DETECTION OF BACTERIA IN URINE OF PREGNANT WOMEN WITH UTI BY USING CHROMOGENIC URINE AGAR MEDIUM

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DEDICATION

To my Parents
With Thankfulness

To my Brothers and Sisters
With great love

To my Friends and Colleagues
With Best Wishes
ACKNOWLEDGEMENTS

First of all, my thanks and praise to almighty Allah, the most beneficent, the merciful, for giving me health and strength to accomplish this work.

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Abstract

One hundred urine samples were collected from pregnant women attending the Department of Obstetrics and Gynaecology of the Military Hospital, at Omdurman. All samples were cultured on chromogenic urine agar medium to detect and identify types of bacteria involved in urinary tract infections.

Eighty three samples (83%) gave positive bacterial cultures and all of them were microscopically positive for bacteria, and seventeen samples (17%) yielded no bacterial growth.

The isolated bacteria from urine belonged to ten genera. Gram negative bacteria, namely: Escherichia coli, Klebsiella pneumonialae, Proteus mirabilis, Proteus vulgaris, Enterobacter cloacae and Pseudomonas aeruginosa; and Gram positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae and Enterococcus faecalis. One yeast species isolated was (Candida albicans). The identification of bacteria on chromogenic urine agar medium was determined by the observation of colony color and subsequent differentiation of bacteria that gave the same colony color by biochemical tests.

The frequency of isolation percentage of bacteria from the samples varied from 45% (E.coli) to 1% (P. vulgaris). From the findings of this study, it can be concluded that E.coli is the prime cause of UTI in pregnant women, other bacteria causing UTI varied in their percentage.

When the bacterial isolates were tested for their sensitivity to eight different antibiotics, all isolates were found to be sensitive to
gentamicin and nitrofurantoin and exhibited varied sensitivity to the remaining antibiotics (streptomycin, colistin, ampicillin, tetracycline, cotrimoxazole and naladix acid).
المستخلص

تمت متابعة عينة بول من النساء الحوامل اللائي تترددين على قسم أمراض النساء والتوليد بالمستشفى العسكري بأم درمان. جمعت العينات زرعت على أجار البول الملون للكشف السريع وتحديد أنواع من البكتيريا المسببة لالتهابات المسالك البولية.

ثلاثة وثمانون عينة (83%) أعطت نتيجة إيجابية للبكتيريا مزرعية وبالفحص المجهرى، و17 (17%) أعطت نتيجة سالية مزرعية ومجهريا.

تتمي البكتيريا المعزولة من البول إلى عشرة أنواع، بكتيريا سلبية الغرام، وهي: الإشريكية القولونية، الكليسيلا الرئوية، المثالية ميرابيليس، المثالية الشائعة، انتروباكتير كلثاكا، الزائفة الزنجارية، والبكتيريا إيجابية الجرام:

المكورات العنقودية الذهبية، المكورات العنقودية البشروية، العقدية، المكورات المعوية البرازية، المكورة السبحية أفلاكشيا و نوع واحد من الخمائر (المبيضة البيضاء).

تعرف على البكتيريا في وسط أجار البول الملون حددت بملاحظة ألوان المستمرات وتفرغ البكتيريا التي تعطي نفس اللون على الاجر بواسطة الاختبارات الكيميائية الحيوية.
اختلاف تواتر نسبة عزل البكتيريا من العينات مختلف من 45% للاشريكية
القولونية إلى 1% للمنتقلة الشائعة من النتائج في هذه الدراسة، يمكن استنتاج

عند النساء الحوامل، والبكتيريا الأخرى التي تسبب عدوى المساكن البولية
مختلفة في نسبتها.

عندما تم اختبار العزلات البكتيرية لحساسيتها لثمانية مضادات حيوية مختلفة،
كل العزلات وجدت حساسة لجنتاميسين و النتروفيورانتوين وتفاوتت حساسيتها
المضادات الحيوية المتبقية (الاستربوميسين، الكولستين، أمبيسيلين،
النتريسيكليلين، الكوتريموكسازول و حمض النالاديكس).
Introduction

The presence of significant number of bacteria (>10^5 organisms per milliliter) in urine reflects urinary tract infection (UTI) (Kass and Finland, 1956). UTI is among the most common infections encountered by physicians (Neu, 1992); it can be asymptomatic or symptomatic (Khan and Shah, 2000).

The UTI are encountered in all ages and both sexes. Urine for bacterial culture is among the most common specimen submitted to the clinical microbiology laboratories and 10 to 20% are subsequently found to be positive for bacteriuria (Aathithan, Plant, Chaudry, and French, 2000). Among the elderly, UTI frequency is roughly of equal proportions in women and men. Neonatal UTI is more common in males than females due to the greater frequency of congenital anomalies of urinary tract of the male infants (Nicolle, 2008). Women are more prone to UTIs than men because in females, the urethra is much shorter and closer to the anus than in males, and they lack the bacteriostatic properties of prostatic secretions (Nicolle, 2008).

Testing for asymptomatic bacteriurias is not usually important, but it is a must during pregnancy and people undergoing urologic surgery, because the condition may lead to a serious infection (Smith, 1994). Diagnosis of urinary tract infection is a frequent microbiological analysis. It usually involves culture of microorganisms on several agar media followed by biochemical tests on different colonies. Urine culture is the gold standard method for diagnosis of urinary tract infection.
The combination of different enzymes and substrates for simultaneous
detection of bacteria in the same medium for the detection of UTI,
minimizes the need for further identification tests and reduce the time
required for diagnosis and initiation of antibiotic therapy compared
with traditional methods (Brown, 2007). A wide range of chromogenic
media is now available commercially for the detection of many
organisms of significance.

A new chromogenic medium was evaluated for the primary isolation
and rapid identification of urinary pathogens (Brosnikoff, Rennie,
Shokoples, and Turnbull, 2005). *Escherichia coli* is the most common
organism associated with urinary tract infections followed by other
Enterobacteriaceae and *Enterococcus* spp. Chromogenic media allows
for the rapid identification of these organisms (Brosnikoff et al., 2005).

**The objective of the study:**

To assess the validity and, hence, the applicability of chromogenic
urine agar medium for the rapid and reliable diagnosis of bacterial
urinary tract infection.
CHAPTER ONE
LETRATURE REVIEW

1.1 Urinary Tract
The soluble wastes are excreted by way of the urinary system, which consists of the kidneys, ureter, urinary bladder, and urethra. The kidneys extract the soluble wastes from the blood, as well as excess water, sugars, and a variety of other compounds. Urine is usually sterile and nearly odorless. Subsequent to elimination from the body, urine can acquire strong odors due to bacterial action; it usually washes the potentially harmful bacteria from the body. The body has other defense mechanisms against urinary tract infection (UTI) e.g. the prostate gland secretes substances that fight infection, the ureters prevent the urine from backing into the kidneys and the immune system fights infection causing organisms (Bates, Bradle, Glen, Melchior, Rowan, Sterling and Hald, 1976).

1.2 Urine composition
Urine is composed of organic constituents such as urea, protein (albumin), ammonia, uric acid, creatinin and inorganic constituents such as sulphur, iron, sodium chloride, phosphate and calcium. All constituents are dissolved in water to form a volume of 600-2500 ml / 24 h. Urine dry weight 55-70 g. The pH of urine ranges between 4.6-8.0 with a mean of 6.1. The cellular constituents in urine are: red blood cells, leukocytes, epithelial cells and casts. Usually microbial presence in normal urine is scanty (Sood, 1994). Although urine is a good medium for multiplication of pathogenic bacteria, it also contains some constituents that decrease the number of bacteria in males and females such as oesterogen, which when present, stimulates
the proliferation of lactobacillus in the vaginal epithelium. Lactobacillus reduces the pH of genitourinary tract and thus interferes with colonization of *E. coli* to the epithelium. Moreover, some lactobacilli strains produce hydrogen peroxide, which also contributes to lower bacterial number in urinary tract. Some compounds including immunoglobulin A (IgA) produced by the prostate give males a kind of protection by preventing bacteria from colonizing the urogenital tract (Garner and Hafez, 2000).

Urine is normally sterile. The body accomplishes this firstly by sphincter muscles that prevent urine from leaking from the bladder to the urethra. Also prevent the bacteria normally colonizing the skin from ascending through the meatus into the bladder. Secondly, the length of the urethra makes it difficult for bacteria to get into the bladder. The shortness of women urethra accounts for five-fold increase of urinary tract infection among women compared to men. Finally, there are valves in the bladder that prevent the reflux of urine, and back up into the kidneys of any bacteria. During urination, the bladder empties completely, so that any bacteria present should be excreted as well. In spite of all these defense mechanisms, infection sometimes occurs (Brooks, Geo, Butel, Ornston, Jawetz, Melnic, and Adellerg, 1995).

**1.3 Infection of the urinary tract**

Urinary tract infections (UTIs); are a variety of clinical entities whose common denominator is microbial invasion of tissues of the urinary tract, extending from the renal cortex of the kidneys to the urethral meatus (Kunin, 1979). UTIs are frequently encountered in
approximately 10% of office visits by women in the United State of America, and 15% of women have UTI at some time during their life in USA. In pregnant women, the incidence of UTI can be as high as 80%. The risk for UTI increase being in week 6 and peak during weeks 22 to 24 (Delzell and Lefevre, 2000).

1.3.1 Types of urinary tract infection
UTI may be categorized as acute urethral syndrome which occurs in women with acute lower urinary tract symptoms with a low bacterial count or without demonstrable bacteriuria or volvovaginal infection (Meares, 1998; Stamm, Wagner, Amsel, Alexander, Turck and Counts, 1980), uncomplicated in otherwise healthy individuals and complicated in patients with residual inflammatory changes following recurrent infection or instrumentation, obstruction, stones, or anatomical or physiological abnormalities or pathological lesions. These interfere with drainage of urine in a part of the tract which stimulates prolonged colonization. Recurrent infection may occur (Warren, 1996).

Urethritis is infection of the urethra, common in both male and female patients and often associated with UTI (Meares, 1998) or occasionally with bacterial prostatitis. Cystitis is infection of the bladder and pyelonephritis is infection of the kidney. Symptomatic UTI may be acute or chronic. The term relapse implies recurrence of infection with same organism. The term reinfection implies infection with another organism (Brooks et al., 1995).
1.3.2 Route of infection
Urinary tract can be infected from an infected source in the body and also can be infected by bacteria entering the urethra and spreading upwards. Infection type (a) is most often seen in new-born babies with systemic infection known as sepsis. Type (b) is much more common and most often seen in small children and adults. In young sexually active women, sex is the cause of 75-90% of bladder infections, with the risk of infection related to the frequency of sex (Nicolle, 2008).

1.3.3 Factors behind recurrent UTI
One factor may be the ability of bacteria to attach cell lining of the urinary tract. In a recent study it was shown that women with recurrent UTI tend to who have certain blood types were more prone to UTIs, because the cells lining the vagina and urethra may allow bacteria to attach more easily (Cheesbrough, 2000).

1.3.4 Casts in urine
Urine casts consists of solidified protein and cylindrical in shape because they were formed in the kidney tubules. The following casts can be found in the urine: Hyaline casts are colorless and empty, associated to glomerular filter membrane. A few may be seen following strenuous exercise during fever (Cheesbrough, 2000). Waxy casts are hyaline and remain in the kidney tubules along time. They are thicker and denser than hyaline casts, often appear dented or twisted, and may be yellow in color. They usually indicate tubular damage and can sometimes be seen in renal failure. Cellular casts contain white or red blood cells. Red cell casts appear red in color. They indicate hemorrhage in renal tubules or glomerular
bleeding. White cells are found when there is inflammation of the kidney pelvis or tubules.
Granular casts: contain granular irregular size originating from degenerated cells and proteins, associated with renal damage (Cheesbrough, 2000).

1.3.5 Incidence of UTI
The incidence of UTIs is influenced by age, sex or by predisposing factors that may impair the wide variety of normal host defense mechanisms (Sobel, 1987).

1.3.5.1 Urinary tract infection in children
UTI is often associated with renal tract abnormalities in children. It is commonest in males in the first three months of life as a result of congenital abnormalities. In older children, females are more commonly affected. Infection in preschool boys is often associated with renal tract abnormality(Sobel and Kaye, 2000). Failure to diagnose and treat UTI quickly and effectively may result in renal scarring and ultimately loss of function. The phenomenon of vesicoureteric reflux, while predisposing children to UTI, may also be caused by UTI(McCracken, 1987; Jakobsson, Berg and Svensson, 1994).

1.3.5.2 Urinary tract infection in adults
The incidence of UTI is highest in young women. Around 10-20% of women will experience a symptomatic UTI at some time. Most infections in adult men are complicated and related to abnormalities of the urinary tract although a low incidence occurs spontaneously in otherwise healthy young men(Krieger, Ross and Simonsen, 1993).
1.3.5.3 Urinary tract infection in elderly

UTI incidence increases with age for both sexes. It is estimated that 10% of males and 20% of females over the age of 65 have asymptomatic bacteriuria; no treatment is indicated for asymptomatic patients except before invasive genitourinary procedures (Nicolle, 1992).

1.3.5.4 Urinary tract infection in pregnant and non-pregnant women

Pregnant women are at increased risk for UTIs. Beginning in week 6 and peaking during weeks 22 to 24, approx. 10% of office visit by women, and 15% of women will have UTI can be as high as 8% (Patterson and Andriol, 1978). Studies in the UK showed that asymptomatic bacteriuria occurs in 2-5% of pregnant women (Little, 1966). Sheikh, Klan, Katoon and Arian (2000) in their study, of the incidence of urinary tract infection during pregnancy, examined 350 urine samples from Pakistani women. Midstream urine was collected from 250 pregnant women and 100 controls and streaked on blood agar. Growth was considered significant if $10^5$ /ml bacteria were present. Among the pregnant women, 28.5% had UTI. Socio-economic status, personal hygiene, education level, postcoital washing, contraceptive use and use of underclothing had significant association with UTI occurrence. A history of past urological problems was associated with increased incidence of UTI in pregnancy.

For pregnant women, 2-4% usually develop UTI which is likely to travel to the kidneys resulting in pyelonephritis due to hormonal
changes and shift in the position of the urinary tract that makes it easy for travel upwards (Nkudi and Afud, 1994).

1.3.6 Symptoms and signs of urinary tract infection
Urinary tract infections are usually untreated because they present little or no symptoms. These symptoms generally depend on the age of the person infected and the location of the urinary tract infected. It includes, burning sensations while urinating, fever, lower abdominal pain, jaundice due to loss of blood, especially in children, dysuria, itching, formation of blisters and ulcers in the genital area, genital and suprapubic pain, dysparelinia (painful coitus) and pyuria (Meadow, 1997).

1.3.7 Diagnosis of urinary tract infection
In straight forward cases a diagnosis may be made and treatment given based on symptoms alone without further laboratory confirmation. In complicated or questionable cases confirmation via urinalysis looking for the presence of nitrites, leukocytes or leukocyte esterase or via urine microscopy looking for the presence of red blood cells, white blood cells, and bacteria may be useful. Urine culture showing a quantitative count of greater than or equal to \(10^3\) colony forming units (CFU) per ml of a typical urinary tract organism along with antibiotic sensitivities is useful to guide antibiotic choice. However women with negative cultures may still improve with antibiotic treatment (Nicolle, 2008).

1.4.7.1 Microscopic examination for UTI
Pfaller, Baum, Niles and Murray (1983) compared the (Bact-T-screen) bacterial detection device for urine of 631 urine samples they
analysed; the urine Gram stain correctly identified 92.1% of all positive cultures. The predictive value of a negative urine Gram stain was 98.4%. Bachman, Heise, Naessens and Timmerman (1993) reported 83.3% sensitivity and 94.9 specificity for this test.

1.4.7.2 Leukocyte esterase-nitrate activity as screen-test for UTI

Smalley and Dittmann, (1983) in their study, using leukocyte esterase-nitrate activity as predictive assay of significant bacteriuria evaluated 484 urine samples in which 113 urine accomplished positive by culture (23.4%). They found that 93 (82.3%) were detected by leukocyte esterase-nitrate tests. Furthermore, 365 of 371 (98.4%) urine samples with negative bacterial cultures were negative in leukocyte esterase and nitrate tests. Urine culture represents a large portion of the specimens processed in most clinical microbiology laboratories. In an effort to provide relevant microbiology data rapidly, many screening tests for the detection of bacteriuria had been developed and evaluated. Bact-T-screen, chemstrip L N and Gram stain, had been used as screening tests for the detection of bacteriuria. Studies that compared these three screening tests with quantitative urine cultures had revealed that tests can detect bacteriuria reliably when $>10^5$ CFU/ml are present (Murray, Smith and Mackinney, 1987). A positive dipstick did not reliably detect significant bacteriuria in 479 (18.9%) of urine samples of women with suspected uncomplicated UTI. On bacteriological culture on artificial media, the samples demonstrated significant bacteriuria and would have been rejected by the laboratory based on a negative urine analysis screen (Semeniuk and Church, 1999).
1.4.7.3 Estimating Bacterial Number

It is necessary to estimate the approximate number of bacteria in urine, because in normal specimens, numbers of all contaminating organisms should be less than $10^4$/ml of urine. Urine from person with untreated UTI contains $10^5$/ml, or more (Nicholson, 1989). The approximate number of bacteria per ml of urine can be estimated by using calibrated loop or measured piece of filter paper. Both methods are based on the assumption that a single colony represents one organism (Cheesbrough, 2000).

1.4.7.4. Detection of bacteria by using chromogenic agar media

1.4.7.4.1. The chromogenic media

Although use of conventional selective, differential media has resulted in a reduction in the volume and extent of confirmatory testing required, their overall specificity remains comparatively limited. Despite their limitations, many of these types of media remain useful microbiological tools and continue to be used extensively today. Given the widespread use of fluorogenic and chromogenic substrates in biochemistry, it was somewhat surprising that their application to microbiology did not really take off until 1980 (Brown, 2007).

1.4.7.4.2 Definition of The chromogenic media

Chromogenic and fluorogenic media are microbiological growth media that contain enzyme substrates linked to a chromogene (color reaction), fluorogen (light reaction) or combination of both. The target population is characterized by enzyme production that metabolizes the substrate to release the chromogen/ fluorogen. This results in color change in the medium and/ or fluorescence under long wave U.V light
A chromogenic substrate is defined as a compound or substance that contains a color-forming group (Ziad, Hughes, Porceddu and Nicholas, 2007).

1.4.7.4. 3 Usage of the chromogenic culture media

Over the last few years, several chromogenic media have been developed and commercialized, allowing more specific and direct differentiation of microorganisms on the primary plates (Collee, Miles and Watt 1996; Ananthanarayan and Paniker, 2000). The catalyst for research in this field was the desire by water microbiologists to develop a rapid screening method for the faecal indicator E. coli (Bovill and Druggan, 2005). Since the work of Feng and Hartman, (1982) that pioneered the use of 4-methylumbelliferone- β, D-glucuronide for the detection of E. coli in water and food samples, an explosion of research and development in the field of chromogenic culture media has ensued.

A new medium containing 5-bromo-4-chloro-3-indolyl-b-D-glucuronide cyclohexylammonium salt (Glu agar) for E. coli and a new medium containing 5-bromo-3-indolyl-b-D-galactoside (Gal agar) for β-galactosidase-positive members of the family Enterobacteriaceae were compared with MacConkey agar in a diagnostic trial with 3562 urine specimens. The isolation rates of E. coli and β-galactosidase-positive Enterobacteriaceae were increased 8.4% and 19.5%, respectively. The sensitivities and specificities of Glu agar and Gal agar were 98.5% and 100% and 99.2% and 99.5%, respectively (Kodaka, Ishikawa, Iwata, Kashitani, Mizuochi, and Yamaguchi 1995).
Several agar media containing chromogenic substrates for *Salmonella*-specific enzymes have recently been developed. Rambach agar (Freydiere and Gille, 1991; Rambach, 1990) and *Salmonella* detection and identification medium (SM ID agar) (Duschand Altwegg (1993); Monnery, Freydiere, Baron, Rouset, Tigaud, Boude-Chevalier, DeMontclos and Gille (1994); Poupart, Mounier, Denis, Sirot, Couturier, and Villeval, 1991) were the first media of this type. Rambach agar uses a chromogenic substrate for β-galactosidase (X-Gal), in conjunction with propylene glycol, which was fermented by *Salmonella* spp.to generate acid (Rambach, 1990).

1.4.7.4.4 Usage of the chromogenic mediafor detection of UTI

The greatest advantages of chromogenic media over cysteine lactose electrolyte-deficient agar (CLED) were easier recognition of mixed growth, shorter analysis duration, workload reduction and higher detection rates. However, chromogenic media from different manufacturers vary in performance and cost (Hengstler, Hammann and Fahr (1997); Perry, Butterworth, Nicholson, Appleby and Orr, 2003). Incorporation of more than one chromogen in a medium can improve its specificity and differential properties. A medium containing 5-bromo-4-chloro-3-indoxyl β-Dglucopyranoside and 6-chloro-3-indoxyl β- D galactopyranoside is useful for the differentiation of potential urinary tract pathogens. Organisms that express β-galactosidase cleave the red/rose-GAL substrate to produce pink/red colonies (*E. coli*), while expression of β-glucosidase results in cleavage of the XGLUC to form green colonies (e.g.*Enterococcus* spp.). Expression of both enzymes results in dark blue-purple colonies.
indicator for *Klebsiella, Enterobacter or Serratia* spp (KES group). Staphylococci (with the main exception of *Staphylococcus saprophyticus*) and streptococci do not produce either enzyme and grow as white or colorless colonies. The specificity of conventional selective media has been improved by the addition of antibiotic supplements to inhibit unwanted organisms (Brown, 2007).

The combination of different enzymes and substrates for simultaneous detection of bacteria in the same medium has been applied successfully for the detection of UTI. CPS ID2 agar (bioMCrieux) allows the identification of *E. coli, Proteae* and *enterococci*, detecting β-glucuronidase (GUD), tryptophane deaminase (TDA), tryptophanase and β-glucosidase (βGLU). Detection of GUD for identification of *E. coli* is an excellent method since more than 95% of all *E. coli* strains produce this enzyme (Hartman, 1989). A number of rapid and convenient commercial tests for *E. coli* detection have been developed for clinical application using GUD activity. Dibb and Bottolfsen (1984) used Rosco tablets with urine samples. Similarly, a dip-slide medium was used by Adler-Mosca, Desgrchamps and Wist (1992) to identify *E. coli* in urinary cultures. Both of these rapid methods were judged as reliable alternatives to conventional biochemical identification of *E. coli*. CPS ID2 was the first medium claimed to reliably detect and partly identify more than one microorganism.

In recent years, a range of chromogenic media has been made commercially available for the improved isolation and identification of urinary tract pathogens. Such media incorporate chromogenic enzyme substrates, which assist in the identification of common
urinary tract pathogens and provide enhanced discrimination of mixed cultures (Perry et al., 2003; Aspevall, Osterman, Dittmer, Sten, Lindback and Forsum, 2002; Carricajo, Boiste, Thore, Aubert, Gille, and Freydiere, 1999). A recently developed medium, Uriselect 4, incorporates two chromogenic substrates for the detection of β-galactosidase and β-glucosidase. Strains that produce β-glucosidase, such as enterococci and the *klebsiella, enterobacter, serratia* (KES group), form colonies that generate a green/blue coloration as a result of hydrolysis of the indoxylic substrate. Strains of *E. coli* appear as pink colonies because of β-galactosidase production. Tryptophan is also present in the medium to detect members of the Proteae group, which generate a diffuse brown coloration as a result of tryptophan deaminase production. Among the bacteria most commonly isolated from urinary tract infections are *E. coli* and β-galactosidase-positive members of the family Enterobacteriaceae (Edberg and Kontnick, 1986; Haines, Covert and Rankin 1993; Kilian and Bu¨low, 1979). The latter include members of the genera *Cedeceea, Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Kluyvera, Rahnella, Salmonella III, Serratia, and Yersinia*. Recently, chromogenic substrate assays based on the enzymatic activities of β-glucuronidase for the rapid identification of *E. coli* in urine (Delisle and Ley, 1989), food (Frampton, Restaino and Blaszko, 1988; Restaino, Frampton and Lyon, 1990; Watkins, Rippey, Clavet, Kelley-Reitz and Burkhardt, 1988) and water (Haines et al., 1993; Ley, Bowers and Wolfe, 1988; Watkins et al., 1988) and that of β-galactosidase for the coliform group in food (Hahn and Wittrock, 1991) and water (Manafi and Kneifel, 1989) has been assessed. However, 5-bromo-4-chloro-3-indolyl- β-D-glucuronide cyclohexylammonium salt (Okrend, Rose
and Lattuada 1990; Restaino et al., 1990; Watkins et al., 1988) for *E. coli* and 5-bromo-3-indolyl-b-D-galactoside (Hahn and Wittrock, 1991; Manafi, and Kneifel. 1989) for β-galactosidase-positive Enterobacteriaceae has not been evaluated directly for use with urine samples.

### 1.5 Bacteria of The urinary tract:

The urinary tract of human maybe infected by a wide range of bacteria includes the following:

#### 1.5.1 *Escherichia coli*

*Escherichia coli* is a Gram-negative, flagellated, rod, motile, non-sporing bacterium (Sojka and Garnaghan, 1961). Uropathogenic *E. coli* is responsible for approx. 90% of UTI (Snyder, Haugen, Lockatell, Maroncle, Hagan, Johnson, Welch and Mobley, 2005). The serogroup most often responsible for UTI are: O1, O2, O4, O6, O7, O9, O11, O18, O39 and O75 (Grunberg and Bettelheim, 1969). These serogroup are predominant in the faeces and colon which was the reservoir of infecting organisms (Sleigh and Duguid, 1989).

Uropathogenic *E. coli* utilizes fimbriae to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D.galactose. D.galactose moieties on the p blood group antigen of erythrocytes and uroepithelial cells (Snyder, *et al* 2005). Approx. 1% of the human population lacks this receptor, to *E. coli* urinary tract infections. It is resistant to drying and chemical disinfectants. Pasteurization usually destroys it although some heat resistant strains may withstand such treatment. In the majority of instances a temperature of 55 ºC for one hour or 60 ºC for 20 minutes is lethal to
these bacteria. They are killed rapidly by autoclaving at 120 °C. Freezing in liquid air for 2 hours destroys 95 percent of cells. Some individual cells resist freezing in ice for six months. It can survive in water for several weeks or months, as well as faeces and dust in animal houses. Their high susceptibility to the efficacy of these disinfectants is reduced in the presence of mucus and faeces (Buxton and Frazer, 1977). However, the antibiotic sensitivities of different strains of \textit{E. coli} vary widely. They are resistant to many antibiotics. Antibiotics which may be used to treat \textit{E. coli} infection include amoxicillin as well as other semi-synthetic penicillin’s, many cephalosporin’s, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin (Johnson, Kuskowski, Menard, Gajewski, Xercavins, and Garau, 2006) and the aminoglycosides (gentamycin, netilmicin) Other antimicrobial agents used against \textit{E.coli} are sulphonamides and nalidexic acid (Sleigh and Duguid, 1989).

\subsection*{1.5.2 \textit{Klebsiella} spp}

They are member of the family Enterobacteriaceae, gram-negative bacteria encapsulated, nonmotile, and rod-shaped. (Baker, 1980) divided the genus \textit{Klebsiella} into six species in accordance to their biochemical reactions. According to these authers \textit{klebsiella pneumoniae} produce gas from glucose and is MR, urease and malonate positive; and VP and KCN negative. On the other hand, Sleigh and Duguid, (1989) defined \textit{klebsiella pneumoniae} as non-motile capsulated, lactose fermenter and VP negative.
*Klebsiellaspp* can cause a wide range of disease conditions, notably pneumonia, urinary tract infections, septicemia, ankylosing spondylitis, and soft tissue infections, (Podschun and Ullmann, 1998) *Klebsiella pneumoniae* is present in the respiratory tract and faeces of about 5% of normal individuals. It causes small proportion (2%) of bacterial pneumonias. *Klebsiella pneumoniae* can produce extensive haemorrhagic necrotizing consolidation of the lung. It occasionally produces UTI and bacteraemia with focal lesions in debilitated patients. *Klebsiella pneumoniae* and *klebsiella oxytoca* cause hospital acquired infections. (Brooks *et al.*, 1995).

*Klebsiella* can survive for several weeks or months at room temperature and are killed at 60 °C for 20 minutes, and they are susceptible to disinfectants.

1.5.3 *Pseudomonasspp*
Gram-negative, slender rods, motile with polar flagella; some are capsulated, aerobic, catalase-positive, urease variable, usually oxidase positive, pigmented and had disinfective smell (Baker, 1980; Barrow and Feltham, 1993; Cheesbrough, 2000). Pathogenic species include *Ps. aeruginosa*, *Ps. oryzihabitans*, and *Ps. Plecoglossicida*. *Ps. aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of human and is the major pathogen of the group. It is commonly present in moist environments in hospitals (Brooks, leo., Butel and Morse, 1998), and is a particular problem in this environment hence it is the second most common infection in hospitalized patients (nosocomial infections). This pathogenesis may in part be due to the proteins secreted by *Ps. aeruginosa*. The
bacterium possesses a wide range of secretion machineries and exports numerous protein substrates considered to be important in the pathogenesis of clinical strains (Hardie, Pommeir, and Wilhilm, 2009).

*Pseudomonas aeruginosa* cause disease in human with abnormal host defences. It is pathogenic only when introduced into areas devoid of normal defences, when mucous membranes and skin are disrupted by direct tissue damage; when intravenous or urinary tract catheters are used (Brooks *et al*., 1998).

Most *Pseudomonas* *spp*. are naturally resistant to penicillin and the majority of related beta-lactamantibiotics, but some are sensitive to piperacillin, imipenem, tobramycin, or ciprofloxacin. (Ryan and Ray, 2004). This ability to survive in harsh conditions is a result of their hardy cell wall that contains porins. Their resistance to most antibiotics is attributed to efflux pumps which pump out some antibiotics before they are able to act. One of the most worrying characteristics of *Ps. aeruginosa* is that it has low antibiotic susceptibility. This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (Anzai, Kim, Park and Wakabayashi, 2000).

**1.5.4 Proteusspp**

Gram-negative, rod shaped, commonly measuring 1-3µm in length and 0.5 µm in width, but often showing considerable poleomorphism, occurring sometimes in coccal forms or as filament measuring 10-20 µm in length. They are show swarming motility with peritrichous
flagellae and the majority produces fimbriae. Spores and capsules are not produced (Buxton and Frazer, 1977).

*Proteus* species do not usually ferment lactose, but have shown to be capable lactose fermenters depending on the species in a triple sugar iron (TSI) test. They are oxidase-negative, but catalase- and nitrase-positive. Specific tests include positive urease (which was the fundamental test to differentiate *Proteus* from *Salmonella*) and phenylalanine deaminase tests. On the species level, indole is considered reliable, as it is positive for *Proteus vulgaris* but negative for *Proteus mirabilis*. (Ryan and Ray, 2004). Many strains of *Proteus mirabilis* and *Proteus vulgaris* produce bacteriocin (protocin) which has lethal effect against other strains (Senior, 1977). Three species *Proteus vulgaris, Proteus mirabilis, and Proteus penneri* are opportunistic human pathogens, responsible for many human urinary tract infections. (Guentzel, 1996). *Proteus mirabilis* is the main *proteus* species of medical importance, common in young boys and males and associated with renal tract abnormalities. In hospitalized patients it may cause chronic infections (Leigh, 1990). It has the ability to produce high levels of urease. Urease hydrolyzes urea to ammonia (NH₃) and thus makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate, and/or apatite. The bacteria can be found throughout the stones, and these bacteria lurking in the stones can reinitiate infection after antibiotic treatment. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure. *Proteus* can also cause wound infections, septicemia and pneumonias, mostly in hospitalized patients.
*Proteus vulgaris* species is occasionally isolated from urine, pus and other specimens. *Proteus mirabilis* infection usually responds better to antimicrobial therapy than those caused by *Proteus vulgaris* and other related organisms (Cheesbrough, 2000).

### 1.5.5 *Enterobacter* spp

Gram negative rods, motile, aerobic and facultative anaerobe, catalase positive, oxidase negative, produce ornithine decarboxylate, citrate positive; attach sugars fermentatively with gas production, VP positive, gluconate positive, gelatin may be liquefied slowly. *Enterobacter aerogenus* has a small capsule (Baker, 1980; Barrow and feltham, 1993). *Enterobacter* species ferment sucrose, arabinose sorbitol and calicin, but do not ferment dulicitol (Baker, 1980). *Enterobacter* strains are mainly fimbriate and slime-forming and they generally do not show definite capsule in wet india ink films. It resembles *klebsiella aerogenes* in many of its biochemical characters. They have the same IMVIC reaction but differ from in producing ornithin decarboxylase. An *E. cloaca* (cloaca A) liquefies gelatin but slowly (after 7 days at 22 °C) (Sleigh and Duguid, 1989). *Enterobacter* species, particularly *Enterobacter cloacae* and *Enterobacter aerogenes*, are important nosocomial pathogens responsible for various infections, including bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. *Enterobacter* spp can also cause various community-acquired infections, including UTIs, skin and soft-tissue infections, and wound infections, among
others (Marce, Antoine, Schaeverbeke, Vernhes, Bannwarth, and Dehais, 1993).

1.5.6 *Citrobacter spp*

Gram-negative coliform bacteria, and rod shaped. The species *C. amalonaticus*, *C. koseri*, and *C. freundii* use solely citrate as a carbon source. *Citrobacter* species are differentiated by their ability to convert tryptophan to indole (Lipsky, Hook, and Smith, 1980). They are motile by peritrichous flagellae and are facultatively anaerobic. They are chemoorganotrophic. D-glucose and other carbohydrates are catabolized with the production of acid and gas.

They are opportunistic pathogens and occasionally isolated from urine, blood, pus and other specimens. *Citrobacter* species can be late or non-lactose fermenters, they require differentiation from *Salmonella*. *C. freundii* is the species most frequently isolated and many of them ferment sucrose.

1.5.7 *Gardnerella spp*

Gram-variable, facultatively anaerobic rods, non-capsulated, none spore forming, non-motile, catalase and oxidase negative, chemoorganotrophic, having a fermentative type of metabolism. They are fastidious in requirement but do not need X or V factors. Optimum growth temperature is 35-37°C. They ferment glucose and some other carbohydrates and produce acid without gas, do not reduce nitrate, and hydrolyze human blood but not sheep blood. They are found in the genital/urinary tract (Holt, 1994). Many studies have demonstrated the relationship of *Gardnerella vaginalis*, with other bacteria in causing bacterial vaginosis (Tabrizi, Fairley, Bradshaw, and Garland, 2006).
1.5.8* Neisseriaspp*

*Neisseriae* are Gram negative diplococci that resemble coffee beans (kidney-shaped) (Ryan and Ray, 2004). The pathogens are more exacting than commensals in their nutritional, atmospheric and temperature requirements, growing poorly on ordinary media unless enriched with serum. Important pathogens of *Neisseria* spp are *neisseria gonorrhoeae* and *Neisseria meningitidis*. The non-pathogens are normal inhibitants of the respiratory tract (Baker *et al.*, 1980). *Neisseriaspp* are catalase positive, oxidase positive, attack sugar by oxidation (Barrow and Feltham, 1993).

1.5.8.1* Neisseria gonorrheae*

*Neisseria gonorrheae* also known as Gonococcus, is responsible for the sexually transmitted disease gonorrhea (Ryan and Ray, 2004). It usually affects the mucus membrane of the urethra in males and the endothevix in female. Infection follows by attachment of the bacterium to non-ciliated epithelial cells via pilli (fimbriae) and production of endo toxin. *Neisseria gonorrheae* produces IgA proteases (Toddar, 1993). *Neisseria gonorrheae* morphologically and culturally resembles *Neisseria meningitidis*. Defferentiation is important because both of them are known to cause urethral infection and cerebrospinal infection (Baker, 1980). *Neisseria gonorrheae* ferments glucose but not lactose, maltose or sucrose. *Neisseria meningitidis* ferments glucose and maltose, but not lactose or sucrose. Both of them do not reduce nitrate (Barrow and Felltham, 1993) or ferment fructose and they do not grow at 22°C and are not pigmented.
1.5.10 *Staphylococci*

*Staphylococci* are spherical Gram-positive bacteria, nonmotile, non-spore-forming, non-capsulated and usually arranged in grape like irregular clusters (Jawetz, Melnick and Adelberge, 1990; Geo, Janet and Stephen, 1998), facultative anaerobes, chemoorganotrophic, with both respiratory and fermentative metabolism. Colonies are usually opaque and may be white or grey and sometimes yellow to orange. They are usually catalase positive and oxidase negative. They grow satisfactorily in nutrient media at an optimum temperature of 37 ºC. Growth may occur more slowly over a wide temperature range of 10-42 ºC (Holt, 1994). They hydrolyze arginine, produce acetoin and attach sugars by fermentation (Barrow and feltham, 1993).

*Staphylococcus aureus* is the primary pathogen in the genus *Staphylococcus*. *Staph.epidermides* and *Staph.saprophyticus* are less commonly found as pathogens. They, act sometimes, as opportunistic pathogens and cause infection in the urinary tract in debilitated or immunocompromised hosts, causing more serious bacteraemic infections (Sleigh and Duguid, 1989). *Staphylococcus aureus* is both catalase and coagulase-positive, all strains of *S. aureus* produce coagulase enzyme: nearly all strains of *S. epidermidis* lack this enzyme; colonies are golden and strongly hemolytic on blood agar, *S. epidermidis* is non haemolytic. Coagulase is a traditional marker for identifying *Staph. aureus* in the microbiology laboratory (Toddar, 1993).

*Staphylococcus saprophyticus* is the second most common cause of UTI in young people. It is responsible for about 20% of urethritis and cystitis in sexually active, otherwise healthy young women. Itadheres
to uroepithelial cells (Leigh, 1990). The majority of staphylococcus strains are killed by temperature at 60 °C for 30 min, although some strains are more resistant and not killed when subjected to temperature of 70 °C for 15 min (Toddar, 1993).

1.5.11 *Streptococci*

*Streptococcus* is a genus of spherical Gram-positive bacteria about 1µm in diameter, arranged in pairs, short, or long chains. Streptococci are oxidase- and catalase-negative, anaerobic and facultative anaerobes, obligatory anaerobes, nonmotile, some may be produce haemolysin and attack carbohydrate fermentatively. Individual species of *Streptococcus* are classified based on their hemolytic properties. Beta-hemolytic *streptococci* are further characterized via the Lancefield serotyping – based on specific carbohydrates in the bacterial cell wall (Facklam, 2002).

1.5.11.1 *Streptococcus agalactiae*

*Streptococcus agalactiae*, causes pneumonia and meningitis in neonates and the elderly, with occasional systemic bacteremia. It can also colonize the intestines and the female reproductive tract, increasing the risk for premature rupture of membranes and transmission to the infant (Schrag, Gorwitz, Fultz-Butts and Schuchat, 2002). *Streptococcus agalactiae* forms a β narrow zone of haemolysis or non hemolytic. It is CAMP test positive, ferments lactose and trehalose and does not ferment sorbitol or inulin, does not hydrolyse aesculin and does not grow at 45°C. It hydrolyses hypurate (Ross, 1989).
1.5.12 Enterococci

There are at least 12 species of enterococci. *Enterococcus faecalis* is the most common. *Enterococcus faecalis* is classified as part of the Group D *Streptococcus* system, is a Gram-positive commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. It can cause life-threatening infections in humans, especially in the nosocomial environment, where the naturally high levels of antibiotic resistance found in *Ent. faecalis* contribute to its pathogenicity (Ryan and Ray, 2004), particularly in intensive care units, and are selected by therapy with cephalosporin and other antibiotics to which they are resistant.

*Enterococci* are transmitted on medical devices. In patients, the most common sites of infection are; the urinary tract, wounds, biliary tract and blood. *Enterococi* may cause meningitis and bacteraemia in neonates. In adults enterococci can cause endocarditis.

*Enterococcus faecalis* is resistant to many commonly used antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semi-synthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole). Resistance to vancomycin is also becoming more common (Amyes, 2007; Courvalin, 2006). It is relatively sensitive to ampicillin and amoxicillin, which may be used in the treatment of urinary tract infections. Its resistant to amino glycosides, but *Strept. faecalis* endocarditis usually respond to high doses of the combination of penicillin and aminoglycoside; vancomycin may be of value for strains that are particularly resistant to other drugs (Ross, 1989).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Samples

2.1.1 Source of the samples

The urine samples investigated in this study were collected from pregnant women attending the Department of Obstetric and Gynaecology at the Military Hospital.

2.1.2 Size of the samples

One hundred urine samples were collected for investigation.

2.1.3 Collection method

2.1.3.1 Collection of urine samples

Pregnant women subject of the study visiting Department of Obstetric and Gynaecology at the Military Hospital for routine checkup. They were asked to submit urine samples. They were instructed to collect mid-stream urine properly in sterile universal bottles. The samples were kept in ice in thermos flasks and transported immediately to the laboratory for bacteriological investigation.

2.1.3.2 Collection of blood

Blood for enriched media was collected aseptically into sterile flask containing glass beads by veinopuncture of jugular vein of healthy sheep kept for this purpose. The blood was defibrinated by shaking the flask after collection. Blood for fibrinolysin was allowed to clot and the serum was removed aseptically. Blood for coagulase test was
aspirated directly from rabbit heart or an ear vein using a sterile vacutainer containing an anticoagulant. Human plasma was also used for the test.

2.2 Sterilization

a. Flaming

It was used to sterilize glass slides, cover slips, needles and scalpels.

b. Red heat

It was used to sterile wire loop, points and searing spatulas by holding them over Bunsen burner flame until they became red-hot.

c. Hot air oven

It was used to sterilize glass wares such as test tubes, graduated pipettes, flasks, forceps and cotton swabs. The holding period was one hour and oven temperature was 160 °C.

d. Moist heat (autoclave)

Autoclaving at 121°C (15lb/ inch²) for 15 minutes was used for sterilization of media and plastic wares.

Autoclaving at 115°C (10lb/ inch²) for 10 minutes was used for sterilization of some media such as sugars containing media.
2.3 Reagents and indicators

2.3.1 Reagents

2.3.1.1 Kovac’s reagent

This reagent composed of para-dimethylaminobenzaldehyde, amyl alcohol and concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath, it was then cooled and the acid was added carefully. The reagent was stored at 4 °C for later use in indole test.

2.4. Reference strains

Reference strains were took from Department of Microbiology in Veterinary Medicine Faculty University of Khartoum namely:

*Escherichia coli* ATCC 25922, *Klebsiella Pneumoniae* ATCC 35657, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923,

ATCC is the American Type Culture Collection.

2.5 Preparations of media

2.5.1 Chromogenic urine agar medium

The medium was prepared by suspending 49.4 g of chromogenic urine agar powder in 1000 ml of cold distilled water and well mixed by boiling, the pH was adjusted to 7.2±02. The mixture was distributed in 5 ml volumes into sterile bottles, and then sterilized by autoclaving at121°C (15 lb/inch²) for 15 minutes (According to Biolife Italiana SrI company, Italy).
2.5.2 Nutrient broth (Oxoid CM 1)

The medium was prepared by adding 13 g of nutrient broth powder to 1 L of distilled water and well mixed. The pH was adjusted to 7.4. The mixture was distributed in 5 ml volumes into clean bottles, and then sterilized by autoclaving at 121°C (15 lb/inch$^2$) for 15 minutes. 20 ml of sterile horse serum were adding to the mixture after cooling to 45 - 50°C. This was mixing gently and distributed into sterile petri dishes.

2.5.3 Peptone(Oxoid CM 9) water

This medium was prepared by dissolving 10 g peptone and 5 g sodium chloride in 1 L of distilled water. The mixture was distributed in 5 ml volumes into clean bottles and sterilized by autoclaving at 121°C (15 lb/inch$^2$) for 15 minutes.

2.5.4 Nutrient agar (Oxoid CM 3) slant

This was prepared by adding 28 g of nutrient agar to 1 L of distilled water and dissolved by boiling. The pH was adjusted to 7.4. The prepared medium was distributed in 10 ml volume into clean bottles, sterilized by autoclaving at 121 °C (15 lb/inch$^2$) for 15 minutes and left to solidify in inclined position.

2.5.5 Nutrient agar (Oxoid CM 3)

This was prepared by adding 28g of nutrient agar to 1 L of distilled water and dissolved by boiling. The pH was adjusted to 7.4, and then sterilized by autoclaving at 121°C (15 lb/inch$^2$) for 15 minutes. The prepared medium was distributed in 20ml volume into sterile Petri dishes. The poured plates were allowed to solidify on flat surface.
2.5.6 Diagnostic sensitivity test agar (Oxoid CM 261)

This medium was prepared as described by Barrow and Feltham (1993). It composed of peptone, veal infusion solid, dextrose, sodium chloride, disodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uricil, xanthine and ion agar. Forty grams of medium was dissolved by boiling in 1L of distilled water. The pH was adjusted to 7.4; and sterilized by autoclaving at 121 °C (15 lb/inch²) for 15 minutes. The sterilized medium was distributed in 20 ml volumes into sterile Petri dishes. The poured plates were allowed to solidify on leveled surface.

2.5.7 Motility medium - Cragie tube medium

Thirteen grams of dehydrated nutrient broth (Oxoid CM 1) were added to 5 g of Oxoid agar No.1 and dissolved in 1 L of distilled water. The pH was adjusted to 7.4. The prepared medium was distributed in 5 ml volumes into clean test tube which containing appropriate Cragie tubes and then sterilized by autoclaving at 121 °C (15 lb/inch²) for 15 minutes.

2.6 Culture Methods

In the laboratory each urine sample was well mixed, then a drop of urine was streaked on chromogenic urine agar plates by using standard wire loop that takes .002 ml (Kodaka et al, 1995).

2.7 Inoculation of the reference strains

The reference strains of bacteria were inoculated by streaking on chromogenic urine agar media and incubated at 37°C for 24 hours.
2.8 Inoculation of the samples

The urine samples were inoculated on chromogenic urine agar media and incubated at 37°C for 24 hours.

2.9 Incubation of culture

For the isolation. The plates were incubated aerobically at 37°C for 24 hours. Other inoculated solid solid and liquid media used for further identification were incubated aerobically at 37°C for 24.

2.9.1 Purification and preservation of culture

Purification of culture was done by sub-culturing part of typical well separated coloured colony on the chromogenic urine agar medium. The purity of the culture was checked by observation of colony color and examining stained smear. Pure culture was then inoculated into nutrient agar slant medium and incubated overnight at 37 °C. The pure culture was then stored at 4 ºC for studying cultures and some biochemical characteristics and sensitivity of the isolates.

2.10 Microscopic examination

Smears were made from each types of colony on primary cultures and from purified colonies. Then fixed by heating and stained by Gram stain method according to Barrow and Feltham (1993) and examined microscopically under oil immersion lens. The smear was examined for cell morphology, cell arrangement and stained reaction.
2.11 Identification of isolates

The purified isolates were identified according to criteria described by Barrow and Feltham (1993). This included staining reaction, cell morphology, growth condition, colonial characteristics on different media, Identification of isolates on chromogenic urine agar medium by colony color (According to Biolife Italiana SrI company, Italy).

2.12 Biochemical methods for identification of isolated bacteria

All biochemical tests were performed as described by Barrow and Feltham (1993). They included:

2.12.1 Motility Test

Motility medium was inoculated by stabbing with straight wire into the center of the Cragie tube and then incubated at 37 °C for 24 h. The organism was considered motile if there was turbidity in the medium in and outside the Cragie tube while the growth of non motile organism confined inside Cragie tube.

2.12.2 Indole production test

The test culture was inoculated into peptone water and incubated at 37 °C for 48 h. One ml Kovac’s reagent was added to the tube. The appearance of a pink color in the reagent layer within a minute indicated positive reaction.

2.12.3 Coagulase test

2.12.3.1 Slide coagulase test

A colony of tested culture was placed on a clean glass slide, emulsified in a drop of normal saline and then a loop-full of human
plasma was added to bacterial suspension. Appearance of coarse microscopically visible clump was recorded as positive result.

2.12.3.2 Tube coagulase test

To 0.5 ml of 1: 10 dilution human plasma in normal, 0.1 ml of an 18-24 hours old broth culture of test organism was added, then incubated in water bath at 37 °C and examined after 4-6 h for coagulation. Definite clot formation indicated positive result.

2.13 Antibacterial sensitivity test

The sensitivity of isolates to antibacterial drugs agents was determined by disc diffusion technique. The isolates were cultured in peptone water and incubated at 37 °C for 2 h. Petri dishes containing diagnostic sensitivity test (DST) agar medium were put in the incubator at 37 °C for 30 minutes to dry and then inoculated with 1 ml volume of the culture. The inoculated culture was evenly distributed by rotation, the excess inoculums were withdrawn by sterile microtitter pipette and the plate was left to dry at room temperature for 15 minutes. Commercially prepared antibiotic discs of Plasmatic laboratory were placed on surface of the medium by sterile forceps and pressed gently to insure good contact with the surface of the culture medium. The plates were then incubated at 37 °C for 24-48 h. The sensitivity of the isolates was examined to the following antibacterial drugs: Ampicillin (25 µg), Co-trimaxazole (25 µg), Tetracycline (30 µg), Gentamycin (10 µg), Naladix Acid (30 µg), Streptomycin (25 µg), colistin (25 µg), Nitrofuratoine (200 µg).
The test organism was considered sensitive if there was a zone of inhibition of 10 mm or more a round the disc according to manufacturer's instructions.
CHAPTER THREE

RESULTS

3.1 The isolates

One hundred urine samples were collected from pregnant women presented to Department of Obstetric of Gynaecology at the Military Hospital during the period May 2009-August 2009. The urine samples were subjected to bacteriological examination by culture, 83 samples (83%) gave positive growth and positive for microscopy, they yielded (130) isolates, most of them (115) were bacterial isolates and the other 15 (15%) samples were yeast isolates, and 17 of these samples (17%) were negative for growth.

3.2 Species of bacteria isolated from urine samples of pregnant women

The type of samples that were tested from the urine samples are shown in table (1). The isolates represent eight different bacterial species, three of which were gram positive and the others were gram negative are shown in table (2) and figure (1) Gram negative bacteria isolated namely: *Escherichia coli*, *Klebsiella pneumoniae*, *proteus mirabilis*, *proteus vulgaris*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and gram positive: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Enterococcus faecalis*.

The frequency of isolation of bacteria from the samples varied from 45% (*E. coli*) to 1% (*P. vulgaris*).
3.3 Characters of reference strains on chromogenic urine agar medium

Reference strains inoculated on chromogenic urine agar medium that were grown with different distinct colors. *Escherichia coli* (ATCC 25922) gave pink colored colonies is shown in figure (2), *Klebsiella pneumoniae* (ATCC 35657) with dark blue colonies is shown in figure (3), *Pseudomonas aeruginosa* (ATCC 27853) gave yellow colonies is shown in figure (4), *Staphylolococcus aureus* (ATCC 25923) grow with white colonies shown in figure (5).

3.4 Characters of isolates on chromogenic urine agar medium

Characters of bacteria isolated from the urine samples of pregnant women on chromogenic urine agar medium are shown in table (3). *E. coli* isolates gave identical morphological characteristics on chromogenic agar medium. These isolates gave pink colonies, *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates gave identical blue colonies and differentiated by the motility. *Enterobacter Cloacae* isolates were motile on semi solid media and *K. pneumoniae* were non motile (table 6). *Proteus spp* isolates were gave brown or beige with brown halo colonies on chromogenic urine agar media (figure 6) and differentiated by their reaction on indole test *proteus mirabilis* gave negative reaction and *Proteus vulgaris* was gave positive reaction is shown in table (7). *Pseudomonas aeruginosa* isolates gave yellow colonies typical to that of reference strain.

Characters of gram positive isolates: *Staph. aureus* and *Staph. epidermidis* isolates were gave white colonies and show different reaction on coagulase test, *staph. aureus* isolates gave positive
reaction and *staph. epidermidis* isolates were gave negative result are shown in table (8). *Ent. faecalis* isolates gave turquoise colonies (figure 7), *Streptococcus agalactiae* isolates gave colorless colonies. *Candida albicans* isolates were gave white colonies and differentiated it from *staphylococcus* spp by Gram stain; *C. albicans* gave positive reaction with large cocci than *staphylococcus* spp are shown in table (9).

The growth of urine samples contain mixed culture with different colonies colors are shown in figure (9).

### 3.4 Sensitivity of bacteria isolated from urine of pregnant women to antibiotics:

Sensitivity of bacteria isolated from urine of pregnant women to eight different antibiotics shown in table (10). All isolates sensitive to gentamicin and nitrofurantoin. Four of the five isolates of *E. coli* were sensitive to ampicillin, gentamicin and colistin; one of these five was resistant to these antibiotics. Three isolates of *Enterobacter cloacae* were sensitive to gentamicin, streptomycin, nitrofurantoin and colistin; one of them was resist to cotrimoxazole.

*Staphylococcus* spp isolates tested were sensitive to nitrofuratoin and gentamicin. *Proteus mirabilis* was resist to tetracyclin, cotrimoxazole and naladix acid.
Table (1) Urine samples of pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of samples</th>
<th>Number % of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for culture</td>
<td>83</td>
<td>83%</td>
</tr>
<tr>
<td>Positive for microscopy</td>
<td>83</td>
<td>83%</td>
</tr>
<tr>
<td>Negative for culture</td>
<td>17</td>
<td>17%</td>
</tr>
</tbody>
</table>
Table (2) bacteria isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Number % from total of samples</th>
<th>Number % from total of bacterial isolates</th>
<th>Number % from total of all isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>45</td>
<td>45%</td>
<td>39.1%</td>
<td>34.6%</td>
</tr>
<tr>
<td><em>Proteus</em> spp</td>
<td>9</td>
<td>9%</td>
<td>7.8%</td>
<td>6.9%</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>8</td>
<td>8%</td>
<td>7%</td>
<td>6.2%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8</td>
<td>8%</td>
<td>7%</td>
<td>6.2%</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>6</td>
<td>6%</td>
<td>5.2%</td>
<td>4.6%</td>
</tr>
<tr>
<td><strong>Gram positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus spp</em></td>
<td>21</td>
<td>21%</td>
<td>18.3%</td>
<td>16.2%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>12</td>
<td>12%</td>
<td>10.4%</td>
<td>9.2%</td>
</tr>
<tr>
<td><em>Sterptococcus agalactiae</em></td>
<td>6</td>
<td>6%</td>
<td>5.2%</td>
<td>4.6%</td>
</tr>
</tbody>
</table>
Table (3) characters of bacterial isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital on Chromogenic urine agar media

<table>
<thead>
<tr>
<th>Isolation species</th>
<th>Colonies color on chromogenic urine agar media</th>
<th>Reaction of Gram stain on microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Pink</td>
<td>Gram negative rods</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Blue</td>
<td>Gram negative rods</td>
</tr>
<tr>
<td><em>Enterobacter cloaceae</em></td>
<td>Blue</td>
<td>Gram negative rods</td>
</tr>
<tr>
<td><em>Proteus spp</em></td>
<td>Brown with halo</td>
<td>Gram negative rods</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Yellow</td>
<td>Gram negative rods</td>
</tr>
<tr>
<td><em>Staphylococcus spp</em></td>
<td>White</td>
<td>Gram Positive cocci</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Turquoise</td>
<td>Gram Positive cocci</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>Colorless</td>
<td>Gram Positive cocci</td>
</tr>
<tr>
<td><em>Yeast</em></td>
<td>White</td>
<td>Gram Positive cocci</td>
</tr>
</tbody>
</table>
Table (4) *Proteus* spp isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Number % from total of samples</th>
<th>Number % from total of bacterial isolates</th>
<th>Number % from total of all isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>8</td>
<td>8%</td>
<td>7%</td>
<td>6.2%</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>1</td>
<td>1%</td>
<td>0.9%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>9%</td>
<td>7.8%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Table (5) *Staphylococcus* spp isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Number % from total of samples</th>
<th>Number % from total of bacterial isolates</th>
<th>Number % from total of all isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
<td>12%</td>
<td>10.4%</td>
<td>9.2%</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>9</td>
<td>9%</td>
<td>7.8%</td>
<td>7%</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>21%</td>
<td>18.2%</td>
<td>16.2%</td>
</tr>
</tbody>
</table>
Table (6) Characters and biochemical reaction for differentiation of *Enterobacter cloacae* from *klebsiella pneumoniae* isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Color of isolates on chromogenic urine agar media</th>
<th>Motility Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Blue</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Blue</td>
<td>+</td>
</tr>
</tbody>
</table>

Table (7) Characters and biochemical reaction for differentiation of *Proteus* spp isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Isolation species</th>
<th>Color of isolates on chromogenic urine agar media</th>
<th>Reaction on indole test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Brown</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Brown</td>
<td>+</td>
</tr>
</tbody>
</table>
Table (8) Characters and biochemical reaction for differentiation of *Staphylococcus* spp isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Isolation species</th>
<th>Color of isolates on chromogenic urine agar media</th>
<th>Coagulase test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>White</td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>White</td>
<td>–</td>
</tr>
</tbody>
</table>

Table (9) Characters and biochemical reaction for differentiation of yeast from *Staphylococcus* spp isolated from urine samples pregnant women presented at Department of Obstetric of Gynaecology at the Military Hospital

<table>
<thead>
<tr>
<th>Isolation species</th>
<th>Color of isolates on chromogenic urine agar media</th>
<th>Gram stain reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>White</td>
<td>+ cocci</td>
</tr>
<tr>
<td>Yeast</td>
<td>White</td>
<td>+ large cocci</td>
</tr>
</tbody>
</table>
Table (10) The antibacterial sensitivity of different bacteria isolated from urine samples of pregnant women

<table>
<thead>
<tr>
<th>Bacterial spp</th>
<th>Antibiotics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td></td>
<td>4(++)</td>
<td>1(-)</td>
<td>+++</td>
<td>2(++)</td>
<td>2(++)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1(-)</td>
<td></td>
<td></td>
<td>1(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclin</td>
<td></td>
<td>2(++)</td>
<td>3(-)</td>
<td>++</td>
<td>3(+)</td>
<td>1(++)</td>
<td>2(-)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td>4(++)</td>
<td>1(++)</td>
<td>+++</td>
<td>3(++)</td>
<td>2(++)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1(++)</td>
<td></td>
<td></td>
<td>1(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>5(++)</td>
<td>+++</td>
<td>3(++)</td>
<td>3(-)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Naladixic acid</td>
<td></td>
<td>5(++)</td>
<td>-</td>
<td>1(++)</td>
<td>2(+)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td></td>
<td>2(++)</td>
<td>3(-)</td>
<td>-</td>
<td>2(++)</td>
<td>1(-)</td>
<td>3(-)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td></td>
<td>+++</td>
<td>++</td>
<td>3(++)</td>
<td>(++)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Colistin</td>
<td></td>
<td>4(++)</td>
<td>1(++)</td>
<td>+++</td>
<td>3(++)</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

1-E.coli  2-k. pneumoniae  3-Enterobacter cloacae  4- Staph.aureus  5- Staph. Epidermidis  6- Proteus mirabilis

The cross corresponds to size of the size of zonal inhibition of the organism according to (Arcomexarab company for medical diagnosis).

* - + 14 mm or less (resistant). ++ 15 _ 16 mm (moderately). +++ 17 or more (sensitive)
Figure. 1 percentage of bacteria isolated from urine samples of pregnant women presented at Department of Obstetric and Gynaecology at the Military Hospital
Figure 2: Growth of *E.coli* (ATCC 25922) on Chromogenic urine agar medium

Figure 3: Growth of *Klebsiella pneumoniae* (ATCC 35657) on Chromogenic urine agar medium
Figure 4: Growth of *Ps. Aeruginosa* (ATCC 27853) on Chromogenic urine agar medium

Figure 5: Growth of *Staph.aureus* (ATCC 25923) on Chromogenic urine agar medium
Figure. 6: Growth of *proteus spp* on Chromogenic urine agar medium

Figure. 7: Growth of *Ent.faecalis* on Chromogenic urine agar medium.
Figure. 8: Growth of different isolates on Chromogenic urine agar medium Klebseilla, E.coli, Enterococcus, Enterobacter, Staphylococcus, candida albicans

Figure. 9: Growth of urine samples contain mixed culture with different colors
DISCUSSION

The principle of chromogenic media is based on the fact that bacteria possess many enzymes for their physiological functions that help them to utilize substrates. They are either species specific or genus specific. This specific feature has been exploited in characterization of bacteria based on biochemical tests. Certain tests are considered as key biochemical identification tests for some bacteria and they have been used in the development of the chromogenic media. In such media, specific chromogenic substrates are specifically broken down by a bacterial enzyme thereby imparting a distinct color to the growing bacterial colony that can be visually observed. (Delisle and Ley, 1989; Merlino, Siarakas, Robertson, Funnell, Gottlieb and Braudbury, 1996; Merlino, Lezoi, Braudbury, Veal and Harbour, 2000; Samra, Heifetz, Talmor, Bain and Bahar, 1998).

The present investigation was designed to study possible bacterial causes of UTI in pregnant women presented at Department of Obstetric and Gynaecology at the Military Hospital.

Urine samples collected from pregnant women were subjected to cultivation on chromogenic urine agar media and some biochemical tests for more differentiation and confirmation. From the 100 collected samples 83(83%) samples were positive for the presence of bacteria by culture on chromogenic urine agar media and 17(17%) samples were negative culture, can be attributed to many factors. Many samples might have been taken from subjects on antibiotic regimen, which result in antibiotic residues in the urine.
Contamination of the samples may occur during the washing post urination process is indicated by the presence of more than one genus of the contaminating bacteria.

Chromogenic media have been compared to traditional urine culture media, e.g., blood agar and MacConkey agar, and were found to be at least as good as traditional media for the isolation of uropathogens (Hengstler et al, 1997; Kodaka et al, 1995; Nunez, Diaz, Lorente, Perez, and Ruiz, 1995; Samra, Heifetz, Talmor, Bain, and Bahar, 1998).

The development of chromogenic agars, combining the medium with various chromogenic substrates, has enhanced the presumptive identification of urinary isolates and enabled mixed cultures to be detected more easily. This enhanced identification of different species facilitates the monitoring of bacterial resistance, thus providing a cost effective mechanism of antibiotic surveillance in the routine diagnostic laboratory (Fallon, Ackland, Andrews, Frodsham, Howe, Howells, Nye, and Warren, 2003).

This study has shown that a substantially higher number of strains can be recovered and rapidly identified using chromogenic media for the isolation of urinary tract pathogens. Chromogenic urine agar media allowed an easy differentiation of the various species from mixed cultures, due to the specificity with respect to color of the colony, similar to the other chromogenic media studied. (Gaillot, Wetsch, Fortineau and Berche, 2000), (Hengstler, et al, 1998) (Kodaka, et al, 1995), (Merlino, et al, 2000), (Samra, et al, 1998).
The results of this study indicate that chromogenic urine agar is a reliable medium for detecting urinary tract pathogens. Nearly all *E.coli* species produced pink-coloured colonies, due to β-D-glucoronidase (GUD) activity, compared with work of Willinger and Manafi, (1995) who found that all *E.coli* strains produced rose-coloured colonies, due to GUD activity, *E.coli* produce the enzyme β-glucuronidase that attacks β-glucuronide chromogenic substrate and grow as distinct pink colored colonies.

Chromogenic urine agar media allowed a definite identification of *E.coli* without the need for further biochemical tests. This makes chromogenic media an attractive primary screening medium, considering that the majority of UTI are caused by *E.coli* and this microbe is responsible for most nosocomial and community acquired UTI (Edberg and Kontnick, 1986), (Edberg and Trepeta, 1983).

*Klebsiella pneumoniae* and *Enterobacter cloacae* produced blue – coloured colonies, compare to these isolates by Lakshmi, Satheeshkumar and Kulkarn, (2004), the KES group (*Klebsiella, Enterobacter, Serratia*) produce the enzyme β-glucosidase that attacks β-glucoside chromogenic substrate and grow as distinct blue colonies. *Proteus spp* produced brown-coloured colonies. All strains of the *Morganella-Proteus-Providencia* group showed brown coloration in the agar indicating tryptophane-deaminase activity. However, further testing was required for exact identification. The rapid indole test also proved to be helpful for the specific identification of *P. mirabilis*. All strains of *P. mirabilis* and onestrain of *Proteus penneri*
were indole negative, whereas other members of the Proteae group were positive (Samra et al, 1998).

*Pseudomonas aeruginosa* produce yellow colonies in this study that agreement with (Lakshmi et al. 2004).

*Enterococcus faecalis* produce turquoise colonies this finding agree with Samra et al, 1998), and constituted of 12% from all samples. In recent studies *Enterococci* represented the third most common cause of hospital acquired bacteremia (Dutka-malen,Evers and conervalin, 1995; Scaberg, Culver and Gynes, 1991).

*Staphylococcus aureus* and *Staph.Epidermidis* produce white colonies and *Streptococcus agalactiae* produce colourless colonies that agree with Brown, (2007) found that *Staphylococci* (with the main exception of *Staphylococcus saprophyticus*) and *streptococci* don’t produce ß-glactosidase or ß-glucosidase and grow as white or colourless colonies.

*Candida albicans* produced white colonies that agree with the finding of Scarparo,piccolli, Ricordi and Scagnelli, (2002).

Species of bacteria isolated from urine samples in this study were mainly enterobacteria, constituting 68% this percentage is similarto (67.9%) obtained by Elsheikh,(2004), and nearly lowerthan (72%) obtained by Hussien(2002) and 77.9% found by Ibrahim, (1994). However, Mohamed (2003) found enterobacteria in males constituting only 20% of his total isolates. The high percentage of enterobacteria in females than males is attributable due to the shortness of the urethra in females which make them prone to faecal contamination.
The isolation incidence of *E. coli* (45%) is lower than that reported by Hussien (2002) who found *E. coli* to constitute 52% of the isolates from urine samples of diabetic women. On the other hand, Elsheikh (2004) isolated *E. coli* in a percentage of 42.9% of his total isolates from urine of pregnant women in maternity hospital in the Sudan, nearly similar to results of this study.

*Proteus spp* and *Klebsiella pneumonia* in this study constituted 9% and 8%, respectively of isolates, nearly similar to Elsheikh (2004) found these two species to constitute 7.1% and 3.6%, Ibrahim (1994) found these species in 6.8% and 15.3%. Prais, Straussberg, Avitzur, Nussinovitch, Harel, Amor, (2003) found *Klebsiella* to constitute 6%.

*Enterobacter cloacae* constituted 6% in this study, Elsheikh (2004) found *enterobacter spp* in 14.3%.

*Staphylococci* constituted 21% of isolates in this study, this finding agree with that of Elsheikh (2004) who found *Staphylococci* to constitute 25%. On the other hand, Ibrahim (1994) reported 14.2% of his isolates were staphylococci.

From the findings in this study, it can be concluded that *E. coli* is the prime cause of UTI, in women, subject of the study.

Bacteria isolated in this study were all sensitive to gentamicin and nitrofuratoin. *E. coli* isolates showed more less the same pattern of sensitivity; they were sensitive to colistin which is mainly active against Gram negative bacteria (Brandes, pugh, Bywater, and jekins, 1993). All staphylococci also showed similer pattern of sensitivity and resistant to the drugs known to act on gram positive bacteria.
Conclusion and Recommendations

Conclusions

The results of the present study demonstrated that:

1- Gram-negative, gram-positive bacteria and yeast \((Candida albicans)\) are associated with UTI of pregnant women.

2- \(Escherichia coli\) was the main agent of urinary tract infection in pregnant women.

3- Chromogenic urine agar medium may be employed to identify bacteria in mixed cultures according to colony color, without the need for further biochemical testing with \(E.coli, Enterococci\) and \(Pseudomonas\), which can be identified directly on this primary isolation medium.

Recommendations:

1- Because the traditional method of culturing the urine is time-consuming, I recommend the use of chromogenic urine agar medium for the detection of bacterial urinary tract infection and thus helping in the rapid diagnosis of the condition.

2- Future studies should be considered to evaluate the sensitivity and specificity of chromogenic urine agar medium.

3- The sensitivity test of bacterial isolates should be conducted using more variable types of drugs to determine the most effective drugs that kill or inhibit the bacterial growth.
References


