CHAPTER ONE

INTRODUCTION & OBJECTIVES

1.1 Introduction

Pregnancy is associated with profound anatomical, physiological, biochemical andendocrine changes that affect multiple organs and systems. These changes are essential to help the woman to adapt to the pregnant state and to aid fetal growth and survival. Kidney size increases by about 1 cm in length. There is marked dilatation of renal calyces, pelvis and ureters. Increase in glomerular filtration rate (GFR) by about 50% reaches maximum at the end of first trimester and is maintained at this augmented level until at least the 36th gestational week. As a result of increased GFR (glomerular filtration rate) there is an increase in the amount of glucose delivered to the kidneys. Associated with this is a reduction in the renal threshold for glucose. During pregnancy about a third of women excrete more than 5.5 mmol of glucose in 24 hours (renal glycosuria) which is significantly higher than that excreted by non-pregnant women (up to 0.55 mmol/24 hours) (Yanamandra and Chandraharan, 2012)

Urinary tract infections (UTIs) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract (Rahimkhani *et al*.,2008). UTIs are very frequent and common pathology that can occur at any age, considering adult hood 48% of women acquired at least one occurrence of UTI in their life, higher susceptibility is due to shorter urethra, closer proximity of anus with vaginal vestibule and urethra and the beginning of sexual activity (John, 2004).

Many factors may contribute to the development of UTIs during pregnancy. One important factor is urethral dilatation, thought to occur due to hormonal effects and mechanical compression from the growing uterus. Urethral dilation can cause bacteria to spread from the bladder to the kidneys, increasing the risk of pyelonephritis. Pregnancy easy contamination of the urinary tract with fecal flora, additionally the physiological increase in plasma volume during pregnancy decreases urine concentration up to70% pregnant women develop glucosurea, which encourages bacterial growth in the urine (Lucas, 1993). UTI in pregnancy is classified into two categories of symptomatic and asymptomatic (Schnar and Smaill, 2008). The involvement of the lower urinary tract, leading to asymptomatic bacteruria is the most common cause of UTI during pregnancy. The involvement of the upper urinary tract can lead to symptomatic bacteruria and is characterized by acute Pyelonephritis (Emamghorshi*et al.*, 2012). If asymptomatic infection is not treated, it leads to some clinical manifestations in mother and newborn (Jido, 2014). Symptomatic UTI occurs in 1% to 2% of pregnancies, while asymptomatic bacteruria has been reported in 2% to 13% of pregnant women.(Dwyer & O'Reilly, 2002). Untreated UTIs can lead to complications, such as pyelonephritis, low-birth-weight infants, premature delivery, and, occasionally, stillbirth(Christensen, 2000); therefore prompt treatment of symptomatic UTIs and asymptomatic bacteruria is warranted in pregnant women.Nearly all antimicrobials cross the placenta, and some of them may exert teratogenic effects. Commonly accepted antibiotics used in treating UTIs during pregnancy, regardless of its period, include derivatives of Penicillin and Cephalosporins, particularly those with low protein-binding ability (such as Cephalexin) (Roganti*et al.*, 2011). Antimicrobial resistance

is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it.

Resistant microorganisms (including bacteria, fungi, viruses and parasites) are able to withstand attack by antimicrobial drugs, such as antibacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist. For several decades antimicrobial resistance (AMR) has been a growing threat to the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. AMR results in reduced efficacy of antimicrobial, making the treatment of patients difficult, costly, or even impossible. For the past two decades, there has been increasing interest in the investigation of different extracts obtained from traditional medical plant as potential source of newantimicrobial agents (Bonjar&Farrokhi, 2004). *Tamarindus indica* is one of the medicinal plants used in traditional medicine as antimicrobial.

1.2 Rationale

Urinary tract infections and its associated complications are the cause of nearly 150 million deaths per year worldwide. The disease can be developed in 40% - 50% of women and 5% of men. UTIs are the second common complications in pregnant women to anemia; hence, it is necessary to regularly screen pregnant women for the presence of infection as many women appear a symptomatic during pregnancy. Knowledge of the causative agents and safe choices of drugs is essential because many antimicrobials could harm the mother and the fetus. Since pregnancy is a known risk factor for UTIs in pregnant women regular screening of pregnant women and determination of the suitable antibiogram could be useful tool in the selection of safe antibiotics for the mothers and babies in various trimesters and reduce the number of emerging resistant strains.

1.3.1 General objective

To determine Symptomatic & A symptomatic urinary tract infection pathogens among pregnant women at Al-Hasahisa Women Hospital and Obsterics and their Susceptibility Towards *Tamarindus indica* Methanolic Extract

1.3.2 Specific objectives

I. To collect, isolate, identify and determine the antimicrobial sensitivity of urinary tract pathogen from pregnant women attending Al-Hasahisa hospital
II. To determine the antimicrobial potential of *T. indica* against selected urinary tract pathogens collected from pregnant women attending Al-Hasahisa hospital.

CHAPTER TWO

LITERATURE REVIEW

Urinary system is the various organs in the body that produce, store and discharge urine. Consist of: two kidneys, two ureters, one bladder and urethra. The upper urinary tract is sterile. Anyone can get a urinary tract infection, but it is most common in women, especially if they are pregnant. In pregnant women, hormones cause changes in the urinary tract, which predispose women to infections. In addition, a growing uterus presses on the bladder, preventing the complete emptying of urine. This stagnant urine is a likely source of infection and if untreated may lead to kidney infection. Urinary tract and kidney infections in pregnant women should be treated to prevent complications. With proper care, urinary tract infections rarely cause serious health problems. Most infections are limited to the bladder and urethra, but sometimes they can lead to a kidney infection. If they do, UTIs may lead to preterm labor and low birth weight.

2.1 Bacteriuriaand Pyuria

Bacteruria refers to the presence of bacteria in urine and does not necessary in infection, bacteruria may be significant or in significant depending on number of bacteria in urine. Significant bacteruria implying infection has traditionally been defined as urine culture yielding more than 10^5 colonies forming unite per ml, bacteruria in pregnancy carries much greater risk of progressing to pyelonephritis than in non pregnant women and is associated with serious risk and costly treatment (John, 2002). Pyuria in medicine is the condition of urine containing white blood cells or pus defined as presence of 6-10 or more neutrophils per high power field of unspun voided mid-stream

urine. It can be sign of urinary tract infection,pyuria may be present in the septic patient or in older patient with pneumonia. Sterile pyuria is urine which contains white blood cells while appearing sterile, it is often caused by sexually transmited infections such as gonorrhea or viruses (Gilbert and Peter. 2015).

2.1.1 Symptomatic bacteriuria

UTI is defined as the presence of at least 100,000 organisms per milliliter of urine in an asymptomatic patient, or as more than 100 organisms/mL of urine with accompanying pyuria (> 7 white blood cells (WBCs)/mL) in a symptomatic patient.

2.1.2 Asymptomaticbacteriuria

Asymptomatic bacteriuria, or asymptomatic urinary infection, is isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection(Lindsay *et al.*, 2005) Untreated asymptomatic bacteriuria is a risk factor for acute cystitis (40%) and pyelonephritis (25-30%) in pregnancy.

2.2 Signs, symptoms and complications of UTI

Pain or burning when urinating, need to urinate more often than usual, feeling of urgency when urinate, blood (haematouria) or mucus (pyuria) in the urine, cramps or pain in the lower abdomen, pain during sexual intercourse, chills, fever, sweats, change in amount of urine either more or less, when bacteria spreads to the kidneys may experience: back pain chills, fever, nausea, and vomiting. Complications of UTIs include Cystitis, pyelonephritis, early labor, low birth weight, cerebral polsy in infant, and might lead to fetal death (Jido, 2014).

2.3 Routes of infection

Infectious organisms enter through two mechanisms ascending mechanism and hematogenous mechanism. The ascending route is primarily the enter route for all UTIs bacteria enter the urinary tract system from the fecal reservoir ascending through the urethra in to the bladder and in the case of pyelonephritis up the ureter to the kidney. Colonization of the urethra occurs commonly in female because of short urethra (Pastore*et al.*, 1999). In hematogenous mechanism the bacteria use blood circulation to reach the urinary tract, enter the tissues and cause infection (Lavanya and Logalakshmi, 2002).

2.4 Recurrent UTI in pregnancy

Twenty percent of women acquired UTI during pregnancy develop infection within 6 months, recurrent infection may occur often 2 weeks after completing antimicrobial therapy and may be cause by same organism or other different one. For pregnant women with recurrent infection the appropriated useful treatment is important to avoid the fetus from the effect of therapy by choosing the least toxic and more effective antibiotics (Lavanya and Logalakshmi, 2002).

2.5 Common bacteria associated with UTI

Most of the UTI pathogens are usually part of bowel normal flora of humans such as *Escherichia coli* and other members of *Enterobacteriaceae*. The major bacteria that cause UTI include *Escherichia coli, Proteus mirabilis, Enterococcus fecalis, Staphylococcus saprophyticus ,Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* (Foxman, 2002).

2.6 Prevention and control of UTI

Plenty of water equivalent six to eight cups every day should be taken to flush bacteria out of urinary system , take Vitamin C (ascorbic acid) cranberry juice makes the urine acidic which discourages the growth of bacteria, Eliminate refined foods, caffeine, alcohol, develop a habit of urinating as soon as the need is felt and empty bladder completely when urinating, urinate before and after intercourse, avoid intercourse while being treated for UTI, after urinating blot dry and keep genital area clean, make sure wipe from the front toward the back, avoid using strong soaps or antiseptic, change underwear every day and visit the doctor in case any symptom like cloudy urine, back pain frequent urination (Tadesse*et al.*, 2007).

2.7 Safety of antibiotic choice for UTIs in pregnantwomen

Some antibiotics should not be used during pregnancy because of their effect on fetus and mothers.Tetracycline hasadverse affects on fetal teeth and bones, the use of fluoroquinolonesaffects fetal cartilage and developmental disorders have been reported in experimental animals, although not in human studies (Addis and Hoe, 1998).Sulfonamides as a group do not appear to pose a serious teratogenicrisk;however, Trimethoprim is a folic acid antagonist and its use during the first trimester has been associated with structural defects, such as neural tube and cardiovascular defects (kova *et al.,* 2003).Cephalosporins are considered safe to use in pregnancy, Cephalexin is not teratogenic and safe(Rockenbauer*et al.*, 2001).Numerous studies has demonstrated the safety of nitrofurantoin in pregnancy many even during the first-trimester use of nitrofurantoin (Einarson*et al.*, 1995) the drug can theoretically induce hemolytic anemia in the fetus or newborn,

particularly in those with glucose-6-phosphate dehydrogenase deficiency (Guillemant*et al*., 2000). Co-amoxiclav can be separated into amoxicillin and clavulanic acid; no adverse effects in newborn or fetus attributed to the combination of Amoxicillin and clavulanic acid during pregnancy.

2.8 Medicinal plants

In recent years herbal medicine has become an integral part of the primary health care system of certain nations, according to the World Health Organization, the use of traditional herbal medicine has spread not only in the developing countries, but also in the industrialized regions. Plants have variety of compounds with potentially significant therapeutic application against human pathogens thus medical practitioners are prescribing herbal medicine teas and herbal extracts as a supplementary type of treatment in everyday problems caused by our modern civilization (Gomes *et al*., 2007).

2.9 Tamarindusindica

Tamarindusindica commonly known as *Tamarind* tree is one of the most important multipurpose tropical fruit tree species in the Indian subcontinent. *Tamarind* fruit was at first thought to be produced by an Indian palm, as the name Tamarind comes from a Persian word "Tamar-I-hind," meaning date of India.*T.indica* is used as traditional medicine in India, Africa, Pakistan, Bangladesh, Nigeria and most of the tropical countries. It is used traditionally in abdominal pain, diarrhea and dysentery, helminthes infections, wound healing, malaria and fever, constipation, inflammation, cell cytotoxicity, gonorrhea, and eye diseases. It has numerous chemical values and is rich in phytochemicals, and hence the plant is reported to possess anti-diabetic activity, antimicrobial activity, antivenomic activity, antioxidant activity, anti-malarial activity, hepatoprotective activity,

antiasthmatic activity, laxative activity, and anti-hyperlipidemic activity (Bhadoriya *et al*., 2011)

Classification

Familia:Fabaceae

Subfamilia: Caesalpinioideae

Tribus: Detarieae

Genus: Tamarindus

Species: *Tamarindusindica*

Tamarindusindica is evergreen tree that can reach 24 m height and 7 m girth that has pale yellow and pink flower (Bhadoriya*et al*., 2011)**.** It needs dry climate and in Africa it coners areas in Senegal in the west, Sudan and Ethiopia in the east and Mozambique and Madagascar in the south. It is also thought that the plant came to India from Africa. Bangladesh, Indonesia in Asia; Mexico, Costa Rica in America are some of the countries in which this plant is mostly encountered (Caluw*et al*., 2010).

Every part of *Tamarindusindica* plant (root, body, fruit, and leaves) not only has rich nutritional value and broad usage area in medicine but also has industrial and economic importance. *Tamarind* can be the most acidic and sweet fruit according to its growing season (caluw*et al.*, 2010). According to World Health Organization report, *Tamarind* fruit is an ideal source of all essential amino acids except tryptophan (Glew*et al.*, 2005). Its seeds also have similar properties so it becomes an important, accessible protein source especially in countries where protein malnutrition is a common problem.

CHAPTER THREE

MATERIALS ANDMETHODS

3.1 Study design

This is a cross sectional study.

3.2 Study area

The study was done in Al-Hasahisa city between 11th May and 9th July.

3.3 Study population

Inclusion criteria: all pregnant women attending Al-Hasahisa women and obstetrics hospital.

Exclusion criteria: all pregnant women using antibiotics.

3.4 Ethical consideration

Permission was taken from the Collage of Graduate Studies Sudan University and a verbal consent was obtained from the recruits after explaining the purpose of sampling and the significance of the study

3.5 Samples collection & processing

Sterile, wide mouthed bottles with screw cap tops were used for the collection of mid stream urine samples. The pregnant women were also informed to clean their hands with water and then cleanse their urethral area to reduce the chances of contamination. Urine specimens were processed in the laboratory within 2 hours of collection (the sample kept in ice bag before processing).Then the samples were cultured in:Cysteine lactose electrolyte deficient agar (CLED). Then the samples were incubated at 37º C for 24 hours.Next day colony morphology described and considered if a significant

growth ($\geq 10^5$CFU/ml)was obtained gram stain and biochemical characterization was done for the identification of the pathogens.

3.6 Identification of isolates

3.6.1 Gram stain

Gram staining (or Gram's method) is a method of differentiating bacterial species into two large groups (Gram positive and Gram negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram positive bacteria (Bergey*et al.*, 1994) Gram positive results in a purple/blue color while a Gram negative results in a pink/red color. The Gram stain is almost always the first step in the identification of bacterial organism.

3.6.2 Biochemical tests

For the identification of Gram positive pathogens the following biochemical tests were aperformed; coagulase test, catalase, culture in Mannitol salt agar and Esculin hydrolysis

For the identification of Gram negative pathogens the following biochemical tests were aperformed; Kligler iron agar, Indole test, Citrate utilize test and Urease test (Monica.2006)

3.7Antimicrobial sensitivity test

The susceptibilities of the clinical isolates to selected antibiotics were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2005) using the Kirby-Bauer disc diffusion method on Muller-Hinton Agar (Cheesbrough, 2006) loop full of bacteria was taken from a pure culture colony and transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube and the inculcating was transferred by sterile cotton swab to Muller Hinton medium. The antimicrobials were used Nitrofurantoin 100 mc g\disc, Cephalexin 30 mc g\disc, Naladixic acid 30 mc g\disc, Amoxicillin-Cavulinic acid 30 mc g\disc.

These antibiotic drug discs were selected based on the availability of these drugs in the study area. After that the antibiotic discs were placed on Muller Hinton Agar and incubated at 37°C for about 18 to 24 hours and the zones of inhibition were interpreted using the interpretive chart either susceptible or resistant. The standard reference strains, *Staphylococcus aureus* (ATCC25923), *Escherichia* coli (ATCC25922) were used to assure testing performance of the potency of antibiotic discs.

3.8 Preparation of the extracts

Extraction was carried out according to the method described by Sukhdev*et al.,* (2008). Hundred grams of each sample (pulp) were grinded using mortar and pestle and extracted with 80 % methanol using soxhelt extractor apparatus. Extraction carried out for about eight hours till the solvent returned colorless at the last siphoning times. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract was

allowed to dry in petri dish and the yield percentage was calculated as followed:

Weight of extract obtained / weight of plant sample * 100

Table (3.1) Yield percent of extracts

Sample	Weight of sample (g)	Weight of extract (g)	Yield %
Tamarindusindica	100	31.067	31.067

3.8 Antimicrobial activity of *Tamarindus indica* pulp against selected clinical isolates

All bacteria showing resistance to three and four antibiotics were screened for antimicrobial activity against methanolic extract of *Tamarindusindica* pulp. The concentrations used were 50, 25, 12.5 and 6.3 (w/v) %. A loop full of bacteria was taken from a pure culture colony and transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it forms a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube and swabbed on nutrient agar media and allowed to dry and then 5 holes were made in media by corkborer (8 mm in diameter), added 100 µl from the methanolic extract(four different concentrations) and methanol was used as control. The plates were incubated in refrigerator at 4ºC for one hour and then incubated at 35-37ºC for 24 hours, next day zone were read.

CHAPTER FOUR

RESULTS

About 100 samples of urine were collected from the pregnant women 72 samples showed significant growth, while 28 samples didn't show significant growth (Table 4.1). Susceptibility test was done for all isolates. All isolates showing resistance to three or more antibiotics were subjected to antimicrobial screening with methanolic extract of *Tamarindusindica.*

Table (4.1) Results of M.S.U inoculation in CLED agar

NO. of M.S.U.specimen	Significant growth	NO growth
100	72	28

M.S.U= Mid-Stream Urine

Table (4.2) Classification of isolatedbacteria according to gram stain

Gram reaction	No. Organism	Percentage (%)
Gram positive cocci	58	81
Gram negative bacilli	14	19
Total	72	100

Results of Gram stain appeared 52 gram positive cocci, 6 *candida* s*pp* and 14 Gram negative bacilli.

Table (4.3) Types and Frequency of isolates microorganism

Pathogen	Frequency	Percentage (%)
S. saprophyticus	22	31
S.aureus	19	26
E.coli	14	20
S. epidermidis	8	11
Candida spp	6	8
Enterococcus faecalis	3	4
Total	72	100

S=Staphylococcus

E=Escherichia

The pathogen isolated in this study*: S. saprophyticus* 22 (31%), *S.sureus* 19 (26%), *E.coli* 14(20%), *S. epidermidis*8(11%), *Candida* spp 6 (8%) and *Enterococcus faecalis* 3 (4%).

Table (4.4) Antibacterial sensitivity tests of clinical isolates against selected antibiotics

Isolates	Antibiotics							
	AMC		NIT		CN		NA	
SS	S 4/22 18%	R 18/22 82%	S 20/22 91%	R 2/22 9%	S 8/22 36%	R 14/22 64%	S 1/22 5%	R 21/22 95%
SA	6/19 32%	13/19 68%	19/19 100%	0/19 0%	11/19 58%	8/19 42%	6/19 32%	13/19 68%
EC	1/14 7%	13/14 93%	13/14 93%	1/14 7%	6/14 43%	8/14 57%	4/14 29%	10/14 71%
SE	5/8 63%	3/8 38%	7/8 88%	1/8 12%	7/8 8%	1/8 12%	1/8 12%	7/8 88%
EF	3/3 100%	0/3 0%	3/3 100%	0/3 100%	2/3 67%	1/3 33%	2/3 67%	1/3 33%

R=Resistance

S=Sensitive

AMC= Amoxicillin/Clavulanate

NIT=Nitrofurantoin

CN=Cephalexin

NA=Naladixic acid

SS=*staphylococcus saprophyticus*

SA=*Staphylococcus aureus*

EC = *Escherichia coli.*

SE= *Staphylococcus epidermidis*

EF= *Enterococcus faecalis*

Table (4.5) Antibacterial activity of different concentrations of *Tamarindus indica* P methanolic extract against *S. saprophyticus*

Organism	**50%(w/v)**	**25%(w/v)**	**12.5%(w/v)**	**6.3%(w/v)**
SS1	24	18	12	8
SS2	27	18	13	8
SS3	21	18	14	8
SS4	27	21	8	8
SS5	18	15	14	8
SS6	22	16	8	8
SS7	34	20	14	8
SS8	30	23	13	8
SS9	30	24	13	8
SS10	34	20	14	8
SS11	29	23	11	8
SS12	29	23	11	8

The zones were measured (mm)

SS= *Staphylococcus saprophyticus*

8=Diameter of cork borer

Table(4.6) Antibacterial activity of different concentrations of *Tamarindus indica* P methanolic extract against *S.aureus*

Organism	50%(w/v)	25%(w/v)	12.5%(w/v)	6.3%(w/v)
SA13	27	18	8	8
SA14	25	20	16	8
SA15	35	22	14	8
SA16	37	30	19	8
SA17	27	20	18	15
SA18	36	22	13	8
SA19	28	19	14	8
SA20	25	20	18	8

The zones were measured (mm)

SA= *Staphylococcus aureus.*

8=Diameter of cork borer

Table (4.7) Antibacterial activity of different concentrations of *Tamarindus indica* P methanolic extract against *E.coli*

Organism	50%(w/v)	25%(w/v)	12.5%(w/v)	6.3%(w/v)
EC 21	19	12	8	8
EC 22	17	11	10	8
EC 23	18	12	8	8
EC 24	27	19	8	8
EC 25	18	12	8	8
EC 26	23	12	8	8
EC 27	27	19	8	8

The zones were measured (mm)

EC= *Escherichia coli.*

8=Diameter of cork borer

Table (4.8) Antifungal activity of different concentrations of *Tamarindus indica* P methanolic extract against *Candida spp*

Organism	50%(w/v)	25%(w/v)	12.5%(w/v)	6.3%(w/v)
Candida spp *	8	8	8	8

The zones were measured (mm)

*N= 6 clinical isolates

8=Diameter of cork borer

Table (4.9) Distribution of patients according to symptoms, past infection and other disorder

Risk factors	Yes	Percentage	NO	Percentage (%)
Symptom	23	32	49	68
Past infection	34	47	38	53
Other disorder	5	7	67	93

About 49 (68%) pregnant women Asymptomatic and 23 (32%) Symptomatic.

About 38 (53%) pregnant had suffering from UTI and 34 (47%) were not

Most infected women did not suffer from other disorder 67 (93%) while just 5 women (7%) had suffering from hypertension and diabetes

Table (4.10) Distribution of Patient according to Trimester

Trimester	No. of patient	Percentage (%)
First	6	8
Second	17	24
Third	49	68

First trimester=Pregnant between first and third month

Second trimester=Pregnant between third and six month

Third trimester=Pregnant between six and nine month

Most infected women in the third trimester 49 (68%), then Those in second trimester 17 (24%) finally Those in first trimester 6 (8%).

Table (4.11) Distribution of Patient according to Hemoglobin Concentration

Hb%	No. of patient	Percentage (%)
G1	2	3
G2	0	0
G3	43	60
G4	22	30
G5	5	7%

G1=Group1=Hemoglobin concentration(%) 40-50.

G2=Group2= Hemoglobin concentration(%) 51-60.

G3=Group3= Hemoglobin concentration(%) 61-70.

G4=Group4= Hemoglobin concentration(%) 71-80.

G5=Group5= Hemoglobin concentration(%) 80-90.

Most pregnant women infected in G3= 43 (60%) then G4=22 (30%)

Table (4.12) Distribution of Patient according to Age

Age with year	No. of patient	Percentage (%)
18-28	47	65
29-38	22	31
39-48	3	4

Most infected women in age less than 28y =47 (65%) and more than 28y =25 (35%).

CHAPTER FIVE

DISCUSSION, CONCOLUSION & RECOMMENDATION

5.1 Discussion

The study aimed to determine pathogenic microorganisms causing urinary tract infection in pregnant women, the collected 100 sample, and 72 growth. out of 72 pregnant women 49(68%) were asymptomatic and 23(32%) were symptomatic, 34(47%) suffered from past infection and 38(53%) didn't suffer, the most affected women 49(68%) were in their third trimester and the hemoglobin (Hb) range was between 60-70% =43(60%) and most affected age was between 18-28 years47(65%) and there was no association between urinary tract infection and or infection with other disease.

Te first pathogen *S. saprophyticus*was the most abundant pathogen 31% (22/72 samples), followed by *S. aureus* 26% (19/72 samples), *E. coli* 20% (14/72 samples), *S. epidermidis* 11% (8/72 samples) yeast 8% (6/72 samples), and *Enterococcus faecalis*4% (3/72 samles).

Ninety one percent (20/22samples) *S. saprophyticus* were sensitive to Nitrofurantoin and 95% (21/22sample) of them resistant to Naldixic acid, Amoxicillin/Clavulanate 82% (18/22sample) and Cephalexin 64% (14/22samples). All *S. aureus* 100% (19/19 samples) were sensitive to Nitrofurantoin, 68% (13/19sample) of them resistance to Naldixic acid 68% (13/19sample) resistance to Amoxicillin/clavulanate. 93% of *E.coli (*13/14 sample) showed sensitive to Nitrofurantoin, 93% (13/14sample) resistance to Amoxicillin/Clavulanate, Naldixic acid 71% (10/14samples) and Cephalexin 57% (8/14samples). 88% (7/8samples) of *S.epidermidis* showed sensitive to Nitrofurantoin, 88% (7/8samples) to Cephalexin and 63% (5/8samples) to Amoxicillin/Clavulanate and resistant to Naldixic acid

88% (5/8samples). All E*nterococcusfeacalis* 100% (3/3sample) showed sensitive to Nitrofurantoin and amoxicillin/clavulanate, and 67% (2/3samples) of them resistence to Naldixicacic and Cephalexin.

In this study the pathogen isolated agree with study of Demilie, *et al*., (2012) The most common isolates detected were *E.coli* (45.7%) followed by coagulase negative *Staphylococcus* (17.1%) and *S.aureus* (8.6%) and differ in percentage of pathogen isolated from study of Alemu, *et al*., (2012) The predominant bacterial pathogens were *Escherichia coli* 47.5% followed by coagulase-negative *staphylococci* 22.5%, *Staphylococcus aureus* 10%, and *Klebsiella pneumoniae* 10% also differ from study of Hamdan, *et al.*(2011) *Escherichia coli* (42.4%) and *S. aureus* (39.3%) this study also agree with study of Emiru, *et al*., (2011) UTI was high among pregnant women in the presence of associated risk factor such as anemia also agree with study of Hamdan, *et al*., (2010) parity and history of UTI in index pregnancy were not associated with bacteriuria.

In this study the antimicrobial activity of methanol extract from the *Tamamrindus indica* pulp methanolic extract was effective against clinical isolates of *Staphylococcus aureus, Staphylococcus saprophyticus* and *Escherichia* coli which gave zones of inhibition (37mm, 34mm, 27mm respectively) by concentration 50% (w/v) and zone decrease with decrease of concentration of extract and no effect showed against *Candida spp*

In india Gupta, *et al*., (2013) study of *Tamarindus indica* plant against different types of bacteria, queous-ethanolic extract was most effective against *S. epidermidis* and *S. aureus* with diameter of zone of inhibition 19 mm and 18 mm respectively and was least effective against *Escherichia coli*

and *Enterobacter aerogenes* with diameter of zone of inhibition of 13 mm each.

In Nigeria Doughari, (2006) study of *Tamarindusindica* plant against different type of bacteria and fungi , in the methanol stem bark extract zone inhibition of *E.coli* , *S.aures* and *Candida spp* 26mm, 25mm 0mm respectively and methanol leave extract 10mm, 11mm, 0mm respectively.

The difference in zone of inhibition may be due to different solvent extract, difference in season, greographic location of plant or the difference in the method of screening i.e, disk paper or agar well diffusion method.

5.2 Conclusions

In conclusion the most frequent bacteria isolated from symptomatic and a symptomatic urinary tract infection in pregnant women attending Al-Hasahisa women hospital was *S. Saprophyticus*. Majority of the patients were asymptomatic, in their third trimester, suffered past infection, the Hb between 60-70%, the patients were between 18-28 years old, and no association was found between urinary tract infection and other disease. The most effective antibiotic against UTI clinical isolates was Nitrofurantoin. *Tamarindus indica* P methanolic extract was effective against *S. Saprophyticus, S. aureus, E. coli* and in effective against *Candida* spp. The results encourage the use of *Tamarindus indica* for treatment of urinary tract bacterial infection after further research.

5.3 Recommendations

Screening pregnant women for urinary pathogens should be done regularly at least once per month. Sensitivity tests for isolated pathogens should be done to find the suitable antibiotics and minimize the chances of drug resistance. Further research needs to be done to validate the results of this study and determine the safest and effective dose of *T.indica* that could be used for therapy against resistant pathogens.

To isolate and identify the active compound of *Tamarindus indica*.

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Appendix (1): CLED Agar

Ingredient	g/l
Peptone	4.00
Lab lemco powder	3.00
Tryptone	4.00
Lactose	10.00
L-cystine	0.128
Bromothymol blue	0.02
Agar	15.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 28 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Mix well and pour into sterile petri plates

Appendix (2): Nutrient Agar

Ingredient	g/l
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50

Yeast extract	1.50
Agar	15.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 28 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Mix well and pour into sterile petri plates.

Appendix (3): Muller Hinton

Ingredients	g/L
Beef infusion form	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00

Preparation

Suspended 38.0 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Mix well before pouring.

Appendix (4): Kligler Iron Agar

Ingredients	g/L
Peptic digest of animal tissue	15.00
Beef extract	3.00
Yeast extract	3.00
Protease peptone	5.00
Lactose	10.00
Dextrose	1.00
Ferrous sulphate	0.20
Sodium chloride	5.00
Sodium trisulphate	0.30
Phenol red	0.024
Agar	15.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 57.52 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Cool the tube slopes with 1 inch butts.

Appendix (5): Peptone water

Ingredients	g/L
Peptic digest of animal tissue	10.00
Sodium chloride	5.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 15.0 gram in 100 ml distilled water Mixed well and dispense into tubes with or without Durham's tubes and sterilize by autoclaving at 15 psi (121ºC) for 15 minutes.

Appendix (6): Simmons Citrate Agar

Ingredient	g/L
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 24.28 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Dispense and desired in tubes or flasks. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes.

Appendix (7): Mannitol Salt Agar Base

Ingredients	g/L
Protease peptone	10.00
Beef extract	1.00
Sodium chloride	75.00
D. mannitol	10.00
Phenol red	0.025
Agar	15.00

Final PH (at 25ºc) 7.4±0.2

Preparation

Suspended 28 gram in 1000ml distilled water, heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Mix well and pour into sterile petri plates.

Appendix (8): Urea agar

Ingredient	g/L
Peptic digest of animal	1.00
Sodium chloride	5.00

Dextrose	1,00
Dipotasium phosphate	1.20
Monopotasium phosphate	0.80
Phenol red	0.012
Agar	15.00

PH (at 25ºc) 7.4±0.2.

Preparation

Suspended 24 gram in 1000 ml distilled water, heat to boiling to dissolve the medium completely. Sterilized by autoclaving at 15 psi (121ºC) for 15 minutes. Cool to 50ºC and aseptically add 50 ml of sterile 40% urea solution and mix well. Dispense into sterile tubes and allow to set on slanting position. Don't over heat or reheat the medium as decomposes very easily.

Appendix (9): Antibiotic disc

Nitrofurantoin	NIT	100mc g/disc
Amoxicillin- clavulanate	AMC	30mc g/disc
Cephalexin	CN	30mc g/disc
Naladixic acid	NA	30mc g/disc
Novobiocin	NV	30 mc g/disc

Appendix (10): Autoclave (Medical Instrumentation MFG CO, Mumbia).

Appendix (11): **Hot air oven** (leader Engineering widness Cheshire, UK).

Appendix (12): Incubator(TorperPicensrdi CCRI, Italy).

All culture media used in this study were purchased from (Hi Media Laboratories Pvt. Ltd. Mumbai, India).

Appendix(13): Soxhlet(Duran UK)

Appendix(14): Rotary evaporator (Buchiswitzerland)

Appendix(15):Methanol (Romile EU)

Table (1) Results of biochemical tests for Gram positive cocci (catalase and coagulase test

Test	Positive	Negative	Total
Catalase	49	3	52
Coagulase	19	30	49

All isolated gram +ve done catalase test and coagulase; 49 isolates +ve for catalase and 3 isolates –ve, 19 isolates showed +vecoagulased and 30 –ve.

Table (2) Distribution of isolates according to Fermentation of mannitol

Mannitol fermentation	Number of isolates	Percentage
Fermenter	41	84%
Non fermenter	8	16%
Total	49	100%

When the isolates catalase +ve, sub cultured in mannitol salt agar showed 41(84%) isolates ferment of mannitol and 8 (16%) non ferment of mannitol.

Table (3) The sensitivity Gram positive isolates to ofnovobiocin disc

Novobiocin	Number of isolates	Percentage
R	22	45%
S	27	55%
Total	49	100%

S=sensitive.

R=resistant

The result of novobiocin disc showed 22 isolates(45%) resistance and 27 isolates(55%) sensitive to novobiocin.

Table (4) Differentiation of mannitol fermenter and non fermenter according tosusceptibility to Novobiocin disc

Mannitol salt	Novobiocin sensitive	Total	Novobiocin resistant	Total
Fermenter	Yes	19	Yes	22
Non fermenter	Yes	8	Yes	0

Twenty two isolates showed ferment manitol and resistant to novobiocin (*S.saprophyticus*), 19 isolates showed ferment and sensitive to novobiocin (*S.aureus*), 8 isolates showed non ferment and sensitive to novobiocin*(S. epidermidis*).

Table (5) Results of biochemical tests of Gram positive cocci with catalase negative (Esculin test)

Esculin	Number of isolates	Percentage
+ve	3	100%
-ve	0	0%
Total	3	100%

The isolated gram positive cocci with catalase negative was done esculine test to differentiation between the *streptococcus* and *enterococcus facials*

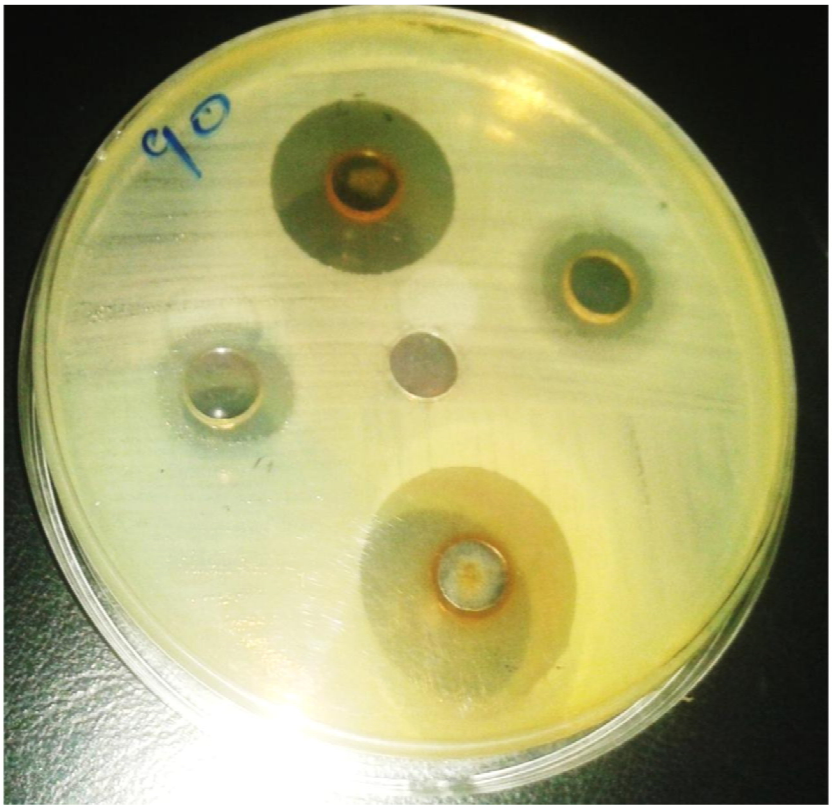
Table (6) Results of Biochemical tests of Gram negative bacilli

Organism	citrate	Urease	Indole	Glucose	lactose	gas	H2S
E11	-ve	-ve	+ve	F	F	No	No
E25	-ve	-ve	+ve	F	F	Yes	No
E23	-ve	-ve	+ve	F	F	No	No
E34	-ve	-ve	+ve	F	F	No	No
E49	-ve	-ve	+ve	F	F	Yes	No
E60	-ve	-ve	+ve	F	F	Yes	No
E70	-ve	-ve	+ve	F	F	No	No
E78	-ve	-ve	+ve	F	F	Yes	No
E104	-ve	-ve	+ve	F	F	No	No
E107	-ve	-ve	+ve	F	F	Yes	No
E156	-ve	-ve	+ve	F	F	Yes	No
E158	-ve	-ve	+ve	F	F	Yes	No
E170	-ve	-ve	+ve	F	F	No	No
E171	-ve	-ve	+ve	F	F	Yes	No

E=*Escherichia coli*+ve=positive -ve=negative

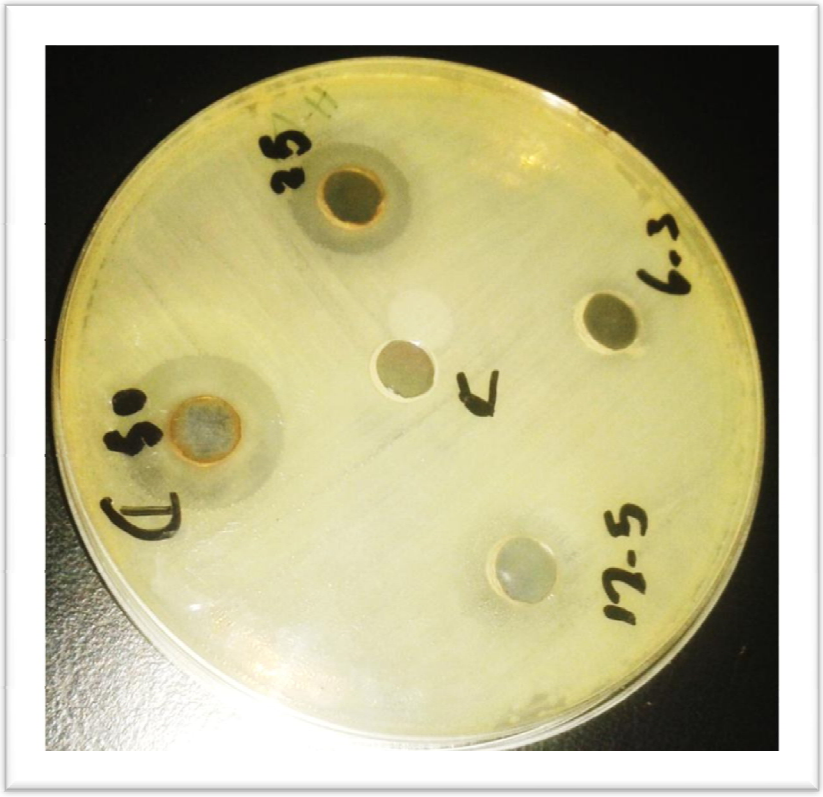
F=fermenteAll isolated gram negative stain showed -ve citrate, -ve urease and +veindole, when inoculated in KIA showed lactose ferment, glucose ferment, gas production and no H2S product.

Appendix (16):



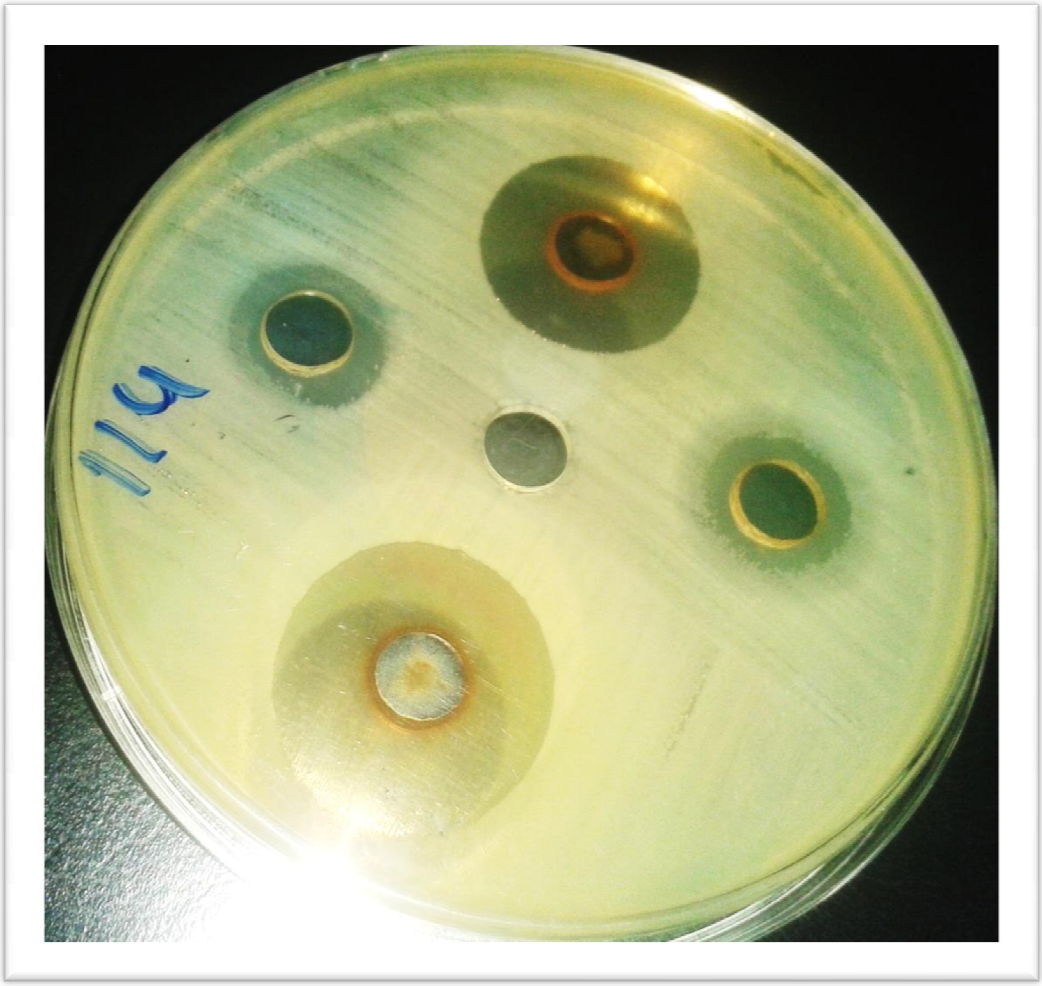
Colour plate (1): The antibacterial activity of different concentration of *Tamarindus indica P* methanolic extraction against *S.aureus.*

Appendix (17):



Colour plate (2): The antibacterial activity of different concentration of *Tamarindus indica P* methanolicextraction against *E.coli.*

Appendix (18):



Colour plate (3): the antibacterial activity of different concentration of *Tamarindus indica P* methanolic extraction against *S.saprophyticus*

Appendix (19):

Color picture No (4): *T.indica* pulp

Sudan University of Science and Technology

College of Medical Laboratory Sciences

Questionnaire

Name:……. Age:…….

Trimester:…… Hb:……..

Symptomatic ( ) Asymptomatic ( )

Using antibiotic(s)

Yes ( ) No ()

Past infection……

Other diseases ……..