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Proportion, etiology and recurrence of urinary tract infections in females of reproductive age attending out-patient clinics in Kwale and Kinango sub-county hospitals

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Abstract
Background information: *Community-acquired urinary tract infections (UTIs) occur mostly in women and are commonly caused by Escherichia Coli.*
Objective and Study design: *This was a cross-sectional study carried out in Kwale and Kinango Sub-county hospitals aimed to determine the proportion and etiology of UTI among female attending the outpatient department for a period of twelve months (September 2014-September 2015).*
Methods: *Mid-stream urine samples (MSU) were collected from 766 no-pregnant female who consented in our study and fulfilled other study criteria. For identification of uropathogens, we employed both quantitative culture techniques and antibiotic sensitivity tests using Kirby-Bauer disc diffusion technique where 14 antibiotics were used.*
Results: *Women aged between 15-29 years old were the most affected, 152(19.8%) with those living in rural area having the disease more than those in urban area. Disease status was high among the married women 141(18.4%), single, 66(8.6%) and divorced, 13(1.6%) than the single or divorced female who took part in the study. Out of the 766 samples collected, 220 (28.9%) had significant bacterial growth, thus having urinary tract infection. Escherichia coli the most predominant uropathogen isolate. Other microbial agent isolated included Pseudomonas aureginosa 28(12.8%)>26(11.8%) Staphylococcus aureus >20(9.1%) Staphylococcus saprophiticus and 20 (9.1%) Proteus mirabilis. Candida albicans was also isolated in 29(13.18%) of the samples suggesting candidiasis. Overall, in vitro susceptibility drug tests were: Doxycycline (85%), Minocycline (83.3%) and Ciprofloxacin (78%) as the most sensitive drugs while the drug with the lowest sensitivity was Ampicillin (31%).An overall urinary tract infection proportions of 28.9% observed in this study shows the clinical and epidemiological significance attained by urinary tract infection.*
Conclusion: *Doxycycline was the most sensitive antibiotic to uropathogens isolated but Ampicillin had the lowest sensitivity. We do recommend for an evidence based management of urinary tract infections, for both asymptomatic and symptomatic cases by making microbial screening including culture as a compulsory investigation for all patients suspected to have urinary tract infections.*

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I. Introduction

Urinary tract infections are among the most common bacterial infections (Aiyegoro et al; 2007). [3]They are non-sexually transmitted diseases and are endemic in developing countries where their incidences have been reported in some studies to be more frequent in females than in males during youth and adulthood (Haider G, *et al*;2010).[4] Infections of the genito-urinary tract are considered a global public health issue and in resource-poor countries, they are among the five most common health problems leading to contact with the health system (Kurtshals *et al.,* 2009).[3] Genito-urinary tract infections are caused by microorganisms most of which are normal commensals in the distal urethra and the adjacent sites (Kolawale *et al*, 2009)[4, 5]. The most common route of infection is by microbial agents ascension to these sites and others such as kidney and urethra causing pyelonephritis, cystitis and other complications (Colgan *et al*; 2011;).[6] Gender differences show some

distinction, with the prevalence of infection being high in females than in males. Community-acquired urinary tract infections (occurring in people not admitted to hospital prior to development of the symptoms of the infection) occur mostly in women and are most commonly caused by *Escherichia Coli (*Mwaka AD, *et al*,2006)[1] . The management of UTIs has been reported to be challenging not only because of the large number of infections but also because the diagnosis of UTI is not always straight forward(Kolawole et al;2009)[5]. A study in India revealed that among Gram-negative bacteria, the most common isolate *E. coli* showed high level of resistance to commonly used empirical antibiotics β-lactams (Ampicillin, Augmentine, Cefaclor, and cefrodoxime), fluoroquinolones (ciprofloxacin and ofloxacin) and co-trimoxazole (Muktikesh et al;2013)[5]. For the past two decades, trimethoprim-sulfametroxazole (SXT) or Trimethoprim alone have been used as empirical therapy for *E.coli* UTI. However, in the United States, resistance to SXT among *E. coli* isolates from persons with community-acquired UTIs has increased substantially over the past decade, with a prevalence exceeding 20% in many parts of the country (Stamm W; 2001)[2]. Globally, *Escherichia coli* cause 75% – 90% of acute uncomplicated cystitis while *Staphylococcus saprophyticus* accounts for 5% to 15%, mainly in younger women (Gupta K 2001; Finkelstein R; 1998)[7]. *Enterococcus species* and aerobic gram-negative rods other than *E.coli*, such as *Klebsiella pneumoniae* and *Proteus mirabilis*, are isolated in the remainder of the cases of UTI (Allan RR; 2001; Fihn SD et al; 2003 ;) [8].

II. Materials And Methods

Study site and subjects

The study was conducted in Kwale and Kinango Sub-county hospitals between February 2013 and September 2015. The respondents recruited were only female patients of the reproductive age (15 – 49 years) attending the out-patient department of Kwale and Kinango Sub-county hospitals during that period of time were consented and included in the study.

Inclusion Criteria

1. All female patients attending the out-patient clinic in Kwale and Kinango district hospitals.
2. Female patients willing and accepted to sign a consent form to participate in the study
3. Women in the ages between 15-49 years
4. Patients from within the two catchment areas of the hospitals

Exclusion criteria

1. Females below 15 years and those above 49 years
2. All women with one or more of the following: pregnancy, puerperium, any vaginal bleeding at the time of the study, long term (14 days) catheterization ending in the last 30 days prior to the study, history of gynecological or recent urological surgery and women with overt vaginal or uterine prolapse on history. The research assistant found from the respondents about their status, ask them if they were in any of this category before recruiting them to the study.
3. Those unwilling to sign the consent form
4. Those outside the catchment areas of the two hospitals

Ethical considerations

This research protocol was approved by Ethical Review Committee of the Kenya Medical Research Institute (SSC protocol: 2762). Permission was also obtained from the administrations of the two hospitals. All participants recruited into the study agreed to a written informed consent after they were given clear explanation of the study rationale.

Sample collection and preparation

Mid-stream urine (MSU) was collected from all 766 participants in order to give those with asymptomatic bacteriuria an opportunity to be tested. Before collecting the urine sample, every respondent was adequately educated on how to collect the mid-stream urine sample and each participant was issued with eight (8) pieces of sterile gauze swabs, four (4) soaked in liquid soap and four (4) soaked in saline and sterile wide mouth screw cap, dry leak proof plastic bottle to collect about 30mls of mid-stream urine, and soon thereafter tightly closed the bottle and handed to the research assistant.

Physical (Visual) Examination of Urine

The aseptically collected clean cached mid-stream urine sample was observed macroscopically for characteristics such as color and turbidity.

Urine Chemistry

The urine test strips Dirui H 10 (Dirui Industrial Company China) was used to determine semi-quantitative characteristics such as leukocytes, proteins, and nitrite, of each urine sample collected. Each urine sample collected was first divided into two portions; one was used for determination of urine chemistry composition and the remaining sample in the sterile bottle spared for culture technique. This was done by dipping a test strip into each urine sample and comparing the observed color changes on the strip to a reference color chart provided on the package of the test strip.

Microscopy

A portion of each urine sample was poured into labeled test tubes and span at 3000 rpm for five minutes using a centrifuge, LW Scientific 800-726-7316 Ultra-8UV. The supernatant was then discarded and the deposits remixed by tapping the bottom of the tube to homogenize it. A drop of the deposit was transferred unto well cleaned and dry glass slide and covered with a cover slip. The slide was then examined under microscope (Olympus, CX 21, Optical Company Limited, Japan) using both low and high power objective lenses (10X and 40X) with the condenser iris closed sufficiently to give good contrast for identification of pus cells, cast, crystals, and motile bacteria.

Urine culture

Using a standard calibrated platinum wire loop sterilized by heating in a Bunsen burner flame, a loopful (0.01ml) of urine sample was taken from each specimen and inoculated on three different culture media namely; CLED, MacConkey and Blood agar and then incubated aerobically at 37 ^{0}C

Identification and counting of bacteria isolates

Only culture plates showing microbial growths were picked and the different bacterial colonies identified on the basis of their colonial morphology, color, and growth size and pattern. Bacterial counts were determined by the product of colony count on agar media. Significant genito-urinary tract infection (significant bacteria growth) was determined by a bacterial colony forming units (CFU) count greater than 10^5.

Gram stain tests, Microscopic Examination and Biochemical Analysis

Biochemical tests were performed for identification and characterization of culture positive organisms isolated. The preliminary tests carried out include Catalase, Oxidase, and Hydrogen sulphide production, Germ tube test and Coagulase. Further confirmatory tests which included oxidation/fermentation test, triple sugar iron agar; nitrate reduction test, Simmon's Citrate medium, Christensen's Urea agar, Sulpher-Indole-Motility medium were performed. Smears were made from culture colonies and Gram's stain technique done to identify and classify isolated organisms as either Gram positive or negative. The dry stained smears were examined under microscope, under oil immersion, using high power (X100) for identification of isolates.

In vitro drug sensitivity tests

Ampicillin (30mcg), Gentamycin (10mcg), Norfloxacin (10mcg), and Nalidixic acid (30mcg Cefotaxime (30mcg), Nitrofurantoin (300mcg), Ceftriaxome, Ceftazidime, Ciprofloxacin, Ceftizoxime, Trimethoprim-Sulfamethoxale, Erythromycin, Minocycline and Doxycycline were obtained from the laboratory. The drug Susceptibility drug assay was used for in vitro drug sensitivity testing (National Committee and Clinical Laboratory Standards). Kirby-Bauer disk diffusion method was used in the antibiotic susceptibility test where Nutrient Agar was used as the media of choice. This was done to record antibiotic reaction(s) against isolated microbial pathogens. The assay was initiated by emulsifying colonies in sterile normal saline using a straight wire. The turbidity was adjusted to the equivalent of 0.5 McFarland Standards. Reconstituted colony from the culture plates was then put on Nutrient agar and by use of a sterile cotton swab was spread to obtain uniform inoculums. Appropriate antimicrobial discs chosen based on the Gram reaction of microorganisms was placed on the plate inoculated with emulsified portion, labeled and left on the bench for 10-15 minutes (to give it time to be adsorbed) with Bunsen burner on to send away microbes in the air before incubating at 37 0C for 24 hours. The plates were observed for growth in the morning and zones of inhibition determined by measuring the diameter of the zones with a ruler. The measurements obtained were compared with a standard chart to determine susceptibility before reporting results, as either sensitive(for those showing zones of inhibition) or resistant(those showing no zones of inhibition). Available stock cultures for all microorganisms were used as controls.

III. Data analysis

Data obtained from this study was entered into SPSS software version 20 (IBM SPSS STATISTICS V20, USA) and then tabulated and cross-checked for out-of- range errors and inconsistencies. Descriptive statistics such as proportions, frequencies and ratios were used. Cross tabulation was used to display relationship between variables. Finally, data was presented in tables, pie charts and histograms. For bivariate analysis, Pearson's ($\chi 2$) survey designs were used for categorical data analysis. Associations between categorical outcomes variables were conducted using Chi squared test ($\chi 2$) at the 95% significant level and symmetric measures to determine the strength of relationship. A two tailed p-value of < 0.05 was considered statistically significant. One way ANOVA was used to test for significance among groups. Logistic regression was used to determine the proportion of those with disease against those without the disease. Ethical approval was sought from the board of postgraduate students, scientific steering committee of KEMRI and submitted to Kwale county department of health. Informed consent were obtained from all participants before the interviews and collection of samples were conducted.

IV. Results

For a period of 12 months, 766 mid stream urine samples collected from non-pregnant female patients in the reproductive age(15-49 years old) attending the outpatient departments, Kwale and Kinango sub-county hospitals were analyzed, of which 220 gave significant bacterial growth, an indication of bacterial urinary tract infection. There were 539 patients from rural area and 227 from urban area who took part in this study. Out of these, 151 from the rural area and 69 from urban area had significant bacteriuria(Figure 1).

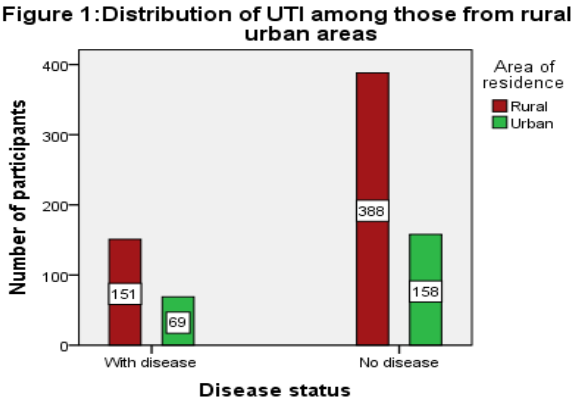

Figure 1: The disease burden is in the rural areas. Few people are not in medication in the urban. There is relationship between people having medication seeking behavior in those leaving in rural as compared to those leaving in Urban. Pearson value < 0.005(P-value

The study participants 509(66.4%), 176 (23.0%) and 81 (10.6 %) aged between 15-29, 30-39 and 40-49 years respectively. The lowest incidence of UTI 26(3.4%) was found in age group 40-49 years while the highest 152(19.8%) were in age group 15-29 years (Table 1).

Table1: Prevalence of UTI amongst different age groups

Age Group	With UTI	Percent
15-29	152	19.8
30-39	42	5.5
40-49	26	3.4
Total	220	28.7

Out of the 381 recruited, significant bacteriuria was demonstrated in only 220, (57.1%) of the samples cultured on the three different media, that is CLED, Blood agar and MacConkey showed significant bacterial growth while 165(42.9%) had no growth obtained even after 72 hours of incubation. Five uropathogens were isolated by culture. Of the five microorganisms isolated Escherichia coli were the most predominant with a prevalence of 97(44.1) %.Other microorganisms isolated include Pseudomonas aureginosa 28(12.7%) Staphylococcus aureus 26(11.8%), Staphylococcus saprophiticus 20(9.1%) and Proteus mirabilis, 20(9%) as is depicted in figure 2.

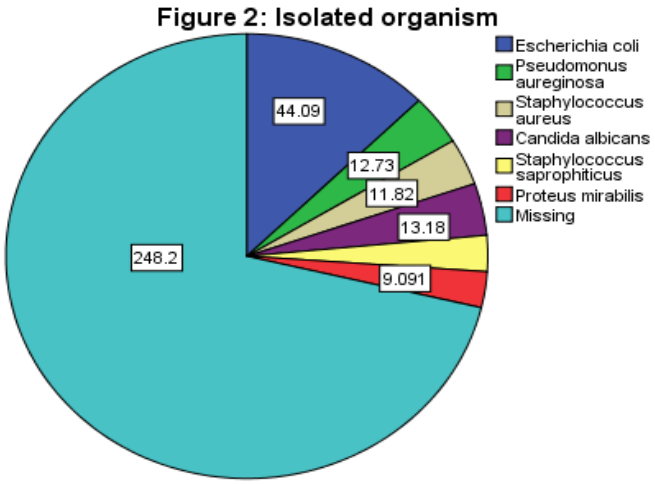

Figure 5 above show the pattern of the different uropathogens as isolated by the culture technique. Of the six uropathogens isolated, Escherichia coli was the most prevalent, 97(44.1%) while *Staphylococcus saprophiticus* and *Proteus mirabilis* had the lowest, with both having 20,(9.1%)

URINARY TRACT INFECTION RECURRENCE

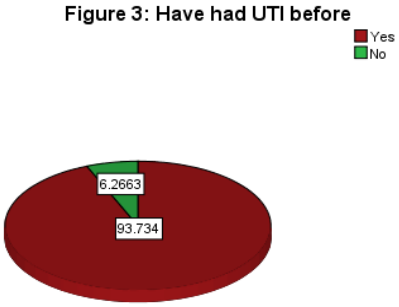

Figure 3 above shows the recurrence of UTI in the participants with 93.7% reporting having an episode of UTI before

REPORTING UTI RECCURRENCE

When asked how many times they had had an episode of urinary tract infection before,328(42.8%) of the participants reported having suffered more than three times same year,323(42.2) twice while only 15% had it once as is shown in the frequency table below.

TABLE 2: NUMBER OF TIMES THIS YEAR

	Frequency	Percent	Valid Percent	Cumulative Percent
Once	115	15.0	15.0	15.0
Twice	323	42.2	42.2	57.2
More than three times	328	42.8	42.8	100.0
Total	766	100.0	100.0	

Figure 3: The incidence of UTI determined by the number of women with positive urine microbial culture when their urine samples were cultured. This indicates the disease status of the female patients attending the two hospitals and who took part in the study

The antimicrobial susceptibility patterns of isolated uropathogens in this study are shown in Table 2. *Escherichia coli* which, the most prevalent pathogen isolated was susceptible to most of the antibiotics with Doxycycline showing the highest antimicrobial activity(86.7%) against *Escherichia coli* while Ampicillin recorded the least sensitivity (31.9%) to most uropathogens.

Table 3: ANTIBIOTIC SUSCEPTIBIITY OF ISOLATES

Antibiotics	**Microbial Isolates**				
	E coli E coli	*Pseudomonas aureginosa*	*Staphylococcus aureus*	*Staphylococcus saprophyticus*	*Proteus mirabilis*
	T(%S)	T(%S)	T(%S)	T(%S)	T(%S)
Cephaloxime	97(80.4)	25(64)	11(36.4)	Nd	20(65)
Ceftriaxome	91(73.6)	23(69.6)	5(80)	10(100)	20(80)
Ceftazidime	91(70.3)	23(73.9)	5(100)	Nd	20(55)
Gentamycin	83(63.9)	25(68)	14(57)	11(81.2)	20(45)
Ampicillin	84(34.5)	23(26)	19(21)	17(35.3)	20(35)
Nitrofurantoin	96(74)	27(74)	20(60)	15(73.3)	20(70)
Nalidixic acid	94(67)	26(69.2)	23(65.2)	19(63.2)	20(75)
Ciprofloxacin	77(75.3)	19(89.5)	14(85.7)	14(82.3)	20(70)
Ceftizoxime	20(50)	Nd	17(47)	11(54.5)	Nd
Trimethoprim-Sulfamethoxale	50(80)	10(100)	16(68.8)	13(76.9)	Nd
Erythromycin	30(60)	20(0)	19(73.6)	14(78.6)	Nd
Norfloxacin	19(60)	17(80)	17(64.7)	18(88.9)	Nd
Minocycline	16(75)	17(88.2)	15(93.3)	10(90)	Nd
Doxycycline	15(86.7)	17(100)	17(94.1)	11(72.7)	Nd

T=Total tested, %S= Percentage Sensitive, Nd=Not done

Table 2: shows the antimicrobial susceptibility patterns of isolated uropathogens in this study. *Escherichia coli* **which was the most prevalent pathogen isolated was susceptible to most of the antibiotics with Doxycycline showing the highest antimicrobial activity against** *Escherichia coli* **while Ampicillin recorded the least sensitivity to most uropathogens.**

V. Discussion

This study reports on the use of evidence based management through microbial analysis of both asymptomatic and symptomatic urinary tract infection for all patients within the ages 15-49 years. Most of the women in the study were in the 15-29 years age group, which constitutes a substantial part of the active reproductive age group and thus susceptible to urinary tract infections (Arul et al., 2012). The study groups were stratified by age distribution, with high prevalence of UTI of 152(19.8%) being observed in participants in the ages 15-29. These findings are similar from previous studies such as 85 % (Alex et al, 2012) 10and in another study that observed a prevalence of 52.6% (Mwaka et al; 2010)1, 4

Prevalence of significant bacteriuria

The proportion of significant bacteriuria/UTI in this study of 28.9 %(220/766) is higher compared to previous studies in the region of 10 % (Mwaka *et al*; 2010)[1, 4] and 17.9% and 13% in asymptomatic and symptomatic participants respectively (Masinde et al, 2009) [1, 4]and 18% (Hannah , *et al*; 2011). However, the results in this study are comparable with 39.5% prevalence of UTI as reported by Moges *et al* in a study that included 70 in- patients.

Uropathogens isolated by culture (significant growths)

Urinary tract infections due to Escherichia coli is a common finding in women and it is associated with microorganisms ascending from the periurethral areas contaminated by fecal flora due to the close proximity to the anus and warm moist environment thereby. Most of the isolated bacteria showed low in vitro sensitivity to Ampicillin and Nalidixic acid. Of the six (6) uropathogen species isolated in this study, Escherichia coli was the most frequent isolate accounting for 44.1%. This is in agreement and comparable with other studies in Africa where Escherichia coli was the prevalent uropathogen isolated,40(10%), Mwaka, et al, 2006), 40-46%(Wanyama, et al;2003; Mayanja , et al; 2005)13, 14. However the prevalence reported in our study was higher than reports from other studies,35.1%(Ayansima,et al,2012),33.3% (Sabrina et al, 2009) and 10 %(Mwaka AD, et al, 2006). A recent study in India similarly isolated high proportion of Escherichia coli (Kothari , et al; 2008). 1, 15 The other bacteria isolated were Staphylococcus aureus with 11.8% isolation rate. The isolation of Staphylococcus aureus as uropathogen is not unique to this study. In other studies, Staphylococcus aureus have been isolated. However, a study in India assessing UTI in general population did not isolate Staphylococcus aureus (Kothari , et al, 2008)15. Staphylococcus aureus has been in the recent time been found as causative agent mainly in complicated UTI (Loren et al, 2004; Wagenlehner et al; 2004) 16causing cystitis. The 26 patients with Staphylococcus aureus significant bacteriuria presented with history of recurrent infection of the urinary tract and had positive leucocytes esterase tests. It is probable that these patients had Staphylococcus aureus bacteriuria with seeding to urinary tract. Similarly high isolation rates of Staphylococcus aureus were demonstrated elsewhere in Africa (Kayima , et al; 1996, Moges, et al, 2002)13, 14. Other isolates include 20(9%) Staphylococcus saprophiticus and Proteus mirabilis respectively. The findings of our study were also in agreement to what Hannah et al, 2011 determined in their study where they isolated Escherichia coli as the most frequent organism at 27%, Pseudomonas aureginosa at 13.6%, Candida albicans, 9% and Proteus species at 9%, although their study was involving in- patients, a group that is predisposed and therefore different from the population considered in our study.

Similar disease recurrence

A recurrence rate of 718(93.7%) was reported among all patients who took part in this study, which is high compared to other previous studies 5% (Henn EW, 2010), 44% Ikaheimo R, et al; 1996)11. Three etiologies of recurrent urinary tract infection (RUTI) have been observed to exist. These etiologies include persistence of original organism, reinfection with the original organism or reinfection with a different strain of bacteria (Rock et al; 2007,Vasquez Y et al; 2004). Russo TA, et al, (1995)12 observed that majority of recurrent UTIs occur as a result of reinfection of the initial bacteria due to bacterial persistence in the fecal flora and subsequently recolonizing of the urethra.

Antimicrobial susceptibility test profiles of the isolated uropathogens *Escherichia coli,* the most common uropathogen reported in this study showed a varying sensitivity rate to antibiotics from 34.5% for Ampicillin to 86.7% for Doxycycline. These findings are similar to previous studies (Forouza *et al*; 2013).;however, *Escherichia coli* exhibited 74% drug sensitivity to Nitrofurantoin and 67% susceptibility to Nalidixic acid which are the most common prescribed antibiotics to treat infection of the urinary tract in the study area. This susceptibility rate was lower than Sevki *et al* (2011) who reported a 95.4% susceptibility rate to Nitrofurantoin in a study carried out in Turkey. *Pseudomonas aureginosa*, the second most common microbial isolate demonstrated no susceptibility to Erythromycin, and a minimal sensitivity rate of 26% to Ampicillin, some of the most common and easily available over the counter antimicrobial agents due to their lower cost. This sensitivity rate was higher to what Amaeze *et al*,(2013) who in their study reported a 100% resistance of *Pseudomonas* species to Ampicillin. *Staphylococcus aureus*, the predominant Gram positive bacteria isolate in this study had a 74% and 69.2% susceptibility to Nitrofurantoin and Nalidixic acid respectively. This is similar to what Okonko (2009) who reported drug susceptibility of 72.7%, while Iram *et al* (2012) reported a susceptibility of 66.7% for Nitrofurantoin. For Nalidixic acid, our study reported a higher susceptibility rate of 36.4% than Okonko (2009), but much lower than what Iram *et al* (2012) reported, (100%). In the present study, *Proteus* species, the third Gram negative uropathogen isolated, the antibiotic resistance to Gentamycin was 45% while resistance to Ampicillin was 35%.

However; our study reports a higher susceptibility activity of 70% for Nitrofurantoin and 75% for Nalidixic acid contrary to Stanley *et al* (2014) and Akobi *et al*(2015)who reported *Proteus* species not being susceptible to Nitrofurantoin. This study also reports a higher drug susceptibility than what Das *et al* (2006) and Onoh *et al* (2013) reported 13.4% and 20.8% . *Pseudomonas* species isolated in this study had a 100% susceptibility to Trimethoprim- Sulfamethoxale and Doxycycline.

Proteus species sensitivity to both Nitrofurantoin and Nalidixic acid respectively. Our study reports a lower rate of 70% of *Proteus* species susceptibility to Nitrofurantoin compared to 100% susceptibility rate as reported by Sevki *et al* (2011) and a 100% Proteus susceptibility to Nalidixic acid Akinola *et al*(2012). In the present study, *Staphylococcus saprophyticus* was isolated at 9.1% which was higher than previous studies, 5%

(Mwaka *et al*, 2011). Ceftriaxome showed a 100% susceptibility to *Staphylococcus saprophyticus*, however the same microorganism was 35% and 54.5% susceptible to Ampicillin and Ceftizoxime respectively.

VI. Conclusion

An overall urinary tract infection proportions of 28.9% observed in this study shows the clinical and epidemiological significance of urinary tract infection. Women in the age bracket of 15-29 years old were the most affected, with Escherichia coli being the most predominant urinary tract pathogen isolated, with high levels of resistance against most antimicrobial agents to most uropathogens being evident. Culture technique is not scheduled as a routine technique in the two hospitals leading to clinicians prescribing antibiotics without laboratory confirmation in treatment of infections of the urinary tract thus leading to increase in uropathogen resistance to most commonly used antibiotics.

VII. Recommendations

We do recommend for an evidence based management of urinary tract infections, both asymptomatic and symptomatic by making microbial screening including culture as a compulsory (routine) investigation for all patients suspected to have UTI. Key stakeholders in the health ministry must come up with measures both short term and long term to address urinary tract infection and its complications in women especially this time when the nation is putting more efforts to meet Millennium Development Goals pertaining Maternal (reproductive health).

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FINANCIAL DISCLOSURE
None reported.
CONFLICT OF INTERESTS
None declared.

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