Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda

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Abstract

Background: Urinary tract infections (UTIs) in women are a common problem in primary health care settings. Resistance of bacterial uropathogens to commonly used antibiotics is common in many places.

Objectives: To determine the prevalence of UTI, associated uropathogens and their antimicrobial susceptibility.

Methods: A cross section study carried out at Mulago hospital outpatients' department. Midstream urine samples (MSU) were collected from 399 women, who gave informed consent and fulfilled other study criteria. Quantitative culture method, identification of uropathogens and antibiotic susceptibility testing using the Kirby-Bauer disc diffusion technique were applied to the isolates.

Results: Out of 399 MSU samples, 40 pure significant bacterial growths ($\geq 10^5$ colony forming units (cfu)/ml of urine) were isolated and these included Escherichia coli, 23 (57.5%), Staphylococcus aureus, 9 (22.5%), Enterococci spp, 6 (15%) and Klebsiella pneumoniae, 2 (5.0%). Overall, sensitivities were: nitrofurantoin (98.3%), cefuroxime (89.3%), and cotrimoxazole (20%) by all uropathogens isolated.

Conclusions: Culture positive UTI among adult non-pregnant women are a common problem, occurring in 10% of the study population. Most bacterial uropathogens showed high sensitivity to nitrofurantoin but low sensitivity to SXT.

Recommendations: Nitrofurantoin should be considered as drug of choice for empirical treatment of community acquired uncomplicated UTI in adult non-pregnant women.

Key words: Bacteriuria, empiric therapy, E. coli, Uganda, urinary tract infections.

African Health Sciences 2011; 11(2): 182 - 189

Introduction

Urinary tract infections describe both microbial colonization of urine and tissue invasion of any structure of the urinary tract[1]. Urinary tract infections are "uncomplicated" when they occur in a normal urinary tract with no structural, functional or underlying host illness to account for the infection, or "complicated" when an underlying abnormality is thought to have enabled the infection to occur[2-4].

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 (E.coli)* [5,6]. For the past two decades, trimethoprim-sulfametroxazole (SXT) or trimethoprim alone have been used widely 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[6].

World wide, *Escherichia coli* cause 75% - 90% of acute uncomplicated cystitis while *Staphylococcus saprophyticus* accounts for 5% to 15%, mainly in younger women[7-9].

Enterococcus spp 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[3,9-11]. In Uganda, we have not found any published studies that have described common uropathogens and their pattern of antibiotic sensitivities in the recent two decades and yet studies elsewhere have shown increasing resistance to commonly used antibiotics in treatment of UTI. A study done in 1963 found a prevalence of symptomless bacteriuria among in-patients in Uganda

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to be 8% in women and 6% in men on medical wards. Out of these, 41% were due to *E.coli*[12].

This study has established the current state of significant bacteriuria, prevalence of the various bacterial uropathogens and their sensitivities to commonly prescribed antimicrobials in patients attending out patient clinics of Mulago hospital. This knowledge will help guide clinicians' prescriptions and ultimately reduce cost of health care.

Methods

This study was conducted at Mulago hospital general out patient clinic where first contact is made by all new and many follow-up patients not attending any specialised clinics of Mulago hospital.

We included all female non-pregnant patients aged 18 years and above, presenting to Mulago hospital assessment centre with various symptoms/complaints and who provided signed informed consent to participate in the study. Exclusion criteria included pregnancy, puerperium, any vaginal bleeding at the time of study, long-term (e"14days) catheterization ending in the last 30 days prior to study, history of recent urologic or gynaecological surgery e.g. uterine/rectal prolapse repair, vaginal hysterectomy in the last 3 years and women with overt vaginal/uterine prolapse on history.

Three hundred ninety nine (399) non-pregnant women were recruited by systematic random sampling, where every fifth adult female patient on the waiting line of the attending clinicians was selected. Patients were selected without consideration to their symptomatology, in particular, irrespective of whether or not they had symptoms suggestive of UTI.

Direct measurements of diameters of zones of inhibition of bacterial growths on agar plates to 8 antibiotics were done with a calibrated ruler. The Clinical Laboratory Standards Institute (CLSI) break points were used for interpretation of susceptibility patterns as sensitive or resistant.

HIV serology testing was done following standard procedures used in the hospital after appropriate counselling of the participants. Patients found to be HIV positive were referred to the HIV/AIDS clinic of Mulago hospital for care. Pregnancy test using the "One-Step Dipstick" method was used to check for urinary *â*-hCG in all urine samples.

We collected 399 midstream urine samples from 399 study participants using a standard procedure. The procedure included; each participant was provided with 8 pieces of sterile gauze swabs – 4 soaked in green soap (Norosept/Norbrook; isopropyl alcohol 2.84%, chlorhexidine gluconate 0.3%, cetrimide 3%), and another 4 – soaked in saline. To collect the urine sample, every woman was requested to sit and set aside the legs, spread apart the vulvae with the left index and middle fingers, cleaned the vulvae from front to back with the sterile gauzes provided, starting with the ones soaked in green soap and then followed by the saline ones, 4 times before starting to pass urine. Midway during voiding, without stopping the urinary stream, she plunged with the right hand a sterile wide mouth screw cap plastic bottle to collect about 30 mls of MSU, and soon thereafter tightly close the bottles and hand them over to the study nurse/research assistant.

The sample size of 399 was arrived at by use of survey formula by Kish Leslie (1965);
$n = z^2 p (1-p)/d^2$. Where: z = Z score for 95% confidence interval = 1.96, p = prevalence, d = acceptable error (5%). We used the prevalence of significant bacteriuria, proportion of *E.coli* isolated and resistance proportion of *E.coli* to SXT, and obtained sample sizes of 234, 366 and 305 respectively using ratios in the study of Ouma and others, 2001[13]. We intended to collect 400 urine samples for adequate power. One questionnaire was incomplete and not included for analysis.

The urine samples were placed in a cool box and transported to the medical school microbiology laboratory within 2 – 4 hours of collection. The medical school laboratory is about 200 meters away from the assessment centre. All urine samples were turned up and down gently to allow proper mixing of urine and were analyzed immediately after they had been entered in a log book on arrival at the laboratory.

We used a standard calibrated wire loop with 4 mm internal diameter to remove 10ìl of urine for inoculation onto Cystine Lactose Electrolyte Deficient (CLED) and MacConkey agar with crystal violet and 5% sheep blood agar.

The streak method was used to uniformly spread the urine onto the agar surface before aerobically incubating the plates at 37^0C for 18 - 24 hours. We used CLED because it gives consistent results and allows the growth of both gram negative and gram positive bacterial pathogens, and as well prevent the swarming of *Proteus species*[5]. Colonies were counted after 18 - 24 hrs of incubation.

Plates with pure growth, and colonies $\geq 10^4$ CFU/ml were further subjected for identification and sensitivity testing. Cut off point of $\geq 10^5$ CFU / ml was used to define urinary tract infections (UTI). Identification of the microorganisms was done following standard procedures, with use of biochemical tests which included triple sugar iron agar, Simmon's citrate agar, lysine decarboxylase, urease, and motility tests[5], and where these could not give conclusive results, API20E and/or APINE20 (Biomeriuex, France) were used.

Sensitivity tests were done using the Mueller – Hinton-2- agar, following the commercial disc diffusion techniques of Kirby – Bauer, against ampicillin (10mcg), sulfamethoxazole-trimethoprim (25mcg and 125mcg), gentamycin (10mcg), ciprofloxacin (5mcg), nitrofurantoin (300mcg), cefuroxime (30mcg), nalidixic acid (30mcg), amoxicillin-clavulanic acid (20/10 mcg)[14].

One to 5 colonies from every plate was picked with a standard wire loop from a purity plate, and emulsified into 5 mls of sterile saline in a test tube. The saline was stirred with the loop to uniformly mix the colony in the saline. The turbidity of the saline was adjusted to match the standard McFarland 0.5, Biomerieux ®.

A sterile cotton swab on a stick was dipped in the colony-saline mixture, excess saline was squeezed out by pressing the swab against the test tube, and then the cotton swab gently applied onto the surface of the Mueller-Hinton-2- agar.

Six antibiotic impregnated discs were gently placed on the agar surface, at a minimum distant of 25 mm from each other, and the plates incubated at 37^0C aerobically for 18 - 24 hours. The zones of inhibition diameters around each disc were measured using a ruler, and compared against the zone diameter interpretative standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS/CLSI), 2003[5]. Results were reported as sensitive or resistant, for each antibiotic used.

Data entry was done on excel spread sheet, and then exported to GenStat (general statistical analysis tool) software for analysis. Chi square test was used for comparisons between significant and non-significant bacteriuria, as well as to demonstrate associations between categorical variables. P value of 0.05 was used to determine level of statistical significance.

Institutional permission to perform this study was obtained from the Department of Medicine, Mulago Hospital Research and Ethics Committee, and the Research and Ethics Committee of Faculty of Medicine, Makerere University, and the Uganda National Council of Science and Technology. Written, informed consents were obtained from every study participants.

Results

The study participants

The mean age (± standard deviation, SD) was 32 ± 12 years, median, 28 years and range of 18-72 years. Most patients, 210 (52.6 %) were young, in the age group 18 – 28 years.

Most of the patients, 355 (89.0%) were from the central region districts of Kampala and Wakiso. The study participants were mainly housewives, 109 (28%), self-employed 101 (26%) and peasant farmers 67 (17%).

Prevalence of significant bacteriuria/urinary tract infections (UTI)

Of the 399 midstream urine samples cultured on CLED, 10% (40/399) of samples showed pure significant growth of bacteria, 50.9% non-significant bacteriuria while 39.1% showed no bacterial growth after 72 hours of incubation.

Presenting symptoms among patients with significant bacteriuria

Only 27.5 % (11/40) of the 40 patients with significant bacteriuria had symptoms suggestive of lower urinary tract infections such as dysuria and frequency.

These symptoms were not significantly associated with presence of significant bacteriuria on culture (p = 0.853).

Other common symptoms among the study patients

Lower abdominal pain, 246 (61.7%) and abnormal vaginal discharges, 186 (46.6%) in various combinations were other common symptoms. Among the patients with significant bacteriuria, 60% (24/40) had history of fever, although there was no significant association between fever and significant bacteriuria (p= 0.249).

HIV status and significant bacteriuria

Of the eighty two patients (20.6%) who tested positive for HIV1/2, 8.5% (7/82) had significant bacteriuria/UTI and this association was not statistically significant (p = 0.918).

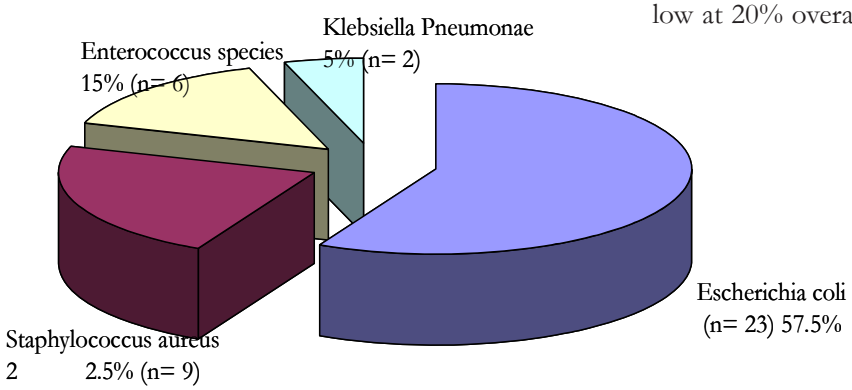
Random blood sugar and significant bacteriuria

Of the 40 patients with significant bacteriuria, 5 (12.5%) had high random blood sugar ranging between 124 – 200 mg/dl. There was no patient with RBS above 200mg/dl.

Uropathogens isolated by culture

Four bacterial uropathogens were isolated from 40 midstream urine samples. Of these, up to 23 (57.5%) had *Escherichia coli,* while others included *Staphylococcus aureus, nterococcus species* and *Klebsiella pneumonae* (Figure 1).

Figure 1: Bacterial uropathogens isolated from 40 pure significant growths



The nine patients with pure significant growth of *Staphylococcus aureus* had characteristic features suggesting systemic and local infections of the urinary tract. Six of these patients (66.7%) reported history of fever at presentation, while 8 patients (88.9%) had positive leucocytes esterase tests.

Antimicrobial sensitivity test profiles

Escherichia coli, the commonest uropathogen isolated showed generally high sensitivity to nitrofurantoin, cefuroxime and ciprofloxacin, at 100%, 85.7% and 66.7% respectively. Most bacteria were more than 50% sensitive to nitrofurantoin and cefuroxime. However, the sensitivities of most of the bacteria to sulfamethoxaole-trimethoprim (SXT) were very low at 20% overall (Table 1).

Table 1: Sensitivity results of isolated uropathogens

Characteristics	Proportions of organisms isolated from pure culture growth of midstream urine samples									
Drugs sensitivity	*Escherichia coli*;		*Staphylococcus aureus;* n/N		*Enterococcus species*; n/N		*Klebsiella*		All *species;*	
organisms	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Nitrofurantoin	28/28	(100)	19/19	(100)	8/8	(100)	2/3	(66.7)	57/58	(98.3)
Cefuroxime	24/28	(85.7)	17/18	(94.4)	0/8	(00.0)	3/3	(100)	51/57	(89.5)
Amoxyclav	10//28	(35.7)	19/19	(100)	8/8	(100)	1/3	(33.3)	38/58	(65.5)
Gentamycin	18/27	(66.7)	13/19	(68.4)	3/8	(37.5)	1/3	(33.3)	35/57	(61.4)
Ciprofloxacin	18/27	(66.7)	13/19	(68.4)	1/8	(12.5)	2/3	(66.7)	34/57	(59.6)
Nalidixic acid	17/28	(60.7)	9/19	(47.4)	0/8	(0)	2/3	(66.7)	28/58	(48.3)
Ampicillin	5/28	(17.9)	11/19	(57.9)	7/8	(87.5)	0/3	(0)	23/58	(39.7)
Sulfamethoxazole-trimethoprim	3/28	(10.7)	6/17	(35.3)	2/7	(28.6)	0/3	(0)	11/55	(20)

Discussion

In this study, the most common bacteria isolated were *Escherichia coli* (57.5%). This is in agreement with most previous studies on community acquired UTI [3,5,11]. Urinary tract infections due to *E.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 sulfamethoxazole- trimethoprim (SXT), which is the first line antimicrobial for treatment of uncomplicated UTI in Uganda[16].

Similarly, low sensitivity to SXT was recently demonstrated in India[15] and in Tanzania where sensitivity of *E.Coli* to SXT was as low 35.3%[17].

Prevalence of significant bacteriuria

The prevalence of significant bacteriuria/UTI in this study of 10% (40/399) is of high concern especially since most of the patients (72.5%) did not have complaints suggestive of UTI. This means that clinicians may miss these cases of asymptomatic bacteriuria/UTI and complications may ensue in women. Previous studies in Mulago hospital found prevalence of significant bacteriuria to range between 6% in asymptomatic patients (256 men and 132 women)[12] and 18.7% (28/150) in diabetic patients attending Mulago hospital diabetic clinic[13]. Among pregnant women aged 15 -44 years in Tanzania, prevalence of significant bacteriuria was found be 17.9% and 13.0% in symptomatic and asymptomatic participants respectively[17]. The higher proportion of UTI in the Tanzanian study is not surprising since their study population were of pregnant women who been more commonly diagnosed with UTI due to the hormonal changes of pregnancy and anatomical predispositions.

Published studies that assessed prevalence of significant bacteriuria/UTI in women in the general out patient population are hard to find.

In Kenyatta hospital in Kenya, Kayima et al, 1996[18] found UTI prevalence of 11.1% amongst 135 asymptomatic diabetic patients (aged 17 - 74 years) at their medical out patient clinic. This proportion is similar to our finding, despite the differences in the population studied.

However, in Ethiopia, Moges et al, 2002[19] found prevalence of UTI of 39.5%, which is about 3 times higher than what we have found. The study by Moges and others included 70 in-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). We had only 86 patients (21.6%) in the age group 40 years and above (3.5% isolation rate) and we did not include patients aged below 18 years in this study to avoid difficulty with attaining consent.

Presenting symptoms and significant bacteriuria

In the general study population, symptoms that are associated with UTI at presentation were lower abdominal pain (61.7%), fever (50.4%), urinary frequency (38.6%), dysuria, (37.8%) and urgency (42.9%) in various combinations. These symptoms were not significantly associated with significant bacteriuria ($p > 0.05$).

Among the 40 patients with significant bacteriuria, only 27.5% (11/40) had symptoms of dysuria and frequency of micturition which suggest lower urinary tract infections. Although fever was a frequent symptom (24/40) among the patients with significant bacteriuria, there was no significance ($p = 0.249$).

The association between significant bacteriuria and symptoms suggestive of either lower or upper UTI has been found to be poor in many other studies. Stamm and colleagues in 1980 found that in the 181 women with symptoms of dysuria and frequency, only 102 (56.4%) had significant bacteriuria (e" 10^5 cfu / ml of MSU) [20].

In Uganda, Ouma et al, 2001 found that only 5 (19.2%) of the 28 patients with significant bacteriuria had symptoms referable to the urinary tract[13].

The lack of significant association between significant bacteriuria and symptoms that would otherwise suggest UTI in this study is in agreement with studies done elsewhere in various populations.

Microoganisms isolated from significant growths.

World wide, *E.coli* has been demonstrated as the most common uropathogen in women.

Of four uropathogens species isolated in this study, *Escherichia coli* was the most frequent isolate accounting for 57.5%. This is comparable with other studies in Africa where *E. Coli* was isolated in 40 – 46%[11,18,19,21]. A recent study in India similarly isolated high proportion of *E. Coli* [15].

The second most frequent bacteria were *Staphylococcus aureus* with 22.5 % isolation rate. The isolation of *S.aureus* as a uropathogen is not unique to our study. Earlier studies in Mulago hospital in 2001 and 2003 isolated similarly high rates of *S.aureus* of 17.1% and 16.7% respectively[11,13]. However, this finding is in variant with an Indian study with similar population of study where *S.aureus* were not isolated from 531 urine samples analyzed[15].

Staphylococcus aureus has in recent time been found as causative agent mainly in complicated UTI[22-24]. Of the 9 patients with *S.aureus* significant bacteriuria in this study, six presented with history of fever while eight had positive leukocytes esterase tests. It is probable that these patients had *S.aureus* bacteraemia,

with seeding to the urinary tract. Similarly high isolation rates of *S.aureus* were demonstrated elsewhere in Africa[18,19,25].

The other isolates in this study included six *Enterococcus species* and two *Klebsiella pneumoniae*. This is in agreement with previous studies in Mulago hospital by Ouma et al 2001 and Wanyama et al, 2003 who isolated *Enterococcus species* at 39.3% and 4.8% respectively[13,19]. More recently in India, *Klebsiella, Proteus,* and *Enterobacter* were isolated at rates 16.9%, 5.5% and 5.3% respectively[15].

HIV Status and significant bacteriuria

In this study, the prevalence of HIV/AIDS (20.6%) among the study population was higher than the Uganda national rates (6.9%) and Kampala / central region of 9%[26]. Our study population was of sick patients who had come for health care thus the higher HIV prevalence is not unexpected. In addition, the predominantly sexually transmitted HIV itself may have been a risk factor for getting the UTI. Sexual intercourse has been associated with getting UTI in women.

The prevalence of HIV/AIDS among patients with significant bacteriuria was 17.5% but this association was not statistically significant (p = 0.918).

Few published studies have assessed prevalence and aetiology of bacteriuira among HIV/AIDS patients. A study in Jos Nigeria by Jombo et al 2005[27], found a significantly higher prevalence of UTI in the AIDS patients (48/200) compared to the control non-AIDS patients (21/200) (p =0.0026).

Antimicrobial sensitivity profiles of the uropathogens.

This study has demonstrated that *in vitro*, nitrofurantoin is the single most efficacious antibiotic amongst those commonly used in Mulago hospital against all the strains of uropathogens isolated, with sensitivity rate as high as 100% against *E. coli, S. aureus* and *Enterococcal spp* albeit lower (66.7%) for *Klebsiella pneumoniae*. This compares well with earlier studies in Africa[11,13,14,17,19,21)]. The efficacy of nitrofurantoin in treatment of upper and /or complicated UTI may not correlate with this high *in vitro* sensitivity pattern since its concentration in tissues and blood are low as this drug is filtered as well as secreted into urine. The drug may only be good for treatment of lower urinary tract infections.

The second most effective antibiotic in this study is cefuroxime, with over 80% sensitivity against *E.coli, S.aureus* and *Klebsiella pneumonae*, a pattern similar to other studies in Africa[11,13,18]. Cefuroxime is however a relatively expensive drug and unaffordable by most Ugandans.

The more widely available and commonly prescribed ciprofloxacin has demonstrated a rather low *in vitro* sensitivity of less than 70% for all the isolated uropathogens. Sensitivity to ciprofloxacin was recently shown to be only 35.8% in India[15].

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[13,19]. Over the counter use of ciprofloxacin in Uganda has probably led to such low degree of sensitivity of uropathogens to this drug. Resting such antibiotic from use by making it not available on the market and/or restricting its use may allow it to recover its potency.

Sulfamethoxazole-trimethoprim (SXT) has shown the lowest sensitivity rate against all 4 uropathogens with less than 40% *in vitro* effectiveness in this study.

In agreement with this study, sensitivity of SXT to all uropathogens isolated ranged from 33% to 67% in other studies in Africa[11,13,16,18,19]. Similarly, low sensitivity (30%) of uropathogens to SXT was recently demonstrated in India[15]. The widespread use of this drug at otherwise subtherapeutic antimicrobial dose in the prophylaxis of *Pneumocystis jeroveci* pneumonia (PCP) in Uganda and elsewhere could have contributed immensely to the low sensitivity of uropathogens to this drug.

Sensitivities to gentamycin and ampicillin which are also commonly prescribed for treatment of UTI are also relatively low, with overall sensitivities of only 61.4% and 40% respectively in this study. Studies done in Kenya and Ethiopia showed similar trends as well[18,19].

However, the earlier studies between 2001 and 2003 in East Africa and Ethiopia showed much higher sensitivities by most uropathogens to ampicillin and gentamycin than in this study[11, 13,18].

Conclusions

The prevalence of significant bacteriuria / UTI among the women aged 18 years or more attending Mulago hospital general outpatient was at 10%, and majority of these (72.5%) did not have symptoms of dysuria and frequency suggesting UTI.

The most common uropathogen isolated was *Escherichia coli*, followed by *Staphylococcus aureus*, *Enterococcus species* and *Klebsiella species*.
Nitrofurantoin was the most efficacious antibiotic to all the uropathogens isolated, whereas SXT had a very low sensitivity profile of less than 40% for each of the uropathogens isolated. The high rate of resistance to ciprofloxacin, SXT and ampicillin, may preclude the use of these commonly used antibiotics for empiric treatment of UTI in Uganda.

Recommendations

The high prevalence of asymptomatic UTI of 72.5% is of concern and an interventional study that follows up this women with significant bacteriuria for at least 14 to 28 days and as well evaluate outcome of treatment (clinical and bacteriological) is recommended. Nitrofurantoin which has shown high overall sensitivity against all the uropathogens isolated should be considered for use in empirical treatment of UTI in women while cefuroxime can be considered as second choice agent for treatment of cases of upper and/or complicated UTI.

The use of SXT 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.

Study Limitations

This study had some limitations such as inability to differentiate lower from upper urinary tract infections, establish risk factors for UTI such as minor urinary tract abnormalities (anatomical and functional), sickle cell disease, recurrent UTI, sexual behaviours, use of diaphragm, use of spermicides and chronic use of antibiotics. Majorly this was due to inadequate resources to demonstrate these risk factors but also due to the sensitivity of issues of sexual behaviours and lifestyles such as anal sex, use of spermicides/diaphragms/condoms, number of sexual partners at a time, new or usual partner.

Due to financial constraints no attempts could be made to study the presence of Chlamydia, viruses and tuberculosis which could actually account for some cases of UTI. It is noteworthy that this was an *in vitro* study and the antimicrobial response may not reflect *in vivo* response since host factors that contribute to antibiotic efficacy and final eradication of pathogens have not been factored into the sensitivity outcome.

Acknowledgements

The authors would like to thank the patients and staff of Mulago hospital assessment centre whose active participations have made this study possible. We are appreciative to Mr. Lapaka T. Odong of Faculty of Agriculture, Makerere University, for his statistical expertise during data entry and analysis. Finally, we thank the Belgian Technical Cooperation (BTC), Kampala Office for the financial contribution towards this research.

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