Prevalence and susceptibility pattern of bacterial Urinary Tract Infections among pregnant HIV positive women in Gucha sub county, Kenya

1Maniga NJ, 2Mogaka G, 3Nyambane L and 1Eilu E

ABSTRACT

Background: The urinary tract infection are the most frequent infection in women and complications with other diseases like HIV/AIDS, diabetes makes its management a top public health concern especially in resource limited setting. Objectives: The main aim of this present study was to determine the prevalence of bacterial UTI among HIV and access the antimicrobial susceptibility of the isolated bacteria uropathogens in HIV positive pregnant patients in Gucha Sub County District. Materials and Methods: A prospective cross-sectional study carried out at Gucha sub county District HIV/AIDS clinic. Mid-stream urine samples were collected from consenting 196 females, who were clinically diagnosed with UTI by the attending Physicians. Standard Microbiological methods were used in the isolation and characterization of bacterial uropathogens. Clinical Laboratory Standards Institutes modifications of Kirby-Bauer disc diffusion technique were adopted. The data was presented as mean ± standard deviation (SD) and compared with student t-test. Results: Out of 200 mid-stream urine samples 35 pure significant bacterial growth (10⁵ colony forming units/ml of urine) were isolated and these included Escherichia coli, 15(43%), Staphylococcus aureus, 7 (20%) S.saprophyticus 5(14%) Enterococci species, 4(11%) and Klebsiella, 4(11%). The uropathogens isolated were more sensitive to Gentamicin, 32(21%) followed with ciproflaxcin, 26(17%), Nitrofurantoin, 20(13%), Erytromcin, 12(12%) and Nilidixic acid, 17(11%) respectively. Conclusion: Escherichia coli were the most dominant bacteria occurring in the pregnant HIV Patients at Gucha Sub County District. Consequently Gentamycin should be considered a drug of choice for empirical treatment of UTI in pregnant HIV/AIDS females.

Keywords: UTI, pregnant women, Antibacterial, HIV, Seropositive females

Introduction

A urinary tract infection (UTI) is a bacterial infection that affects part of the urinary tract (1) and rarely the urine may appear bloody (2) or contain visible pyuria (3). It has already been established that HIV/AIDS patients suffer UTIs more than the immunocompetent patients due to altered immunity. UTI is one of the major cause of morbidity and mortality in the pregnant women in Africa with Kenya reporting UTI prevalence rate of 23% in the year of 2014. This is influenced by the treatment failure by the commonly used antibacterial agents as a result of resistance (4). HIV infections and AIDS continue to devastate the local population in developing countries especially in people with underlying medical and physiological conditions. Reduction of hospital stays, aseptic care of catheterized patients, selective use of antibiotics and strict follow up of hospital disease controls are some UTI prevention options currently being used by many health units to prevent UTI. However this has not influenced the increasing trend of UTI, complicated by factors such as: high costs of antibiotic therapy, emergence of multidrug-resistant bacteria and unsatisfactory therapeutic options in urinary tract infection (UTI) in resource limited settings. This calls for more indebt research and continued surveillance and advanced medical solutions. Right now, no adequate means to successfully prevent painful and disabling UTI has been found. Even though long-term oral antibiotic treatment has been used with some success as a therapeutic option, this is no longer secure due to the development of bacterial resistance, probably induced by factors such as previous antibiotic exposure, urinary catheterization and hospitalization. On the other hand, pregnancy causes numerous changes in the woman’s body. Hormonal and mechanical changes increase the risk of urinary stasis and vesicoureteral reflux. These changes, along with an already short urethra (about 3-4 cm in females) and difficulty with hygiene due to a distended pregnant belly, increase the incidence of urinary tract infections (UTIs) in pregnant women. Pregnant patients are generally considered immunocompromised UTI hosts because of the physiologic changes associated with pregnancy. These changes increase the risk of serious infectious complications from symptomatic and asymptomatic urinary infections even in healthy pregnant women.

Unfortunately in Gucha sub-county, HIV patients have limited access to the national HIV clinic and there is no surveillance on UTIs in HIV/AIDS patients especially at the grass root level. From existing research databases in the sub-counties and mainstream search, information about UTI and bacteria susceptibility in pregnancy and HIV/AIDS are not available for healthcare providers. This study was therefore designed to assess bacteria UTI prevalence and their susceptibility pattern among pregnant HIV/AIDS patients in Gucha sub-county Kenya.

Materials and Methods
The study was a prospective cross sectional by design involving collection of urine specimen from HIV/AIDS seropositive women attending Kenenya District Hospital and Ogembo District Hospital. The study included 196 seropositive females attending the HIV/AIDS clinics. The 196 participants were confirmed to be pregnant by using HCG test and HIV positive by using Indirect Elisa Test. They were adult patients aged 18 years and above. The 196 participants qualified to be included in this study by showing urinary tract clinical manifestations as confirmed by the medical officer in charge. The participants were included if they had used antibiotics for the last 3 weeks prior to the study. The participants were excluded if they were seropositive females not attending the HIV/AIDS clinics in Kenenya District Hospital and Ogembo District Hospital. Consequently ant participants who were in their menstruation period and those who did not consent were excluded from the study.

Approval was obtained from Kisii University Research and Ethics Committee. Informed consent was sought and obtained from the patients and from the HIV clinics of the hospitals where the patients were receiving treatments. All results were treated with utmost confidentiality. Each sample was having a number and the results of the findings returned to the medical officers in charge of patients. The Medical Officers was responsible for releasing results to the patients. The participants who were confirmed to be infected with UTI benefitted by being treated free of charge. The sampling technique used random sampling method whereby urine samples were collected randomly after a sequence of two patients respectively. Questionnaires were used to ascertain the age, occupation and clinical history of the participants. Morning Mid-Stream Urine collection followed Kenya’s Ministry of health standard operating procedures for specimen collection, packaging (5). The specimens were collected aseptically using sterile wide mouthed and leak proof universal glass screw capped bottles (Human diagnostics- Kenya). The specimens were sent to the laboratory within 2 hours and cultured immediately.

The collected urine clinical sample was cultured in the Chromogenic agar media (Oxoid, UK) by plating 0.001ml of urine on the media using calibrated wire loop to deliver the needed volume (6). The cultured plates were then incubated for 18-24 hours and media with growth not prominent enough to allow for decision making regarding the identity of the bacteria isolates we re-incubated for another 24 hour and any suspect colony was taken for further identification. All samples which had a microbial load of 10^5 CFU/ml were considered to be positive for UTI Infections and taken as significant bacteurea (7). For the identification of each bacteria, subsequent biochemical and staining methods were used and recommended by the Cowan and steel’s scheme for the identification of medical bacteria (8). The isolates were preserved in the microbiology lab by using cryopreservation method.

The antibiotic susceptibility test method used was the Kirby Bauer disc diffusion method as modified by the clinical laboratory standards institute (9). Briefly: three well-isolated pure colonies were selected from the pure culture and transferred into a tube containing 4–5ml of sterile normal saline and mixed to form a turbid suspension. The turbidity of the suspension was adjusted to match 0.5 McFarland standards using standard guidelines. The adjusted turbid bacteria suspension were aseptically inoculated on to the surface of the Muller Hinton agar plates previously prepared according to manufacturer’s instructions, using the surface spreading method for a uniform surface distribution of inoculums (10). The standard antibiotic discs (Oxoid, UK, Human diagnostics Limited) were placed in the inoculated at the surface of the inoculated culture plates at a distance of 2 cm apart. The set up was incubated at 36 degree centigrade for a period of 18 hours to 24 hours. Re-incubation for another 24 hours was done where more growth was needed to make a definite decision while reading results. The diameter of Zones of inhibition were measured and recorded in millimeter. This was compared with the standard reference chart of CLSI as detailed in the previous study of Jorgensen and Turnidge (11).

Data Analysis
The data was analyzed using SPSS version 16 software and tables were used to explain the data generated from the software. The antibacterial activity was reported in terms of diameters of the zones of inhibition (mm). The data was presented as mean ± standard deviation (SD). Comparison of means of zones of inhibition was done using student t-test since there was more than one variable in consideration and values of (p<0.05) were regarded as significant.

RESULTS
This present study recruited 196 pregnant female HIV/AIDS female patients. As observed 20(10.2%) of the patients turned out with negative UTI. A total of 176(89.8%) of the patients had UTIs and the isolates per Hospital HIV/AIDS clinic were as follows; E. coli had a frequency of 9(47.3%) from ODH and 10 (52.7%) from KDH. Enterococcus species had a frequency 1 (16.6%) isolated from ODH and 3(3.4%) from KDH.Klebsiella species had a frequency of 4(100%) isolated from ODH,S saprophyticus had a frequency of 2 (40%) isolated from ODH and 3 (60%) isolated from KDH. Lastly, S. aureus had a frequency of 4(50%) isolated from ODH and 4 (50%) isolated from KDH.

Table 3 below shows the mean zone diameter for each microbial isolates in response to tested antibiotics. According to clinical laboratories standard institute guidelines, zone diameter of 20mm and above is sensitive (S) to tested antibiotics, 15-19.9mm is interpreted as sensitivity depends on dose (SDD) while 10-14.9mm and above is resistant (R). The sensitivity of E coli to gentamycin was clearly the best with respect to zone diameter followed by Enterococcus species and S saprophyticus. (30±0.58, 29±0.58 and 28.05 mm respectively. Nitro, Cipro, cephratrazine and gentamycine showed the overall highest sensitivities to tested Antitiotics (Table 3).This study observed that the isolates from the urine specimen showed some resistance to the antibiotics that were used for susceptibility testing. They were as follows; Cotrimoxazole (4%), Ampicillin (5%), Tetracycline (2%)
In this study, the most common bacteria isolated were Escherichia coli (9.7%). This is in agreement with previous studies on community acquired UTI (12, 13, 14). Urinary tract infections due to E. coli is a common finding in women and it is associated with microorganisms ascending from the urethral 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 Cotrimoxazole, which is the first line antimicrobial for treatment of uncomplicated UTI in Uganda (15). Similarly, low sensitivity to cotrimoxazole was recently demonstrated in India (16) and in Tanzania where sensitivity of E. coli to cotrimoxazole was as low as 35.3% (17).

The prevalence of significant bacteriuria/UTI in this study which was of 19.7% is of high concern compared to the Previous studies in Mulago which found the prevalence of significant bacteriuria to range between 6% in asymptomatic patients (256 men and 132 women) (18) and 18.7% (28/150) in diabetic patients attending Mulago hospital diabetic clinic(19).The previous study done Among pregnant women aged 15 – 44 years in Tanzania, prevalence of significant bacteriuria was found to be 17.9% and 13.0% in symptomatic and asymptomatic participants respectively(20). This was a higher proportion of UTI in the Tanzanian study since their study population was of pregnant women who had been more commonly diagnosed with UTI due to the hormonal changes of pregnancy and anatomical predispositions.

In Kenya, a hospital in Kenya, recent report (21) found that UTI prevalence was 11.1% amongst 135 asymptomatic diabetic patients (aged 17 – 74 years). However, in Ethiopia it was reported that prevalence of UTI was 39.5%, which is about 2 times higher than what was found in this study (22). The study by Moges et al included 70 patients and this could account in part for the difference in prevalence of UTI. They isolated more uropathogens in the age groups 1 – 4 years (38.5% isolation rate) and those 50 years and above (54.7% isolation rate). This study had only 9 patients (5.1%) in the age group 46 years and above and patients aged below 18 years were included but consent was sought for from the adult relatives. Worldwide, E. coli has been demonstrated as the most common uropathogen in females. Among the uropathogens species isolated in this study, Escherichia coli were the most frequent isolate accounting for 43%. This present study is comparable with other studies in Africa where E. coli was isolated in 40 – 46% of the participants (23-26).

The second most frequent bacteria were Staphylococcus aureus with a frequency of 8(4.0%). Staphylococcus aureus has in recent time been found as causative agent mainly in complicated UTI (27,28). The possible reason for this observation could be that the studied poor

**DISCUSSION**

In this study, the most common bacteria isolated were *Escherichia coli* (9.7%). This is in agreement with previous studies on community acquired UTI (12, 13, 14). Urinary tract infections due to *E. coli* is a common finding in women and it is associated with microorganisms ascending from the urethral 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 Cotrimoxazole, which is the first line antimicrobial for treatment of uncomplicated UTI in Uganda (15). Similarly, low sensitivity to cotrimoxazole was recently demonstrated in India (16) and in Tanzania where sensitivity of *E. coli* to cotrimoxazole was as low as 35.3% (17).

The prevalence of significant bacteriuria/UTI in this study which was of 19.7% is of high concern compared to the Previous studies in Mulago which found the prevalence of significant bacteriuria to range between 6% in asymptomatic patients (256 men and 132 women) (18) and 18.7% (28/150) in diabetic patients attending Mulago hospital diabetic clinic(19). The previous study done Among pregnant women aged 15 – 44 years in Tanzania, prevalence of significant bacteriuria was found to be 17.9% and 13.0% in symptomatic and asymptomatic participants respectively(20). This was a higher proportion of UTI in the Tanzanian study since their study population was of pregnant women who had been more commonly diagnosed with UTI due to the hormonal changes of pregnancy and anatomical predispositions.

In Kenya, a hospital in Kenya, recent report (21) found that UTI prevalence was 11.1% amongst 135 asymptomatic diabetic patients (aged 17 – 74 years). However, in Ethiopia it was reported that prevalence of UTI was 39.5%, which is about 2 times higher than what was found in this study (22). The study by Moges et al included 70 patients and this could account in part for the difference in prevalence of UTI. They isolated more uropathogens in the age groups 1 – 4 years (38.5% isolation rate) and those 50 years and above (54.7% isolation rate). This study had only 9 patients (5.1%) in the age group 46 years and above and patients aged below 18 years were included but consent was sought for from the adult relatives. Worldwide, *E. coli* has been demonstrated as the most common uropathogen in females. Among the uropathogens species isolated in this study, *Escherichia coli* were the most frequent isolate accounting for 43%. This present study is comparable with other studies in Africa where *E. coli* was isolated in 40 – 46% of the participants (23-26).

The second most frequent bacteria were *Staphylococcus aureus* with a frequency of 8(4.0%). *Staphylococcus aureus* has in recent time been found as causative agent mainly in complicated UTI (27,28). The possible reason for this observation could be that the studied poor pregnant women devastated by the HIV pandemic could have had contaminated their urinary tract with *Staphylococcus* from the skin where the organism is very common. The other isolates in this study included five *Enterococcus* species, four *S. saprophyticus* and four *Klebsiella* species. This is in agreement with previous studies in Mulago hospital Whereby *Enterococcus* species at 39.3% and 4.8% respectively. More recently in India, *Klebsiella*, Proteus, and *Enterobacter* were isolated at rates 16.9%, 5.5% and 5.3% respectively (29). Poor hygiene simply explains female urinary tract contamination by enterobacteria. Most local women after defecation clean from back to front thereby raising the chance of loading the urogenital system with stool samples heavily loaded with entero-bacteria (30).

In Table 4, this study demonstrated that Gentamycin was the single most efficacious antibiotic amongst those commonly used against all the strains of urogenital pathogens isolated, with sensitivity against *E. coli*, *S. aureus*, *S. saprophyticus*, *Enterococcus*, *Klebsiella* species and *Chromobacterium violaceum* in West Africa (31). The earlier studies between 2001 and 2003 in East Africa and Ethiopia agreed with this study with high sensitivities by most uropathogens to gentamycin. The second most effective antibiotic in this study was Ciprofloxacin, with high sensitivity against *E. coli*, *S. aureus*, *S. saprophyticus*, *Enterococcus*, and *Klebsiella*. In Africa however, earlier studies by Ouma and Moges showed very high sensitivity of uropathogens to ciprofloxacin, with over 90% sensitivity against all uropathogens isolated (32, 33). The population studied have low per capita income and may not afford the cost of quinolones and quinolones are used as second line of drugs after penicillins or cephalosporins in Pharmacies and hospitals. This makes quinolones unavailable and cost-prohibitive to the local users who merely use drugs purchased from drug stores in rural settings and invariably may impact the susceptibility to quinolones.

*Cotrimoxazole* has shown the lowest sensitivity rate against all 5 uropathogens in *vivo* in this study. In agreement with this study, sensitivity of Cotrimoxazole to all uropathogens isolated ranged from 33% to 67% in other studies in Africa (34-38). Similarly, low sensitivity (30%) of uropathogens to *Cotrimoxazole* was recently demonstrated in India (39). The possible explanation may be over-prescription of this drug as both broad spectrum antibiotic and as maintenance therapy in the management of HIV/AIDS patients in both urban and rural setting. In Uganda where there is no specific law restricting the prescription of antibiotic to qualified medical or Pharmaceutical personnel, prescription by drug dispensers and other unqualified health care providers and use of sub lethal dose is very common.

Sensitivities to ampicillin which is also commonly prescribed for treatment of UTI were also relatively low in this study and may signify to emergence or re-emergence of penicillin resistance to common uropathogens and warrants further in-diet laboratory investigation to assist in the control of resistance spread.

There is need to mention at this point that the observation of 78% of no bacteria growth may indicate that bacteria was rarely involved in *in Urogenital infections and underscores the need for laboratory investigation* as a criteria for commencement of treatment of clinically diagnosed UTI in pregnancy especially among HIV/AIDS pregnant patients. Inclusion of mean zones of inhibition seen in Table 3 above was necessary to outline the detailed activity of selected antimicrobials against urinary bacterial isolates in this settings with poor resources for minimum inhibitory concentration (MIC).

**Conclusion**
The most common uropathogens isolated in this present study was *Escherichia coli*, followed by *Staphylococcus aureus*, *Enterococcus species*, *S. saprophyticus* and *Klebsiella species*. Gentamycin was the most efficacious antibiotic to all the uropathogens isolated, whereas cotrimoxazole had a very low sensitivity profile for each of the uropathogens isolated. The high rate of resistance to tetracycline, cotrimoxazole and ampicillin, may preclude the use of these commonly used antibiotics for empiric treatment of UTI in Uganda. The high prevalence of asymptomatic UTI of 19.7% is of concern and an interventional study that follows up these women with significant bacteriuria for at least 14 to 28 days and as well evaluate outcome of treatment (clinical and bacteriological) is recommended.

Gentamycin which has shown high overall sensitivity against all the uropathogens isolated should be considered for use in empirical treatment of UTI in women while ciprofloxacin can be considered as second choice agent for treatment. The use of cotrimoxazole as first line agent for the treatment of community acquired UTI in Uganda may need to be revisited by the policy makers since it has shown very low sensitivity against all the uropathogens isolated in this study especially as regarding to HIV/AIDS seropositive patients.

**Acknowledgements**

We hereby wish to thank the participants of this study, Kenyanya District Hospital and Ogembo District Hospital management at large. This work was solely financed by the authors.

**Conflict/Disclosure of interests**

The authors hereby declare that there is no any competing interest in relation to this study.

**Table 3: Antibiotics susceptibility pattern of urinary isolates from study population (MEAN STANDARD Diameter of Zone of inhibition (mm))**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ODH number (%) positive</th>
<th>KDH number (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>Ent spp</td>
</tr>
<tr>
<td>Ampi</td>
<td>14±0.58</td>
<td>13±0.58</td>
</tr>
<tr>
<td>Cipro</td>
<td>25±0.58</td>
<td>24±0.58</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>27±0.58</td>
<td>21±0.58</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>17±0.58</td>
<td>17±0.58</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>19±0.58</td>
<td>19±0.58</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>21±0.58</td>
<td>21±0.58</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>14±0.58</td>
<td>14±0.58</td>
</tr>
<tr>
<td>Tetra</td>
<td>14±0.58</td>
<td>14±0.58</td>
</tr>
</tbody>
</table>

n=number sampled, ODH=Ogembo District Hospital, KDH=Kenyanya District Hospital. Ampi= Ampicillin, Cipro=Ciprofloxacine, Genta=Gentamycin, Ceftria=Ceftriazone, Erythro=Erythromycin, Na=acid=Nalidixic acid, Nitrof=Nitrofurantoin, Cotrim = Cotrimoxazole, Tetra=Tetracycline, E=Escherricia, Ent=Enterococcus, spp=species, Klieb=Klebsiella, S.=Staphylococcus, Sapro=Saprophyticus(p<0.05).

**Table 4: Antibiotics susceptibility pattern of urinary isolates from study population (MEAN STANDARD Diameter of zone of inhibition (mm))**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ODH number (%) positive</th>
<th>KDH number (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
<td>Ent spp</td>
</tr>
<tr>
<td>Ampi</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Cipro</td>
<td>8(8.9)</td>
<td>1(100)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>9(100.0)</td>
<td>1(100)</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>1(11.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1(11.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1(10.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>1(11.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Tetra</td>
<td>1(11.0)</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

n=number sampled, ODH=Ogembo District Hospital, KDH=Kenyanya District Hospital. Ampi= Ampicillin, Cipro=Ciprofloxacine, Genta=Gentamycin, Ceftria=Ceftriazone, Erythro=Erythromycin, Na=acid=Nalidixic acid, Nitrof=Nitrofurantoin, Cotrim = Cotrimoxazole, Tetra=Tetracycline, E=Escherricia, Ent=Enterococcus, spp=species, Klieb=Klebsiella, S.=Staphylococcus, Sapro=Saprophyticus(p<0.05).
References