

**THE SPECTRUM OF BACTERIA CAUSING URINARY TRACT
INFECTION AMONG PREGNANT WOMEN ATTENDING SABASABA
CLINIC AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL
AGENTS**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
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ABSTRACT

Urinary tract infections (UTI) during pregnancy can lead to serious obstetric complications, poor maternal and perinatal outcomes. The purpose of this study was to determine the prevalence of bacterial UTI in pregnant women in Morogoro municipality, Tanzania. This study was conducted at SabaSaba Health Centre from May 2014 to July 2014 involving 196 pregnant women. Mid stream of urine samples were obtained and analyzed by dipstick and processed for culture. Out of 196 pregnant women studied 13.8% had significant bacterial UTI. *Escherichia coli* were the most prevalent isolated bacteria with 12 isolates (44.4%). Other isolated organisms were *Klebsiella* species (3; 11.1%), *Proteus* species (2; 7.4%), *Enterococcus faecalis* (3; 11.1%), Coagulase negative *Staphylococcus* (5; 19%) and *Staphylococcus aureus* (2; 7.1%). Antimicrobial sensitivity test results showed that out of 12 *E. coli* isolates, 33%, 42%, 50%, 50%, 67%, 83%, were resistant to ciprofloxacin, ampicillin, tetracycline, amoxicillin, cotrimoxazole and erythromycin respectively. Out of three *Klebsiella* species, 33% were resistant to ciprofloxacin, 67% were resistant to ampicillin, amoxicillin and tetracycline and 100% were resistant to cotrimoxazole and erythromycin. Two *Proteus* species isolated showed 100% resistance to tetracycline and cotrimoxazole and 0% resistance to amoxicillin, ampicillin, ciprofloxacin and erythromycin. Two *Staphylococci aureus* isolates showed 50% resistance to ampicillin, amoxicillin, tetracycline, ciprofloxacin, cotrimoxazole and erythromycin. Five Coagulase negative *Staphylococci* isolates showed 20% resistance to ampicillin, amoxicillin and ciprofloxacin, 60% to tetracycline, cotrimoxazole and erythromycin. Three *Enterococcus faecalis* isolates showed 100% resistance to ampicillin, amoxicillin, tetracycline, ciprofloxacin,

cotrimoxazole and erythromycin. Using univariate analysis there was no significant association of age, parity, gestational age, education, marital status, and history of UTI with bacteriuria. The study showed that only ciprofloxacin could be the drug of choice for treatment UTI, but it is contraindicated during pregnancy. Further studies of antimicrobial sensitivity of isolates from UTI during pregnancy are recommended.

DECLARATION

I, **RAMADHAN ALLY MATALINGANA**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

.....

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.....

Date

The above declaration is confirmed by:

.....

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(Supervisor)

.....

Date

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DEDICATION

This Thesis is dedicated to my father Ally Matalingana, my mothers' Fatimah Mbombwe and Ashura Ngonyani.

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Source: [http://en.wikipedia.org/wiki/Morogoro_Region] site visited 1/10/

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ABBREVIATIONS

A	Amoxicillin
ANC	Antenatal clinic
AP	Ampicillin
ATCC	American Type Culture Collection
°C	Centigrade
CFU	Colony Forming Unit
CLSI	Clinical Laboratory Standard Institute
Cip	Ciprofloxacin
Cot	Cotrimoxazole
E	Erythromycin
HIV	Human immune deficiency virus
mg	Milligram
MDR	Multi Drug Resistance
MMC	Morogoro Municipal Council
MOHSW	Ministry of Health and Social Welfare
N	Number
NIMR	National Institute for Medical Research
(O)	Orally
P	Probability
PMTCT	Prevention Mother to Child Transmission
SUA	Sokoine University of Agriculture
SD	Standard Deviation
T	Tetracycline
UTI	Urinary Tract Infections

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Screening for urinary tract infections (UTI) is one of the interventions in antenatal clinic (ANC) of the National strategy plans to accelerate the reduction of maternal, newborn and child deaths in Tanzania where the estimated number of annual maternal deaths is 13 000 (MOHSW, 2008). The government of Tanzania is expecting to reduce maternal mortality rate by three-quarters by 2015. UTI are common health care problems among pregnant women with the global prevalence ranging from 13%-33% with asymptomatic bacteriuria occurring in 2-10% and symptomatic occurring in 1-18% during pregnancies (Samad, 2007; Agersew *et al.*, 2012; Wamalwa *et al.*, 2013). Previous studies that have been done in Tanzania, the prevalence of UTI among pregnant women was found to be 16 % in Hanang and Mwanza (Olsen *et al.*, 2000; Masinde *et al.*, 2009) and 21% in Dar es Salaam (Moyo *et al.*, 2010). UTI occur more frequently in women due to the anatomical structure whereby the female urinary tract is shorter and near to the anus which serves as a rich reservoir of microbial pathogens. Also the level of oestrogen in women decreases with menopause which tends to increase the possibility of suffering from UTI due to loss of protective vaginal flora (Dielubanza and Schaeffer, 2011).

As reported by Schnarr and Smaill (2008), the incident of acute pyelonephritis in pregnant women is more significant than is the case with non pregnant women. This is because pregnancy is a unique state with changes in anatomical and physiological status of the urinary tract. During pregnancy, progesterone level becomes high causing

a decrease in muscle tone of the ureters and bladder leading to an increased reflux of urine back to the ureters and the kidneys (Dielubanza and Schaeffer, 2011). When a pregnant woman is diabetic or has low immunity, the infection in the upper urinary tract is considered as a more complicated case than is the case with similar infection to an infected healthy woman (Colgan and William, 2011). The presence of UTI during pregnancy if undetected or left untreated can lead to serious obstetric complications, poor maternal and perinatal outcomes such as intrauterine growth restriction, pre-eclampsia, caesarean delivery and preterm deliveries (Mazor *et al.*, 2009). Screening for and treatment of asymptomatic bacteriuria in pregnancy has become a standard procedure of obstetric care and it has been included in most antenatal guidelines (Nicolle *et al.*, 2005; MOHSW, 2008).

1.2 Problem statement and study justification

Pregnant women are vulnerable to UTI which may lead to complications such as, hypertensive diseases, anaemia, chronic renal failure, premature delivery and foetal mortality (Moyo *et al.*, 2010). The understanding of the epidemiology and antimicrobials susceptibility pattern of UTI during pregnancy is fundamental care for pregnant women and health care providers in guiding intervention strategies. Literature on UTI such as prevalence of bacterial isolates and antimicrobials sensitivity during pregnancy in Morogoro municipality is limited. As a result, most of clinicians continue treating UTI on the basis of probability which may increase antimicrobials resistance. The aim of this study was to determine the prevalence of UTI and antimicrobials susceptibility pattern of the bacterial isolates in pregnant women diagnosed with UTI in Morogoro municipality. The study results are expected

to help health administrators in formulating guidelines on how to control and treat UTI leading to safeguarding the health of pregnant women and the newborns. The guidelines such as performing antimicrobials sensitivity tests before prescribing antimicrobial will help to reduce antimicrobial resistance in pregnant women.

1.3 Objectives

1.3.1 Overall objective

The main objective was to determine the prevalence of bacterial urinary tract infections and antimicrobial sensitivity of the bacterial isolates in pregnant women in Morogoro municipality.

1.3.2 Specific objective

Specifically the study intended to

- i. Identify the most prevalent bacterial isolates from UTI in pregnant women in Morogoro municipality.
- ii. Assess and establish antimicrobials susceptibility profiles among identified bacterial isolates in Morogoro municipality.
- iii. Assess the awareness and frequency of checking UTI in pregnant women in Morogoro municipality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of urinary tract infection

UTI is the term that includes either asymptomatic microbial colonization of urine or symptomatic infection with microbial invasion and inflammation of the urinary tract. However, microbiologically urinary tract infection exists when pathogenic microorganisms are detected in the urine, urinary bladder, urethra, kidney, or prostate (Kumar *et al.*, 2013).

2.2 Symptoms

Lane and Takhar (2011) classified UTI as either asymptomatic or symptomatic. Schnarr and Smaill (2008) classified symptomatic UTI into lower tract (acute cystitis) or upper tract (pyelonephritis) infections. The acute cystitis is defined as significant bacteriuria with associated bladder mucosal invasion with the presence of symptoms such as dysuria, urgency, frequency, nocturia, haematuria and suprapubic discomfort in febrile women without evidence of systemic illness and is distinguished from pyelonephritis with associated inflammation of the renal parenchyma, calices and pelvis in the presence of systemic illness (McCormick *et al.*, 2008; Schnarr and Smaill, 2008).

Symptoms of pyelonephritis include flank or renal angle pain, pyrexia, rigor, chills, nausea and vomiting (McCormick *et al.*, 2008; Schnarr and Smaill, 2008).

2.3 Aetiological agent

The common cause of UTI is *Escherichia coli* whereas *Staphylococcus saprophyticus* is the second isolated pathogen (Nicolle, 2008). *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter* are uncommon and typically related to abnormalities of the urinary system or following catheterization (Salvatore *et al.*, 2011). The UTI caused by *Staphylococcus aureus* is typically occurring due to secondary blood borne infections (Lane and Takhar, 2011).

2.4 Transmission

Transmission of UTI in women in most cases occurs because members of the colonic microbiota, for example certain strains of *Escherichia coli* move a short distance from the anus to the vagina where they become established. They then proceed from the vaginal area to the urethra and then to the urinary bladder. The bacteria may be cleared from the bladder by antibiotic therapy; however, if they remain in the colon or vagina, they may reinfect the urethral and the bladder (Salyers *et al.*, 2001).

2.5 Diagnosis

In some cases, the diagnosis of UTI is based on the symptoms alone such as dysuria, urgency, frequency, nocturia, haematuria and suprapubic discomfort in a febrile woman without laboratory confirmation (Salyers *et al.*, 2001). When the case is complicated it is important to confirm through urinalysis to detect the presence of nitrites, white blood cells (leukocytes), specific gravity, blood, protein and pH (Salyers *et al.*, 2001). Microscopic technique is a routine method for detecting UTI which identifies the presence of red blood cells, white blood cells, epithelial cells and casts

cells (Cheesbrough, 2006). According to Nicolle (2008), urine culture is a useful technique in the isolation of organisms; and the antimicrobials sensitivity test can show which antimicrobial will be most effective in controlling a microbe infection. The diagnostic methods of UTI at SabaSaba health center observed during the study period were based on symptomatic, urinalysis and microscopic examination (MMC, 2014).

2.6 Treatment

Currently, UTI during pregnancy is treated by amoxicillin 500mg (O) every 8 hourly for 5 days as first line drug while nitrofurantoin 100mg (O) every six hours for 5 days plus amoxicillin + clavulanic acid 625mg (O) 8 hourly for 5 days as second line treatment (MOHSW, 2013). According to prescriptions for treating UTI for one hundred individuals at SabaSaba health center, 4%, 12%, 16%, 30%, 38% were given doxycycline, erythromycin, ciprofloxacin, amoxicillin and cotrimoxazole respectively (MMC, 2014)

2.7 Prevention

There are a number of general guidelines and suggestions that will help women avoid UTI in most instances. These may conveniently be divided into five categories namely hygiene, clothing, diet, activities, and medication (Brusch, 2013).

(i) Hygiene

Women are always required to wipe from the front to the back after using the bathroom and they should not try to reach from behind because germs from the rectum can be transferred to the hand and tissue.

(ii) Clothing

Pregnant women are not required to wear tight-fitting undergarments made of non-breathing materials. Such fabrics make the moisture accumulates leading to maceration of the skin and can cause bacterial over growth adjacent to the opening of the bladder.

(iii) Diet

Drinking more water started with one extra glass with each meal is suggested. Increased fluid intake will minimize UTI.

(iv) Activities

Women who are engaged in physical activities and exercise must empty their bladder frequently and drink plenty of water and other fluids. Also they are required to take special precautions after sexual activity because such an activity may increase the risk of bacterial infection into the bladder area.

(v) Medication

The use of estrogen vaginal cream may help increase resistance against bladder infections. Estrogen cream for the vagina may be advised to women after menopause even if an oral estrogen supplement or patch has already been prescribed. The cream helps to keep the tissues around the bladder healthy and more resistant against infections.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study setting

This study was conducted at SabaSaba Health Center located about one kilometer from Morogoro municipal central precinct. The Health Center was chosen due to location where many clients and patients come for seeking health services. The Health Centre was meant to serve two wards of SabaSaba and Uwanja wa Taifa; but due to its geographical location it serves about 19 wards found in the municipal area and the neighbouring villages in other districts.

Morogoro regionis located on the eastern part of Tanzania (Fig. 1) and lies between latitudes 5°58' and 10°00' south of Equator and between longitudes 35°25' and 38°30' east of Greenwich meridian. The total area of the region is 73 039 km², of which 531 km² are found in the municipal council. The municipal of Morogoro is located about 195 km west of Dar es Salaam city. The climate of Morogoro municipal is warm between November and December with temperatures ranging between 28°C and 30°C. There are two rain seasons: long rainy season which occurs between March and May (with an average rainfall of between 821 – 1 505mm) and short rainy season which occurs between October and December (with an average rainfall of 400mm). According to the population census of 2012, Morogoro municipal has 315 866 people, among these 151 700 were men and 164 166 were women (URT, 2012).

3.2 Study design

A cross sectional study was conducted at SabaSaba Health Center in order to establish the prevalence of bacterial UTI. The study was carried out for the period of three months from May 2014 to July 2014. Systematic random sampling method was used to identify qualified pregnant women. At least five pregnant mothers were selected daily from ANC by serial interval of five. Demographic information, symptoms, previous infection, awareness and frequency of checking UTI were gathered by using questionnaire (Appendix 1). The information from the questionnaire was translated in Swahili language by health care provider to all pregnant mothers enrolled. During this period, urine samples were collected and analyzed from all pregnant women who fulfilled the inclusion criteria. The inclusion criteria were all pregnant mothers attended, registered, consented and willing to be included in the study while exclusion criteria were all pregnant mothers used antibiotics for the past seven days.

3.3 Patient population

The Health Centre was meant to serve two wards of SabaSaba and Uwanja wa Taifa with a total population of 9 586 people. The number of clients and patients served per day at the health center is above 300 where about 30 clients are pregnant women (MMC, 2014). The services provided at the ANC are prevention of mother to child transmission (PMTCT) of HIV, vaccination, and family planning.

3.4 Sample collection

The required sample size for this study was calculated using the formula generated by Kish and Leslie (1965).

$$n = z^2 \times p(1-p) / d^2 \dots\dots\dots(1)$$

Where n = required sample size;

z = score for 95% confidence interval = 1.96;

p = estimated prevalence;

d = tolerable error = 5%.

Using the prevalence of 15% of Mwanza reported by Masinde *et al.* (2009) since the prevalence of Morogoro was not known, the required sample size estimated was 196 pregnant women. Ten milliliters of mid stream urine was collected in a sterile universal container from each pregnant woman after been given some instructions on the procedures of collecting sterile urine. The women were instructed to clean the area around the urethral opening with clean water, and were asked to wait to for the area to dry and then collect the urine with the labia held apart. The pregnant women used their fingers to open the labia so as to release a small amount of urine into the toilet bowl then to the universal container.

3.5 Laboratory investigation

Some of the urine specimens were analyzed by using multistik (CYBOW™ Republic of Korea) at the Health Center and the remaining samples were stored at 4 - 6 °C in a cool box which was then transferred to Sokoine University of Agriculture (SUA) laboratory for culture and sensitivity analysis. On the same day of the collection of specimens, a calibrated wire loop of 0.001 ml was used to inoculate a loop full of urine on MacConkey and blood agar (OXOID England) plates. The plates were incubated for 24 hours at 37°C aerobically and observed on the following day (Plate

1A). Significance of UTI was determined according to Hamdan *et al.* (2011) as the presence of bacterial count of 10^5 colony forming unit (CFU) /ml or more. The identification of bacteria was done according to Cheesbrough (2006) method of observing the morphology of bacteria in the culture media, Gram stain and biochemical tests.

After the identification of bacteria pathogens, disk diffusion (Bauer *et al.*, 1966) was used to perform antimicrobial susceptibility tests as recommended by Clinical and Laboratory Standard Institute (CLSI, 2012). The antimicrobial discs which were chosen for all the isolates were ciprofloxacin, erythromycin, cotrimoxazole, amoxicillin, tetracycline and ampicillin. The choice of antimicrobial depended on the pathogens isolated, the range of locally availability and the local prescribing policies. Individual colonies were inoculated on Muller Hinton agar and antimicrobial discs were distributed (Plate 1B). The *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains (Hamdan *et al.*, 2011; CLSI, 2012). By using sterile wire loop three isolated bacteria colonies of similar appearance and control separately were emulsified in 3 ml sterile physiological saline, compared to 0.5 McFarland standard and separately inoculated into Muller Hinton agar. The appropriate antimicrobial discs were correctly placed on the inoculated plate by using sterile forceps. After incubation for 24 hours at 37°C, the test plates and control were examined to determine growth. Measurement of the diameter of each zone of inhibition was done underside the plate by using a ruler and was interpreted using a CLSI interpretive chart.

3.6 Data analysis

Data storage and analysis were done using Epi info version 7 statistics software which is applied in epidemiological studies where descriptive statistics and univariate analysis were performed. Means and proportions of the social demographic characteristics were calculated and compared between the growth of positive and negative groups by using student t- test and chi square test respectively. Probability values of <0.05 were considered as statistically significant for all the results. The prevalence of UTI was calculated using the formula described by (Thrusfield, 2005).

Prevalence of UTI = Number of cases / Population size.....(2)

3.7 Ethical approval

Ethical approvals were obtained from National Institute for Medical Research (NIMR) and district health authorities of Morogoro municipality (Appendix 5). A consent form was used to invite the pregnant women to participate into the study (Appendix 2 for Kiswahili and 3 for English). For those who were diagnosed with UTI, results of laboratory investigation were given and appropriate management conducted.



Figure 1: Tanzanian map showing the location of study area, Morogoro Region.

Source:[http://en.wikipedia.org/wiki/Morogoro_Region] site visited

1/10/ 2015.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence

During this study, a total of 196 pregnant women were examined for bacterial UTI. Using bacteriological technique (culture with more than 10^5 CFU), Twenty seven out of 196 were found to have bacterial UTI. The prevalence of bacterial UTI from pregnant women studied in Morogoro municipality was found to be 13.8%. Fifty six (28.6%) of the pregnant women in the study had symptoms associated with UTI while 140 (71.4%) had no symptoms associated with UTI. With regards to symptoms, 21 (37.5%) were most reported symptom to dysuria and oedema while 2 (3.6%) as the least reported symptom to haematuria and incontinence (Table 1). The prevalence of symptomatic and asymptomatic bacteriuria was 16.1% and 12.9% respectively. There was no statistically significant difference between symptomatic and asymptomatic pregnant women with bacteriuria ($p = 0.5563$).

4.2 Bacteria isolation

Routine bacteriological culture was carried out in order to isolate bacteria from mid stream urine in horse blood agar and MacConkey agar (Plate 1 A). Gram stain and biochemical tests were carried out for identification of bacterial isolates (Plates 1 C - D; Plate 2 E - H). Out of 196 urine samples collected 27 showed significant growths. The frequency of Gram negative bacteria causing UTI was higher than was the case with Gram positive bacteria. The causative bacteria isolated were 17 Gram negative rods and 10 Gram positive cocci. *Escherichia coli* were the most prevalent bacteria isolated with 12 isolates (44.4%). Other isolated bacteria were *Klebsiella* species (3;

11.1%), *Proteus* species (2; 7.4%), *Enterococcus faecalis* (3; 11.1%), Coagulase negative *Staphylococcus* (5; 19%) and *Staphylococcus aureus* (2; 7.4%). The quantity of bacterial isolates is displayed on (Table 2).

Table 1: Common symptoms of urinary tract infection among pregnant women at SabaSaba Health Center, Morogoro, Tanzania

Symptoms	Number of pregnant women with respect to symptom of UTI (%)
Frequency of urination	18 (32.1%)
Dysuria	21 (37.5%)
Urgency of urination	3 (5.4%)
Haematuria	2 (3.6%)
Nocturia	15 (26.8%)
Incontinence	2 (3.6%)
Back pain	20 (35.7%)
Oedema	21 (37.5%)

NB: Total number of pregnant women with symptoms of UTI, N = 56

Table 2: Distribution of bacteria isolated from urine among pregnant women at SabaSaba Health Centre, Morogoro, Tanzania.

Gram positive bacteria	Number of isolates	Gram negative bacteria	Number of isolates
<i>Enterococcus faecalis</i>	3	<i>Escherichia coli</i>	12
Coagulase negative <i>Staphylococcus</i>	5	<i>Klebsiella spp</i>	3
<i>Staphylococcus aureus</i>	2	<i>Proteus spp</i>	2

4.3 Antimicrobial sensitivity test

Sensitivity test was carried out in order to assess the resistance of isolates to a particular drug (Plate 1B). The antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method following the definition of the Clinical Laboratory Standards (CLSI, 2012) for agar diffusion tests. The interpretation was based on the size of the zone of inhibition in which the organism reported as susceptible, intermediate, or resistant to the agents that have been tested (Appendix 4). The antimicrobial discs used for all isolates were ampicillin, amoxicillin, tetracycline, ciprofloxacin, cotrimoxazole and erythromycin. Antimicrobial sensitivity test results showed that out of 12 *E. coli* isolates, 33%, 42%, 50%, 50%, 67%, 83%, were resistant to ciprofloxacin, ampicillin, tetracycline, amoxicillin, cotrimoxazole and erythromycin respectively. Three *Klebsiella* species isolates showed 33% resistance to ciprofloxacin, 67% to ampicillin, 67% to amoxicillin, 67% to tetracycline and 100% resistance to cotrimoxazole and erythromycin. Two *Proteus* species isolates showed 100% resistance to tetracycline, 100% to cotrimoxazole and 0% resistance to amoxicillin, ampicillin, ciprofloxacin and erythromycin. Two *Staphylococci aureus* isolates showed 50% resistance to ampicillin, amoxicillin,

tetracycline, ciprofloxacin, cotrimoxazole and erythromycin. Five Coagulase negative *Staphylococci* isolates showed 20% resistance to ampicillin, 20% to amoxicillin, 20% to ciprofloxacin, and 60% resistance to cotrimoxazole, tetracycline and erythromycin. Three *Enterococcus faecalis* isolates showed 100% resistance to ampicillin, amoxicillin, tetracycline, ciprofloxacin, cotrimoxazole and erythromycin.

An isolate was considered multidrug resistant (MDR) if it was resistant to at least three of the antimicrobial agents tested (Santo *et al.*, 2007). The multidrug resistance phenotype of Gram negative bacteria was shown by the *Escherichia coli* 75% and *Klebsiella* species 100%, while for Gram positive bacteria the MDR was mostly detected in *Enterococcus faecalis* 100% (Table 3). All these bacteria (*Escherichia coli*, *Klebsiella* and *Enterococcus*) showed MDR to tetracycline, erythromycin, cotrimoxazole, ciprofloxacin, ampicillin and amoxicillin.

Table 3: The antimicrobials resistance phenotypes showing multidrug-resistance of Gram positive and Gram negative bacteria isolated from urine of pregnant women at SabaSaba Health Centre Morogoro, Tanzania

Antimicrobial resistance phenotype	Number of isolates
<i>Escherichia coli</i> N = 9	
T, E, Cot	2
E, A, AP	1
T, E, Cot, Cip	1
E, Cot, A, AP	2
T, E, Cip, A	1
E, Cot, Cip, A, AP	1
T, E, Cot, A, AP	1
<i>Klebsiella</i> species N = 3	
T, E, Cot, Cip	1
E, Cot, A, AP	1
T, E, Cot, A, AP	1
<i>Enterococcus faecalis</i> N = 3	
A, AP, T, E, Cot, Cip	3

Note: AP Ampicillin, A Amoxyciline, Cot Cotrimoxazole, E Erythromycin, T Tetracycline, Cip ciprofloxacin

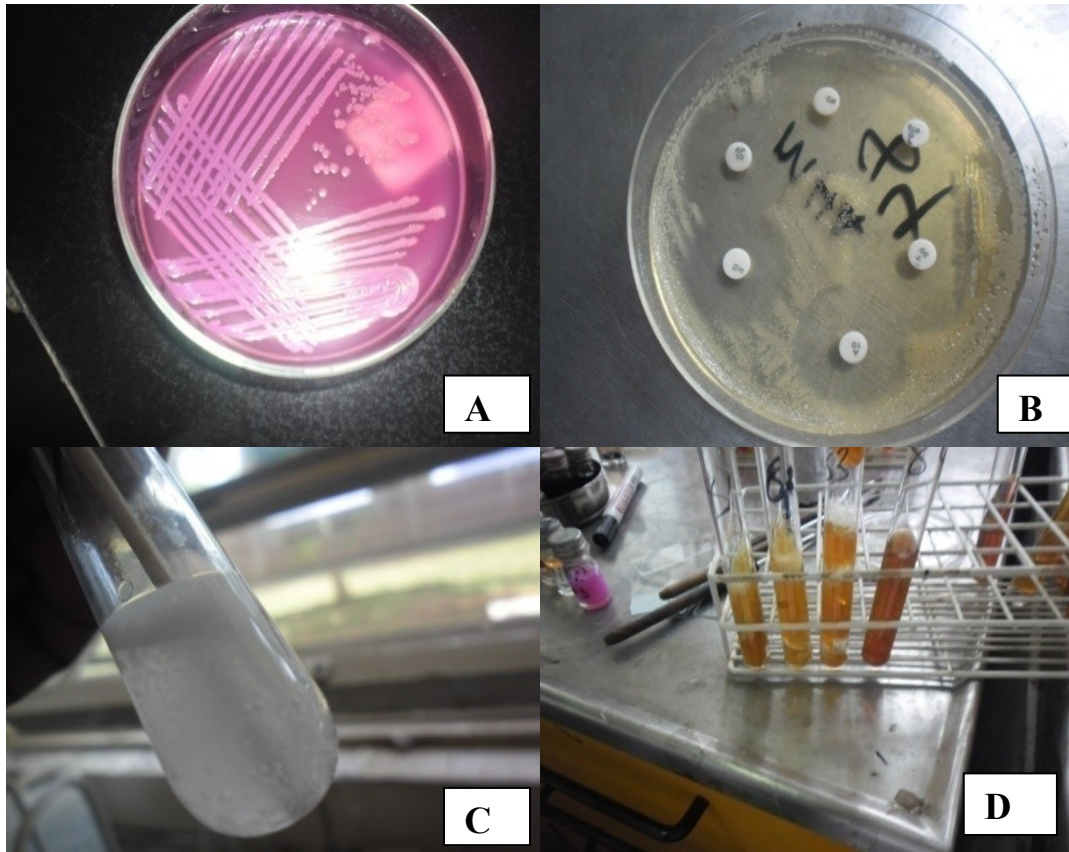


Plate 1: Bacterial isolation (A); Antimicrobial sensitivity test (B); Coagulase test(C); Triple Sugar Iron (D)..

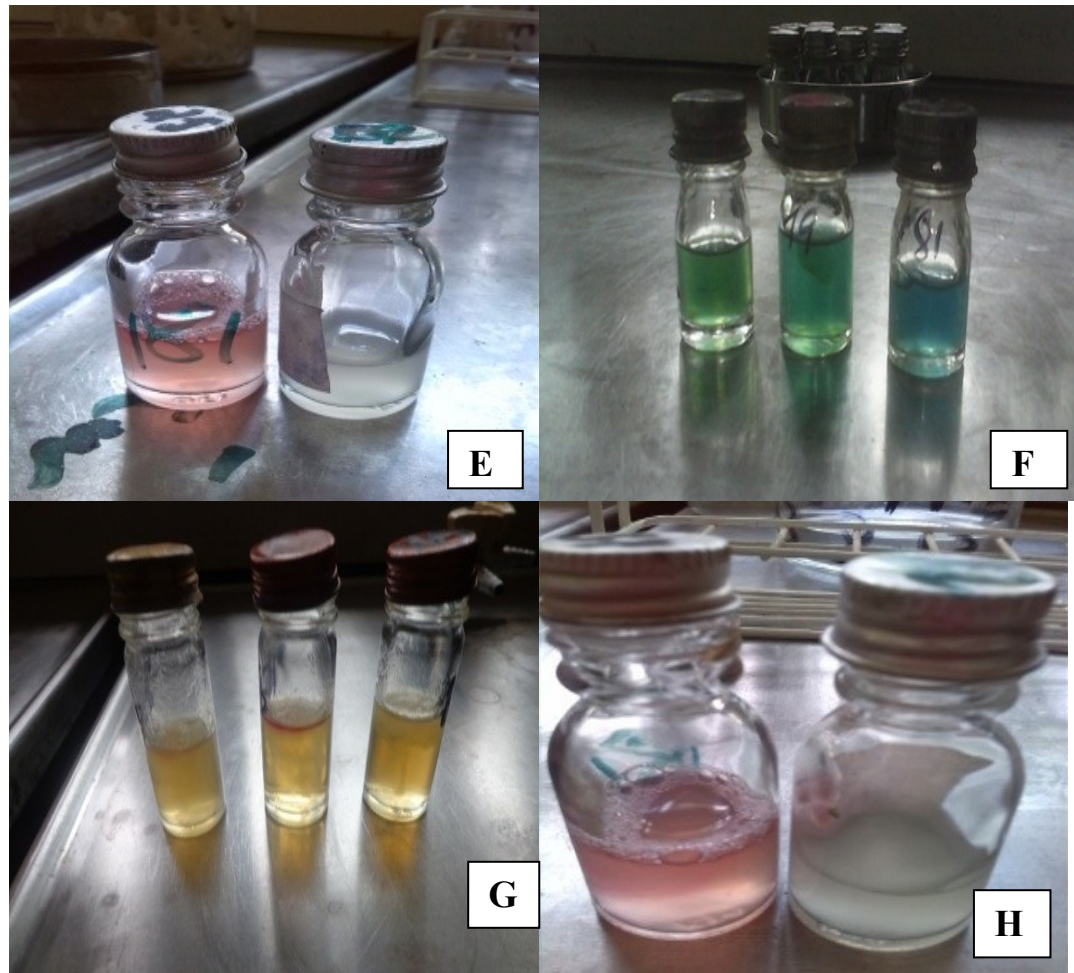


Plate 2: Voges – Proskauer test (E); Citrate test (F); Sulphur Indole Motility test (G)
;Methyl red test (H) .

4.4 Assessment of demographic characteristics associated with the urinary tract infection among pregnant women.

4.4.1 Maternal age

The ages of pregnant women enrolled in this particular study were between 16 and 46 years old. The mean and standard deviation (SD) of the age were 26 and 5.4 respectively. Of all individuals enrolled, 21 - 25 years old had higher frequency 71 (36.2%), while those aged between 46 - 50 years (0.5%) had the least frequency. There was no statistical association between the ages of pregnant women and bacterial UTI ($p = 0.1763$).

4.4.2 Gestation age

The mean and standard deviation of gestation age in weeks of pregnant women enrolled were 20.4 and 7.8 respectively. Thirty six (18.4%) of pregnant women examined were in the first trimester while 96 (50%) were in the second trimester and 64 (32.6%) in the third trimester. Four (11.1%), 13 (13.5%) and 10 (15.6%) were detected with bacterial UTI in the first, second and third trimesters respectively. There was no statistically significant difference between the gestation ages of pregnant women and bacterial UTI ($p = 0.5216$).

4.4.3 Parity

Ninety seven (49.5%) of all pregnant women enrolled were prime gravid, 38 (19.4%) were found in gravid one, 36 (18.4%) were in gravid two and 25 (12.7%) were in gravid three and above. The frequency of bacterial UTI regarding to gravid was 15 (15.5%), 5 (13.2%), 4 (11.1%) and 3 (12.0%) respectively. There was no statistically

association between the number of gravid in pregnant women and bacterial UTI ($p = 0.8299$).

4.4.4 Education

One hundred and eighteen (60.2%) pregnant women had completed primary education, among of them 15 (12.7%) were diagnosed with bacterial UTI. Sixty six (33.7%) had completed secondary school, four (2%) had completed post-secondary school and the remaining eight (4.1%) had not attended any formal education. Ten (15.2%), none (0%) and two (25%) were diagnosed with bacterial UTI from secondary, post-secondary, and among those with no formal education respectively. There was no statistically significant difference between the level of education of pregnant women and bacterial UTI ($p = 0.3874$).

4.4.5 Marital status

One hundredth and sixty seven (85.2%) pregnant women examined were married while 24 (12.2%) were single, 5 (2.6%) cohabiting, 0 (0%) divorced and 0 (0%) widowed. Twenty two (13.2%) married and five (17.2%) single were tested significant bacterial UTI. There was no statistical association between the marital status of pregnant women and bacterial UTI ($p = 0.3874$).

4.5 Assessment on the awareness and frequency of checking urinary tract infection

A questionnaire was used to collect information on awareness and frequency of checking urinary tract infection during pregnancy (Appendix 1).

4.5.1 Assessment of importance of checking urinary tract infection

Although majority of pregnant women infected with urinary tract, most of them were unaware about the problem. The results of the questionnaire conducted on knowledge related to UTI showed that nine (4.6%) of the respondents knew the risk of becoming infected during pregnancy. Seven (3.6%) explained that UTI may cause miscarriage while one (0.5%) each cited the risks of becoming anaemic and have eclampsia.

4.5.2 Assessment of pregnant women who checked for urinary tract infection before being enrolled into the study

Thirty seven (18.9%) of the pregnant women were tested for UTI using microscope, dipstick or both microscope and dipstick during pregnancy before enrolled into this study. Among pregnant women who were tested for UTI, eight (21.6%) were in the first trimester, while 14 (37.8%) were in second trimester and 15 (40.5%) were in third trimester.

4.5.3 Assessment of pregnant women with a history of urinary tract infection

Thirty seven (18.9%) respondents had a history of UTI before they were engaged into the study and had received antibiotic for treating this infection. Among the respondents who had a history of UTI in the last months, two (5.4%) respondents had bacteriuria while 25 (15.7%) individuals had bacteriuria from this particular study but had no history of this infection in the past months. Thirty three (89.2%) out of 37 individuals who had history of UTI were prescribed for amoxicillin, one (2.7%) respondent was prescribed for ciprofloxacin while three (8.1%) respondents were

prescribed for erythromycin. There was no statistically significant difference between the history of UTI and bacterial UTI ($p= 0.1018$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence

The study has shown that the prevalence of bacterial urinary tract infections among pregnant women in Morogoro municipality is 13.8%. This was found to be similar to what has been observed in other parts of Tanzania (Olsen *et al.*, 2000; Masinde *et al.*, 2009; Moyo *et al.*, 2010), and in other East African countries such as Kenya (14.2%) (Wamalwa *et al.*, 2013) and Uganda (13.0%) (Andabati and Byomugisha, 2010). Similar prevalence of UTI among pregnant women were found in other African countries such as Ethiopia (11.0 %) and Sudan (14.0%)(Assefa *et al.*,2008; Hamdan *et al.*, 2011).

The prevalence of symptomatic bacteriuria observed was 16.1% with highest frequency in dysuria and oedema, while that of asymptomatic bacteriuria was 12.9%. Asymptomatic bacteriuria is common during pregnancy and if left untreated can lead to the development of symptomatic cystitis in approximately 30 percent of patients and pyelonephritis in up to 50 percent of patients (Kass, 1970). However, the prevalence of asymptomatic and symptomatic bacteriuria observed in our study is significantly higher than those reported in developed countries and this is likely to be attributed to low socio-economic status (Schnarr and Smaill 2008; Gayathree *et al.*, 2010; Wamalwa *et al.*, 2013). The presence of association between low social economic statuses with UTI especially in pregnant women could be related to poor nutrition and low immunity (Dimetry *et al.*, 2000; Jalali *et al.*, 2014).

5.2 Bacterial isolation and antimicrobial sensitivity

In this study, 63% of Gram negative rod and 37% of Gram positive cocci were isolated giving high prevalence of *Escherichia coli* followed by *Staphylococci*. Similar results about *Escherichia coli* and *Staphylococci* being the main pathogens causing UTI have been reported in other parts of Tanzania (Masinde *et al.*, 2009; Moyo *et al.*, 2010) and elsewhere (Schnarr and Smaill, 2008; Almushaitet *et al.*, 2013).

Escherichia coli is considered a lead uropathogen due to a number of virulence factors specific for colonization and invasion of the urinary epithelium, including P and S fimbrial adhesins (Moyo *et al.*, 2010). Among 75% *Escherichia coli* isolates showed multidrug-resistance mainly to erythromycin, cotrimoxazole and amoxicillin. An increased trend of resistance for the commonly used antibiotics such as cotrimoxazole, erythromycin, ampicillin, and tetracycline in Tanzania has been reported by Mshana *et al.* (2013), where the resistance rates of cotrimoxazole in *Escherichia coli* and *Staphylococci* from urine ranged from 50% - 97% and 31% - 73% respectively. In other countries like Brazil, it was found that the multi-drug resistance (MDR) for *Escherichia coli* were 73%, 65%, 58%, 58% and 31% for tetracycline, ampicillin, cephalothin, cotrimoxazole and norfloxacin, respectively (Santo *et al.*, 2013). Sixty seven percent of *Escherichia coli* and 80% of Coagulase negative *Staphylococci* isolates were susceptible to ciprofloxacin. Similar findings have been reported (Gupta *et al.*, 1999; Farrel *et al.*, 2003) that many common UTI pathogens are susceptible to ciprofloxacin. This may be due to the direct relation between the frequency of antibiotic used and the resistance strains of microbial pathogens in human beings (Karlowsky *et al.*, 2003; Nadia Gul *et al.*, 2004).

However, the study showed high MDR in *Escherichia coli*, *Klebsiella* and *Enterococcus faecalis* to the commonly used antibiotics among the Gram negative bacteria and Gram positive bacteria. The observed resistance of these bacteria to the drugs is a probable indicator of previously exposure of the isolates to these antibiotics, which may have enhanced resistant development (Joseph, 2003).

5.3 Assessment of demographic characteristics with the presence of urinary tract infection among pregnant women in Morogoro municipality

The maternal age, marital status, education, gestation age, parity and history of UTI were not associated with bacterial UTI. Similar findings have been reported elsewhere (Masinde *et al.*, 2009; Moyo *et al.*, 2010; Wamalwa *et al.*, 2013). However, the present study shows that there was higher frequency of bacteriuria in the third trimester than was the case with the first and second trimesters. Some studies have shown that UTI developed in the third trimester due to urethral dilatation which started as early as six weeks and reaching the maximum during 22-24 weeks (Dalzell and Lefevre, 2000; Tugrulet *et al.*, 2005; Wamalwa *et al.*, 2013).

5.4 Assessment for awareness and frequency of checking urinary tract infection during pregnancy

Majority (95.4%) of the pregnant women did not know the importance of checking for UTI during pregnancy. In Kenya, studies about prevalence and risk factors for UTI among pregnant women showed that 67.3% of the mothers knew the importance of urine testing during pregnancy (Wamalwa *et al.*, 2013). Lack of awareness on the importance of checking for UTI during pregnancy might have resulted into few

(40.5%) pregnant women up to third trimester actually checking for UTI. As reported by MOHSW (2008), about 41% of pregnant women in the country actually do urinalysis during pregnancy. More health education on UTI during pregnancy should be provided by nurses and midwives during ANC visit.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Although risk factors for urinary tract infection in pregnant women are documented, there is a need of strengthening health education for pregnant women about the awareness of regular checkup for urinary tract infection. This will reduce maternal deaths and antibiotics consumption especially because UTI is the second cause for unnecessary antibiotic prescription all over the world (Almushait *et al.*, 2013). Since UTI is a preventable disease, it could be easily controlled through the provision of health education pertaining to its nature, causes, risk factors and preventive measure to the target groups.

The rate of resistance to Amoxicillin as first line drugs for treating bacterial UTI during pregnancy is high; therefore, there is a need for revising treatment guidelines. The present study showed that only ciprofloxacin could be the drug of choice for treatment of UTI, but it is contraindicated during pregnancy (MOHSW, 2013). Ampicillin, tetracycline, cotrimoxazole and erythromycin were found not effective for management UTI during pregnancy; thus further studies of antimicrobial sensitivity of isolates from UTI during pregnancy are recommended.

To reduce unnecessary prescription for antibiotics and multi drug resistance, urine specimen for dipstick, microscopy, culture and sensitivity test for all pregnant women should be carried out whenever possible. Regular antibiogram is suggested to minimize antibiotic resistance and reminding the prescribers about the use of correct antimicrobials in treating UTI. As urinalysis is recommended for all pregnant women

at the time of enrollment at SabaSaba health center; results for leucocytes and nitrites tests should also be included in the antenatal card for reminding health care providers about the status of urinary tract infection. Currently, only urine for sugar and protein are indicated and documented in the antenatal card at the health center. Microscopic examination to be done for those samples showed positive multistik results.

Genotyping of the isolates and molecular studies of the resistance determinants are recommended in order to set more insights about the isolates and antimicrobial resistance. This will help to understand which genes are circulating in the isolates causing UTI in pregnant women. Nurse midwives and other health care providers should provide health education about urinary tract infection at ANC.

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APPENDICES

Appendix 1: A questionnaire presenting demographic information, symptoms and awareness of urinary tract infection

CONFIDENTIAL

PART A

Name:Identification number Date:.....Religion:Tribe.....

Age: ...Gestation age: ...Number of parity:Gravid.....Abortion.....

Education: none, primary, secondary, above secondary.

Marital status: Married, Single, Cohabiting, Divorced, widowed

Temp:Blood pressure.....Weight: Height:

PART B - Please answer questions 1 through 5

1. Please circle the symptoms you are experiencing and fill the blank

Frequency: How many times an hour do you urinate?

Dysuria: (Burning or pain on urination)**Haematuria:** (Blood in urine)

Urgency: (sudden need to urinate) **Nocturia:** (awakening during sleep to urinate)

How many times during your sleep? **Incontinence:** (loss of control)

Back pain: if yes, right side, left side or both?

Oedema: Yes or No **Fever:** Yes or No

2. How long (days) have you had these symptoms?.....

3. Have you had a previous urinary tract infection (UTI)? Yes or No

If yes, this month or previous months

- 4. Have you ever had an infection of the kidney? Yes or No
- 5. Have you taken any medications for current UTI symptoms? Yes or No
- 6. List all prescriptions, over the counter medication, or herbs that you
have taken in the last 2 days:
- 7. Do you know the importance of checking UTI during pregnancy? Yes or No
- 8. Were you told to check UTI by health care providers in the last visit? Yes or No

PART C: Information available in the antenatal card

- 9. Did the client test for UTI? Yes or No
- 10. If yes which method was used? Clinistix, Microscopic, Culture

Appendix 2: Consent form Kiswahili

Kibali cha ujulisho wa kufanya mahojiano

Namba ya mtafitiwa na mwaka:.....

Ruhusa ya kukubali kufanya mahojiano kwenye utafiti huu

Salam ! Mimi naitwa Ramadhan Mataligana, ni mwanafunzi wa chuo kikuu cha Sokoine (SUA) ninayesomea shahada ya uzamili katika fani ya microbiolojia. Nia ya kufanya utafiti huu ni kuchunguza chanzo cha bakteria pamoja na tiba yake katika maambukizi ya njia ya mkojo kwa wanawake wajaawazito katika manispaa ya Morogoro Tanzania. Unaombwa kushiriki katika utafiti huu kwa sababu kuwepo kwako kutatusaidia kugundua chanzo na kutatua tatizo la maambukizi ya njia ya mkojo.

Kukubali kushiriki:Kama utakubali kushiriki katika utafiti huu

- Mahojiano ya takuwa yasiri na yatachukua dakika 15.
 - Mahojiano yatahusu maambukizi ya njia ya mkojo hivyo utatakiwa kuleta mkojo wako kwa ajili ya upimaji
 - Taarifa zako zitatunzwa katika sehemu ya usiri katika kituo hiki cha afya.
- Jina lako halitatumika na watafiti

Faida: Hakutakuwa na faida ya moja kwa moja kwako kutokana na utafiti

huu. Taarifa tutakazokusanya kwako zitasaidia kuzuia na tiba ya maambukizi ya njia ya mkojo.

Usiri:Taarifa zako zitakuwa za siri ila zinaweza kutumika na wahudumu wa afya katika matibabu ya njia ya mkojo.

Mambo yahatari: Tunategemea kutokuwepo viashiria vya hatari katika huu utafiti. Mahojiano na sampuli ya mkojo vitatumika.

Ruhusa ya kujitoa katika utafiti na mbadala wake: Utafiti huu ni wa hiari, kama hutapenda kuendelea na utafiti unaruhusiwa kujitoa.

Kujitoakwakohakutakuathirikupatahudumayoyotekatikahikikituo.

Endapo utaumia: Hatutegemei kupatwa na athari yoyote katika utafiti huu.

Nani wa kuwasiliana naye: Kama utakuwa na maswali au wasiwasi katika utafiti huu, tafadhali fanya mawasiliano na mimi mwenyewe au mganga mkuu wa afya wa kituo hiki au wasimamizi wa utafiti Dr Samweli Laizer wa Halmashauri ya wilaya Morogoro vijijini P. O. BOX 1862 Morogoro na Dr Huruma N. Tuntufye wa chuo kikuu cha kilimo Sokoine (SUA) P.O. BOX 3019 Morogoro.

Cheti cha ruhusa: Nimekaribishwa katika utafiti huu wa uchunguzi wa bakteria na tiba yake katika njia ya mkojo katika manispaa ya Morogoro. Nimesoma na kuelewa taarifa za utafiti huu. Nimekubali kushiriki katika huu utafiti

Jina la mshiriki (Katika herufi Kubwa)..... Sahihi.....

Sahihi ya shahidi endapo mshiriki hawezi kusoma au kuandika.....

Sahihi ya mtafiti..... Tarehe.....

Je unakubali kuhusishwa katika taarifa zijazo? ndio.....au hapana.....

ANUANI: P. O. BOX.....Mtaa.....Kata.....

Wilaya.....Mkoa.....

Appendix 3: Consent form English

Respondents Identification number.....

Consent to participate in this study

A greeting, my name is Ramadhan Matalingana, a postgraduate student at Sokoine University of Agriculture pursuing a master of Applied microbiology. The purpose of my research is to investigate the prevalence of bacterial urinary tract infections and antimicrobials susceptibility pattern of the isolates from pregnant women in Morogoro Municipal council. You are invited to participate in this study because your presence will assist us to identify and solve a particular problem.

Participation involvement: If you agree to participate in this study the following will take place

- The interview will be confidential and conducted for 15 minutes.
- You will be interviewed about urinary tract infection and required to bring your urine specimen for analysis.
- Your information will be stored in the confidential place in this health facility. No names will be used by researchers.

Benefits: There will be no direct benefit to you in this study but the information collected will help to control and management of UTIs.

Confidentiality: The information collected from you will be confidential but the findings from this study may be used by medical staff for management of infection.

Risks: There are no risks associated with this study. Only interview and collection of urine specimen will be required.

Right to with draw and alternatives

This study is voluntary; if you are not interested you may with draw in any time.

This will not affect you in obtaining health services.

In case of injury: We do not expect to cause any harm to you as a result of participation in this study.

Who to contact: If you have any question or any doubt in this study, please ask me or you may contact medical officer in charge of this facility or my supervisors Dr. Samwel Laizer of Morogoro rural district council P.O.BOX 1862 Morogoro and Dr. Huruma N. Tuntufye of Sokoine University of Agriculture P.O.BOX 3019 Morogoro.

Certification of consent: I have been invited to take part in the study titled Prevalence of bacterial urinary tract infections and antimicrobials susceptibility pattern among pregnant women in Morogoro municipality. I have read and understand the information in this study. Therefore I agree to participate in this study.

Name of participant (In capital letters).....Signature.....

Signature of researcher.....Date consent signed.....

Are you willing to be contacted for future research? Yes.....or... No...

ADDRESS: P.O.BOX.....Street.....Ward.....

District.....Region.....

Appendix 4: Interpretative chart of zone sizes

	Disc Content	Zone Diameters Nearest Whole (mm)			Equivalent MIC Breakpoints ($\mu\text{g/ml}$)	
		R	I	S	R	S
Ampicillin	10 μg	≤ 13	14-16	≥ 17	≥ 32	≤ 8
Amoxicillin	10 μg	≤ 13	14-16	≥ 17	≥ 32	≤ 8
Tetracycline	30 μg	≤ 11	12-14	≥ 15	≥ 16	≤ 4
Ciprofloxacin	5 μg	≤ 15	16-20	≥ 21	≥ 4	≤ 1
Trimethoprim/ Sulfamethoxazole	1.25/ 23.75 μg	≤ 10	11-15	≥ 16	$\geq 8/52$	$\leq 2/38$
Erythromycin	15 μg	≤ 13	14-22	≥ 23	≥ 8	≤ 0.5

Source: Clinical and Laboratory Standards Institute 2012

Appendix 5: Ethical clearance

THE UNITED REPUBLIC OF
TANZANIA



National Institute for Medical Research
P.O. Box 9653
Dar es Salaam
Tel: 255 22 2121400/390
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NIMR/HQ/R.8a/Vol. IX/1708

Ministry of Health and Social Welfare
P.O. Box 9083
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04th April, 2014

Ramadhan Matalingana
Sokoine University of Agriculture
Department of Microbiology and Parasitology
P O Box 3006 MOROGORO

**CLEARANCE CERTIFICATE FOR CONDUCTING
MEDICAL RESEARCH IN TANZANIA**

This is to certify that the research entitled: The Prevalence of Urinary Tract Infection and Antimicrobials Susceptibility Patterns among Pregnant Women in Morogoro Municipal Council, (Matalingana R *et al*), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:


1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Site: Sabasaba Health Centre in Morogoro Municipal Council, Morogoro

Approval is for one year: 04th April 2014 to 03rd April 2015.

Name: **Dr Mwelecele N Malecela**

Signature 
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: **Dr Donan Mmbando**

Signature 
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL
WELFARE

CC: RMO
DED
DMO