropicalis*
f35	-	-	-	-	+	-	*Candida krusei*
f39	+	+	-	+	+	-	*Candida tropicalis*
f40	+	+	-	-	+	+	*Candida albican*
f41	+	-	+	+	+	-	*Candida pseudotropicals*
f42	-	-	-	-	+	-	*Candida glabrata*
f43	+	+	-	+	+	-	*Candida tropicalis*
f44	+	+	-	-	+	+	*Candida albican*
f45	+	-	-	-	+	-	*Candida Parasililosis*
f46	-	-	-	-	+	-	*Candida glabrata*
f48	+	-	-	+	+	-	*Candida guilliermondiI*

- **GTT = Germ Tube Test**

The table 3 above shows the sugar test used for the identification of the candida species and Germ tube test used for its confirmation for *Candida albican*. It was observed that out of the 50 subjects used in this study only 35 (70%) of the samples has candida infection while 15 (30%) were free of candida infection.

Table 4: Frequency of Occurrence of Candida species

Candida specie	Frequency of Occurrence (%)
C. guiliermondii	6 (17.1)
C. krusei	2 (5.7)
C. prarasiliosis	2 (5.7)
C. tropicalis	7 (20)
C. albican	14 (40)
C. glabrata	2 (5.7)
C. pseudotropicals	2 (5.7)
Total	35 (100)

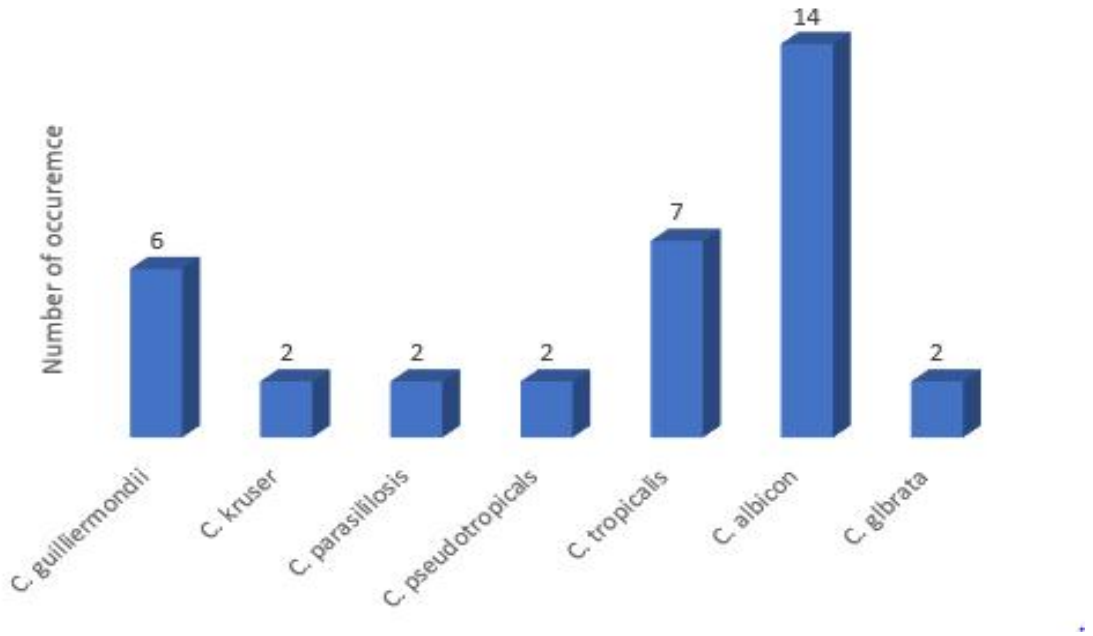

Figure 4.1: Frequency distribution of Candida species

Result in table 4 and figure 1 revealed that *Candida albican* was the most frequently occurring candida species in the urine samples studies. *Candida albican* had an occurrence of 14 (40%), followed by Candida tropicals and Candida guilliermondii with occurrence of 7 (20%) and 6 (17.1%). While C. Krusei, C. parasiliosis, C. pseudotropical and C. glabrata made up the least occurring species with 2 (5.7%) occurrence each.

Table 5: Frequency Occurrence of Candida Species within Age Groups

Fungi	Freq. occurrence within Age 17-20	Freq. occurrence within Age 21-24
C. guiliermondii	4	2
C. krusei	**0**	2
C. prarasiliosis	**1**	1
C. tropicalis	4	3
C. albican	6	8
C. pseudotropicalis	2	0
C. glabrata	1	1
Total	18	17

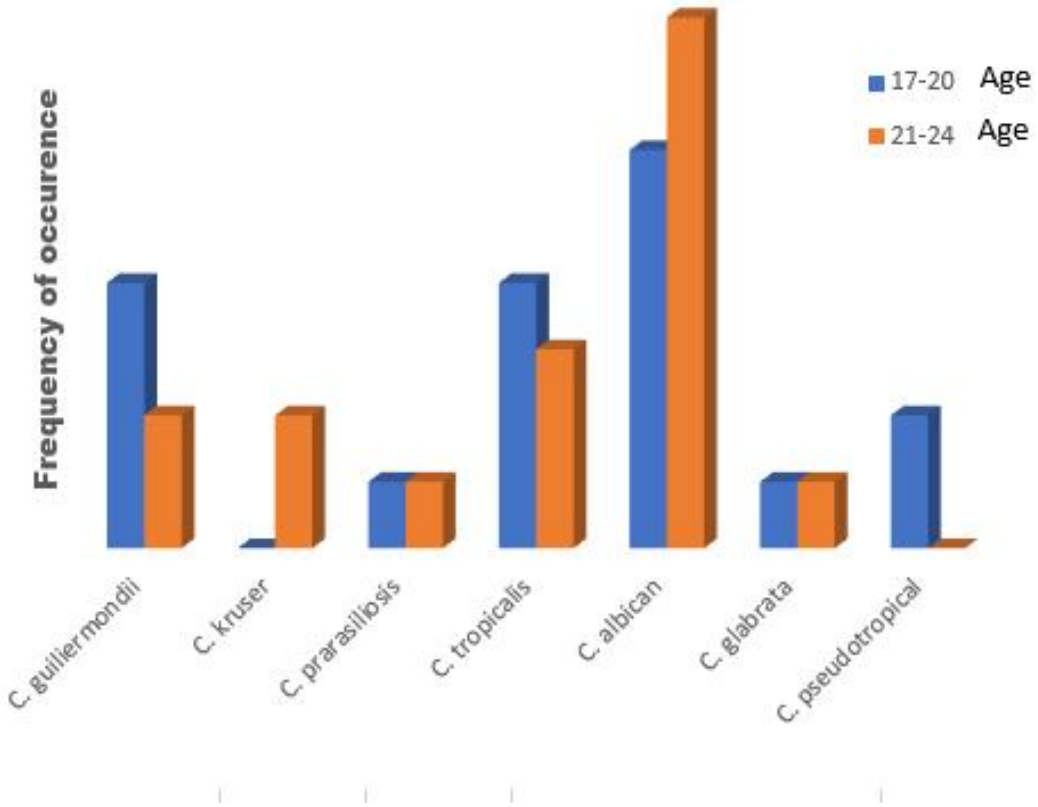

Figure 4.2: Frequency distribution of Candida species within age groups

Table 5 and figure 2 show the frequency distribution of Candida species withing two age range groups 17-20 and 21-24. The result revealed that *Candida albican* was most abundant in the higher age group 21-24 with a value of 8 count (57.1%) and least in lower age group with 6 count (42.9%). *C. kruser* was absent in the lower age group, while *C. pseudotropical* was absent in the higher age group. *C. guilliermondii* and *C. tropicalis* were found to be more abundant in the lower age group than they are in the higher age group.

CHAPTER FIVE

DISCUSSION OF RESULT

The isolation and distribution of candida in the human urine (female) in University of Benin is an important discovery which exposed array of candida which is an opportunistic microorganism to humans and animals.

In this study, a total of 50 human urine sample were taken from female student in university of Benin. Physical screening was first carried out on the various sample which revealed that 40% of the collected sample appeared clear, 38% of the sample were light yellow in colour while 22% of the collected samples were amber coloured. It was observed that the appearance of the urine was not a determining factor if there's candidas present or not as growth was found in all type of colour. However, Amber colour tend to have the highest percentage of candidas growth with a percentage occurrence of 81.8% followed by the yellow-coloured urine with and occurrence of 68.4% while the clear urine had the last occurrence of candida growth with an occurrence rate of 65%. It is tempting to conclude that the darker the urine colour has the higher possibility of candida presence. But it's known from literature that other factors affect the colour of the urine such as dehydration, use of drugs etc.

Culturing of the samples reveals that out of the 50 subjects, only 35 (70%) of the sample had candidas growth. The morphological examination of the various isolate showed that the various organism isolated from the 35 sample were either Oval or Circular in shape. With most of the organism being Oval in shape with a percentage occurrence of 68.6% and 31.4% occurrence for those with circular shape this is consistent with the findings of Mahmoudabadi *et al*. (2012) who discovered a larger amount of oval candida species in urine samples. Microscopic examination of

the various culture showed that sample with candida count greater than a bench mark of 5 x 10^3 cfu/ml were from subject of 20 years and above except for sample f4 which was slightly above the bench mark with a value of 5.1 x 10^3 cfu/ml and from a subject of 18 years of age.

The identified fungal organisms associated with female urine in this study include *Candida guilliermondii*, *Candida tropicalis*, *Candadi pseudotropical, Candida kruser, Candida albican, Candida glabrata and Candida parasiliosis.* suggesting that these fungal organisms could be responsible for toilet infections and other types of infections affecting both the female and male gender. This finding is in conformity with previous works of Kauffman (2014) and Sardi *et al*. (2013) which reported isolation of *Candida tropicalis*, *Candida pseudotropical, Candida kruser* and *Candida albican.*

Initial screening by sugar test and Germ tube test and other conventional methods led to the isolation and identification of Candida strains. Out of 35 (70%) subjects with positive urine culture test, all were positive for Candida strains *Candida albicans* (n=14, 40%), followed by *Candida tropicalis* (n=7, 20%) and *Candida guiliermondii* (n=6, 17.3%). *While Candida kruser, Candida glabrata, Candida pseudotropicals* and *Candida parasiliosis* had (n=2, 5.7%) each as shown in table 4.

Table 5 and figure 2 show the frequency distribution of Candida species withing two age groups 17-20 and 21-24. The result revealed that *Candida albican* was most abundant in the higher age group 21-24 with a value of 8 count (57.1%) and least in lower age group with 6 count (42.9%). *Candida kruser* was absent in the lower age group, while *Candida pseudotropical* was absent in the higher age group. *Candida guilliermondii* and *Candida tropicalis* were found to be more abundant in the lower age group than they are in the higher age group. The mean age of the studied subject was 20.46±1.951 years.

Out of the 50 subjects 24 were within 16-20 years of age while 26 were within 21-24 years of age. However, it was later observed that out of the 35 subjects with candida, only 17 were within the age of 21-24 and 18 within the age of 17-20. This shows that there was more prevalence of candida species in the higher age group than the lower one. This is partially contrary to the findings of Salehi *et al*. (2016) who discovered increased in candida species in elderly women than in younger women as the prevalence rate of UTIs was higher in elderly people. Regarding this, aging, is accompanied by the appearance of glucose in the urine. The growth of Candida strains begins when urine glucose rises to more than 150 mg/dl (Emami *et al*., 2016). According to Emami *et al*. (2016), it is impossible to say with certainty that our findings contradict those of Salehi *et al*. (2016) because we only took into account females under the age of 25. Therefore, the trend in our age group statistics may just be a result of chance.

CONCLUSION

In summary, the obtained results demonstrated that despite a trend was found between the colour of urine and the prevalence of candida species it can't be categorically ascertained to an indicator and more research is needed to fully understand the relationship between the appearance of urine and the prevalence of candida species. Furthermore, despite the increase in the number of Urinary Tract Infection cases caused by non-*Candida albicans* species, this species still ranks first for fungal Urinary Tract Infection as it was found to be the most abundant in this study. In addition, such infections rarely occur in the absence of any predisposing factors. More research in therefore needed to fully understand the various factors that predisposes the female gender to candida infections such as Urinary Tract Infection.

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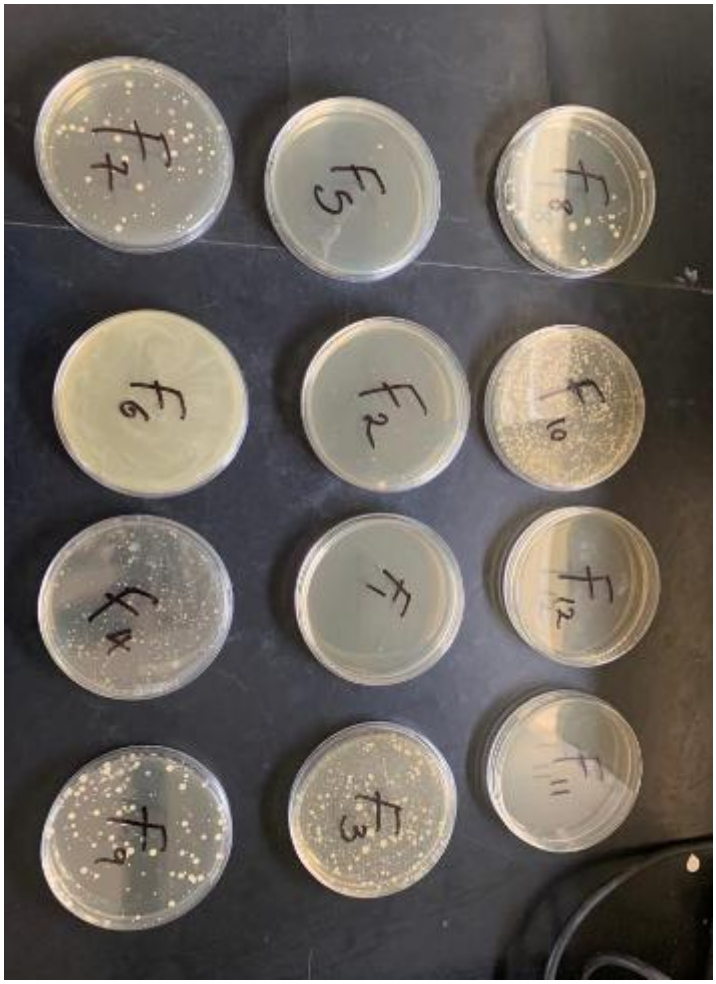
Appendix I

Growth from the cultured urine

Showing the positive and negative appearance of a sugar test

Growth from cultured plate

Growth from cultured urine



Growth from cultured urine.