

Antimicrobial Susceptibility Patterns Of Escherichia Coli Isolates Causing Urinary Tract Infections Among Pregnant Women At Kisii Teaching And Referral Hospital

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Abstract: Urinary tract infection is a major public health problem in terms of morbidity and financial cost and represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide. It remains a commonly diagnosed infection both in community as well as in hospitalized pregnant mothers and *Escherichia coli* was found to be the most common pathogen of UTI both in community and hospital acquired infections. The main objective determines antimicrobials susceptibility pattern on *Escherichia coli* isolates causing urinary tract infection in pregnant women attending Kisii Teaching and Referral Hospital. The study was a Cross-sectional descriptive study where systematic sampling was used to recruit the respondents that meet the inclusion criteria 80 pregnant women from trimester 1, 2 and 3 respectively participating in the study. Demographic and risk factors details were obtained through a structured Check List interviews. Data was analysed using Microsoft Excel and presented in tables and graphs presentation. Coding and verification of the data was done before data analyzed. Analysis was done using SPSS version 15. Chi-square test (2x2) or Fishers Exact Test was applicable at P-value derivation for socio-demographic and risk factors to identify variables associated with UTIs. Antimicrobial susceptibility pattern rate of gram negative bacteria ranged from 27% to 92% to be accurate. Susceptibility was as Ceftriaxone 74 (92%), Ciprofloxacin 56 (70%), Gentamycin 56 (70%), co-Trimoxazole 55 (69%), Ampicillin 50 (63%), Chloramphenicol 40 (50%), Streptomycin 32 (43%) and Tetracycline 22 (27%) respectively. This study recommends that effective identification, isolation and sensitivity tests of uropathogens in pregnant mothers such as *E. coli* associated with UTI should be done as SOP and the government should expand the existing maternal health programs and put more emphasis on uti treatment components targeting mothers who were established to be at a higher risk. The study was a hospital based study and may not truly reflect findings in the rural areas and the entire state. The antibiotic sensitivity test against bacteria in the laboratory is an in vitro activity and may not exactly reflect the in vivo activity.

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I. Introduction

1.0 Introduction

The introduction of antimicrobial therapy has contributed significantly to the management of UTIs. However the main problem with current antibiotic therapies is the rapid emergence of antimicrobial resistance in hospitals and the community (Habte, *et al.*, 2009).

The aetiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years, both in community and hospital acquired infection (Manges, *et al.*, 2006) and (Kahn, *et al.*, 2006). Current knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy. However, there is not much information available on the aetiology and resistance pattern of community and hospital acquired UTIs. Therefore, this prospective study is aimed to determine and compare the antibiotic susceptibility patterns of most common uropathogens i.e., *E. coli* isolated from pregnant mothers with community and hospital acquired UTIs (Kahn, *et al.*, 2006).

This study in particular was of more importance for clinician practicing in the community in order to facilitate the empiric treatment of pregnant mothers and management of pregnant mothers with symptoms of UTIs. Moreover, the data would also help authorities to formulate antibiotic prescription policies (Manges, *et al.*, 2006).

In studies that have been done regionally, the prevalence of UTIs among pregnant women was found to be 14 % in Sudan (Hamdan, *et al.*, 2011), 15.5% in Tanzania (Masinde *et al.*, 2009) and 13.3% in Uganda (Andabati and Byamugisha, 2010). Screening for and treatment of UTI in pregnancy has become a standard of

obstetric care and most antenatal guidelines included Prime routine screening for asymptomatic bacteriuria, Urinary Tract Infections during pregnancy are among the commonest health problems worldwide, especially in developing countries (Delzell, and Lefevre, 2000). The economic burden of UTI in adult women is significant. The health care direct and indirect costs associated with UTIs in terms of bed occupation, staff and supply are also large and included substantial out-of-pocket expenses for the pregnant mothers (Bell, 1999).

Hormonal effects especially during pregnancy and post-menopausal period increases the risk for UTI due to lack of estrogen. Estrogen loss thins the walls of the urinary tract and reduces its ability to resist bacteria. It also reduces certain immune factors in the vagina that help block *E. coli* form adhering to vaginal cells (Harvey, 2009). Estrogen is essential to maintain the normal acidity of vaginal fluid. This acidity is critical to permit the growth of Lactobacillus in the normal vaginal flora, which acts as a natural host defense mechanism against symptomatic UTI (Smita and Ravi, 2012).

Differences in urine pH and osmolality and pregnancy induced glycosuria and Aminoaciduria may facilitate bacterial growth (Jeyabalan and Lain, 2007). During pregnancy, due to the abdominal distension, women find it difficult to clean their genitalia well and this may highly contribute to the occurrence of UTIs in pregnancy (Bell, 1999).

1.1 Background Information

Urinary Tract Infection (UTI) is the presence of bacteria in urine or bacterinuria and defined as “the growth of a single pathogen of $\geq 10^5$ colony forming units/ml from a properly collected mid-stream urine sample” (C.D.C., 2004).

Urinary tract infection is a major public health problem in terms of morbidity and financial cost and represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide (Karlowsky, *et al.*, 2002). It remains a commonly diagnosed infection both in community as well as in hospitalized pregnant mothers and *Escherichia coli* was found to be the most common pathogen of UTI both in community (Smita and Ravi, 2012) and hospital acquired infections (Amdekar, *et al.*, 2011).

Hospital acquired infections (HAIs) are important public health problems both in developed and developing countries. It is defined as “an infection which is acquired during hospital stay and that appears within 48-72 hrs after admission in the hospital and the patient was not incubating this infection at the time of admission” (Amdekar, *et al.*, 2011).

1.2 Statements of the Problem

The prevalence of UTI among pregnant women has been reported to be high regardless of the women's age, parity and gestational age and *E. coli* is suspected to be the commonest isolated organism with multi resistance toward different antibiotics. Previously the prevalence of UTI among pregnant women in the neighbor countries has been indicated to be 14.6% and 11.6% in Tanzania and Ethiopia (Masinde, *et al.*, 2009).

It has been shown that anti-microbial resistance to one drug does not always correlate to the consumption of the same drug or closely related drugs (Kahlmeter, *et al.*, 2003). Inappropriate antimicrobial use can lead to inadequate therapy and contribute to further drug resistance (Fluit and Schmitz, 2001). The inappropriate use of antimicrobial in low income countries is perhaps due to the lack of adequate knowledge about drugs and non-availability or non-accessibility of guidelines for therapy (Mathai, *et al.*, 2004), or to the availability of antimicrobials without prescription and perhaps it was prescribed by non-skilled practitioners (Yilmaz, *et al.*, 2009).

1.3 Objectives of the Study

1.3.1 Broad Objective

To determine antimicrobials susceptibility pattern on *Escherichia coli* isolates causing urinary tract infection in pregnant women attending Kisii Teaching and Referral Hospital.

1.3.2 Specific Objectives

- i. To characterize UTI causing pathogens from urine samples in pregnant women attending Kisii Teaching and Referral Hospital.
- ii. To determine antimicrobial resistance of *Escherichia coli* isolates from urine samples in pregnant women attending Kisii Teaching and Referral Hospital.
- iii. To compare antimicrobials susceptibility pattern on *Escherichia coli* isolates in inpatient and out-pregnant mothers.

1.4 Research Questions

- i. What's the criterion of characterizing *Escherichea coli* isolates from urine samples in pregnant women attending Kisii Teaching and Referral Hospital.
- ii. What's the level of antimicrobial resistance of *Escherichea coli* isolates from urine samples in pregnant women attending Kisii Teaching and Referral Hospital.
- iii. Is there any significance in antimicrobials susceptibility pattern on *Escherichia coli* isolates in inpatient and out-pregnant mothers?

1.5 Justification

Prevalence rate of UTI in pregnant women in America is 2.5-8.7% and 12-40% in developing countries in Africa with high mortality rate on mothers and newborn due septicemia and Lower abdominal pain which is a common problem among antenatal women and has a number of causes among them UTI. In Kisii Teaching and Referral Hospital pregnant women with lower abdominal pain and not in labor are commonly treated as urinary tract infection in pregnancy. This leads to over-use of antibiotics unnecessarily and causing antibiotic resistance. Therefore, there was need to establish the prevalence of UTI among antenatal women who present with lower abdominal pain in order to justify this practice. Therefore, it is important to establish a very sensitive and specific was of diagnosing UTI and determine the involved bacterium and their sensitivity pattern in the institution (Assefa, *et al.*, 2008).

UTI which presents with lower abdominal pains is associated with serious and poor obstetric outcomes like preterm labor, low birth weights and intrauterine growth restriction, hypertension and maternal anemia hence the need to treat it fast and rightly.

In order to achieve Millennium Development Goals 4 and 5, which is to reduce the less than 5 mortality rate and improve maternal health respectively, we should be able to prevent and reduce preterm births, which is the commonest cause of perinatal deaths and maternal morbidity by promptly and adequately treating UTI. And we should also avoid overuse and abuse of antibiotics to minimize development of resistance this prompted the research to be undertaken.

1.6 Conceptual Framework

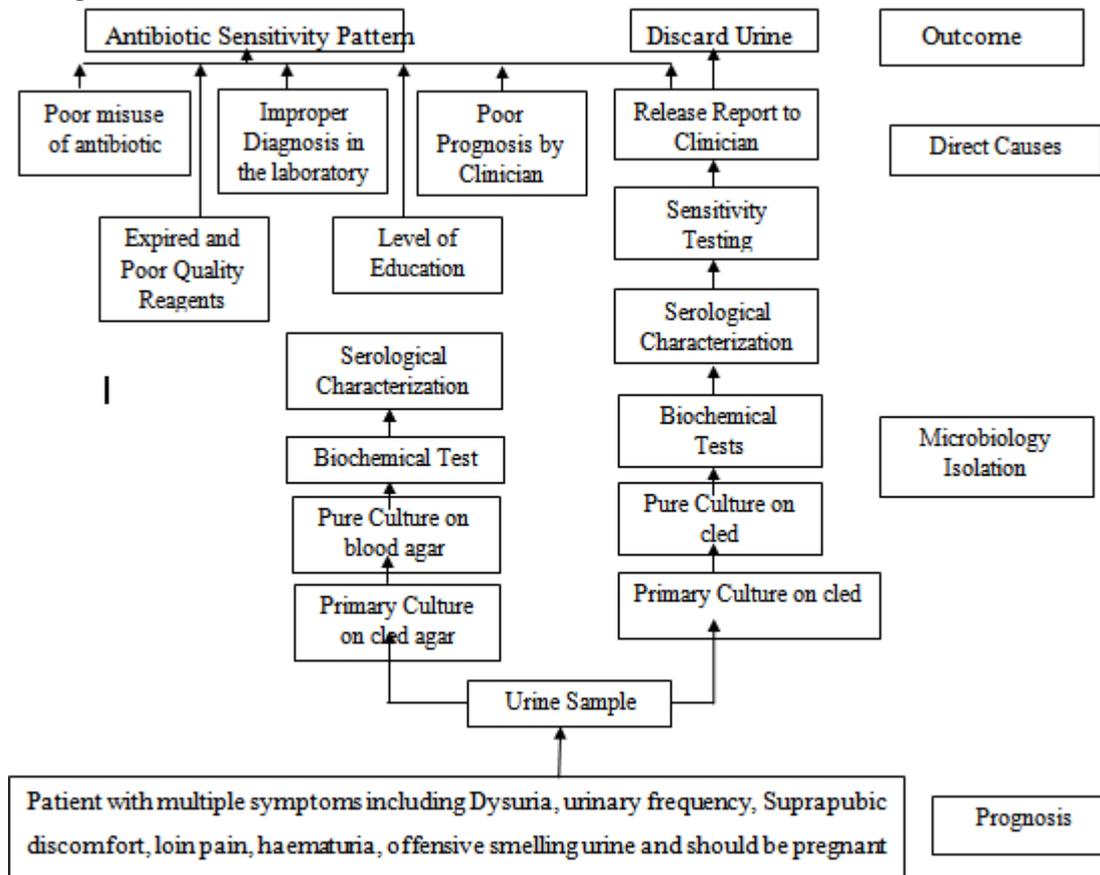


Figure 1: Conceptual Framework

II. Literature Review

2.0 Urinary Tract Infection in Pregnant Women

Urinary tract infection is the commonest bacterial infection in pregnancy occurring more frequently in developing countries among the low socio-economic populations including Kenya, in the USA surveys estimated that there about 8 million cases of UTI annually with huge economic implications, no similar surveys have been done in Africa and developing countries, Fox man. B (Mikhail and Ayaegbunam, 1995) found prevalence rate of UTI in pregnant women in America to be 2.5-8.7% estimated the prevalence of UTI in pregnancy to be 12-40% in developing countries in Africa. This was due to the differences in the socio-economic levels and standards of living (Okonko, 2012).

UTI is said to be about 4-10 times more common in pregnancy than in the non-pregnant women (Pooja and Deborah, 2005). Due to change in urine chemical composition it increases in glucose and amino acids which facilitate bacterial growth in urine (Mohamed, 2000). Its high frequency is also due to physiological, anatomical and functional changes that occur in the urinary tract during pregnancy. It also tends to be recurrent in association with urinary tract anomalies. Its management is mostly empirical and local microbial pattern and sensitivities ought to be adhered to in prescription as urine culture's and blood cultures are not always done or important (Gilst and Ramin, 2001).

2.1 Antimicrobial Susceptibility of *Escherichia coli*

Escherichia coli, is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation. It is the leading pathogen causing urinary tract infections and is among the most common pathogens causing blood stream infections (Biedenbach, *et al.*, 2002) wounds, otitis media and other complications in humans (Gebre-Sellassie, 2007). *E. coli* is also the most common cause of food and water-borne human diarrhea worldwide and in developing countries, causing many deaths in pregnant mothers under the age of five years (Tesfaye, *et al.*, 2006).

Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (Bell, 1999). A rise in bacterial resistance to antibiotics complicates treatment of infections. In general, up to 95% of cases with severe symptoms are treated without bacteriological investigation (Dromigny, *et al.*, 2005). Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations as well as significant differences in various populations and environments (Erb, *et al.*, 2007). In Ethiopia, a number of studies have been done on the prevalence and antimicrobial resistance patterns of *E. coli* from various clinical cases (Bharathi, *et al.*, 2002).

In all clinical samples, *E. coli* has high resistance rates of > 80% to erythromycin and amoxicillin and > 60% to tetracycline, *E. coli* isolates are sensitive to gentamicin, nitrofurantoin, ciprofloxacin and chloramphenicol (Tesfaye, *et al.*, 2009). High sensitivity to ciprofloxacin and gentamicin and norfloxacin has been recorded from previous studies conducted in Nigeria and India (Wariso and Ibe, 2006). In this study, norfloxacin, ciprofloxacin, gentamicin and chloramphenicol was found to be the most effective antimicrobials against *E. coli* isolates (Bharathi, *et al.*, 2002).

Antimicrobial susceptibility of *E. coli* was tested by the disk diffusion method according to the CLSI recommendations, using the Mueller-Hinton agar (Clinical and Laboratory Standards Institute, 2012). Antimicrobial agents tested ampicillin, amoxicillin-clavulanic acid, aztreonam, cephalothin, cefoxitin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefipime, piperacillin, piperacillin-tazobactam, imipenem, gentamycin, tobramycin, norfloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and tetracycline. The CLSI-ESBL phenotypic confirmatory test with ceftazidime, cefotaxime, ceftriaxone, and cefixime performed for all the isolates by disk diffusion method on the Mueller-Hinton agar plates with and without 10 µg of amoxiclav. Susceptibility test results interpreted according to the criteria established by the Clinical & Laboratory Standard Institute (CLSI). A minimum of 5 mm increase in the zone of diameter of third-generation cephalosporins, tested in combination with amoxiclav versus its zone when tested alone, considered indicative of ESBL production. *E. coli* ATCC 25922 used as ESBL-negative and *K. pneumoniae* 700603 used as ESBL-positive reference strain. Statistical analysis: variables expressed as percentages (Bell, 1999).

2.2 Urinary Tract Pathogens

E. coli is the cause of 80–85% of community-acquired urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–10% (Nicolle, 2008). Rarely may they be due to viral or fungal infections (Amdekar, *et al.*, 2011). Healthcare-associated urinary tract infections (mostly related to urinary catheterization) involve a much broader range of pathogens including: *E. coli* (27%), *Klebsiella* (11%), *Pseudomonas* (11%), the fungal pathogen *Candida albicans* (9%), and *Enterococcus* (7%) among others. Urinary tract infections due to *Staphylococcus aureus* typically occurs secondary to blood-borne infections. *Chlamydia trachomatis* and

Mycoplasma genitalium can infect the urethra but not the bladder. These infections are usually classified as a urethritis rather than urinary tract infection (Lane and Takhar, 2011).

2.3 Isolation of Escherichia coli

Samples for culture tested within half an hour of sampling. All samples inoculated on blood agar as well as MacConkey agar and incubated at 37°C for 24 hours, and for 48 hours in negative cases. A specimen is considered positive for UTI in the light of the number of yielded colonies ($\geq 10^5$ cfu/mL) and the cytology of the urine through microscopic detection of bacteriuria and PMNs (≥ 8 leukocytes/mm³). However, lower colony counts associated with significant pyuria or low PMN count associated with significant colony counts is considered and analyzed in the light of the clinical picture and the patient's immunological status. Bacterial identification was based on standard culture and biochemical characteristics of isolates (Lane and Takhar, 2011).

Gram-negative bacteria identified by standard biochemical tests. Gram-positive microorganisms identified with the corresponding recommended laboratory tests (Lane and Takhar, 2011).

2.4 Impact on Hospitalization

Impact of hospitalization on the prevalence of resistance E.coli in pregnant mothers of three affiliated hospitals in Netherland determined on admission at time Of discharge 1and 6months discharge have been (Brussma, *et al.*, 2005).

For total patient population no significant difference of prevalence of resistance observed at different sampling intervals except father discharge (10% to 3%) p less than 0.05.this decrease mainly observed in pregnant mothers of the university hospital Maastricht (Bruishma, *et al.*, 2002).

II. Materials And Methods

3.1 Study Site

The study was carried out at Kisii Teaching and Referral Hospital a government institution that serves Kisii municipality and its environs between august 2015 and October 2016.

The municipality constitutes of a cosmopolitan affluent, middle class and slum based population. The target population included all women who attend outpatient and antenatal clinic at Kisii Teaching and Referral Hospital Kisii.

KTRH is in Kisii. Kisii lies in highland equatorial climate and receives rainfall almost throughout the year. The average rainfall received is over 1500mm per annum. The temperature ranges from 10-30 degrees centigrade. It is boarded by Nyamira County, Narok, Bomet and Migori counties.

Kisii County has a population of 1,152 m people as recorded in 2014 census. The rate of economic growth is high due to high population density and majority people practice farming and business despite limited land. The local climate is hit and wet with flat terrain, altitude of 1240-2000m above the sea level and have two rainfall seasons during the year that's march-April and august-September. Planting season is in March while harvesting season is in august for subsistence

3.2 Study Design

This was a Cross-sectional descriptive study where systematic sampling was used to recruit the respondents that meet the inclusion criteria. . The data was collected and/or obtained at one point in time for different groups. Cross sectional studies described the distribution of certain variables in a study population at a certain time.

3.3 Study Population

The study population included all women who seek treatment services at the hospital at any gestational stage of the pregnancy and other women who consent or assent to participate in the study.

3.4 Sample Size Determination

Prevalence of urinary tract infection among pregnant women is 10% (Masinde, *et al.*, 2009) and the expected total population of pregnant women in Kisii Teaching and Referral Hospital in two months is 1000 which give 10% of 100 as a population size (N) presenting with UTI.

Formula that was used to calculate sample size was Harper's formula (Israel, *et al.*, 2000).

Equation:

$$n = \frac{N}{1+N(e)^2}$$

n = Sample size

N= Population size

e = Level of precision

I = Desired confidence level
 Estimated N= 100 in 3 months
 $e = 1 - 0.95$ (confidence level)
 $e = 1 - 0.95 = 0.05$
 Hence $e = 0.05$
 $N = 100 \div 1.25 = 80$

The study population included all pregnant women who seek treatment and antenatal services at the hospital and consented to participate. Simple random and Stratified sampling methods was employed in selecting respondents until the expected number was reached. To avoid over representation from one trimester the sample size was stratified to the three trimesters with an aim of getting 80 pregnant women from trimester 1, 2 and 3 respectively participating in the study. Demographic and risk factors details were obtained through a structured Check List interviews. To obtain the prevalence, Midstream urine specimen was collected aseptically and cultured. A diagnosis of UTI was done when at least 10⁵ colony forming units (CFU/L).

3.6 Sampling procedure

The study population was conveniently selected and clustered into three study groups based on characteristics presented in table 3.1. Following this clustering, simple random sampling technique was used to select participating patients according to probability proportionate to sample size

Table3. 1: Cluster of study groups

No	Group	Characteristics
1.	Outpatient pregnant women	<ul style="list-style-type: none"> ▪ Dysuria, urethritis, fever, febrile illness ▪ Honey moon cystitis ▪ Outpatient number ▪ gestation ▪ parity ▪ age ▪ residence ▪ marital status
2.	inpatient pregnant women	<ul style="list-style-type: none"> ▪ Dysuria, urethritis, fever, febrile illness ▪ Honey moon cystitis ▪ inpatient number and in the ward ▪ gestation ▪ parity ▪ age ▪ residence ▪ marital status
3.	Uti re infection	<ul style="list-style-type: none"> ▪ Dysuria, urethritis, fever, febrile illness ▪ Honey moon cystitis ▪ inpatient number and ward ▪ gestation ▪ parity ▪ previous diagnosed and antibiotic treated uti ▪ age ▪ residence ▪ marital status

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Consenting women presenting with UTI's while attending Kisii Teaching and Referral Hospital.

3.4.2 Exclusion Criteria

- Unwilling to participate and non- consenting women presenting with UTI's while attending Kisii Teaching and Referral Hospital.
- Mothers presenting with non UTI's and are attending Kisii Teaching and Referral Hospital.

3.5 Sampling Procedure

Urine samples (n=80) was collected from pregnant mothers in different inpatient wards (n = 40) and outpatient department (n=40) from Kisii Teaching and Referral Hospital Samples was centrifuged and sediments

was cultured primarily on CLED by spread plate technique. Bacterial colonies having different morphology was selected, purified and was identified by their biochemical profiles.

3.5.1 Isolation of Enteric Bacteria from Urine Samples

For isolation of enterobacteriaceae from urine samples was inoculated in CLED a universal media from urine culturing. All inoculated media was incubated at 37°C for 24 hours.

3.5.2 Selection of Colonies

For further isolation of colonies was selected according to growth and colony morphology. *E. coli* on CLED media appeared as opaque, yellow colonies with slightly yellow centres. *Klebsiella* appeared as yellow to whitish-blue colonies extremely mucoid.

3.5.3 Escherichia Coli Strains

Each of the specimens was streak plated onto MacConkey agar (Oxoid) and incubated at 37°C overnight. Colonies suspected to be *E. coli* was isolated from the MacConkey agar plates and identified using biochemical tests, confirmed by API 20E strips (Himedia KB2). The isolates were stored at -70°C in microvials for further analysis (Kariuki, *et al.*, 1999).

3.5.4 Gram Staining

To characterize the isolates, gram staining was done to differentiate gram negative from gram positive bacteria. First, a smear of a colony was made on a drop of distilled water on a slide using a loop sterilized in flame. The smear then be dried by passing over the flame to fix and placed on a staining rack and flooded with crystal violet for 1-2 minutes. The stain was pitched using forceps and the slide flooded with Grams Iodine for 1-2 minutes and pitched off. Degrowthization then follows by washing of the slide briefly with acetone for 2-3 seconds. The slide then be thoroughly washed with water to remove acetone without delay and then flooded with safranin counter stain for 2 minutes followed by washing with water. Excess water was blotted and the slide dried in hand cover Bunsen flame. Gram positive bacteria retain the dark purple crystal violet stain while the gram negative bacteria not retain the crystal but rather the peptidoglycan layer was stained with the pink safranin counter stain.

3.5.5 Biochemical Tests for E. coli

3.5.5.1 Indole Production

Two to five pure colonies was inoculated using a sterile wire loop in 2 ml of peptone water in bijoux bottles and incubated overnight at 35°C. 0.5 ml of Kovac's reagent was added and examined after 1 minute. Presence of rose red growth on upper layer was considered positive (+), while absence of rose red or pale growth was considered negative (-).

3.5.5.2 Methyl Red Test

Five millilitres of Methyl red, Voges-Proskauer broth was distributed in bijoux bottles and inoculated with pure colonies of test organisms. The bijoux bottles was incubated at 35°C for 48h, followed by addition of 0.5ml or 5 drops of methyl red and observed for growth change. The bijoux bottles with red growth was considered positive (+), while those which developed yellow growth was considered negative (-).

3.5.5.3 Voges-Proskauer (VP)

In each bijoux bottle, 2.5ml of Methyl red-Voges Proskauer broth was added and inoculated with pure colonies of test organisms. The bijoux bottles was then incubated at 35°C for 48h, followed by addition of 0.6 ml or 6 drops of VP reagent A (α -naphthanol 34 solution), then 0.2ml (2 drops) of VP reagent B (40% KOH). The bijoux bottles was shaken and allowed to stand for 15 minutes. Pink red growth (reddish pink) of the broth culture in the bijoux bottles was considered positive (+), while growthless (pale) was considered negative (-).

3.5.5.4 Simmons Citrate

Simmons Citrate agar slants in bijoux bottles were stabbed using a sterile wire loop and incubated for 48h at 35°C. Positive (+) growth for example citrate utilization produce an alkaline reaction and the medium change growth from green to blue, while no growth change (no citrate utilization) was considered negative (-).

3.5.5.5 Triple Sugar Iron Agar

TSI slopes with a butt of about 1 inch (3.5cm and 2.5cm) was inoculated by stabbing the butt and carefully streaking of slant using a sterile inoculating needle after slightly touching the centres of a discrete colony on selective media. The tubes were incubated overnight at 35°C. Production of acid (yellow) slant and acid (yellow) butt, gas, without production of H₂S (blackening of agar) was considered positive for *E. coli*.

3.5.5.6 Confirmation by API 20E

Two millilitres of bacterial suspension of one single colony of the isolate was prepared in Mueller Hinton broth and incubated overnight at 37°C. The kits were opened aseptically, and 50ul of bacterial suspension added to each compartment and incubated at 37°C for 18-24 h. The results was interpreted as positive (growth change of medium) or negative (no growth) in accordance with IMViC Biochemical identification test kit KB001 (Appendix II).

3.6 Serological Test

Serological tests were performed on sterile glass slides. Using a sterile wire loop, a portion of growth from an overnight culture on TSI was suspended in normal saline on a slide and then mixed with a drop of serum using the wire loop. The slide was rocked to ensure uniformity. The slide was then observed under X40 on a microscope for agglutination controls containing standard *E. coli* ATCC 25922.

3.7 Antimicrobial Susceptibility Testing

3.7.1 Escherichia Coli Strains

All isolates was routinely tested by the single-disk diffusion method. Mueller Hinton Agar was prepared according to the manufacturer's instructions (Oxoid). With a sterile wire loop, the tops of five isolated colonies of similar morphological type were transferred to a tube containing 5 ml of Mueller Hinton broth medium. The broth was incubated at 35 °C until its turbidity exceeded that of the 0.5 McFarland standard within 15 minutes of adjusting the density of the inoculums using sterile distilled water, a sterile cotton swab on a wooden applicator stick was used to streak the dried surface of Mueller-Hinton plates in three different planes. The inoculated plates was allowed to remain on a flat surface for 3 to 5 minutes for absorption of excess moisture, and then antimicrobial disks Antibiotic sensitivity pattern of *E. coli* isolates was determined on Muller Hinton agar plates by Kirby-Bauer disc diffusion (Wayne, 1996) was declared as sensitive or resistant on the basis of zone of inhibition following the criteria of Clinical Laboratory Standards Institute CLSI guidelines (2012) and interpreted accordingly⁸. Following disks was used: amikacin (30µg), ampicillin (25µg), cefraxozonee (30µg), cefepime (30µg), cefoxitin (30µg), ceftazidime (30µg), cefuroxime (30µg) ciprofloxacin (5µg), imipenem (10µg), meropenem (10µg), piperacillin-tazobactam (100/10µg), and trimethoprim/sulphamethaxzole (1.25/23.75µg) (Wayne, 1996).

3.8 Data Analysis

Data was analysed using Microsoft Excel and presented in tables and graphs presentation. Coding and verification of the data was done before data analysis. Analysis was done using SPSS version 15. Chi-square test (2x2) or Fishers Exact Test was applicable at P-value derivation for socio-demographic and risk factors to identify variables associated with UTIs. Binary logistic regression analysis was carried out to generate the adjusted odds ratio with 95% confidence interval for the associations between variables and UTIs. An alpha of less than 0.05 (P<0.05) was considered statistically significant.

3.9 Ethical Considerations

There were no invasive procedures on the study subjects. Consent to carry out research on the specimens was administered on subjects who was on routine medical check-ups. Report on any enteropathogenic bacteria and antibiotic sensitivity was availed for therapeutic purposes. The study was approved by the accredited Maseno University Research Institute Scientific and Ethical review committee.

IV. Results and Findings

4.1 Socio-Demographic Characteristics

A total of 80 pregnant women were enrolled in this study with maternal characteristic on maternal age distribution, clinical features, marital status, education, occupation and water source as illustrated on table 4.1.

Table 4.1: Maternal Characteristics

Characteristic	Frequency	Percentage
Maternal Age		
15 - 19 years	20	25%
20 - 24 years	20	25%
25 - 29 years	17	21%
30 - 34 years	12	15%
35-39 years	11	14%
Clinical Features		
Dysuria	80	100%
Frequent Suprapubic Pain	72	90%

Loin Pain and Tenderness	60	75%
Fever	54	67.5%
Urethritis	36	45%
Cystitis	30	37.5%
Recent Febrile Illness	22	27.5%
Maternal Marital Status		
Married	50	62.5%
Single	30	37.5%
Highest Level of Education		
Primary	2	2.5%
Secondary	18	22.5%
Tertiary	60	75%
Maternal Occupation		
Formal Employment	54	67.5%
Self Employed	14	17.5%
Subsistence Farmer	12	15%
Water Source		
Treated Tap Water	36	45%
Well	34	42.5%
River	10	12.5%

4.2 Characterizations of UTI Causing Organisms

Table 4.2.1: Characterizations in Gram Negative Rods

Test	E. coli	Proteus Mirabilis
Motility	+	+
TSI	A/A	Alk/A
Gas from Glucose	+	+
Acid from Lactose	+	-
Indole	+	-
MR	+	+
VP	-	+/-
Citrate	-	+/-
Hrs	-	+
Lysine Decarboxylase	+	-
ONPG	+	-
Urease	-	-
Oserotypes	01, 02, 04, 06, 07, 018, 075 (Nephritogenic Strains)	010, 038, 09, 036 (Liposaccharide Antisera)
Gelatinase	-	+
Total No. of Micro-Organisms Isolated	80	27

Table 4.2.2: Characterizations in Gram Positive Rods

Test	Staphylococci Aureus	Streptococci Pyogen
Gram Stain Arrangement	Cluster	Chains
BA Heamolysis	Alpha/Beta	Alpha
Bacitracin Test	-	+
Catalase	+	-
Sorbitol	-	+
Trehalose	-	+
Maltose	-	+
Mannitol	+	+
Hydrolysis Pyrolydonyl Naphthylamidepyr Test	-	+
Hydrolyse Urea	+	-
Liquefy Gelatin	+	-
MR	+	-
VP	+	-
Phosphotase	+	-
Reduction of Potassium Tellulite	+	-
Coagulase	+	-
Total No. of Micro-Organisms Isolated	19	28

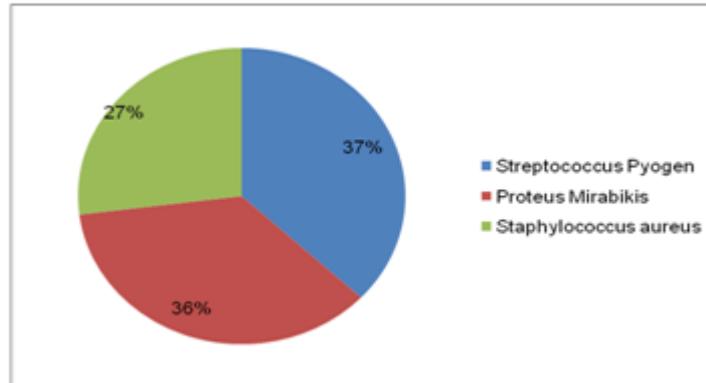
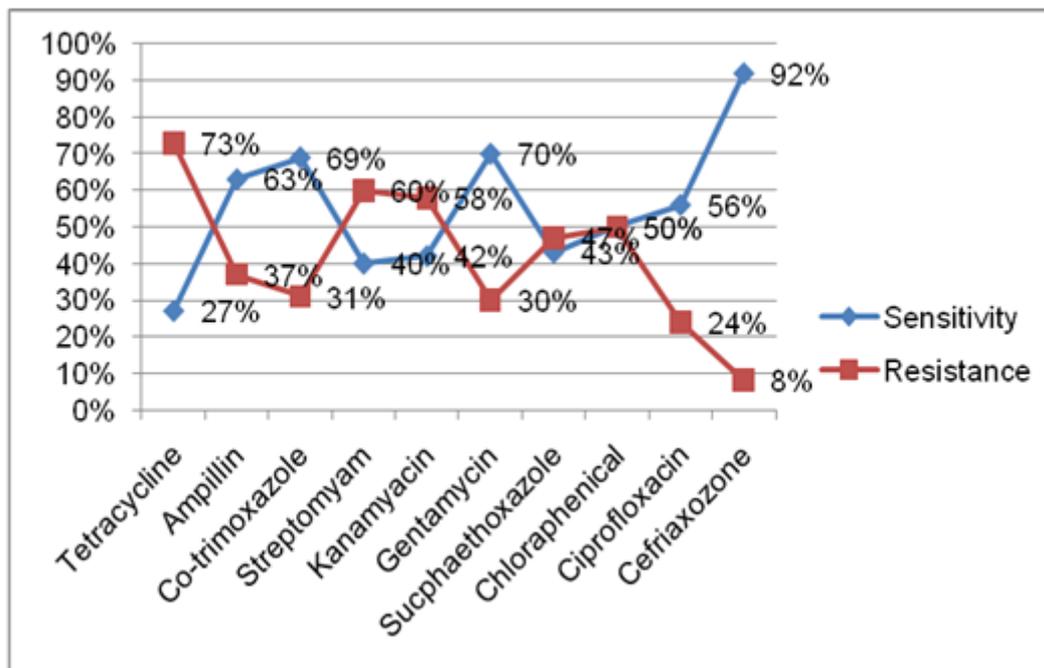


Figure 4.2.3 Other UTI causing organisms

Table 4.3: Antimicrobial Susceptibility Pattern of *Escherichia coli* in Pregnant Women attending in Kisii Teaching and Referral Hospital

Antibiotic	Sensitivity		Resistance	
	Population (n)	%	Population (n)	%
Tetracycline	22	27	58	73
Ampicillin	50	63	30	37
Co-trimoxazole	55	69	25	31
Streptomycin	32	40	48	60
Kanamycin	34	42	46	58
Gentamycin	56	70	24	30
Sulphamethoxazole	30	40	40	50
Chloraphenical	40	50	40	50
Ciprofloxacin	56	70	24	30
Cefriaxozone	74	92	6	8

Figure 4.3.1: Antimicrobial Susceptibility Pattern



Antimicrobial susceptibility pattern rate of gram negative ranges from 27% to 92% to be accurate. Susceptibility was as Cetriaxozone 74 (92%), Ciprofloxacin 56 (70%), Gentamycin 56 (70%), co-Trimoxazole 55 (69%), Ampicillin 50 (63%), Chloraphenical 40 (50%), Streptomycin 32 (43%) and Tetracycline 22 (27%) respectively.

The resistance pattern was Tetracycline 58 (73%), Streptomycin 48 (60%), Keramycine 46 (58%), sulphamethoxazole 40 (57%), Chloraphenical 40 (50%), Ampicillin 30 (37%), Co-trimoxaloe 25 (31%), Gentamycin 24 (30%), Ciprofroxacin 24 (30%) and Cefriaxozolen 6 (8%).

4.3.2 Antibiotic Resistance in repeated antibiotic use.

Table 4.3.2: Antibiotic Resistance

	Ciprofloxacin	Streptomycin
Previous Treatment	8	12
Present Sensitive	3	1
Present Resistance	5	11

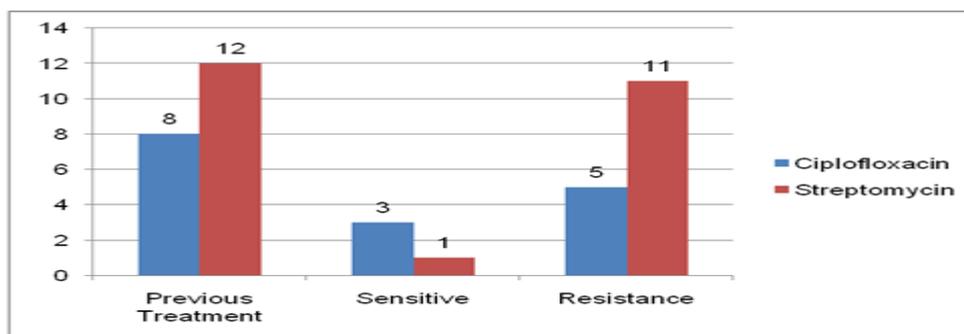


Figure 4.3.2: Antibiotic Resistance

Multidrug resistance was seen in more than two drugs in 80% of the isolate and an average of 43.9 resistance in all drugs resistance of tetracycline being at 73% Sulphame Throxazole 62%, Streptomycins 60% Kanamycin at 58% and Chloraphenical 50% among, the antibiotics having more resistance, Cefriaxozone was the lowest resistance antibiotic at 8%.

After multiple use of drugs by same pregnant mothers and a follow-up done Ciprofroxacin n=8 in a previous sensitive drugs test produced n = 5 resistance and n = 3 sensitive and streptomycin after a previous use n = 12. It showed resistance of n = 11 and n = 1 sensitive in the second use.

4.4. Inpatient and outpatient

Urine samples (n=80) was collected from pregnant mothers in different inpatient wards (n = 40) and outpatient department (n=40) from Kisii Teaching and Referral Hospital

Table 4.4:1 Inpatient and Outpatient

Drug	Inpatient			Outpatient		
	Sensitivity	Resistance	p-value	Sensitivity	Resistance	p-value
Tetracycline	65%	35%	0.0092	64%	36%	0.0151
Ampicillin	23%	77%	<0.0001	52%	48%	0.729
Co-trimoxazole	35%	65%	0.0092	34%	66%	0.0054
Streptomycin	58%	42%	0.1655	64%	36%	0.0151
Kanamycin	71%	29%	0.0003	43%	57%	0.2251
Gentamycin	32%	68%	0.0017	39%	61%	0.0564
Sucphamethoxazole	68%	32%	0.0017	57%	43%	0.2251
Chloraphenical	45%	55%	0.3863	59%	41%	0.1187
Ciprofloxacin	26%	74%	<0.0001	34%	66%	0.0054
Cefriaxozone	10%	90%	<0.0001	18%	82%	<0.0001

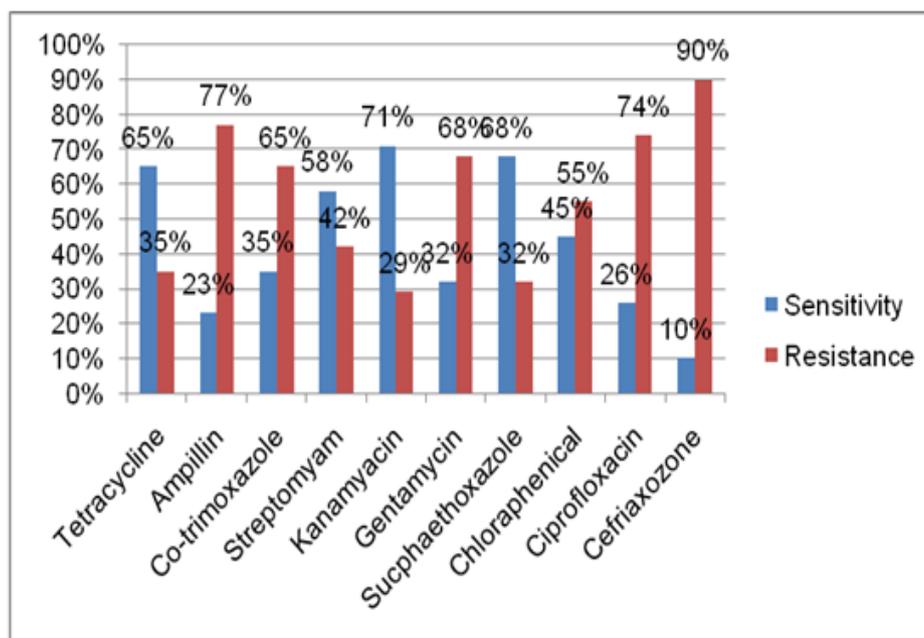


Figure 4.4.2: Inpatient

In the inpatient, drug resistance was Tetracycline 65%, Ampicillin 23%, Co-trimoxazole 35%, Streptomycin 58%, Kanamycin 71%, Gentamycin 32%, Sulphamethaxazole 68%, Chloramphenicol 45%, Ciprofloxacin 26% and Ceftriaxone 10%.

In the inpatient, drug sensitivity was Tetracycline 35%, Ampicillin 77%, Co-trimoxazole 65%, Streptomycin 42%, Kanamycin 29%, Gentamycin 68%, Sulphamethaxazole 32%, Chloramphenicol 55%, Ciprofloxacin 74% and Ceftriaxone 90%.

4.3 Outpatient

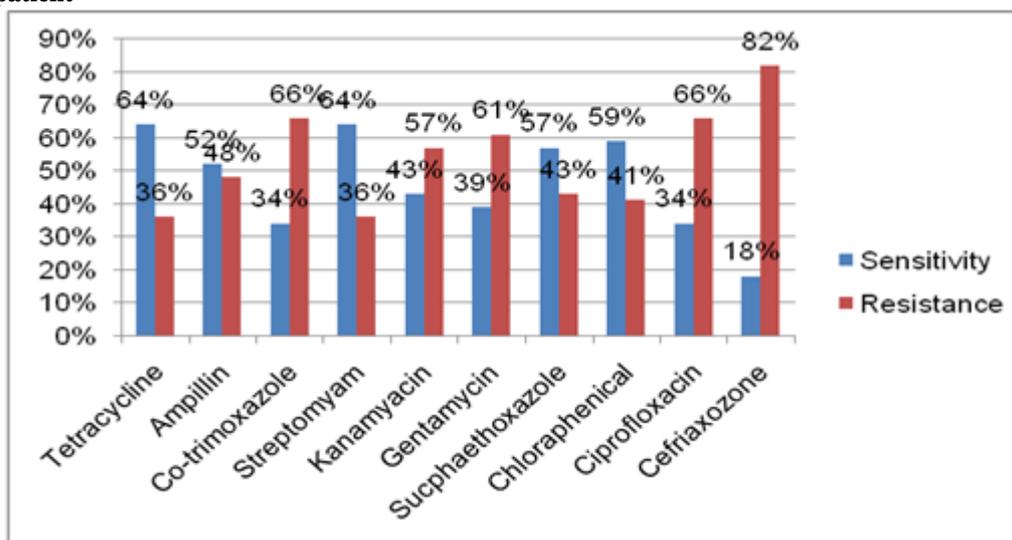


Figure 4.4.3: Outpatient

In the inpatient, drug resistance was Tetracycline 64%, Ampicillin 52%, Co-trimoxazole 34%, Streptomycin 64%, Kanamycin 43%, Gentamycin 39%, Sulphamethaxazole 57%, Chloramphenicol 59%, Ciprofloxacin 34% and Ceftriaxone 18%.

In the inpatient, drug sensitivity was Tetracycline 36%, Ampicillin 48%, Co-trimoxazole 66%, Streptomycin 36%, Kanamycin 57%, Gentamycin 61%, Sulphamethaxazole 43%, Chloramphenicol 41%, Ciprofloxacin 66% and Ceftriaxone 82%.

V. Discussion, Conclusion And Recommendations

5.1 Discussion

Pregnant women with symptomatic UTI were subjects for the case controlled study; most of the clients were literate and understood the inclusion and exclusion criteria. Also the research assistants, laboratory personnel, and the pregnant women were well trained and they observed all the aseptic procedures from specimen collection to specimen analysis. The predominant social classes in the study done were social classes III and IV.

These are mainly elites and urban dwellers. This may differ from the prevalence in the rural area in this Kisii County. Also the study was on pregnant women with symptoms of UTI hence asymptomatic pregnant mothers would have been missed. All trimesters were also included in this study and the pregnancy hormonal effect on the urinary system may not be well established or pronounced in the early trimester.

E. coli was the most common bacteria isolated in this study and this is similar to most other studies, (Ezechi OC, *et al*). this supports the fact that most organisms causing UTI are from the lower gastrointestinal tract which acts as a reservoir for organisms like *E. coli*. Patterson TF and Andriole VT 28% *Enterococci* which have been noted as a significant bacterial isolate from women with UTI in pregnancy, (Onuh S.O., *et al*.) This is in support of the postulation that there tends to be a shift in the percentages of micro organisms 27 % *P. mirabilis* and 19% *S. aureus* common bacteria isolated in this study accounting for 19% of cultures.

In this study, cephalosporins had a remarkable antibiotic sensitivity pattern of 92% of ceftriaxone. Cephalosporins, although expensive, are safe in pregnancy. Currently, most cephalosporins have both oral and parenteral combinations and have been noted to be the first line drug for pyelonephritis and the most commonly used antimicrobials for symptomatic UTI in hospital settings (Duft p, 2002).

The antibiotic second overall highest sensitivity pattern in this study was ciprofloxacin which is a quinolone. This is similar to other reports where quinolones was the most effective and sensitive antibiotics to the organisms causing UTI, (Aziz MK, *et al*) all quinolones used in this study had good antibiotic sensitivity pattern: 70% for ciprofloxacin. Quinolones are expensive and have been associated with teratogenicity in first trimester and risk of auditory and vestibular toxicity in the fetus in later trimesters, and are therefore contraindicated in pregnancy. However, for recurrence and persistent UTI, quinolones could be used with caution in late pregnancy or postpartum after counselling, especially if it is the only sensitive drug, as it is also secreted in breast milk.

The poor antibiotic sensitivity pattern of the above commonly available drugs could be due to the practice of self-medication, visiting pharmacy shops manned by non-professionals leading to under dosage and indiscriminate abuse of drugs in the environment. The above practice leads to emergence of resistant strains among the UTI causing bacteria. This increases the cost of treatment because the quinolones and cephalosporins, which have excellent sensitivity patterns, are expensive.

Stratified urinary isolates in visit setting, *E. coli* was the most commonly cultured organism in pregnant mothers in each setting, followed by *streptococcus pyogen*, *proteus mirabilis* and *staphylococci aureus*

The different resistance patterns in outpatient and inpatient settings affect empirical antibiotic use when treating pregnant mothers with a UTI. Clinicians must choose an antibiotic with a high likelihood of coverage while considering potential adverse effects and minimizing unnecessary overuse of broad-spectrum antibiotics. Since antibiotics are generally prescribed before the return of culture results, antibiogram are published to guide empirical antibiotic choice.

Antibiogram are designed by a set of guidelines using antimicrobial resistance and sensitivity data. At most hospitals combined outpatient and inpatient laboratory data are currently used to create an antibiogram. Halstead dc et-al

Along these lines, (Boggan, *et al*), reported that when practitioners are provided with antibiogram that target the specific diagnosis of the patient, physicians select narrower spectrum antibiotics. The investigators asked physicians to choose an empirical antibiotic for theoretical pregnant mothers with a UTI of different ages with UTIs. When no antibiogram was available, physicians chose an effective antibiotic of the time. When an antibiogram was available, that combined adult uropathogens data; physicians chose an effective antibiotic of the time. The study support the fact that when clinicians are aware of resistance and prevalence patterns, more informed and effective antibiotic choices can be made for empirical treatment of UTIs.

A patient who presents to a clinic as pregnant mother with fever and urine studies suggestive of a UTI most likely requires empirical antibiotics. Usually the patient is on empirical antibiotics for at least 48 hours before culture results are available. A local antibiogram based on urine cultures can be an important tool to aid in the selection of an antibiotic with a high likelihood of treating the uropathogens.

When urine cultures are stratified to specifically represent the patient being treated, they are most useful. These stratifications can reflect inpatient or outpatient status, and patient age. For example, it would be clear that Ceftriaxone is an excellent empirical antibiotic for pregnant mothers with a UTI since the most common bacteria in that age and gender group is *E. coli*, which is sensitive to Ceftriaxone 92% of the time.

5.2 Conclusion

In this study, cephalosporins had a remarkable antibiotic sensitivity pattern of 92% of ceftriaxone, constituted a significantly high proportion of bacterial isolates in this study. Quinolones and cephalosporins were the most sensitive drugs to all the micro organisms isolated from symptomatic cases in this study. Cephalosporins, like ceftriaxone, could be administered empirically because of their high sensitivity and wide spectrum of activity against micro organisms causing UTI in pregnancy seen in this study. Thereafter, the cheap and readily available antibiotics like nitrofurantoin could be used to replace the cephalosporins if the antibiotic sensitivity of the offending bacterial isolates shows susceptibility to these cheap readily available antibiotics. However, considering the morbidity and mortality that may complicate UTI in pregnancy if not adequately managed, the cephalosporins, even though expensive, are cost effective and safe when appropriately used in pregnancy. However, the cost implication could be addressed by effective implementation of ministry of health. Through the government chemist and poisons board and national health insurance scheme, cephalosporins could be made available at no financial burden to these pregnant women with symptomatic UTI. However, in the interim, the government could subsidize the cost of this potent antibiotic to reduce perinatal and maternal morbidity and mortality. This research will be used for empirical therapies and improvement of appropriate infection control strategies.

We found significant differences in resistance patterns between outpatient and inpatient urinary isolates for several of the most commonly prescribed antibiotic agents. We also noted that uropathogens prevalence varies by patient visit setting. These findings substantiate the need for separate inpatient and outpatient based antibiogram to optimize empirical antibiotic selection for pregnant mothers with UTI treatment.

5.3 Recommendations

This study recommends that effective identification, isolation and sensitivity tests of uropathogens in pregnant mothers such as *E. coli* associated with UTI.

- i. Pregnant mothers treatment and safe practices which reduce pathogen carriage such as pathogen clean water, increased hygiene hospital, health education efforts to protect public health and continued implementation of safe motherhood. This will minimize pregnant mothers' auto infections and contamination with these pathogens that can occur.
- ii. Routine surveillance and timely reporting of antibiotic resistance patterns among uropathogens should become a high priority to establish possible sources of bacterial resistance and provide data that can be used to select appropriate treatment.
- iii. Establishing National Committee of Clinical Laboratory Standards on antibiotic sensitivity testing on uropathogens.
- iv. The government should expand the existing maternal health programs and put more emphasis on uti treatment components targeting mothers who were established to be at a higher risk.
- v. The government should support the private sector and NGOs that deals antibiotic regimes program through enhanced private partnerships.
- vi. Health policy makers should encourage unified and well-coordinated mechanisms with other relevant stakeholders such as Ministry of Public Health and Sanitation, Ministry of Housing, Local Government, Ministry of Finance and Planning to incorporate free maternal

5.4 Limitations

The study was a hospital based study and may not truly reflect findings in the rural areas and the entire state. The antibiotic sensitivity test against bacteria in the laboratory is an in vitro activity and may not exactly reflect the in vivo activity. There may be some observational error, especially with the error due to parallax in measuring the inhibition zone diameter.

This study should be interpreted in the context of some database limitations. It was not possible to determine whether urinary isolates collected in the inpatient setting actually represented infections acquired in the inpatient or outpatient setting. If a patient was admitted to the hospital, the urine culture obtained on hospital day 0 was counted as an inpatient culture. Clearly, the timing of that culture should be considered outpatient but in the data set it was counted as an inpatient culture. While the data prevented us from determining this with certainty, pregnant mothers from whom urine cultures was obtained during inpatient admission tended to have different resistance patterns than those treated in the outpatient setting.

There is no information in the TSN data set on associated clinical signs or symptoms that would support a UTI diagnosis. Thus, it was not possible to determine whether some positive cultures were related to asymptomatic bacteriuria.

Another limitation of the data set was the inability to identify the specimen sthence, i.e. clean catch versus catheterized specimen. Despite this limitation it is unlikely that these cultures represented contamination.

Investigators at participating laboratories are instructed to submit only culture data that they deem clinically positive and we only chose specimens with single growth bacteria to minimize the concern for contamination.

Lastly, these aggregate data do not necessarily reflect resistance patterns in specific communities since they were not separated regionally. However, this information is essential to evaluate overall uropathogens resistance patterns and trends in the United States.

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Appendices

Appendix III: Checklist

Antibiotic Susceptibility Patterns on Women from Urinary Isolates of at *Escherichia coli* at Kisii Teaching and Referral Hospital

A: DEMOGRAPHIC INFORMATION:

1. Name/Identification No.:

2. Nationality: a) Kenyan b) non Kenyan c) hers (specify) _____

3. Age of the Patient : _____ Gender: Female

4. Date of hospital admission/visit:

5. Name of admission ward: _____

B: CLINICAL INFROMATION:

6. Clinical diagnosis: UTI

C: LABORATORY DATA:

7. Clinical specimen examined: Urine

8. Organism isolated: _____

9. Antibiotic Sensitivity Pattern:

Name of Antibiotic	Sensitivity Results (CLSI, 2012)				
	Disk content	Zone diameter breakpoints nearest to whole mm			
		Susceptible (S)	Intermediate (I)	Resistant (R)	Zone Readin g(mm)
Tetracycline	30µg	≥17	15-16	≤14	
Ampicillim	25µg	≥17	14-16	≤13	
Co-trimoxazole	30µg	≥23	20-22	≤19	
Streptomycin	30µg	≥18	15-17	≤14	
Kanamycin	30 µg	≥18	15-17	≤14	
Gentamycin	30µg	≥21	18-20	≤17	
Sucphamethoxazole	30 µg	≥23	15-22	≤14	
Chloraphenical	5 µg	≥21	16-20	≤15	
Ciprofloxacin	10µg	≥23	20-22	≤19	
Cefriaxozone	10µg	≥23	20-22	≤19	
Tetracycline	100/10µg	≥21	18-20	≤17	
Ampicillim	(1.25/ 23.75 µg)	≥16	11-15	≤10	

Appendix IV: Consent Form

DATE.....PATIENT STUDY NUMBER.....

STUDY TITLE: To Determine Antimicrobials Susceptibility Pattern on *Escherichia coli* Isolates Causing Urinary Tract Infection in Pregnant Women attending Kisii Teaching and Referral Hospital. CONSENT FORM 1A CLINICAL URINE MICROBIOLOGY TESTING OF INDIVIDUALS.

Purpose:

This is a microbiology study looking at antibiotic susceptibility pattern of *E. coli* in pregnant women both outpatient and inpatient I Mr. Abel Onchiri am requesting for permission from you, so that you can participate in this research study.

The goal of this study is to understand antibiotic susceptibility of *E. coli* in pregnant women and can do this by examining the urine specimen. The information obtained was used to improve on antibiotic treatment of *E.coli* in pregnant mothers. I request for the small urine sample (midstream) on sterile urine bottle it is a non invasive procedure with no harm.

The sample was examined macroscopically, microscopy ,biochemical content, culture, and isolation of *E.coli* was done on the specimen and finally sensitivity testing while the remaining specimen was discarded if you was found to be having U.T.I due to *E.coli* you was treated and or this procedures was done in Kisii teaching and referral hospital microbiology laboratory.

I understand that if any test results are found it's important you was given a confidential report and you have the full rights of terminate the agreement anytime for any reason and the sample was discarded and reports destroyed. The sample not be used for any other use not described in this study procedure. If you agree circle YES and if you disagree circle NO.

Yes

No

Signature.....*date*.....

Signature of witness.....*date*.....

Confidentiality:

The result was assigned a stud nber to preserve confidentiality database linking you and the personal identity to the study was kept by the investigator and relevant key personnel under key and lock.

Risks and Benefits:

There are minimal risks to having additional urine sample if required and no additional risks to the fetus. The results of this study may benefit the community and you have an opportunity to get the best treatment for the

condition it also improve the current recommended treatment of the condition to provide appropriate medication and the study cover any cost related to the laboratory diagnosis procedure.

Summary:

This research study is voluntary refusing to participate will not alter the usual health care or involve any penalty. Contact information ABEL ONCHIRI has described what is going to be done the risks benefits involved and can be contacted on moiabel@yahoo.com or +254 722-146 692.

Signature:

Signing bellow indicates that you have been informed about the research study in which you voluntarily agree to enroll.

.....
Signature of Participant

.....
Date

.....
Signature of Investigator

.....
Date

Appendix V: Ethical Approval



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariat@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 30th March, 2016

TO: Abel O. Onchiri
H/152/4463/2013
School of Health Sciences
Jaramogi Oginga Odinga University of Science and Technology
P. O. Box, 210 - 40601 Bondo – Kenya

REF: MSU/DRPI/MUERC/00272/16

RE: Antimicrobial Susceptibility Patterns of *Escherichea Coli* Isolates Causing Urinary Tract Infections in Pregnant Women Attending Kisii Teaching and Referral Hospital. Proposal Reference Number MSU/DRPI/MUERC/00272/16

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 30th day of March, 2016 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 29th March, 2017. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 28th February, 2017.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 28th February, 2017.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advise MUERC when the study is completed or discontinued.

Thank you.

Yours faithfully,

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

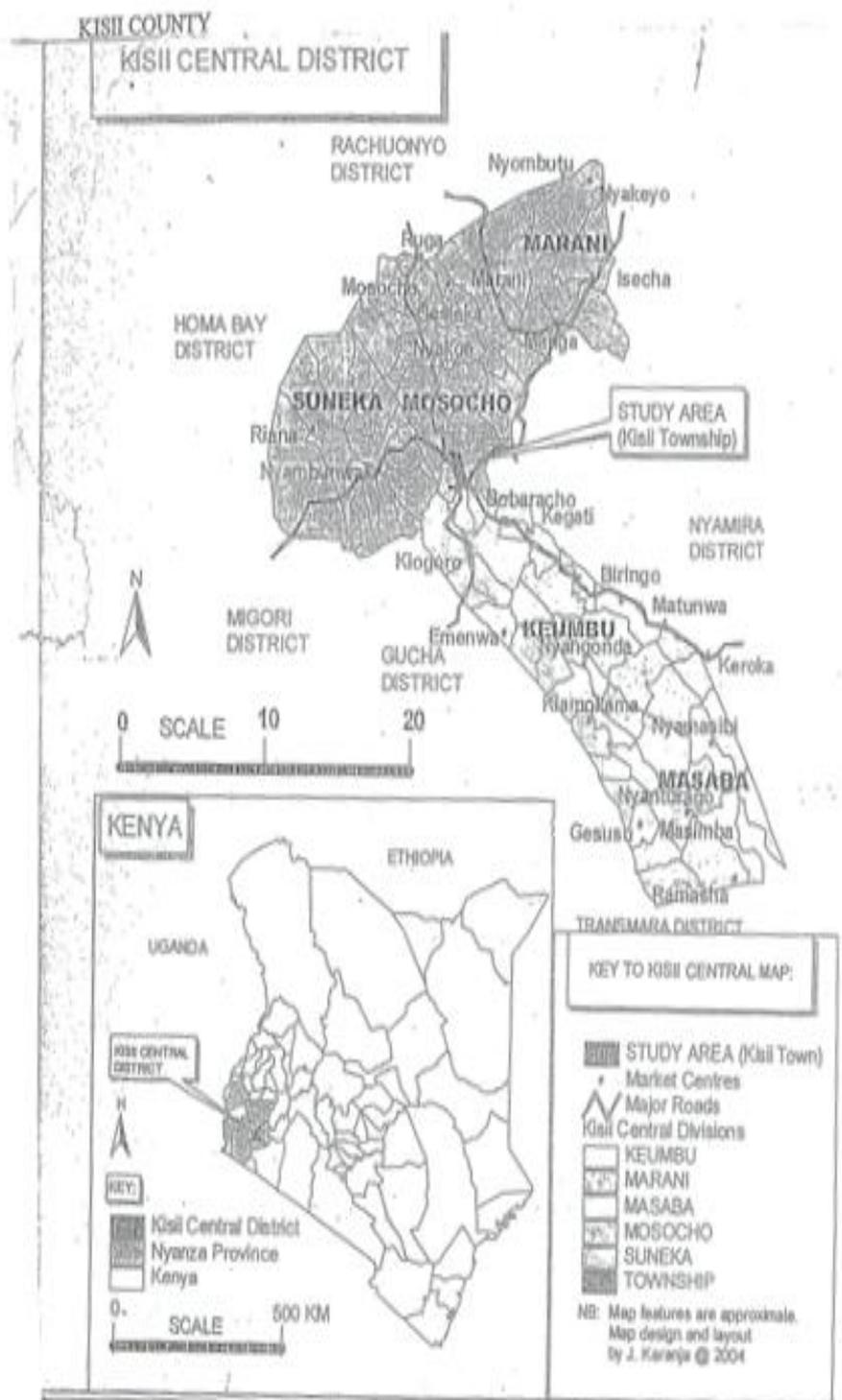


Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED



Appendix VI: Map of Study Area



Abel Orero Onchiri " Antimicrobial Susceptibility Patterns Of Escherichea Coli Isolates Causing Urinary Tract Infections Among Pregnant Women At Kisii Teaching And Referral Hospital." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.4 (2018): 44-62